THE CLINICAL PHARMACOLOGY OF NOVEL ANTITHROMBOTIC COMPOUNDS

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voor Iris

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SECTION I

GENERAL INTRODUCTION

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CHAPTER 1

INTRODUCTION

Haemorrhage, the loss of the vital fluid blood, is a potential threat to life. Under normal circumstances, this is averted by the system of blood coagulation. This is a dynamic process occurring to a limited degree physiologically throughout the overall circulation, as evidenced by circulating activation peptides associated with the coagulation proteins. The coagulation system becomes activated with great efficacy in response to vascular damage and involves a large number of plasma proteins and multiple cell-cell and cell-matrix interactions. Owing to the complexity and the biological potential of the blood coagulation system, strict regulation is required in order to avoid uncontrolled clotting or bleeding. Several important control mechanisms have been unravelled. Coagulation and fibrinolysis exist in a balanced equilibrium [1,2]. Despite control mechanisms, disruption of this balance between clot formation and dissolution, in order to achieve haemostasis while preventing either excessive thrombosis or bleeding, may develop. This results in a spectrum of diseases, many of which are life threatening and often fatal. On one extreme, if essential components of the coagulation system are deficient or dysfunctional, clot formation is inadequate and (further) bleeding ensues, leading to conditions in which mucous membrane or joint space bleeding is common and dangerous. On the other extreme, in favour of coagulation, lies excessive thrombosis, resulting in blockage of blood vessels and eventual ischaemia of tissues [3]. In between these extremes are patients who have mild disease and develop clotting or bleeding problems only after some inciting event.

COAGULATION

Haemostasis is a physiologic mechanism that maintains blood within the circulation. The body achieves haemostasis by forming a clot when blood vessel injury occurs, stopping propagation of

the clot at the right time, and then dissolving the clot when the vessel has healed. This is due to interplay between a series of biochemical reactions. The constituents of this coagulation cascade are plasma proteins, with the exception of tissue factor, which is an integral membrane protein expressed on non-vascular cells. Physiologically, most of these proteins are enzymes in an inactive form. Once activated, these enzymes are proteases, and hence cleave other specific protein(s) in the cascade. The complex interactions between blood cells, specific soluble plasma proteins and vascular surface maintain the fluidity of blood.
Damage to the endothelial wall exposes tissue underlining to blood components, leading to an explosive reaction. The blood-coagulation cascade has the ability to transduce a small initiating stimulus into a large fibrin clot rapidly terminating blood loss and initiating the healing process. The ultimate extent of the coagulation reaction depends on the amount of activated membrane that can be provided by the damaged tissue and the aggregated platelets that are accumulated in the region of the wound. The coagulation reaction is terminated by a collection of stoichiometric and enzymatic reactions to yield an ultimate system in which procoagulant complexes are destroyed and the residual enzymes inhibited. This occurs by means of a dynamic regulation system. Under physiologic conditions, clot formation will only occur in the region of vascular damage where the membranes are available for procoagulant complex formation.

THE CASCADE

In the past two distinct pathways of blood coagulation were recognised, an intrinsic and an extrinsic pathway, respectively. These pathways were thought to proceed through a series of ordered steps in which inactive zymogens are transformed into active enzymes [4, 5]. As such, the classic cascade has traditionally been taught in textbooks. The intrinsic pathway occurs by physical chemical activation and the extrinsic pathway is activated by tissue factor released from damaged cells. Both pathways are thought to be activated simultaneously to initiate and sustain clot formation (Figure 1).

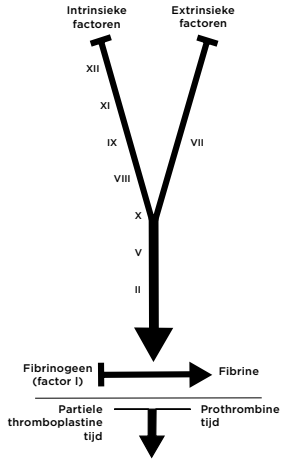


FIGURE 1 **Vascular injury initiates the margination of platelets to the site of injury.**

The coagulation cascade requires the presence of phospholipids, which are provided by platelets, i.e. much of the coagulation cascade occurs on the surface of platelets. Initially, platelets adhere to the damaged endothelial cells via von Willebrand Factor and aggregate to surround the site of vascular damage. In addition, damaged endothelial cells also contribute phospholipids. At this same time, blood is exposed to TF. It is upon this mass of perivascular cell tissue, damaged endothelial cells, and platelet membranes that the coagulation reactions occur and propagate the generation of thrombin; figure from [6].

The so-called Intrinsic Pathway begins with trauma to the blood vessel, exposure of blood to collagen in a damaged vascular wall, or exposure of the blood to a wetable surface such as glass. In the so-called Extrinsic Pathway, the initial step is a traumatised vascular wall or extravascular tissue. Non-vascular tissue cells contain an integral membrane protein called TF. Damage to the vessel wall or extravascular tissue exposes the plasma to TF. Factor VII is a circulating plasma protein that then binds to TF, creating a complex. In doing so, Factor VII is activated to Factor VIIa. This complex, in the presence of Ca^{2+} and phospholipids, activates Factor X to Factor Xa.

In the Combined Pathway (the outcome of both the intrinsic and extrinsic pathway), the inactive molecule prothrombin is converted to the active thrombin by activated Factor X, requiring

the 'prothrombinase complex'. Thrombin cleaves fibrinogen to fibrin, which then polymerises to form fibrin strands.

The current hypothesis is slightly different from the traditional waterfall/cascade hypothesis. The extrinsic pathway is considered the initiator of events. Under normal circumstances, blood and its constituents are not exposed to TF. When either blood vessel or tissue injury occurs, the plasma becomes exposed to TF (only present in non-endothelial cells) initiating coagulation [7]. It is felt that the extrinsic 'pathway' is paramount in generation of enough thrombin to initiate coagulation and that this thrombin then activates the intrinsic 'pathway', which in turn maintains coagulation [8].

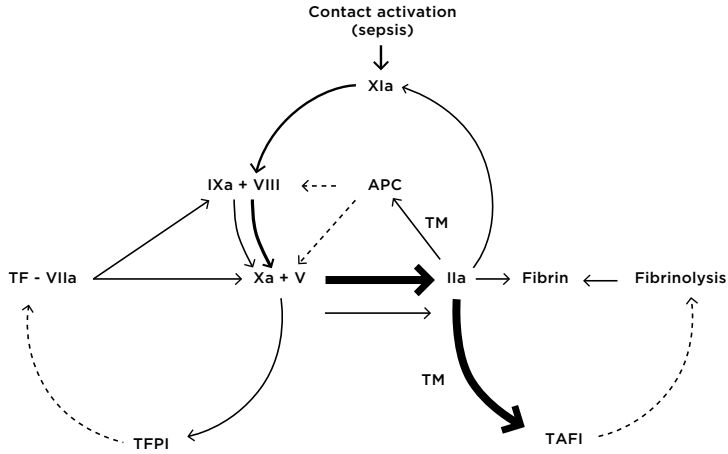


FIGURE 2 **Graphical representation of a revised model for blood coagulation, without the distinction between intrinsic and extrinsic pathways; figure from [8].**

Regulation

The regulatory mechanisms of the coagulation cascade serve two main functions. Limit the amount of fibrin clot formed to avoid ischaemia of tissues. Localise clot formation to the site of tissue or vessel injury, thereby preventing widespread thrombosis.

Physiologically, coagulation is a continuous process in which the anticoagulants are in excess to the procoagulants in blood. The improper functioning of these coagulation regulators can cause thromboembolic disease. The main regulators are Tissue Factor Pathway Inhibitor (TFPI), Antithrombin (AT, formerly known as AT-III), activated Protein C (APC) and Protein S, Thrombomodulin (TM) and the fibrinolytic system (Figure 2). To summarise, the net effect of all the coagulation reactions is that the wound site is plugged with a platelet-fibrin clot and the overall reaction is terminated. The clot represents a temporary barrier to blood flow and must be replaced by reconstitution of the vascular architecture via cellular proliferation and regeneration of the connective tissue of the intima.

THROMBOSIS

Venous thromboembolism (VTE), which includes deep vein thrombosis (DVT) and pulmonary embolism (PE), is a common clinical problem. It is the result of a delicate interplay between three pro-coagulative factors: the so-called Virchow's triad [9]. Alterations in the elements of this triad are major contributors to (venous) thrombosis, depending on specific risk factors that are present in a given patient [10]. Thrombosis is a multicausal disease; hypercoagulable states (congenital and acquired) arise when there is an imbalance between the anticoagulant and prothrombotic activities of plasma in which the prothrombotic activities predominate. These factors may act independently of each other or in concert [11,12].

Antithrombotic Compounds

The primary aim of VTE-treatment is to prevent recurrent thrombotic events at a minimal risk of bleeding [13]. All currently available antithrombotic therapies with either anticoagulants or platelet-active drugs are prophylactic, since these agents interrupt progression of the thrombotic process, but (unlike thrombolytic agents) do not actively resolve it [14,15].
Until recently, unfractionated heparin, vitamin K-antagonists (i.e. coumarins) and aspirin were the only anticoagulant agents

in wide use. The antithrombotic properties of these drugs were identified before their mechanisms of actions were fully defined [16,17,18] and the indications, optimal dose and duration of treatment for these agents were uncertain. The principal reason for this uncertainty was the lack of evidence-based data, because these antithrombotic agents were introduced before the well-designed clinical trial became the accepted standard for therapeutic decision making [19,16]. For instance, only in the 1990s it became clear that that the use of UFH in the initial treatment of venous thromboembolism (VTE) is necessary to prevent pulmonary embolism (PE) and recurrent thrombosis [20]. Nevertheless, in general, clinicians were comfortable with the use of these compounds.

Heparin

More than 80 years after its discovery the sulphated polysaccharide heparin remains an important tool in clinical practice, and has been the most widely used anticoagulant drug over the past 40-50 years. It functions in life as a component of the heparans that line the inner walls of the microvascular system, but unfractionated heparin (UFH), as a drug is a heterogeneous extract, from either porcine or bovine sources, administered by injection to circulate in the bloodstream.
The anticoagulant effect of UFH is mediated largely through its interaction with the plasma proteinase inhibitor AT. Binding and activation of AT by heparin-like GAGs depends on a specific pentasaccharide fragment [21]. For the inhibition of thrombin, UFH must form a bridge between thrombin and AT. It has been shown that molecules of UFH with fewer than 18 saccharide-units are unable to bind thrombin and AT simultaneously and, as a result cannot catalyse thrombin inhibition. For the inhibition of factor Xa this bridging is not necessary and UFH fragments with smaller numbers of saccharide units are capable of catalysing the inhibition of factor Xa by AT, providing the high-affinity pentasaccharide sequence is present [22].

ANTITHROMBIN

Antithrombin (AT, formerly known as AT-III) is a protein synthesised by liver and endothelial cells. It is a member of the serpin (serine proteinase inhibitor) superfamily of proteins. It is a single chain glycoprotein with a molecular weight of approximately 58,000 Da. The normal human plasma level is about 2-3 μM. The half-life of the AT in plasma is about 70 hours. [23]. Two isoforms of AT exist: AT-α has four glycosylation sites, whereas AT-ß, which constitutes 10% of plasma AT, lacks one of these glycosylations sites, resulting in the ß-isoform having a naturally higher affinity for heparin [24]. AT is an important physiological inhibitor of coagulation factors in plasma [25,26]. The uncatalysed reaction between the serine proteases and AT is relatively slow. The serine proteases still have time to generate thrombin and fibrin before becoming inactivated. However, in the presence of particular sulphated heparin-like GAGs, the reaction between AT and the serine proteases is virtually instantaneous [27]. For instance, the binding of UFH to AT causes a conformational change in AT that accelerates its ability to inactivate the coagulation enzymes thrombin and factor Xa by about 1000 times [28].

TABLE 1 Serine proteases inhibited by AT*

COAGULATION	FIBRINOLYSIS	KININ SYSTEM
Factor XIIa	Plasmin	Kallikrein
Factor Xia		
Factor Ixa		
Factor VIIa		
Factor Xa		
Thrombin		

*AT also inhibits activated enzymes involved in fibrinolysis and the kinin system

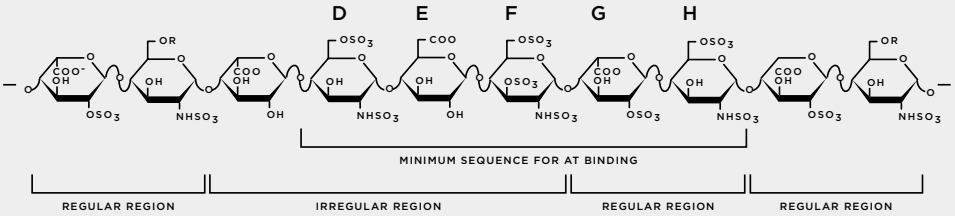


FIGURE 3 **Especially the sulphate groups in the heparin pentasaccharide are critical for tight binding to AT.**
It is the pentasaccharide sequence that confers the molecular affinity for AT [29]. Whereas the AT conformational change is necessary and sufficient for the inhibitor to accelerate the inactivation of factor Xa, it is not sufficient for accelerated thrombin inhibition. The latter acceleration additionally requires a longer heparin chain to bridge the proteinase and the inhibitor in a ternary complex.

Next to its mode of action via AT, heparin can inactivate thrombin by binding to HC-II, although much higher doses of UFH are required. And it releases TFPI from endothelium, which also contribute to its antithrombotic effect. It is believed that heparin primarily acts through interactions with these endogenous cofactors, however, many additional factors may be responsible for the anticoagulant and antithrombotic effects of this compound. Much remains uncertain about UFH: particularly the non-anticoagulant properties are poorly understood [30,31]. Heparin has pharmacokinetical, biophysical and biological limitations [32,33]. This has the following implications for its clinical use: UFH has a poor subcutaneous bioavailability (especially at low doses), an unpredictable dose response, a relatively short plasma half-life, a need for close laboratory monitoring, and a narrow benefit/risk ratio [34]. Therefore, continuous administration of UFH by the intravenous route is the current standard practice. And treatment or prophylactic administration of UFH requires hospitalisation, which limits the mobility of the patients, exposes them to the risks of hospital-acquired infections and increases management costs [35,36]

HEPARINS

The anticoagulant response to a standard dose of UFH varies widely between patients, because UFH is heterogeneous with respect to molecular size, anticoagulant activity, and pharmacokinetic properties.

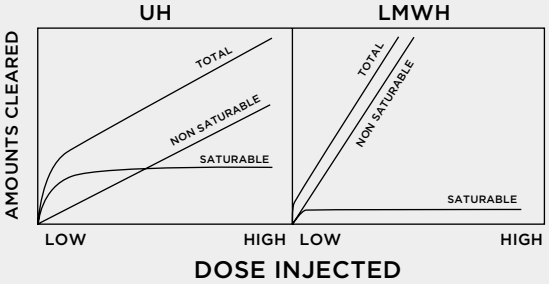
Heparin represents a heterogeneous mixture of polysaccharides and oligosaccharides with a molecular weight range of 1200 to 40,000 D (mean molecular weight of 15,000 D, approximately 50 monosaccharide units). Depending upon the source and method of manufacturing, 10 to 30 molecular species may be present in a given heparin preparation. The heterogeneity in a molecular structure of heparin presents a complex problem because conventional pharmacologic concepts are not applicable to the study of heparin. For instance, radiolabelling procedures typically used for many other drugs are very difficult, making that the pharmacokinetics of UFH are mostly expressed in terms of its pharmacodynamic activity [37].

The pharmacokinetics of UFH are rather complex:

- The clearance of UFH chains is influenced independently by two properties: affinity towards AT and length of the polysaccharide chains. High-affinity material has a slower clearance than low-affinity material and higher-molecular-weight species (the longer chains) are cleared from the circulation more rapidly than the lower-molecular-weight species. After parenteral administration, the UFH composition is progressively changed: there is a progressive increase in the anti Xa/anti-IIa ratio after the injection, resulting from the faster clearance of the anti-IIa activity [38].
- UFH is metabolised by the combination of two mechanisms, which operate in conjunction with each other of a non-dose-related saturable cellular mechanism (rapid zero-order clearance), followed by a slower dose-related renal mechanism (first-order clearance). The saturable mechanism of UFH-clearance represents the endothelial and reticuloendothelial cells (mainly reflecting hepatic uptake). The non-saturable and linear removal mechanism of UFH is represented by renal

elimination. The relative contribution of the two mechanisms to the clearance of UFH varies with the dose and the molecular weight of the UFH-preparation injected. The amounts of UFH cleared per time unit via the saturable mechanism initially increase rapidly with the dose delivered but tend to plateau at higher doses. In contrast, the amounts of UFH cleared via the non-saturable mechanism remain linearly correlated to the dose. Low doses of UFH are mainly removed by the highly efficient saturable mechanism while, at higher doses, the relative contribution of the non-saturable mechanism becomes pre-eminent (see Figure) [39,40].

- Existing non-specific binding to plasma proteins (see later).



Following properties also play an important role in the heterogeneous, unpredictable anticoagulant response of UFH:

- Only one-third of the UFH molecules administered to patients have AT mediated activity. The remaining two thirds of UFH has minimal anticoagulant activity at therapeutic concentrations that are used clinically.
- The chain length of the molecules influences the anticoagulant profile of UFH.
- Non-specific binding of UFH to plasma proteins reduces its anticoagulant activity, because less is available to interact with AT; there is a wide variability in plasma concentrations of heparin-binding proteins, even more complicated is the fact that some of these proteins are acute-phase reactants, whereas others are released during the clotting process [22].

There are important pharmacokinetic differences between UFH and LMWHs. The contribution of the saturable mechanism to the clearance of LMWHs is negligible and elimination of LMWHs is primarily achieved via renal filtration (see Figure). This explains the dose independence of the pharmacokinetic-parameters of LMWHs, the excellent sc bioavailability at any dose, and the much more prolonged duration of biological activity in patients with renal insufficiency [41].
However, because LMWHs still carry some risk of bleeding and HIT, it has been suggested that agents with an even higher anti-Xa/anti-IIa ratio may exhibit an even more favourable benefit/risk ratio (e.g. leading to the investigation of the pentasaccharides).

In addition, some patients develop relative UFH resistance and require a large dose of UFH to achieve a response in the APTT. And a rebound thrombin generation phenomenon was reported when UFH is abruptly stopped [42,43].

The rules for optimal utilisation of UFH have been established empirically and confirmed relatively recently using animal models. Unfortunately, due to its heterogeneity, there is no proportionality between the dose of UFH injected and the anti-coagulant effect. Unless a prescriptive heparin nomogram is used, many patients receive inadequate UFH in the initial 24–48 h of treatment [44]. This inadequate therapy has been shown to increase the incidence of venous thromboembolism during follow up. Thus, frequent monitoring of the dose is required and the dose must be adapted for each patient. Monitoring is usually done by the APTT, a test that is sensitive to the inhibitory effects of UFH on thrombin, factor Xa, and factor IXa. However, there is only a moderate correlation between APTT-levels and UFH-concentrations and a diurnal variation in the APTT response in patients on a constant infusion of intravenous UFH [45,46].

LMWHs

It was discovered that UFH was composed of different fractions with anticoagulant activity fractions with high affinity and low affinity to AT; only about one third of the UFH-molecules contains the high-affinity pentasaccharide and its distribution among the UFH-molecules seems to be random. In addition, it was observed that low molecular weight components of UFH, progressively lose their potency to prolong APTT while retaining their ability to inhibit activated factor X, and that LMWHs induce less bleeding in animal models than UFH [47,48]. Hence the attempts to obtain fractions of UFH with more favourable properties.

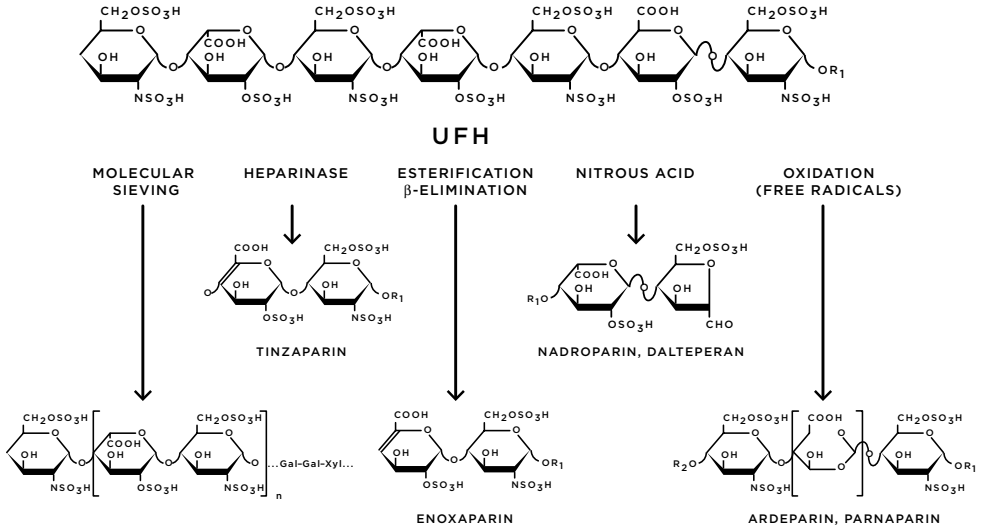


FIGURE 4 **LMWHs are fragments of UFH are prepared by different techniques (controlled processes).**

Currently, preparations of LMWHs in general use have a variable molecular weight distribution (mean molecular weight of about 5000) and, therefore, are likely to have different pharmacokinetic properties. Like UFH the LMWHs are polysulphated uronic acid-amino sugar derivatives and, as such, polyanionic electrolytes. From [49].

It is about 20 years since the first LMWH preparation was reported and, since then, several products have been introduced. Although with a narrower range than UFH, LMWHs still present a certain degree of structural and functional heterogeneity. But it is a class of anticoagulants that has pharmacokinetic and biological advantages over UFH. Comparable to UFH, LMWHs exert their anticoagulant activity by activating AT. But as most of the saccharide chains are composed of less than 18 saccharides, they have a reduced ability to catalyse thrombin inhibition relative to their ability to inhibit factor Xa [38]. The LMWHs have a predictable lower clearance (enabling once- or twice-daily injection), a prolonged half-life, a sc bioavailability close to 100%, and a predictable antithrombotic response based on body weight permitting treatment without laboratory monitoring. These advantages are translated clinically into (1) greater convenience afforded by the ability to administer LMWHs by sc injection and the associated cost reduction resulting from reduced hospital stay and (2) a lower incidence of HIT and possibly of osteoporosis.

The LMWHs have shown to be at least as effective as UFH in the management of patients with VTE [50,33]. And currently, due to the advantages over UFH combined with the increased potential for outpatient treatment [51,52], the LMWHs have established their niche as an important class of antithrombotic compounds licensed all over the world [22]. Because LMWHs are more convenient for the patient and the nursing staff they are becoming more and more widely used in clinical practice [48]. This has also prompted studies exploring the safety and efficacy in other settings than VTE (e.g. patients with acute coronary syndromes and after percutaneous coronary interventions) [53].

A greater understanding of structure activity relationships has led to further modifications in the LMWH-manufacturing process. This resulted in so-called second generation LMWHs with a lower mean molecular weight and a more precisely defined composition of polysaccharide chains, leading to compounds with a optimised and balanced molecular and biochemical profile [54].

In meta-analysis, no convincing difference was found between LMWH and UFH in terms of recurrent VTE, major bleeding, or associated thrombocytopenia [50]. However, it was found that fewer patients who were treated with LMWH died during follow-up. The validity of this mortality is uncertain. Because this survival advantage seems to be greatest in patients with an underlying malignancy, an anti-cancer effect of LMWH has been proposed [55]. Other unresolved issues related to the use of LMWHs are the need for monitoring in certain patient subgroups, the cost-benefit relative to UFH, the interchangeability of the different LMWH preparations and the reversal of the anti-coagulant effect (an antidote) [53].

SEARCH FOR NEW ANTITHROMBOTICS

Rationale

Despite an enormously increased knowledge of the blood coagulation mechanism and many advances in therapy, treatment of VTE is still far from optimal and hampered by the disadvantages of the current antithrombotic therapies compromising safety and efficacy [56].

CURRENT STATUS OF TREATMENT

In recent years, great strides have been made in defining the optimal intensity and optimal duration of anticoagulant therapy. The need for initial treatment with heparin was demonstrated [20]. It became also clear that a 4- to 5-day course of heparin was as effective as a 9- to 10-day course of heparin [57,58]. The latter is important because it allows patients to be discharged from the hospital earlier and reduces the risk of adverse effects.

After initial treatment with heparins, there is a need for long-term anticoagulant therapy to prevent recurrent VTE. Oral anticoagulants can be started within 24 hours of initiating UFH or LMWHs at doses sufficient to achieve moderate intensity anticoagulation (INR of 2.0 to 3.0), which is as effective in preventing recurrent VTE and produces less bleeding than an

INR of 3.0 to 4.5. [59,60]. All patients with VTE should receive at least 3 months of secondary prophylaxis with vitamin K antagonists. In certain patients, this might be prolonged to 6 months or even to 2 years. These optimal durations are likely to be even longer in patients with PE or thrombophilia [13]. Long-term therapy is only indicated for patients with multiple episodes of VTE, certain hypercoagulable states and those with continuing risk factors for recurrence (hereditary thrombophilia, or active cancer) [61].
Additionally, elastic compression stockings should be considered as a treatment for patients with DVT to prevent the development of the post-thrombotic syndrome. If vitamin K antagonists are contraindicated, therapeutic doses of UFH or LMWHs should be considered. Currently, the use of caval vein filters can only be defended in very specific patients [13].

Although LMWHs overcome the pharmacokinetic and some of the biological limitations, both these classes of heparin share the same biophysical limitations [33]. The need for improvement of the current treatments has prompted ongoing research into developing novel agents) [29]. These should differ from established therapies in their biochemical actions, conferring a greater risk/benefit ratio due to inducement of a stable and predictable anticoagulated state (periodic episodes of excessive or inadequate anticoagulation can be avoided. Convenience and ease of dosing are also key requirements. In addition, a rapid achievement of a therapeutic intensity is necessary for maximum efficacy.

Because of the increased demand for these compounds, the increase in bovine spongiform encephalopathy (BSE) and stricter agriculture controls, it can be anticipated that the supply of animal source tissue will reach a limitation. Both UFH and LMWHs are obtained from mammalian tissues. The availability of synthetic antithrombotic agents will provide a timely source to fill the gap. Chemically synthesised compounds are free of viral

contaminants, they can be used universally, and quarantine regulations may not be applicable to the production and international transport of these agents. Furthermore, due to the defined chemical and biological properties, the biological/pharmacological differences as seen amongst the heparins will not be seen with these compounds.

HAEMOSTATIC BALANCE – FOCUS ON THROMBIN

Considerable information has been gleaned since the 1950s about the various components of both the coagulation and fibrinolytic systems and evidence has accumulated to support mechanistically the global concept of a haemostatic balance [1,2]. It appears procoagulants have the capacity to act as anticoagulants under certain circumstances. The most notable example of these activities, which seem to alternate between being procoagulant and anticoagulant is the terminal expression of the coagulation system: thrombin.

Once generated, thrombin is a powerful procoagulant. It catalyses the further conversion of factors V and VIII to their activated forms through a positive feedback mechanism and converts more prothrombin to thrombin (see Figure 2). In this manner, thrombin is able to accelerate the entire cascade once generated, resulting in the formation of large amounts of fibrin. Thrombin generation is much like an explosion: when the cascade is activated, the amount of product formed in the individual reactions increases logarithmically as the cascade progresses. On the other hand, thrombin also activates protein C, which attacks components of the clotting system (factors Va and VIIIa) reducing the amount of thrombin formed. In addition, thrombin is an agonist for the vascular secretion of t-PA and urokinase, thus positively influencing plasminogen activation and fibrinolysis. The efficiency of dissolution of the fibrin clot is enhanced by the binding of plasmin to lysine-binding sites that are generated during the fibrinolysis process. In contrast, thrombin also behaves as a fibrinolysis antagonist by its activation of TAFIa.

TAFIa is derived from the inactive precursor TAFI by thrombin bound to TM [62]. In addition to these roles in haemostasis, thrombin generation is also important in other contexts. It is capable of affecting a variety of cells and e.g. enhances endotoxin-induced cytokine release, which contributes to the mediation of septic shock [63]. The exploration of the complex biological role of thrombin as multifunctional enzyme still reveals more roles [64].

To summarise, thrombin is required for clot generation, clot stabilisation, clotting inhibition, and both fibrinolysis enhancement and inhibition, which makes the choreography of thrombin expression is a complex and highly regulated process [65]. Nevertheless, once a tiny amount of thrombin is produced, massive thrombin generation erupts during coagulation. Thrombin generation resulting in platelet activation and fibrin formation is an important pathogenetic mechanism involved in the development of thrombosis.

Directions

The central role of thrombin has prompted newer antithrombotic strategies to inhibit thrombogenesis by focusing on the inhibition of thrombin or preventing thrombin generation [66,67]. With the assumption that a safe and effective level of anticoagulation could be achieved without excessive bleeding, thrombin-specific inhibitors were developed as anticoagulants. Results form early clinical trials have shown that the therapeutic range of thrombin inhibitors may be limited by bleeding complications [68,69,70]. Furthermore, thrombin inhibitors will not by themselves prevent the continuing conversion of prothrombin to thrombin as long as the activity of factor Xa in the prothrombinase complex is uninhibited.

Because intervention at early stages of the coagulation cascade will already prevent the generation of thrombin, factor Xa has become increasingly of interest as an alternative target for the development of new anticoagulant/antithrombotic drugs [66,67]. To control the activity of this activated coagulation factor

eliminates the continued production of thrombin by either extrinsic or intrinsic pathways without interfering with a basal level of thrombin activity necessary for normal haemostasis. This would provide a slower more regulated control with minimal bleeding risk because some clot formation is still possible under treatment. [71,72]. In addition, the thrombin catalysed activation of the protein C-pathway is still possible. And indeed, inhibitors of factor Xa showed a more favourable antithrombotic/bleeding ratio in experimental models [66,67]. This has led to the development of numerous compounds that cause an inactivation of factor Xa, either indirectly via the potentiation of endogenous anticoagulant mechanisms or directly.

ANTI-XA COMPOUNDS

Heparin successors – indirect inhibitors

UFH is the prototypical glycosaminoglycan (GAG) antithrombotic with multiple biological actions, which contribute to both its therapeutic and adverse effects. Research efforts into indirect thrombin inhibition have been directed at developing agents that exhibit the favourable attributes of UFH while eliminating actions that jeopardise clinical safety.

Inhibition of the cascade at the earliest stage possible without altering normal haemostasis is a highly rational approach to thrombosis prevention. Standard UFH inhibits factor Xa and factor IIa to the same degree, but because low doses of UFH can prevent thrombosis with only little effect on coagulation, it was suggested that factor Xa inhibition may be more relevant than factor IIa inhibition in therapeutic anticoagulation. In addition, since the ability of heparin fragments to reinforce AT-mediated factor Xa inhibition appeared to be independent of their size; it was logical to look for the smallest fragments able to catalyse AT-mediated factor Xa inhibition. Initially, the study of the structure-activity relationship of heparin has brought about the development of heparin derivatives (LMWHs or non-heparin GAGs) with an increased anti-Xa/anti-IIa ratio. The most unique heparin derivative that has been identified is a pentasaccharide that binds to AT to elicit a factor Xa inhibitory response.

The original pentasaccharide sequence was identified from natural heparin by fractionation procedures. A specific pentasaccharide of a predetermined sequence based of these findings was subsequently synthesised by glycosaminoglycan synthesis. Later the α-methyl pentasaccharide (Org31540 / SR90107A) was synthesised with identical biological properties [73,74,75].

TABLE 1 Comparison UFH, LMWH and pentasaccharide

DETERMINANT	UFH	LMWH	PENTASACCHARIDE
Mean molecular weight	12000 – 15000	4000 – 6500	1700
Saccharide units	40 – 50	13 – 22	5
Anti Xa:anti IIa activity	1:1	2:1 – 4:1	Specific anti-Xa act ≈ 1000 U/mg; no anti-IIa act.
SC bioavailability	Low	High	100%
Dose-dependent clearance	+	-	-
Inhibited by Platelet Factor 4	+++	+	-
Inhibits platelet function	++++	+	-
Increases vascular permeability	+	-	-

Direct inhibitors

Another approach for more effective antithrombotic agents has focused attention on the potential limitations of indirect thrombin inhibition. The antithrombotic effects of heparins/heparinoids depend on the presence of endogenous cofactors. It also requires a sufficient proportion of molecules with the pentasaccharide moiety to bind to AT and inhibit factor Xa, or a sufficiently high concentration of larger molecules to inhibit factor IIa effectively. The heparins may be inactivated by heparinase and PF4, and bound by plasma proteins, limiting its availability for therapeutic action. Even more significant is the inability of the UFH-AT complex to inhibit clot-bound thrombin, which may therefore act as a protected, ongoing source of thrombogenesis at sites of pathological thrombus formation. In contrast, specific direct antithrombin agents are able to

inactivate clot-bound thrombin as well as free thrombin [76]. So there has been keen interest in the development of specific, direct inhibitors of factor Xa. When engaged in the prothrombinase complex, this factor proteolytically cleaves prothrombin to generate thrombin, whereas thrombin cleaves a variety of substrates that have key roles in haemostasis and thrombosis. Targeted inhibition of coagulation by selective factor Xa may yield superior antithrombotic efficacy, safety, or both, compared with the less selective conventional antithrombotic agents [56].

Summary

The group of synthetic Xa-inhibitors represents an emerging new class of drugs, which are derived either from natural sources or are synthesised chemically and are chemical and functional very heterogeneous [56]. All have a low molecular mass and some may be available for oral administration. In preclinical studies, the efficacy-safety ratios with these agents were better than those of UFH and antithrombin agents. Compared with direct antithrombin agents, factor Xa-inhibiting agents seem safer and may not induce bleeding and a fibrinolytic deficit.
However, a clear clinical developmental approach is not evident, which hampers a direct comparison of the compounds.
The pentasaccharides were developed for the prophylaxis of deep venous thrombosis with orthopaedic surgery. Most advanced in clinical development is the 'natural' pentasaccharide, Org31540 / SR90107A [73], which is recently registered (Arixtra®, fondaparinux) [77]. Because of its prolonged duration of action, the methylated derivative of this pentasaccharide is being considered for extended prophylaxis of thromboembolism in various conditions [78]. Most of the non-heparinomimetic anti-Xa drugs are in development for cerebrovascular and cardiovascular indications such as acute coronary syndromes and thrombotic / ischemic stroke. However, only limited data are available on the use of these agents in specific indications. Preclinical data must be validated to justify their use in specific thrombotic disorders.

Major differences can be expected between the heparinomimetic and non-heparinomimetic anti-Xa agents (in terms of pharmacodynamics and toxicology). The synthetic heparinomimetics factor Xa inhibitors, like UFH and LMWHs, produce their antithrombotic actions indirectly by binding with AT and exhibit sustained anti-Xa effects. An equimolar amount of AT is required for full expression of the antifactor Xa effects of these compounds. At plasma concentrations higher than 3mg/mL (upper limit of 'normal' for AT in plasma) no additional anticoagulant effect can be obtained. Once bound to AT, a macromolecular complex is generated that cannot penetrate the formed thrombus and endovascular lesions.
In contrast, the direct-acting anti-Xa agents produce a short-duration effect, but these small agents can penetrate the clot and significantly inhibit clot-bound Xa. On the other hand, these peptidomimetics do not activate endogenous vascular-bound AT. As yet, the clinical implications of these differences is unknown. Within each subclass, individual drugs might also have quite different characteristics, just like the LMWHs. Each drug therefore requires individual dose-finding studies for each indication [79].

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CHAPTER 2

OUTLINE OF THESIS

Some major developments in the area of antithrombotic therapy have occurred during the past decades as an increased understanding of the molecular mechanisms underlying thrombogenesis, combined with the biotechnology revolution of the 1980s, has resulted in new anticoagulants appearing at a breath-taking pace [1,2]. The current campaign to identify novel direct inhibitors of thrombin and factor Xa is largely fuelled by the perceived limitations and shortcomings of the only orally applicable group of antithrombotics, the coumarins. Despite some major advances in the use of vitamin K-antagonists during the 1980s, their control can be difficult. In particular, the intensity of the coumarin-induced anticoagulant state is affected by numerous food and drug interactions. Consequently, patients must be monitored in order to avoid periods of excessive or inadequate anticoagulation. This poses a major inconvenience to the patient and contributes to the costs of treatment. Furthermore, there is biochemical evidence suggestive of a rebound hypercoagulable state after therapy with oral anti-coagulants is stopped [3], and oral anticoagulants are one of the few medication classes for which an age-related increase in sensitivity has been demonstrated [4].

Outline

Circumventing the need for frequent patient monitoring by using inhibitors of thrombin and factor Xa, would represent a welcome advance in the treatment of chronic thrombotic disorders [5]. This thesis takes you by the hand and guides you through a part of the search for such compounds. The thesis is divided in five different sections.

In section I, **CHAPTER 1** is a general introduction and covers the current hypothesis of coagulation. Thereafter, an introduction is given about the currently available anticoagulant drugs with

a focus on the heparin-like compounds. Based on the shortcomings of these compounds, the rationale is given why there is a tremendous search for newer compounds. At this point the attention turns to factor Xa, together with thrombin one of the key enzymes in coagulation. The current chapter, CHAPTER 2, begins with a introduction, which clarifies the need for more convenient routes of administration (other than iv/sc). Thereafter the general outline of this thesis is described.
Section II describes the findings of several Phase I studies with new antithrombotic compounds. This section starts with the 'natural pentasaccharide' fondaparinux and contains three studies. This pentasaccharide is mainly cleared by the renal route, therefore a study was conducted in subjects with various degrees of renal function impairment (chapter 3). The next two studies are interaction studies, because it could be anticipated that simultaneous administration with fondaparinux could occur in clinical practice: one study was performed with the oral anticoagulant warfarin, and one with the non-steroidal anti-inflammatory drug (NSAID) piroxicam (CHAPTERS 4 and 5).
It is more and more recognised that for certain indications people need to be treated for longer periods of time. The use of a safe drug with an extended elimination half-life allowing a low dosing frequency would be attractive. Therefore, a longer-acting pentasaccharide was developed, the methylated derivative of fondaparinux. Four phase I studies are described in this section with this compound, SanOrg34006. The first entry-into-man study was a single rising dose study in healthy young male volunteers (CHAPTER 6). However, as most of the target population will be of older age, this study was more or less repeated in a population of healthy subject over 60 years of age (using three selected doses of the compound; CHAPTER 7).
In both these studies, investigation of the subcutaneous (sc) bioavailability was implemented in the study design. Based on the findings of these studies, the third study was conducted, in which the pharmacokinetics and pharmacodynamics of sc multiple-doses in subjects, recently treated for VTE, were investigated (CHAPTER 8). The fourth study was a study to

investigate the interaction between this compound and warfarin (CHAPTER 9).

Whereas these pentasaccharides selectively inhibit coagulation factor Xa, a compound obtained by full chemical synthesis, combining both modes of action of UFH (anti IIa, as well as anti-Xa activity) might be advantageous, especially in arterial thromboembolic diseases. The glycoconjugate Org 36764 consists of two carbohydrate domains interconnected via an inert polyethylene glycol spacer. By means of its two domains it mimics heparin in selective acceleration of the inhibition of Xa, as well as providing the template required for bringing AT and thrombin together, resulting in the interactions that lead to thrombin inhibition. In CHAPTER 10, a first entry-into-man study is described with this compound. As described in section I, thrombin is the final key enzyme in the coagulation cascade, on the crossroad of extrinsic and intrinsic coagulation; therefore, it is an attractive target for an antithrombotic compound. The final chapter in this section covers an interaction study of the direct-acting thrombin inhibitor napsagatran (formerly known as Ro 46-6420) and warfarin (CHAPTER 11).

Development of an orally active anticoagulant with minimal bleeding side effects, and sufficient duration of action to allow once or twice daily dosing, would be essential if a compound is to compete favourably with the coumarins. Such a compound could provide benefits in various indications (e.g. the treatment and prevention of DVT, acute and chronic restenosis following angioplasty, endotoxin-induced DIC, and in long-term out-patient care) [6]. However, as yet a suitable replacement for the coumarins has not been found, although orally active thrombin and factor Xa inhibitors are under development [1].

Section III describes studies performed to investigate the possibilities of other routes of administration of anticoagulant drugs than the conventional iv or sc injection. Currently, the Low-Molecular-Weight heparins (LMWHs) are emerging as the antithrombotic agents of choice instead of unfractionated heparin (UFH) in clinical practice. This section therefore starts with a study performed with pentosan polysulphate, which is a

semi-synthetic LMWH that was supposed to be orally applicable (CHAPTER 12). Because the longer-acting pentasaccharide SanOrg34006 had shown SL bioavailability in dogs, this route of administration was investigated in a group of healthy young male volunteers (CHAPTER 13). YM 466 is a synthetic compound acting directly against activated coagulation factor X (Xa). The next chapter in this section describes the first Phase I experiences with this compound and covers the first entry-into-man study with single rising oral doses, an investigation of the absolute oral bioavailability, as well as investigation of the influence of food on this oral bioavailability (CHAPTER 14).

Despite an enormously increased knowledge of the blood coagulation mechanism and many advances in therapy, treatment of VTE is still far from optimal and hampered by the disadvantages of the present antithrombotic therapies compromising safety and efficacy. The group of newly developed antithrombotic drugs represents a marked structural and functional heterogeneous group of agents that are targeted to modulate different biochemical pathways leading to thrombosis. Additionally, the assessment of the efficacy of these new anticoagulant treatments in patients with symptomatic thrombosis is impeded by the low incidence of the outcome measures of choice (symptomatic venous thromboembolic complications). This has provided incentives to develop approaches for monitoring the alterations of haemostatic mechanisms induced by new agents. Therefore, there is a scope in finding models that can serve as alternative efficacy outcome to relate the pharmacokinetics to the pharmacodynamics in order to evaluate the safety and efficacy in early phase thrombo-prophylactic studies.
In section IV it is tried to evaluate these items based on characteristics of the earlier described compounds in Phase I-studies. In order to get an early idea on the compounds, these were compared based on their selectivity towards coagulation factor Xa (CHAPTER 15), based on their effects on the currently used conventional coagulation assays: the activated partial thromboplastin time (APTT) and prothrombin time (PT)

(CHAPTER 16). During the Phase I studies with anticoagulant drugs with various mechanisms of action on the coagulation cascade, a bedside monitor (Coagucheck Plus® – formerly called Biotrack 512®) coagulation monitor (Roche Diagnostics, Mannheim, Germany) was used to determine the APTT and PT as initial safety measure. As it is essential to know the reliability of the results obtained by such a bedside monitor, these were compared with the 'golden' standard laboratory assays for APTT and PT (CHAPTER 17).

The last section of this thesis, section V, summarises the findings described in earlier chapters and is an overall discussion with regard to these results.

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SECTION II

NEW COMPOUNDS IN HEALTHY VOLUNTEERS

CHAPTER 3–11

CHAPTER 3

THE INFLUENCE OF RENAL FUNCTION ON THE PHARMACOKINETICS AND PHARMACODYNAMICS OF THE NOVEL ANTITHROMBOTIC FONDAPARINUX

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ABSTRACT

Aims

To assess the safety/tolerability and the pharmacokinetics/pharmacodynamics of fondaparinux administered as a single intravenous bolus injection to four groups of subjects with varying degrees of renal impairment.

Methods

Twenty subjects (12 females / 8 males) with varying degrees of renal failure were administered fondaparinux 4 mg intravenously. The pharmacokinetics of the drug were evaluated using plasma and urine concentrations. The relationship between the pharmacokinetic parameters and creatinine clearance (calculated using Cockroft & Gault) was investigated using linear regression analysis.

Results

No clinically relevant differences in vital signs, APTT and bleeding time were noted. C_{max} and V_{ss} of fondaparinux were independent of creatinine clearance (used as measure for renal function). Clearance of fondaparinux was decreased and its terminal elimination half-life was significantly prolonged in subjects with renal function impairment.
The clearance of fondaparinux showed a proportional increase with the creatinine-clearance ($r^2 = 0.90$).

Conclusion

The clearance of fondaparinux is highly correlated with the creatinine clearance, calculated from a single plasma creatinine assessment. This allows simple dose regimen adaptation.

INTRODUCTION

Fondaparinux is a fully chemically synthesised pentasaccharide, identical to the antithrombin (AT, formerly called AT-III) binding sequence in heparin. Fondaparinux inhibits factor Xa selectively, while the compound is devoid of anti-thrombin activity and does not affect platelet function. These properties of the drug are

reflected in very low bleeding enhancement demonstrated in *in vitro* and *in vivo* experiments [1,2,3]. In clinical practice, subcutaneous administered fondaparinux is intended to substitute (LMW-)heparins used concurrently with vitamin K-antagonists in the treatment and prevention of deep vein thrombosis and pulmonary embolism, and probably the prevention of clotting (or coagulation) occurring at thrombogenic surfaces as in haemodialysis or other forms of extra-corporal circulation. It is therefore likely that this compound will be administered to subjects with reduced renal function, as a result of the composition of the target subject population or when the drug is used during haemodialysis. In both cases, knowledge of the pharmacokinetics and pharmacodynamics in relation to renal function is essential.
Therefore, a study was performed to assess the safety, tolerability and the pharmacokinetics and pharmacodynamics of a single intravenous (iv) dose of fondaparinux administered to subjects with different degrees of renal function, varying from normal to severely impaired.

METHODS

Subjects and study-design

The study had an open design. Twenty subjects participated in the study. These subjects were categorised in one of four groups on the basis of their actual renal function as reflected by a creatinine clearance (CL_{CR}). Fifteen subjects had an impaired renal function as follows: group I, severe renal impairment (CL_{CR} 10-30 mL/min); group II, moderate renal impairment (CL_{CR} ≥30-60 mL/min); and group III, mild renal impairment (CL_{CR} ≥60-90 mL/min). Five subjects had a normal renal function. This group of young healthy volunteers (group IV) served as a control group. The creatinine clearance was calculated from the plasma creatinine by applying the formula of Cockroft and Gault [4].
The subjects with renal function impairment were recruited from the outpatient clinic of the department of Nephrology of Leiden University Medical Center. These subjects had stable renal

function as reflected by fluctuations in serum creatinine level of less than 30% over the last 6 months before study entry (measured regularly at the outpatient clinic). The control subjects were recruited from the Leiden student population. The control-subjects were younger than the patient-subjects but previous studies demonstrated no obvious effect of age on pharmacokinetics [5]. The study was conducted according to the principles of the 'Declaration of Helsinki'. Approval was obtained from the Ethics Committee of the Leiden University Medical Center and all subjects gave written informed consent prior to inclusion in the study.

Treatments

Subjects were admitted to the research unit after an overnight fast. Following arrival, a light breakfast was provided. After iv cannulation (one cannula in each arm) baseline blood samples were taken and vital signs were measured. Each subject was administered a single dose of 4 mg fondaparinux as a slow iv bolus injection over 30 seconds using a calibrated infusion-pump. Blood sampling, measurements and urine collection took place at regular time intervals. Adequate urine flow was maintained by administration of oral fluids in relation to the urine volume passed. Lunch and dinner were provided at approximately 5 and 10 hours after drug administration. After 24 hours the subjects were discharged from the research-unit but returned for sampling at 36, 48 and 72 hours. Additional blood sampling was done at 120, 144 and 168 hours for patients in the two groups with the poorest renal function.

Sample collection

No tourniquet was applied when blood was collected. The iv cannula used for blood sampling was kept patent by intermittent flushing with of 0.9% saline. The blood was taken after discarding the contents of the cannula. Blood samples for fondaparinux-quantification were drawn pre-dose and at 5, 10, 15, 30, 45 minutes, and 1, 2, 3, 4, 6, 9, 12, 18, 24, 36, 48, 72, 120, 144 and 168 hours relative to drug administration. Blood samples for the APTT

assay were drawn pre-dose and at 15 minutes and 24 hours. Urine was collected over the 0–6, 6–12, 12–24 and 24–48 hour intervals. Concentrations in plasma and urine were determined using a validated, anti-Xa based, amidolytic assay with S2222 as substrate at the department of Drug Metabolism and Kinetics of NV Organon (Oss, The Netherlands). APTT was assessed by standard procedure [6] on a STA coagulation analyser (Boehringer Mannheim, Diagnostica Stago) with the STA APTT-reagent (Boehringer Mannheim).

Safety Analysis

Blood samples for routine haematology and serum biochemistry were collected pre-dose and at 72h after drug administration. A general physical examination was performed at 72 hours after dose administration. Blood pressure and heart rate were measured using an automated blood pressure monitor (Nihon Kohden MPV-7201, Tokyo, Japan) pre-dose and at 15 min, 2, 12, 24, 48 and 72 hours after dose administration. A twelve-lead electrocardiogram was made pre-dose, 1 and 24 hours after dose administration using an electrocardiograph (CardiofaxV ECAPS12, Nihon Kohden, Tokyo, Japan).

Statistics

The drug-concentration time profiles were analysed using non-compartmental. The following kinetic parameters were derived: the peak concentration (C_{max}), the elimination half-life associated with the terminal elimination phase ($t_{½}$), the AUC extrapolated to infinity using the linear trapezoidal rule ($AUC_{0-\infty}$), and the clearance (CL) calculated using $AUC_{0-\infty}$. In addition, the steady-state volume of distribution (V_{ss}) was estimated using a two-compartment open model with weighting equal to 1/(predicted value)^2. Renal clearance of fondaparinux was calculated as the cumulative urinary excretion of the compound over the first 48 hours of urine collections divided by the corresponding plasma AUC. Calculations were performed, using WinNonlin V1.1 software (Scientific Consulting, Inc., Apex, NC).

The relationship between the creatinine clearance and drug

clearance and the relationship between plasma clearance and renal clearance were investigated using linear regression. In order to investigate the possible influence of fondaparinux on renal function, the creatinine clearance values were compared pre-dose and post-dose, using paired samples t-tests. Creatinine clearance was calculated according to Cockroft & Gault [4] from the serum biochemistry samples taken approximately 60 minutes before and 72 hours after fondaparinux administration. Statistical analysis was performed, using SPSS for Windows V6.1.2 (SPSS, Inc., Chicago, IL). With regard to the pharmacodynamic parameters assessed during the study, no formal statistical analysis was performed.

RESULTS

A summary of the demographics is giving in Table 1. Except for the abnormalities related to renal impairment, none of the subjects had any significant abnormalities in medical history, physical examination and routine laboratory tests, including coagulation screen. Controlled hypertension was present and treated by their specialists in eight subjects. One subject occasionally suffered from migraine. All subjects completed the study without serious adverse events. No changes were seen in renal function during the study as the creatinine clearance showed no significant difference comparing pre-dose with 72 hours after drug administration (paired t-test, p=0.92). Minor haematomas around venipuncture sites were reported for 2 subjects and one subject noted a trace of blood after sneezing. One subject reported a mild headache and three subjects reported light-headedness. Two subjects reported minor gastrointestinal complaints. All adverse events were mild and of single occasion and no actions were taken, except for treatment of headache in one subject.

Pharmacodynamics

The APTT value of each subject was in the normal range and no obvious changes due to drug administration were detected. No obvious changes were detected in the post-dose samples for the routine laboratory parameters compared to the pre-dose values.

Pharmacokinetics

The mean plasma concentration-time curves for the four groups following the iv-bolus injection are shown in Figure 1. A summary of the pharmacokinetic analysis results is given in Table 2. These data indicate that the maximal plasma levels and volume of distribution did not differ between the groups. However, the parameters reflecting drug elimination were influenced by the degree of renal dysfunction. The individual estimates of drug clearance were linearly related to the assessed creatinine clearance and correlated well ($r^2 = 0.898$; Figure 2). This was also reflected by the urinary excretion of fondaparinux over the 48-h period, which decreased with the degree of renal function (Table 2).

The renal clearance of the compound was greatest in healthy subjects and declined in subjects with impaired renal function. The relationship between total clearance and renal clearance can be described as CL_{total} (in mL/min) = 0.73 + 1.24 * CL_{renal} ($r^2 = 0.966$). Since the intercept of this relationship was significantly different from zero, the compound is partly cleared by other routes.

DISCUSSION

The main objective of this study was to assess the pharmacokinetics of fondaparinux after administration of a single intravenous bolus injection to subjects with normal renal function and different degrees of renal failure. The peak concentration was similar in all four groups of subjects, as was the volume of distribution. However, substantial differences between the groups were found for terminal elimination half-life, AUC and clearance of fondaparinux. The plasma fondaparinux clearance was linearly related to the creatinine clearance (as a measure for renal function) estimated with the formula of Cockroft and Gault [4]. This confirms that the compound is cleared almost entirely through the kidney.

The other objective was to assess the safety and tolerability of fondaparinux in these groups of subjects. It was shown that the drug had no significant effects on the vital signs, ECG, and

routine laboratory parameters. These observations confirm the results of pre-clinical studies and the good safety/tolerability-profile observed in studies involving healthy subjects.
The effects of a single dose of fondaparinux on secondary haemostasis as measured by APTT in relation to renal function were also assessed. The APTT did not change following drug administration. However, some caution should be taken to make this finding synonymous with the conclusion that secondary haemostasis is not influenced by administration of fondaparinux. The finding that APTT is not greatly influenced by the very specific action of fondaparinux may also reflect unsuitability of this marker to monitor the effect of the drug. Although the relation between the anti-Xa activity and the probability of bleeding is unknown, this measure is a better predictor for the amount of fondaparinux in the circulation. The iv administration of a single dose of 4 mg fondaparinux was well tolerated and no significant adverse events were seen in this study-population consisting of young healthy volunteers and elderly subjects with different degrees of renal dysfunction.
It can be concluded that the pharmacokinetics of fondaparinux are strongly dependent on renal function. The drug clearance correlated linearly with the creatinine clearance allowing an easy calculation to adapt the dose-regimen of fondaparinux in accordance with the renal function in patients with renal dysfunction.

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LEGENDS TO TABLES

TABLE 1 Subject Characteristics

	Renal Function			
VARIABLE	GROUP I (10–30 mL/min)	GROUP II (≥30–60 mL/min)	GROUP III (≥60–90 mL/min)	GROUP IV (≥90–140 mL/min)
female/male	2/3	4/1	4/1	2/3
age (yrs)	54.8 (51–61)	53.6 (48–59)	47.0 (34–53)	23.2 (21–26)
body-weight (kg)	75.6 (63.5–92.0)	64.9 (63.5–76.0)	72.9 (59.6–81.0)	70.2 (50.0–88.1)
height (cm)	168.6 (158–178)	164.0 (158–175)	164.4 (157–175)	178.6 (170-186)

Reported as mean (ranges)

TABLE 2 Mean (SD) pharmacokinetic parameters derived from Non-Compartmental analysis) for fondaparinux

GROUP	CL_{CR} (mL/min)	C_{MAX} (ng/mL)	$AUC_{0-\infty}$ (mg*hr/L)	$t_{1/2}$ (h)	CL (mL/min)	V_{SS} (L)	EXCRETION (mg)	CL_{RENAL} (mL/min)
I	20.8 (7.9)	1160 (200)	50.2 (10.0)	71.5 (11.7)	1.37 (0.29)	6.78 (0.98)	0.68 (0.30)	0.54 (0.27)
II	46.6 (10.3)	1210 (205)	21.0 (5.4)	28.7 (7.5)	3.35 (0.85)	7.02 (0.69)	1.87 (0.22)	2.26 (0.59)
III	82.2 (5.5)	1220 (275)	13.2 (2.2)	17.9 (0.9)	5.22 (1.15)	7.06 (1.31)	2.45 (0.29)	3.77 (1.25)
IV	126.3 (33.7)	1050 (237)	8.7 (1.3)	13.1 (3.6)	7.82 (1.21)	8.16 (2.44)	2.65 (0.24)	5.51 (0.54)

$AUC_{0-\infty}$ = Area under the concentration-time curve extrapolated to infinity
CL_{CR} = Creatinine Clearance calculated predose (according to Cockroft & Gault)[4]
CL = Clearance
CL_{renal} = Renal clearance
C_{max} = Peak concentration
Excretion = Cumulative urinary excretion over sampling period (48 hours)
$t_{½}$ = Elimination half-life
V_{ss} = Steady state volume of distribution (calculated using model-dependent analysis)

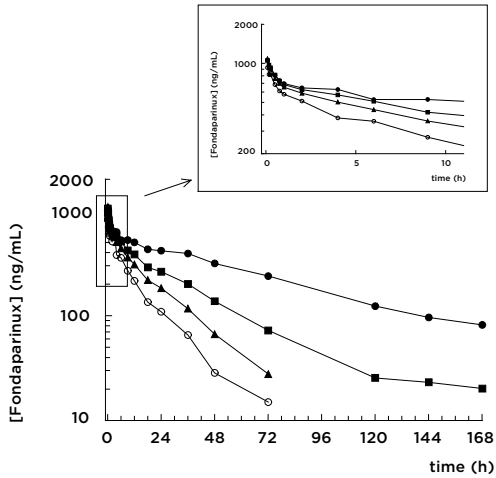

FIGURE 1 **Average plasma concentration-time curves per group; ● group I; ■ group II; ▲ group III; ○ group IV**

(Group I: severe renal impairment (CLCR 10-30 mL/min); group II: moderate renal impairment (CLCR≥30-60 mL/min); group III: mild renal impairment (CLCR≥60-90 mL/min); group IV: young healthy volunteers (CLCR≥90-140 mL/min)).

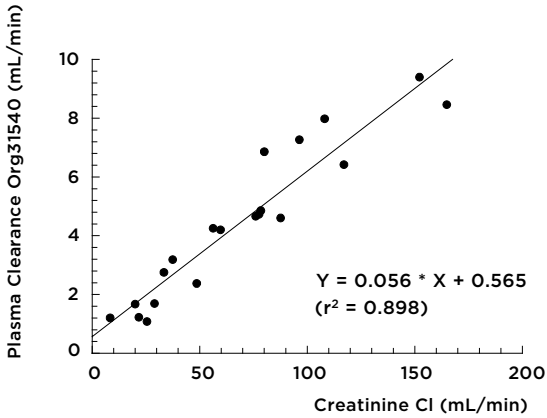

FIGURE 2 **Relationship between plasma clearance of fondaparinux and creatinine clearance.**

CHAPTER 4

ABSENCE OF AN INTERACTION BETWEEN THE SYNTHETIC PENTASACCHARIDE FONDAPARINUX AND ORAL WARFARIN

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ABSTRACT

Aim

To investigate the interaction between the antithrombotic pentasaccharide fondaparinux (subcatneously) and oral warfarin in healthy subjects.

Methods

This study was performed using a randomised, 3-way cross-over, placebo-controlled, double-blind design in 12 male subjects. The treatment consisted of five subcutaneous (sc) injections of fondaparinux (4mg) or placebo at 24 h intervals. Oral warfarin or placebo was administered at the time of the fourth (15mg) and fifth (10mg) sc injection. Blood samples for pentasaccharide assay, prothrombin time (PT) and activated partial thromboplastin time (APTT) were drawn before the first sc dose of pentasaccharide and over a 6-day period thereafter.

Results

Fondaparinux administered alone or in combination with oral warfarin was well tolerated and no serious adverse events were observed. No differences were found in the AUC (43 vs. 44 mg/l*h), C_{max}(645 vs. 678 ng/mL) or elimination half-life (13.8 vs. 14.1hr) of fondaparinux administered as single drug or in combination with warfarin. The effect of warfarin on PT (mean maximal increase: 8.2 sec.) was not influenced by the presence of fondaparinux (mean maximal increase in PT: 9.1 sec.). After all treatments a small insignificant rise in APTT occurred. No further differences could be detected in the effects after the three treatments.

Conclusions

The co-administration of warfarin did not influence the pharmacokinetics of fondaparinux. If these findings can be extrapolated to patients, PT can still be used to monitor the effect of oral anticoagulants during the switch from antithrombotic treatment with pentasaccharide to full oral anticoagulant therapy.

INTRODUCTION

The pentasaccharide fondaparinux (formerly known as Org31540 / SR90107A) is a fully synthetic antithrombotic. The chemical structure of the compound is identical to that of the antithrombin (AT) binding domain of heparin. The drug selectively inhibits factor Xa, is devoid of anti-thrombin activity and does not affect platelet function. As a consequence the drug exhibits a low bleeding tendency, as demonstrated *in vitro* and *in vivo* [1–3]. Assessment of the safety and efficacy of fondaparinux in the prophylaxis and treatment of deep venous thrombosis is an integral part of the clinical development of the compound [4]. Simultaneous administration of subcutaneously (sc) administered pentasaccharide and oral anticoagulants can be anticipated in clinical practice. The change from pentasaccharide to oral anticoagulants would normally occur using a loading dose of oral anticoagulants followed by regular prothrombin time (PT) assessments after 48–72 hours. Combination treatment could potentially effect this measurement and lead to erroneous dose adjustment of the warfarin.

The objectives of this study were to evaluate the possible pharmacokinetic and pharmacodynamic interaction between sc administered fondaparinux and orally administered warfarin.

METHODS

Subjects and Design

The Ethics Committee of Leiden University Medical Center approved the protocol. The study was conducted according to a randomised, 3-way cross-over, placebo-controlled double-blind study design. Wash-out between the study periods was 2 weeks. Twelve healthy males with a normal coagulation screen (age: 19–27 yr., body weight: 61–87 kg) participated in this study after written informed consent was obtained.

Treatments

Subjects received the following treatments during the study: pentasaccharide (Penta-only), oral warfarin (Warf-only) or the combination (Penta+Warf). The Penta-only treatment consisted

of five 4mg sc injections of fondaparinux (Arixtra®) at 24 hr intervals and oral placebo tablets concomitant with the 4th and 5th injection. The Warf-only treatment consisted of five sc placebo (0.9% saline) injections at 24 hrs. At the time of the 4th sc dose, 15 mg of oral warfarin sodium (Coumadin®) was administered followed by 10 mg of warfarin at the 5th injection. During combination treatment the subjects received 5 sc injections of the pentasaccharide and oral warfarin as indicated for the single drug treatment. All subjects received a single oral dose of 10mg of vitamin K (Konakion®) at 132h after the first sc injection, to reverse any residual effect of warfarin (see figure 1 for a schematic view of the study design). Subjects were studied after an overnight fast. They were admitted to the research unit on the evening before first administration of the pentasaccharide and remained there for 12h after each sc injection. The subjects stayed in the unit from the fourth sc injection and first oral administration until at least 36h after the fifth sc injection.

Blood Sampling

Blood was collected without the use of a tourniquet from an iv cannula which was kept patent by intermittent flushing with of 0.9% saline. Blood samples for baseline values were taken before dosing. Blood samples for drug analysis, PT and activated partial thromboplastin time (APTT) were drawn at regular time-intervals until 144h after the first sc dose of the pentasaccharide. Blood samples for routine haematology and serum biochemistry were taken prior to dosing and at the end of each study period.

Laboratory Tests

Anti-factor Xa activity in plasma (reflecting the pentasaccharide concentration) was assessed using a validated amidolytic photometric assay at the Department of Drug Metabolism and Kinetics of NV Organon. Briefly, a calibration curve for the relationship between pentasaccharide concentration and anti-Xa activity is prepared allowing expressing the measured anti-Xa activity in concentration units of the pentasaccharide.
The detection limit of the assay is 2.4 ng/ml, accuracy varied

between 94–104% and the precision ranged between 3.1–6.7%. The PT and APTT assays were performed following standard procedures with a STA coagulation analyser (Boehringer Mannheim, Diagnostica Stago) using the reagents provided by the manufacturer.

Statistical analysis

The drug-concentration time profiles were analysed using non-compartmental pharmacokinetic techniques with the WinNonlin program (Version 1.1, Scientific Consulting, Inc.). Points included for calculation of terminal half-life were automatically determined by the program and visually checked for adequacy. The following parameters were derived: AUC up to the last measurable concentration using the linear trapezoidal rule ($AUC_{0\text{-last}}$), AUC extrapolated to infinity ($AUC_{0\text{-}\infty}$), terminal half-life ($t_{½}$), clearance/F (calculated using $AUC_{0\text{-}\infty}$ and assuming a total dose of 20mg fondaparinux), AUC from 72h (from the first administration of warfarin) up to the last measurable concentration ($AUC_{72h\text{-last}}$), peak concentration (C_{max}) and time to reach peak concentration (T_{max}). The last two parameters were calculated relative to the final sc administration.

APTT and PT were analysed using the uncorrected area under the effect curve (AUEC) and on baseline-corrected data (AUEC above average pre-value) divided by the corresponding time span. This pre-value correction results in a weighted average increase above baseline for which 95% confidence intervals were calculated to test whether a significant increase from baseline occurred. Maximal effect (E_{max}) and the time to reach this maximal effect (T_{max}) on the basis of observed data were calculated for APTT and PT during the Warf-only and Penta+Warf treatments. All contrasts were calculated using paired t-tests on untransformed measures except for AUC and C_{max} parameters that were log-transformed. Log-transformed contrasts were back-transformed, resulting in parameters that can be interpreted as percentage increase due to the addition of warfarin along with 95% confidence intervals. Statistical analysis and calculations were performed using SPSS for Windows (SPSS, Inc., Chicago, IL).

RESULTS

All subjects completed the study without serious adverse events. Two subjects reported mild gastrointestinal complaints after intake of placebo-tablets. Mild headaches were reported by 4 subjects (a total of six episodes) and three subjects reported an episode of malaise. Minor haematomas around venipuncture sites were reported for 4 subjects. One subject reported a bleeding episode of approximately 10 minutes, after he cut himself while shaving and one subject had a spontaneous epistaxis between two study periods.

Pharmacokinetics

The mean plasma concentration-time curves for the treatments are shown in figure 2. No statistically significant difference between Penta-only and the combination treatment was found. Co-administration of warfarin led to a non-significant increase in C_{max} of 5.1% (95% CI: -1.1, +11.7%), $AUC_{0-\infty}$ of 2.5% (-1.1, +6.2%) and $AUC_{72h\text{-}last}$ of 2.8% (-2.8, +8.7%).

Pharmacodynamics

The mean PT-time curves for the different treatments are shown in figure 2. The treatment with Warf-only as well as Penta+Warf resulted in a statistically significant increase in the time-corrected AUEC and E_{max} compared to the Penta-only treatment. The difference was 2.6 seconds (95% CI: +1.9, +3.3s) and 2.9 seconds (+1.8, +3.9s), respectively for the time-corrected AUECs and 8.2 seconds (+3.6, +12.7s) and 9.1 seconds (+4.0, +14.2s) for E_{max}. No statistically significant difference was found between the Warf-only treatment and the combination treatment for the time-corrected AUEC (both with and without pre-value correction), E_{max} and T_{max}. The APTT increased above baseline after Penta-only (mean: 3.1 seconds; 95% CI: +0.7, +5.5s) and after Warf-only (3.2 seconds; 95% CI: +0.3, +6.2s). The combination treatment resulted in a greater increase (5.0 seconds; 95% CI: 4.0, 5.9), which however was not significantly higher than the summed increase after single drug treatment (figure 2). This suggests a possible additive effect of both drugs on APTT.

DISCUSSION

The objective of this study was to investigate the possible pharmacokinetic and pharmacodynamic interaction of sc administered pentasaccharide fondaparinux and oral warfarin in healthy male volunteers. For warfarin a loading dose regimen was chosen to avoid the cumbersome procedure of attaining stable oral anticoagulant concentrations in healthy volunteers.

It is recognised that with this dose-regimen a full suppression of all vitamin K-dependent clotting factors is not reached. However, it provides answers for the clinical situation during which the pentasaccharide and oral anticoagulants are administered concomitantly. This is of particular importance for the effects of the combination of the two drugs on the PT at the time the switch from pentasaccharide treatment to oral anticoagulants occurs clinically. In addition, previous studies using a single loading dose of 25 mg of warfarin have been proven to provide useful information on this relevant issue [5–8]. The pharmacokinetic profile of pentasaccharide given in combination with warfarin was identical to that of pentasaccharide alone. Hence, no pharmacokinetic interaction was detected.

PT is the most commonly used test to monitor orally administered anticoagulants [9]. In this study PT did not change from baseline with the pentasaccharide-only treatment. Treatment with warfarin alone or in combination with the pentasaccharide prolonged PT as expected. However, the presence of the pentasaccharide did not influence the effect of warfarin on the PT. It can thus be concluded that PT can remain to be used to monitor the effect of oral anticoagulants during the switch from anticoagulant treatment with pentasaccharide to oral anticoagulant therapy.

The administration of pentasaccharide or warfarin alone resulted in a small (approximately 3 second) rise in APTT. The increase in APTT after the combination treatment was only slightly more than that after the monotherapy.

In conclusion, concomitant oral administration of warfarin does not affect the pharmacokinetics of the novel pentasaccharide fondaparinux in healthy volunteers. In addition, warfarin-

induced increase in PT (INR) was not influenced by concomitant subcutaneous pentasaccharide treatment. Accordingly, if this finding can be extrapolated to patients, PT can safely be used to monitor the effect of warfarin during the switch from pentasaccharide treatment to warfarin.

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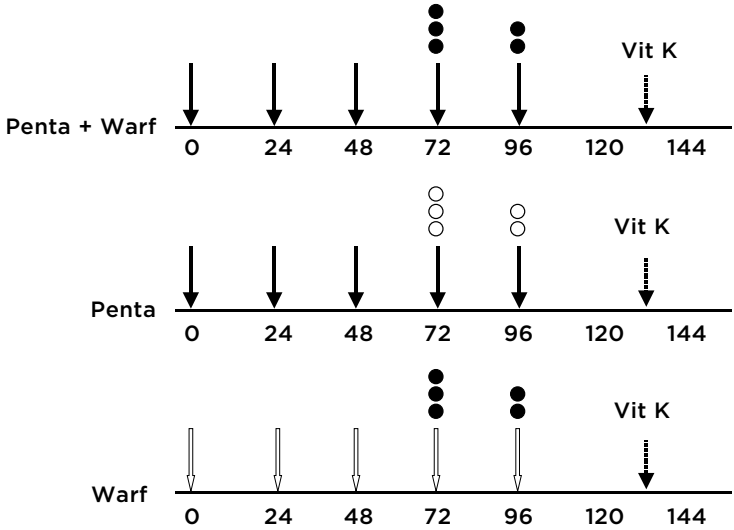

FIGURE 1 **Schematic view of the study design.**
Closed symbols indicate active treatment with pentasaccharide (arrows) or warfarin (circles), and open symbols indicate corresponding placebo treatment. K indicates vitamin K administration.

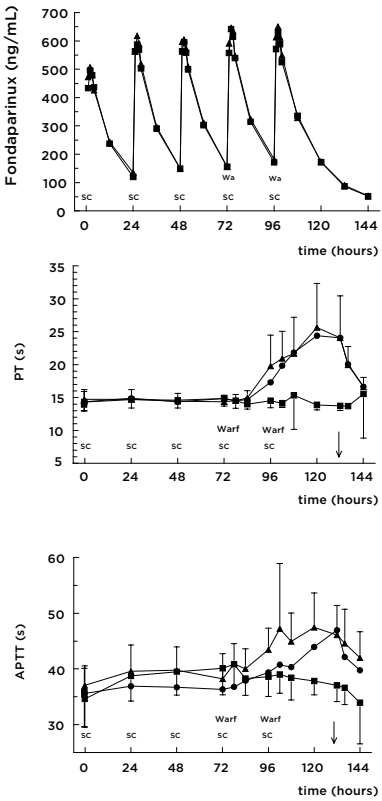

FIGURE 2 **Average pentasaccharide plasma concentration-time profile (upper panel), average PT-values (middle panel), and average APTT-values (lower panel) per treatment ■: pentasaccharide; ▲: pentasaccharide plus warfarin; ●: warfarin).**

The sc pentasaccharide injections and oral warfarin administrations are indicated. The arrow indicates the timepoint at which vitamin K was administered.

CHAPTER 5

ORAL PIROXICAM DOES NOT INTERACT WITH THE NOVEL SYNTHETIC ANTITHROMBOTIC PENTASACCHARIDE FONDAPARINUX

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2. Diagnostic Center SSDZ, Delft, The Netherlands.

ABSTRACT

Objective

To investigate possible pharmacokinetic and pharmacodynamic interactions between the subcutaneously (sc) administered novel pentasaccharide fondaparinux and oral piroxicam as a representative of the class of non-steroidal anti-inflammatory drugs (NSAIDs).

Methods

The study was performed in 13 healthy subjects in a 3-way crossover, randomised, double-blind study with a three-week washout period between occasions. After pre-treatment with 6 oral doses of piroxicam (20 mg at 24-h intervals), the subjects received sc injections of 10 mg pentasaccharide at 0, 24, 48 and 72 hours while piroxicam was continued. Blood samples for drug assay for pentasaccharide, collagen induced platelet aggregation (CIPA), and APTT were drawn pre-dose and over a 5-day period after the first dose of the pentasaccharide. Gastrointestinal blood loss was estimated by fecal porphyrin content. Comparisons were made using paired t-tests and are reported as mean difference and 95% confidence intervals.

Results

The combination treatment was well tolerated. Over the period that the chance for an interaction was maximal (from the 4th sc injection onwards) no differences were noted in the pharmacokinetics of the pentasaccharide with or without co-administration of piroxicam, as indicated by the absence of significant differences between the treatments for the main parameters for the pentasaccharide (C_{max} (11; CI: -92,+115 ng/mL), elimination half-life (0.3; CI: -0.44, +1.1 hr) and $AUC_{72-\infty}$ (0.59; CI: -0.87, +2.06 mg*h/L). Compared to the treatment with the pentasaccharide alone, piroxicam decreased CIPA in a similar fashion when given alone (5.5Ω; +0.3,+10.7Ω) or in combination with the pentasaccharide (5.0Ω+1,6,+8.4Ω). Also, no differences were observed in gastrointestinal blood loss.

Conclusion

This study has shown that no pharmacokinetic or pharmacodynamic interactions occur during concomitant use of the pentasaccharide fondaparinux and the NSAID piroxicam.

INTRODUCTION

The antithrombotic pentasaccharide fondaparinux (formerly called Org31540/SR90107A) is fully synthetically prepared and potentiates the anti-factor Xa activity of antithrombin. The compound is devoid of anti-factor IIa activity and does not affect platelet function, which is reflected in a low bleeding enhancement. The drug is safe and well tolerated after single subcutaneous (sc) doses up to 30mg in young subjects and 18mg in elderly healthy subjects. The drug shows linear kinetics and is renally cleared. Following multiple dosing, steady state is reached after 3 days [1]. Recently, data have became available that fondaparinux has the potential to improve significantly the risk-benefit ratio for the prevention of venous thromboembolism (VTE), as compared with low-molecular-weight heparin in patients who underwent total hip replacement [2].

The selectivity and good tolerability of the pentasaccharide appears to represent an improvement over the presently available drugs, although this is currently based on limited information currently in the public domain from a large phase III program in prevention of VTE in orthopedic surgery [3-6].

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most widely used classes of drugs [7]. This is particularly true for patients with chronic joint disorders as rheumatoid arthritis or arthrosis. Patients with these conditions are frequently candidates for joint-replacement surgery, a condition known to be associated with a high incidence of VTE. As pentasaccharide may become a new therapy in the prevention of post-surgery venous thrombosis, it is highly likely that the combination of this drug and NSAIDs will be encountered frequently during clinical care. Among the NSAIDs, piroxicam is the most frequently prescribed, probably because its relatively long elimination half-life (approximately 50 hours) and its relatively low ulcerogenic

potential compared to other NSAIDs [8]. This makes the drug attractive for use in chronic conditions as rheumatoid arthritis and related disorders.
Therefore a study was undertaken to investigate the possible pharmacokinetic and pharmacodynamic interactions between fondaparinux and piroxicam as a model NSAID.

METHODS

The study was performed in 13 healthy male volunteers (aged: 19–27 yr., normal weight for height), using a randomised, 3-way crossover, placebo-controlled, double blind design. The washout period between treatments was 3 weeks. The experiment was conducted according to the principles of the 'Declaration of Helsinki' and in accordance with the Guideline for Good Clinical Practice. The Medical Ethics Committee of Leiden University Medical Center (LUMC) approved the study protocol and all subjects gave written informed consent.

Treatments

For this study a dose-regimen of four 10mg of fondaparinux to be administered sc in the abdominal skin region, every 24 hours during 4 days was selected. The pentasaccharide was supplied by NV Organon as pre-filled syringes containing 1.0mL in which the drug was dissolved. Placebo sc injection was a similar volume of 0.9% saline. Pre-treatment with oral piroxicam (20 mg) started 6 days before the first dose of pentasaccharide and was continued during pentasaccharide administration. Thus, piroxicam and matching placebo (obtained via the hospital pharmacy of LUMC) were administered at 24 hr intervals for 10 days. As TxA_2-production is almost completely (but reversibly) blocked after single dose piroxicam above 20mg [9], the study design was such that that the concentrations of piroxicam and pentasaccharide (even for possible arterial indications) were in a clinically relevant window and complete blockade of TxA_2-production over the entire duration of the experiment was achieved.

Study Periods

During the pre-treatment period subjects reported to the research-unit every morning. After a check for adverse events and compliance to the study protocol restrictions, piroxicam was administered and subjects could leave. The evening before the first sc drug administration, the subjects were admitted to the unit. At arrival a brief medical history was taken and a physical examination took place. The next morning, prior to the zero time point, an intravenous cannula was inserted and bleeding time measurement (Ivy method) took place. Then blood samples were drawn for drug assay, collagen-induced platelet aggregation (CIPA) and activated partial thromboplastin time (APTT). Subsequently, the subjects were administered sc fondaparinux and oral piroxicam. Drug administration was repeated at 24, 48 and 72 hours. During the stay at the unit subjects collected a random sample from every portion of fresh feces. In addition, routine hematology, biochemistry and urinalysis was performed prior to and upon completion of each treatment period, and bleeding time (Ivy) was measured at 2 and 48 hrs after the last dose of the pentasaccharide. The subjects remained in the unit up to 48h after the last sc injection.

Sampling and assays

The intravenous cannula used for blood sampling was kept patent by intermittent injection of 0.9% saline and blood was taken without using a tourniquet. After the first sc injection, blood samples for Pentasaccharide concentration, CIPA and APTT were taken at regular time-intervals. Blood samples for drug assay and APTT were centrifuged and the separated plasma was stored at –40° until analysis. The pentasaccharide plasma concentrations were assessed using a validated, anti-Xa based, amidolytic assay with the substrate S2222. The detection limit of the assay was 2.4 ng/ml, accuracy varied between 94–104% and the precision ranged between 3.1–6.7%. APTT assay was performed, using standard procedures with a STA coagulation analyser (Boehringer Mannheim, Diagnostica Stago) with the STA APTT reagent (Boehringer Mannheim).

CIPA was measured in 5mL of free flowing blood collected in citrate containing tubes (1 part citrate: 9 parts venous blood) immediately after blood collection with an impedance method. Briefly, a very small electric current is passed between electrodes that are put into the blood sample. During the initial contact of the blood, the electrodes become coated with a monolayer of platelets. When collagen is added, platelets aggregate on the monolayer thereby increasing the impedance (expressed in Ω). The assays were performed using collagen 1 µg/ml as aggregating agent with a Chronolog Aggregometer (model 590), according to the manufacturer s instructions.
Gastrointestinal blood loss was quantified by measuring the cumulative porphyrin content of the collected fecal samples using HPLC with spectrofluorometric detection using coproporphyrin as internal standard [10]. This methodology allows to calculate the ratio of the sum of deuteroporphyrin and pemptoporphyrin over coproporphyrin (ratio I), which is a biomarker for the total amount of hemoglobin lost in the gastrointestinal tract. In addition, the ratio of chemproporphyrin minus protoporphyrin over coproporphyrin (ratio II) can be calculated, which is a measure of intact heme, which can be present in the case of extensive blood loss in the gastrointestinal tract [11-13]

Pharmacokinetic analysis
Non-compartmental pharmacokinetic analysis was performed on the pentasaccharide concentration-time profiles using standard techniques and commercially available software (WinNonlin, version 3.1, Pharsight Corp., USA). Most parameters were calculated across the four doses of the compound. The peak concentration (C_{max}) and the time to reach these peak concentrations (T_{max}) were determined. From the time course profiles the following parameters were derived: the area under the concentration-time curve for the intervals from zero time point up to the last measurable concentration (C_{last}) using the trapezoidal rule ($AUC_{0\text{-}last}$), the half-life associated with the terminal elimination phase ($t_{½}$), the AUC extrapolated to infinity ($AUC_{0\text{-}\infty}$), and the apparent volume of distribution.

The C_{max} and T_{max}, and AUCs were also determined for the last administration of pentasaccharide ($AUC_{72h\text{-}last}$ and $AUC_{72\text{-}\infty}$). The terminal half-life was calculated using log-linear regression on the terminal part of the curve. Points for inclusion in the regression equation were automatically determined by the program, and subsequently visually checked for adequacy. The apparent clearance of the pentasaccharide was calculated as total dose administered divided by the $AUC_{0\text{-}\infty}$. This provides a measure of the average clearance over the entire study period. The differences in pharmacokinetic parameters between the combined treatment of fondaparinux + piroxicam and fondaparinux-only were compared.

Pharmacodynamic analysis

For each subject-occasion combination the area under the effect curve (AUEC) for the amplitude in the aggregometry test (CIPA) and APTT was calculated using the linear-trapezoidal rule for the period from zero time up to the last measurement and from 72 hours up to the last measurement. These AUECs were analysed with and without correction for baseline values. Contrasts were calculated for the AUEC corrected for baseline of the CIPA and APTT for the combination treatment versus the two single drug treatments, and versus the summation of the effects of the single drug treatments. Without baseline correction the AUECs for these parameters were compared between the three different treatments. Fecal blood loss was analysed by calculating contrasts for ratio I and II for the combination treatment versus the pentasaccharide and versus piroxicam. Bleeding time values were analysed by comparing the observed values (with and without pre-value correction) between treatment groups. For all parameters, contrasts were evaluated using paired Student t-tests and presented with 95% confidence intervals. However, the parameters used for fecal blood loss were not normally distributed and hence analysed non-parametrically using Wilcoxon's signed ranks test. Statistical analysis and calculations were performed with SPSS for Windows V9.0.1 (SPSS, Inc., Chicago, IL).

RESULTS

General

Due to a dispensing error at the pharmacy, one subject received sc placebo-injections instead of active pentasaccharide during one study-period. This only came to light after he had completed all his study-periods, and therefore, he was replaced. As such, this study was carried out with 13 subjects. The results of the replaced subject were used as much as possible.

None of the changes in assessed safety indices was of clinical relevance. All adverse experiences were mild in severity and none required medical intervention. The most frequently reported adverse event was development of hematoma(s) at puncture sites; the incidence was highest following the combination of pentasaccharide and piroxicam (11 times) compared to 5 times during the mono-treatments.

Pharmacokinetics

Mean plasma concentration time profiles are presented in Figure 1. The pharmacokinetic parameters for fondaparinux during both treatments are summarized in Table 1. Piroxicam did not influence the pharmacokinetics of the pentasaccharide. A small, but significant difference was found in the maximum drug plasma concentration after the first administration. Furthermore, none of the other pharmacokinetic parameters was statistically significant between the treatments.

Pharmacodynamics

Administration of piroxicam (either alone or in the combination treatment) resulted in lower impedance in CIPA test (figure 2). The mean decrease was 5.5Ω (95%CI: 0.3-10.7Ω) between pentasaccharide and piroxicam monotherapy, and 5.0Ω (1.6-8.4Ω) between pentasaccharide monotherapy and the combination therapy. No significant differences between treatments were found for the fecal porphyrin excretion rates over the study period of 5 days (table 2).

Following both treatments with the pentasaccharide a small, but significant increase was found in the average APTT. This increase

was comparable for both treatments, because no significant difference was found in the average APTT between the combination treatment and the pentasaccharide mono-treatment. This was confirmed by t-tests; the mean difference for the combination treatment vs. piroxicam was 5.5 seconds (95%CI: 3.6-7.4), and the mean difference between pentasaccharide monotreatment and piroxicam was 5.2 seconds (95%CI: 2.6-7.7). For the pharmacodynamic measures (APTT and CIPA), the analysis for the period after the last administration ($AUEC_{72h\text{-}last}$ and $AUEC_{72\text{-}\infty}$) gave similar results as found for the entire study period. For both parameters, the paired samples test after correction for pre-value confirmed the results for the uncorrected (observed) data. Moreover, no difference was found between the combination treatment and the summation of effects of the mono-treatments, indicating absence of a synergistic interaction between the pentasaccharide and the NSAID on these parameters. The average (SD) pre-dose bleeding time was 183 (76), 179 (41), 164 (47) seconds for the pentasaccharide only, piroxicam only, and for the combination treatment respectively. At 2 hours after the first administration of pentasaccharide non-significant increases were noted for the respective treatments. After the last pentasaccharide administration the bleeding times were at the same level as the pre-treatment values, hence no prolongation of bleeding time was noted in this study.

DISCUSSION

Fondaparinux administered subcutaneously to healthy male volunteers at the dose 10 mg in combination with the oral NSAID piroxicam was well tolerated and no serious adverse events were observed. The dose of pentasaccharide studied was in the high range of the therapeutic doses (comprised between 2.5 mg and 12 mg considering all phases II and III studies both in venous and arterial thrombotic disorders including prevention and curative treatments). This pentasaccharide alone, or in combination with piroxicam, had no obvious effects on vital signs and routine laboratory parameters in blood. These observations confirm results from pre-clinical and prior clinical studies. The occurrence

of hematoma at puncture sites was highest following the combined treatment, but all adverse events were mild in severity and none required medical intervention.

The co-administration of piroxicam did not influence the pharmacokinetics of fondaparinux. A statistically significant effect on C_{max} after the first dose of the compound was observed, however, taken into account the effect size (70 ng/mL, approximately 6%) this can be considered not clinically relevant. Platelet aggregation was not influenced by the single administration of the pentasaccharide. The administration of the pentasaccharide in combination with piroxicam was followed by a small decrease in platelet aggregation similar to the CIPA-decrease after the single administration of the NSAID. As the main mode of action of all NSAIDs is by interfering with the prostaglandin synthesis, it is likely that concomitant use of 10 mg of pentasaccharide and NSAIDs does not result in any relevant interaction on platelet aggregation. This was further confirmed by the absence of an interaction on bleeding time and gastrointestinal blood loss. As orally administered NSAIDs may affect the gastroduodenal mucosa, [14], all subjects underwent a fecal occult blood test at screening, commonly used for diagnostic purposes. This confirmed the absence of gastro-intestinal blood loss before the study. No differences between treatments in this study were found with regard to fecal porphyrin excretion (used as measure for gastrointestinal blood loss). It can be concluded that the co-administration of piroxicam and fondaparinux does not result in an increase in fecal porphyrin excretion in comparison to piroxicam alone.

The administration of fondaparinux alone, or in combination with piroxicam was followed by only a small rise in APTT, which confirms the high selectivity of the anti-Xa pentasaccharide.

In conclusion, sc administered fondaparinux in combination with oral piroxicam well tolerated and no pharmacokinetic or pharmacodynamic interactions were found between the pentasaccharide and piroxicam used as a representative of the group of NSAIDs.

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TABLE 1 Mean (SD) pharmacokinetic parameters of the pentasaccharide fondaparinux in the absence or presence of piroxicam.

Parameter	PENTASACCHARIDE + PIROXICAM N=13) Mean (SD)	PENTASACCHARIDE (N=12) Mean (SD)	PAIRED DIFFERENCES* Mean (95%CI)
$AUC_{0\text{-last}}$	74.1 (10.5)	75.7 (10.7)	0.82 (-3.35 , 1.72)
$t_{½}$	13.9 (1.9)	13.6 (1.5)	0.31 (-0.44 , 1.07)
$AUC_{0\text{-}\infty}$	76.8 (11.5)	78.3 (11.5)	-0.66 (-3.39 , 2.08)
V_z/F	9.2 (0.9)	8.8 (1.0)	0.22 (-0.20 , 0.64)
Cl/F	464 (72)	455 (68)	4.5 (-12.9 , 22.0)
C_{max} 1st dose	1068 (134)	1139 (144)	-70 (-114 , -26)
T_{max} 1st dose	2.27 (0.60)	2.54 (0.49)	-0.26 (-0.65 , 0.13)
$AUC_{72\text{-last}}$	24.3 (4.3)	24.3 (4.2)	0.43 (-0.89 , 1.76)
$AUC_{72\text{-}\infty}$	27.0 (5.38)	26.8 (5.07)	0.59 (-0.87 , 2.06)
C_{max} 4th dose	1453 (214)	1459 (292)	11 (-92 , 115)
T_{max} 4th dose	2.01 (0.41)	2.25 (1.43)	-0.24 (-1.02 , 0.54)

AUCs in mg*h/L; $t_{½}$ in h; Cl/F in mL/h; C_{max} in ng/mL; CT_{max} in h; V_z/F = apparent volume of distribution (in L). * Paired Samples Test (degrees of freedom=11).

TABLE 2 **Mean fecal porphyrin excretion rate.**
Ratio I(the sum of deuteroporphyrin and pemptoporphyrin over coproporphyrin) and ratio II (ratio of chemproporphyrin minus protoporphyrin over coproporphyrin)

RATIO	TREATMENT	MEAN (SD)	MEDIAN	RANGE	INTERQUARTILE RANGE
I	Fondaparinux	6.78 (5.78)	4.22	0.72 - 18.32	10.52
	Piroxicam	6.10 (5.32)	4.26	1.23 - 20.30	3.07
	Fondaparinux + piroxicam	6.80 (5.63)	6.03	1.48 - 17.89	6.25
II	Fondaparinux	33.06 (43.70)	22.53	0.70 - 148.73	26.00
	Piroxicam	31.85 (33.82)	21.69	1.46 - 112.47	25.39
	Fondaparinux +piroxicam	18.96 (15.07)	16.84	1.01 - 44.51	26.24

Reference values: ratio I < ratio II, and ratio II < 32.

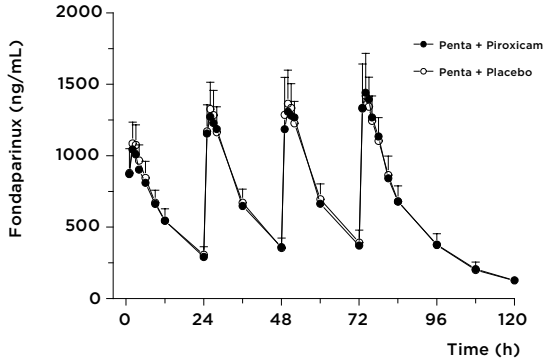

FIGURE 1 **Mean (SD) concentration time profiles after four sc doses of pentasaccharide with or without co-administration of piroxicam.**

Doses of the pentasaccharide were given at 0, 24, 48 and 72 hours. Piroxicam was administered at 24-hr intervals for 10 days starting 6 days before pentasaccharide dosing.

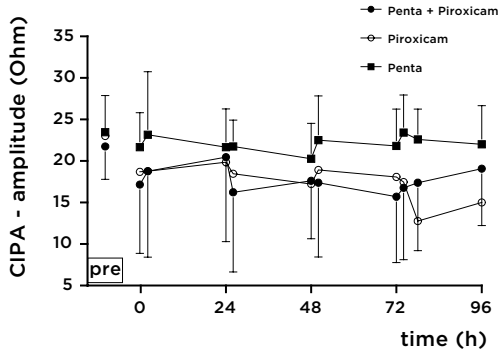

FIGURE 2 **Mean (SD) profiles of the amplitude in the collagen-induced platelet aggregation test for each treatment period.**

Doses of the pentasaccharide were given at 0, 24, 48 and 72 hours. Piroxicam was administered at 24-hr intervals for 10 days starting 6 days before pentasaccharide dosing. Pre: indicates value before piroxicam administration.

CHAPTER 6

A PHASE I SINGLE RISING DOSE STUDY TO INVESTIGATE THE SAFETY, TOLERABILITY AND PHARMACOKINETICS OF IDRAPARINUX SODIUM IN HEALTHY YOUNG MALE VOLUNTEERS

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ABSTRACT

Objectives

Idraparinux is a selective coagulation protein Xa inhibitor, designed to be long acting. Objective of the current study was to investigate the pharmacokinetics and tolerability, safety of consecutive rising doses of intravenously (iv) administered idraparinux in healthy young volunteers and to estimate the absolute bioavailability of subcutaneous (sc) idraparinux at two doses.

Methods

The study had a double-blind single rising iv dose design with an included sc bioavailability protocol. Five subjects received idraparinux and one subject placebo per dose level. For the sc bioavailability protocol six subjects received idraparinux and one placebo. The dose-increments were 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12 and 14 mg as twenty seconds iv bolus injections. In the sc study 2 or 10 mg was given. Blood sampling was done up to 336 h for pharmacokinetics and pharmacodynamics (APTT/PT, AT, anti-IIa activity). Urine was collected up to 168 h.

Results

Idraparinux sodium was well tolerated and no significant adverse events were noted. AUC and Cmax increased in a dose-proportional manner, indicating linear pharmacokinetics. Total plasma clearance was independent of dose, whereas terminal half-life estimates reached a plateau of about 120 hours (following doses of 6 mg or higher; range of means: 116–127 h). Indications of an even longer elimination half-life were found. Idraparinux sodium plasma clearance correlated with body weight (r=0.503; p=0.003). The sc bioavailability was 93% after 2 mg idraparinux sc and 99.8% after 10 mg sc with good consistency of the pharmacokinetic parameters between the sc and iv route of administration. Following one week of urine collections, almost 20% of idraparinux was cleared by the renal route (range: 14.3-23.3% for dosages of 1 mg or higher). Renal clearance and mean urinary excretion rate increased with dose. A small, clinically insignificant effect in APTT and PT was noted.

Conclusions
Idraparinux sodium is an antithrombotic compound with linear pharmacokinetics and an absolute bioavailability after sc administration of almost 100% and a terminal half-life of about 5 days. It is therefore anticipated that idraparinux may be a useful new drug for long-term anti-thrombotic use.

INTRODUCTION

Idraparinux sodium (Figure 1) is a novel synthetic pentasaccharide compound with anticoagulant properties *in vitro* and *in vivo*. Preclinical studies indicated that it works by activation of antithrombin (AT), the naturally occurring inhibitor of the blood clotting process. The main activity of AT is against factor Xa and IIa (thrombin). Heparin-like drugs accelerate the reaction that forms complexes between AT and factor Xa or factor IIa. These complexes dissociate very slowly relative to their target protein in the coagulation cascade. Recently, synthetic analogues of the heparin pentasaccharide sequence that binds to AT have become available, which selectively inhibit factor Xa. One of these compunds (fondaparinux) has been registered. Most anticoagulants presently available need to be administered at least once daily. However, it is increasingly recognised that people at risk of thrombotic disorders need to be treated over an extensive period of time (months, years, or even life-long). Prolonged antithrombotic treatment ideally requires a dose regimen with a drug with an extended elimination half-life, enabling a low dosing frequency. On the basis of the slow elimination rate of idraparinux compared to fondaparinux observed in pre-clinical studies, it was anticipated that idraparinux sodium could be a useful new drug for prolonged antithrombotic treatment. Therefore this study was performed to investigate the safety, tolerability and pharmacokinetics of intravenously (iv) administered idraparinux. As it can be envisaged that for clinical use this compound will be administered subcutaneously (sc), the absolute bioavailability of sc idraparinux was investigated in the same experiment. Since animal data suggested that the compound was partly renally cleared, investigation of its renal clearance was also part of the analysis.

METHODS

Subjects and Design

This study was conducted according to a single rising dose, randomised placebo-controlled design. Parallel dose-level groups started consecutively with at least one-week intervals, double blind within each dose level. Subjects were allowed to participate more than once, provided a washout period of at least 4 weeks was observed. The bioavailability part of the study was also double blind regarding placebo administration. Iv and sc administration of idraparinux within relevant dose levels was not blinded; no dummy treatments were used, but the order of administration was randomised.

All rising dose groups consisted of six subjects, with dose levels 0.25, 0.5, 1, 4, 6, 8, 12 and 14 mg idraparinux. Five subjects were randomised to active treatment and one subject to placebo. All iv doses were administered in an identical volume of 5 mL as a 20 seconds bolus injection, and the sc doses consisted of a volume 0.53 and 2.63 mL for administration of the 2 and 10 mg doses. Syringes for iv or sc injection used as placebo, contained an equal volume of 0.9% saline solution. All study medication was prepared by the Leiden University Medical Center pharmacy.

The bioavailability part of the study included six subjects receiving active treatment plus one receiving placebo for the dose levels involved (2 and 10 mg). Thus, in total fourteen subjects participated in this part. First subjects were randomised to receive idraparinux iv or sc and either idraparinux or placebo. The order was reversed after a washout period of at least 4 weeks. Subjects receiving placebo the first time also received placebo the second time.

Procedures

All subjects participated in this study after written informed consent was obtained. The Ethics Committee of Leiden University Medical Center approved the investigational protocol. The subjects were studied after an overnight fast. After arrival at the CHDR unit a short medical history and a physical exam were performed to ensure compliance to the protocol restrictions. Subjects were exposed to no more than four single injections.

Sampling

No tourniquet was applied when blood was collected during the study days. An iv cannula used for blood sampling was kept patent by intermittent flushing with 0.9% saline. Blood was taken after discarding the contents of the cannula. Blood samples for baseline values were taken pre-dose.

Pharmacokinetic Assessments

Blood samples for drug-assay were drawn frequently until 336 h after administration of idraparinux. Urine was collected for measurement of drug concentration over the following time-intervals: 0-4, 4-12, 12-24 h, and subsequently in 24-hour periods until maximally 168 h (1 week) after dosing in pre-weighted plastic containers. In view of the fact that a physicochemical method for assessing plasma and urine concentrations of idraparinux is not available, a detection method based on the anti-factor Xa activity of the idraparinux-AT complex was used. Since there is no evidence of the formation of active metabolites of the drug in vivo (data on file) the specificity of this assay can be assumed. The validated assay in plasma and urine was performed at the Department of Drug Metabolism and Kinetics of Organon Development GmbH (Waltrop, Germany). In the sample preparation the presence of sufficient AT is secured either by dilution of samples with plasma or the addition of AT (in urine samples). Idraparinux sodium binds to AT to form a complex which inactivates factor Xa by irreversible binding. Subsequently, factor Xa is added in excess and the remaining free factor Xa catalyses the generation of paranitroalanine (pNA) by the hydrolysis of the peptide pNA conjugate. The liberated pNA has a yellow color that can be measured spectrophotometrically at 405 nm. The measured color is inversely proportional to the idraparinux concentration in the sample. Lower limits of quantification were 16 ng/mL in plasma and 6 ng/mL in urine.

Pharmacodynamic Assessments

Blood samples for APTT/PT, AT and anti-factor IIa-activity assays were drawn at regular time intervals until 336 h relative to the

dose of idraparinux. Bleeding time (Ivy method) was done at screening (baseline value) and at 15 min and 48 hrs after dosing.

Laboratory Parameters

APTT and PT assays were performed using standard procedures [1]. AT in plasma was assessed using a photometric assay with S-2765 as a chromogenic substrate and is based on the inhibition of factor Xa (Comatic© Antithrombin, Mölndal, Sweden). The heparin anti-IIa activity was determined using a photometric assay with thrombin substrate H-D-HHT-L-Ala-L-Arg-pNA.AcOH (Spectrolyse® Heparin anti-IIa, Biopool, Umea, Sweden). An aliquot of the corresponding idraparinux dosing formulation was used as calibrator.

Statistical analysis

Non-compartmental pharmacokinetic analysis was performed using WinNonlin V1.1 (Scientific Consulting, Inc., Apex, NC). The following parameters were derived from the time course profiles: the peak concentration (C_{max}) and the time to reach these peak concentrations (t_{max}), the AUC from the time intervals from zero time point up to the last measured concentration using the lineair trapezoidal rule ($AUC_{0\text{-last}}$), the elimination rate constant associated with the terminal elimination phase (λ_z) and its associated half-life ($t_{1/2}$) the AUC extrapolated to infinity ($AUC_{0\text{-}\infty}$), the plasma clearance (CL) and apparent volume of distribution (Vz). The number of points used for λ_z calculation were automatically determined by the pharmacokinetic-program and were visually checked for adequacy. The sc bioavailability was calculated by dividing the $AUC_{0\text{-}\infty}$ for the sc administration by the $AUC_{0\text{-}\infty}$ for the iv administration. Non-zero pre-values were dealt with by subtracting from the total AUC, the AUC attributable to these non-zero pre-values (calculated as pre-value/λ_z, where λ_z is the estimated elimination rate constant for that particular curve).

The pharmacokinetic parameters resulting from iv administration were compared between doses using one-way analysis of variance with factor dose; AUC and C_{max} were dose-normalised. Between-dose variability was further investigated by estimating a linear trend over dose. Primary analysis was performed for the non-extended profiles (see further).

The urinary excretion of idraparinux was calculated using

cumulative excretion and percentage of dose excreted. For each collection interval, the excretion rate was calculated by dividing the amount excreted over the interval by the corresponding time period. The renal clearance was calculated by dividing the cumulative excretion by the plasma AUC with endpoint closest to the end of the urine collection period ($AUC_{0\text{-}t}$).For repeatedly measured pharmacodynamic variables (APTT/PT, AT and anti-IIa activity), the Areas Under the Effect Curves (AUECs) using the linear trapezoidal rule on protocol times were calculated after subtraction of pre-values, both for the periods 0–6 h as well as 0–24 h post-dosing using BMDP/Dynamic Version 7.0 (BMDP Statistical Software, Inc., Los Angeles, CA). Subsequently, these areas were divided by the corresponding time span to result in a weighted average response (AUEC/time). Additionally, the change from pre-value to E_{max}(delta E_{max}) and the change from pre-value to t=24 h post-dosing (delta 24h) were calculated. Pharmacodynamic parameters (iv administration) were compared between doses using one-way analysis of variance with factor dose. Between-dose variability was further investigated by estimating a linear trend over dose.
Statistical analysis and calculations were performed using SPSS for Windows V8.0.1 (SPSS, Inc., Chicago, IL).

RESULTS

The study was performed in 40 volunteers. Nine received placebo treatment during at least one occasion. Two subjects, treated with placebo were given idraparinux on a second occasion. Thus, a total number of 33 subjects were treated with at least one dose of idraparinux sodium. The compound was well tolerated and no significant adverse events were noted. Most of the reported adverse events were local hematoma at the injection or sampling site. There was no apparent dose-relationship in the incidence of adverse events and there was no difference between idraparinux and placebo concerning safety. There were no discontinuations. It was decided to introduce bedside APTT/PT-measurements with the Biotrack® 512 Coagulation Monitor of Ciba Corning® (Coagu-Chek® *Plus*). This monitor provides an integrated system for

immediate measurement of PT and APTT in one drop of whole blood [2]. This technique was used for initial safety measurements only. Measurements were within normal ranges for the subjects at all timepoints. However, since effects on bedside measurements were seen at dosages of 10 mg, the number of timepoints at which these measurements were taken was increased. For instance at the 14 mg-group a consistent and distinct increase of the bedside-APTT of 13 sec (approximately 165% of baseline values was found; Figure 2). This and the fact that it appeared that the drug showed a very long elimination half-life, and it could be expected that the chance on drug-related AEs would increase at higher doses, made that it was decided to abandon dosing beyond the 14 mg dose.

Pharmacokinetics

Terminal half-life, $AUC_{0-\infty}$, clearance and Vz are not reported for doses below 1 mg because of poor definition of the terminal phase. All non-zero pre-dose concentrations were obtained when previous drug-administration had occurred. The percentage of the total AUC attributable to these non-zero pre-values was about 4% maximally (for the iv 10 mg dosage-group, less for the other groups).

A summary of the results is given in Figure 3 and Table 1. The data indicate that the maximal plasma levels (C_{max}) and $AUC_{0-\infty}$ increased linearly with the dose (Figure 4). Dose-linearity was confirmed by one-way analysis of variance. Plasma clearance was independent of dose. Terminal half-life increased with dose ($p=0.001$), but reached a plateau of about 120 hours based on 2 week profile for dosages 6 mg iv and higher. Mean sc bioavailability was 93.0% (SD: 8.36%; range: 81.8-105.2%) after 2 mg idraparinux sc and 99.8% (SD: 5.85%; range: 89.2-104.6%) after 10 mg sc. Additionally, pharmacokinetic parameters were determined for those occasions where non-zero pre-values of the next occasion were added as if they were a final measurement. Samples at 4 weeks post-dosing or beyond were also included in the curve fitting. Results of this analysis indicated that idraparinux has a very long terminal half-life of approximately 300 hours, when sampling is continued for a sufficiently long time.

The urinary excretion increased with dose. Mean cumulative urinary excretion was about 20% of the administered dose (range of means: 14.3–23.3% for dosages of 1 mg or higher). At higher plasma concentrations a greater part was cleared by the renal route ($p<0.001$); average renal clearance ranged from 0.17 mL/min for the 0.5 mg dose group to 0.30 mL/min for the 14 mg dose group (Table 1). The dose-corrected renal excretion rate calculated for the first urine-collection portion (0–4 h post-dosing) increased with dose (Figure 5).

Pharmacodynamics

APTT and PT AUEC versus dose plots are presented in Figure 6. Maximum individual increase observed in this study was 17.3 seconds for the APTT and 2.7 seconds for the PT, both observed in subjects after 14mg idraparinux iv. The mean increase in this dose-group was 9.1 seconds for the APTT and 1.9 seconds for the PT, respectively.

With regard to the analysis concerning the between-dose variability for the APTT the p values for linear trend with dose were just above 0.05 for all measures. There was no statistically significant increase with dose for APTT (Figure 6). The slope of the regression-line calculated for the $AUEC_{0\text{-}6h}$ for APTT is 0.436 s/mg (95%CI: 0.17-0.70). For the PT a significant increase with increasing dose was observed for the weighted average response: $AUEC/time_{0\text{-}6h}$ and $AUEC/time_{0\text{-}24h}$ (p values 0.002 and 0.021, respectively).

For PT the slope of the regression-line for the $AUEC_{0\text{-}6h}$ was 0.094 s/mg (95%CI: 0.05, 0.14) (Figure 6). For AT the relationship from the regression of the $AUEC_{0\text{-}6h}$ on dose can be described as AT $AUEC_{0\text{-}6h}$ = –2.24 + 0.533*dose (95%CI for the slope: 0.05, 1.02).

Anti-IIa activity remained virtually unchanged after dosing idraparinux at the dose-levels tested in this study and none of the response measures reached statistical significance.

Bleeding times remained within the clinically acceptable range for all doses. There was a weak dose-response relationship (increase of approximately 2% per mg idraparinux at the t=15 min assessment).

Additional Analysis

The relationship between body weight and body mass index (BMI) and the pharmacokinetic parameters of the compound idrapari-

nux was investigated by calculating correlation coefficients with C_{max}, elimination half-life, clearance, and the apparent volume of distribution. Only parameters for iv administered doses higher than 0.5 mg were used. For subjects participating on multiple occasions, the average parameter for the occasions was used. Clearance was significantly correlated with bodyweight (Pearson's (parametric) correlation coefficient r=0.503; p=0.003).

DISCUSSION

Because pre-clinical studies had shown that idraparinux sodium, a novel synthetic pentasaccharide compound selectively inhibiting factor Xa, was eliminated at a slow rate, it was anticipated that idraparinux could be a potential new drug for long-term antithrombotic use. This study confirms the favorable safety/tolerability-profile found in preclinical studies and studies conducted with another pentasaccharide (the shorter acting fondaparinux sodium) [3]. Comparable to data found in idraparinux-treated animals and studies conducted with fondaparinux an almost complete absolute SC bioavailability was found, with a good consistency of pharmacokinetic parameters between the IV and SC route of administration. With regard to the pharmacokinetics, plasma concentrations were determined by using an anti-Xa activity assay, since a direct method to measure idraparinux levels does not exist.

The peak concentration and AUC linearly increased with the dose. Elimination half-life estimates increased with dose, but reached a plateau of about 120 hours based on a 2 week profile for dosages higher than 6 mg iv. This is most likely caused by the extended measurement period at higher doses, and thus a poorer definition of the terminal elimination phase at lower doses. As it is known that low endogenous anti-Xa levels can be measured (albeit with substantial variability), this may have further complicated the assessment of the elimination phase of the lowest doses.

After a washout period of at least 4 weeks some of the subjects still showed detectable plasma concentrations of idraparinux sodium prior to a next administration. Non-compartmental pharmacokinetic parameters were derived from extended profiles

obtained by adding these non-zero values observed just before the next dosing occasion to the profile associated with the previous dosing occasion. Comparing plasma concentrations of idraparinux sodium at timepoints up to 2 weeks and up to 4 weeks (or beyond) it can be concluded that the elimination half-life estimate increased from 120 to 300 hours. This half-life was associated with low concentration levels in the extended portion of the idraparinux sodium plasma profiles. Supported by the concept that unfractionated heparin disappears as free heparin from the equilibrium heparin-AT [4], idraparinux sodium released as a result of endogenous AT turn-over can possibly bind to newly produced AT, and the half-life of idraparinux sodium can be greater than the elimination half-life of AT (which is about 70 h). Therefore, it is conceivable that the prolonged half-life is associated with redistribution of idraparinux sodium.
The clinical relevance of this prolonged half-life and its relevance in terms of accumulation during repeated dosing is yet to be assessed.
In an *in vitro study*, undertaken to determine if the plasma concentrations of AT would be the rate limiting factor for the potency of this group of pentasaccharides, it was determined that an equimolar amount of AT was required for full expression of the anti-Xa effect of fondaparinux [see 5]. In this study approximately 20% of idraparinux was cleared within a week by the renal system with a tendency towards a higher renal clearance rate at higher plasma concentrations (i.e. following dosages exceeding 6 mg). This phenomenon can best be explained by concentration-dependent renal clearance of the pentasaccharide. Even though the mean plasma AT concentration in healthy human subject is approximately 200 µg/mL [6], it seems after administration of higher dosages of the compound the capacity for idraparinux binding by AT is exceeded, resulting in a higher free fraction which is renally excreted. Nevertheless, after one week of complete urine-collections, mean maximum percentage of the dose excreted in the urine was already 23.3 % (SD: 2.46) and it can be anticpated that over longer collection periods this fraction may increase. Nevertheless, it seems that the total plasma clearance

remains stable at approximately 0.9 mL/min for doses above 2 mg. The effects of a single dose of idraparinux on secondary haemostasis as measured by APTT and PT were also assessed. The bedside assessment in whole blood seemed to overestimate the effects. For the more reliable laboratory assessments, small increased in mean APTT and PT were noted. This finding confirms that the conventional coagulation assays are not greatly influenced by the very specific action of this pentasaccharide. In addition, the changes in APTT/PT seem to be clinically unimportant. Though the relation between the anti-Xa activity and the probability of bleeding is unknown, this parameter is a better predictor for the effect of idraparinux in the circulation. In conclusion, idraparinux sodium is at least partly renally cleared. Its long elimination half-life is expected to allow once per week dosing in the prophylactic treatment of thromboembolic disorders.

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Summary of pharmacokinetic results

(data points included up to two weeks)

TABLE 1

TREATMENT	C_{MAX} (ng/mL)	$AUC_{0-\infty}$ (mg/L*h)	$t_{1/2}$ (h)	CL (mL/min)	VZ (L)	CL_{RENAL} (mL/min)	FRACTION renal
IV 0.25mg (n=5)	84.3 (5.2)	*	*	*	4.93 (1.7)		
IV 0.5mg (n=5)	147 (27)	*	*	*	7.72 (1.9)	0.17 (0.10)	
IV 1mg (n=5)	309 (42)	16.7 (1.5)	82.4 (16.8)	1.00 (0.09)	7.08 (1.1)	0.19 (0.06)	0.20 (0.06)
IV 2mg (n=6)	582 (64)	37.7 (5.6)	102.2 (17.0)	0.90 (0.13)	7.91 (1.4)	0.20 (0.03)	0.22 (0.03)
IV 4mg (n=5)	1320 (120)	78.7 (7.3)	99.6 (6.1)	0.85 (0.08)	7.32 (0.04)	0.20 (0.04)	0.23 (0.04)
IV 6mg (n=8)	1860 (230)	117.3 (7.7)	116.3 (27.1)	0.86 (0.06)	8.64 (2.2)	0.19 (0.05)	0.23 (0.06)
IV 8mg (n=5)	2560 (240)	149.1 (22.0)	122.0 (28.8)	0.91 (0.13)	9.48 (2.1)	0.23 (0.09)	0.25 (0.09)
IV 10mg (n=6)	2850 (270)	205.1 (13.0)	127.3 (15.7)	0.82 (0.05)	8.69 (1.3)	0.29 (0.05)	0.35 (0.04)
IV 12mg (n=5)	3430 (720)	223.9 (30.9)	122.9 (22.7)	0.91 (0.12)	9.54 (2.0)	0.25 (0.04)	0.28 (0.04)
IV 14mg (n=5)	3840 (360)	261.5 (13.6)	119.6 (22.1)	0.89 (0.05)	9.13 (1.2)	0.30 (0.07)	0.33 (0.07)
SC 2mg (n=6)	426 (43)	34.8 (4.3)	95.9 (10.8)	0.97 (0.12)	8.09 (1.6)	0.20 (0.04)	0.21 (0.04)
SC 10mg (n=6)	2180 (132)	204.4 (12.6)	134.3 (28.2)	0.82 (0.05)	9.14 (1.8)	0.29 (0.06)	0.35 (0.07)

Results are from non-compartmental analysis, corrected for non-zero pre-values and reported as mean (SD). *Terminal $t_{½}$, $AUC_{0-\infty}$ and clearance are not reported for doses below 1 mg because of poor definition of the terminal phase.

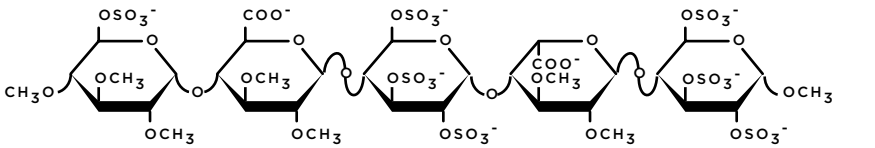

FIGURE 1 Chemical Structure idraparinux

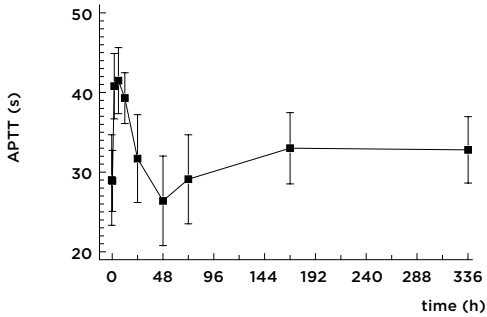

FIGURE 2 Mean 'bedside' APTT-results following administration of 14mg idraparinux iv.

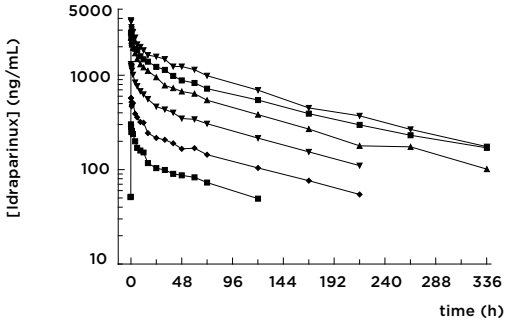

FIGURE 3 Average concentration-time curves per group following intravenous administration

(logarithmic scale; ■ 1mg, ◆ 2mg, ▼ 4mg, ▲ 8mg, ■ 10mg, ▼ 14mg are the administered dosages group).

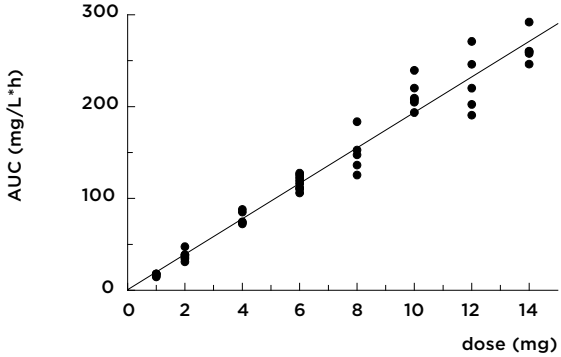

FIGURE 4 AUC extrapolated to infinity versus dose

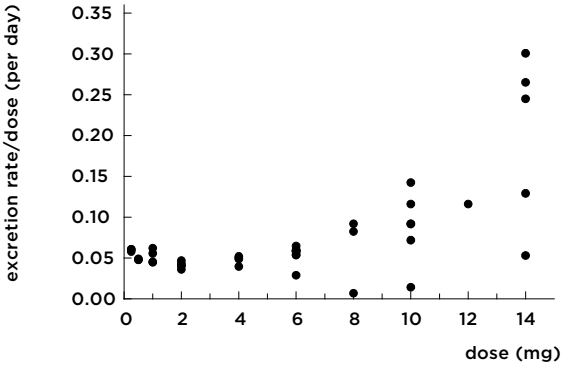

FIGURE 5 Urinary excretion rate/dose versus dose calculated for the first urine-sampling period: 0–4 hours post-dosing.

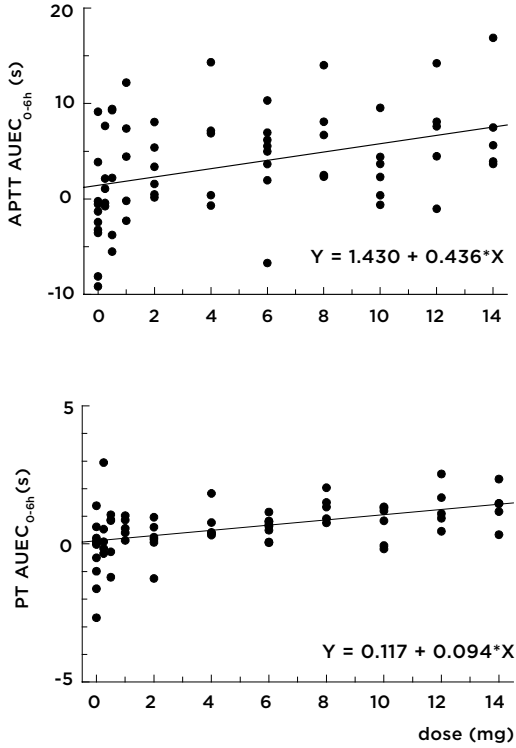

FIGURE 6 **APTT and PT AUEC above baseline (calculated for the period 0–6 hours post-dosing).**
Predicted increase after 14mg idraparinux iv is 7.53 seconds for the APTT and 1.44 seconds for the PT.

CHAPTER 7

THE EFFECTS OF AGE ON THE PHARMACOKINETICS AND EFFECTS OF THE LONG-ACTING SYNTHETIC PENTASACCHARIDE IDRAPARINUX SODIUM

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ABSTRACT

Objectives

To investigate the safety, tolerability and pharmacokinetics and absolute bioavailability of subcutaneously (sc) administered idraparinux in elderly volunteers.

Methods

Eighteen volunteers (8F/10M; 3 groups of six subjects) were included in an open label study. The first group received 2 mg idraparinux sc. The second group received 6 mg sc and intravenously (iv) in a randomised crossover fashion. The third group received 10 mg sc. Blood sampling was done up to 336 hrs for pharmacokinetics and pharmacodynamics (APTT/PT). Urine was collected up to 168 hrs. Individual plasma concentration-time profiles were analysed model-independently. Urinary data were used to calculate the renal clearance. The pharmacodynamic response was analysed by maximal effect values and the time-integrated AUEC responses.

Results

Idraparinux sodium was well tolerated and no significant adverse events were noted. Plasma clearance was independent of dose and correlated with body weight. The drug demonstrated linear pharmacokinetics, and the sc bioavailability was almost 100%. The extrapolated theoretical urinary excretion of idraparinux was almost 50%. The mean (SD) terminal half-life was 136 (27) hr. The drug induced a maximal (SD) increase in APTT of 8.3 (2.9) sec and in PT of 1.2 (0.3) sec.

Conclusions

In elderly subjects idraparinux is almost 100% bioavailable after sc administration, shows linear pharmacokinetics and elicits only minor changes in APTT/PT . The data from this study are comparable to data in young subjects, indicating absence of influence of age on the pharmacokinetics of this highly selective anti-Xa compound. It is anticipated that idraparinux sodium can be a useful new drug for long-term anti-thrombotic use.

Introduction

Idraparinux is a novel synthetic pentasaccharide compound with anticoagulant properties *in vitro* and *in vivo*. Pre-clinical studies indicate that it works by activation of antithrombin (AT), the naturally occurring inhibitor of the clotting process. The main activity of AT is against factor Xa and thrombin. Heparin-like drugs accelerate the reaction that forms complexes between AT and factor Xa or factor IIa. These complexes dissociate very slowly relative to the complexes with the target protein in the coagulation cascade. Recently, synthetic analogues of heparin pentasaccharide sequence that binds to AT have become available, which selectively inhibit factor Xa. One of these compunds (fondaparinux) has been registered. Unlike heparin, the pentasaacharides are selectively active against factor Xa and idraparinux has a specific activity of about 1400 anti-Xa units/mg. Previous experience with idraparinux sodium and other pentasaccharides suggests that a highly selective anti-thrombotic effect can be elicited with this group of compounds [1,2]. If thrombotic risk or complications require prolonged treatment, a safe drug with an extended elimination half-life allowing a low dosing frequency would be preferable. Idraparinux has an elimination half-life of approximately 130 h in young subjects and a subcutaneous (sc) bioavailability of almost 100%.

The present study was carried out to investigate the safety, the tolerability and pharmacokinetics in healthy elderly subjects. As it can be envisaged that for clinical use idraparinux sodium will be administered sc in this target population, three sc doses were employed in this study. The absolute bioavailability of the compound was also estimated for one dose in this population.

METHODS

Subjects and Design

Eighteen healthy subjects (8F/10M; age: 60-71 years; body weight 56.5-91.8 kg) with a normal coagulation screen participated in this study after written informed consent was obtained. The Ethics Committee of Leiden University Medical Center approved the investigational protocol.

This open label study was conducted according to a single rising dose design. Three groups (six subjects each) were dosed with 2, 6 or 10 mg idraparinux sodium sc. The 2 and 10 mg dose were only sc administered. The 6 mg dose was administered twice (once intravenously (iv) and once sc) in a randomised crossover fashion. For this group the washout period between study-occasions was at least 4 weeks.
For the 6 mg IV dose, disposable syringes were filled as close as possible to 5 mL with diluted drug solution (diluted with 0.9% saline solution). For the sc doses, disposable syringes were filled with undiluted medication solution (as provided by the sponsor) as close as possible to the minimum required volume (i.e. 0.53, 1.58, and 2.63 mL for the 2, 6 and 10 mg doses, respectively).
All drug preparations were done by Leiden University Medical Center pharmacy.

Study Days
Subjects were studied after an overnight fast. Upon arrival at the CHDR unit a short medical history and a physical exam were performed to ensure compliance to the protocol restrictions. Then the subjects voided and an iv cannula for blood sampling was inserted in a forearm vein. During the iv study days an additional cannula was inserted in the other arm. Idraparinux was administered sc in an abdominal skinfold or as a slow iv bolus injection.

Sampling
Free flowing blood from an iv cannula that was kept patent by intermittent flushing with 0.9% saline was sampled. Blood was taken after discarding the contents of the cannula. Blood samples for base-line values were taken pre-dose. Blood samples for drug-assay were drawn frequently until 336 h after drug administration (264 h for sc 2 mg). The subjects participating in the 2 and 10 mg group collected urine for measurement of drug excretion (time intervals: 0-12, 12-24 h and subsequently in 24-hour periods until 168 h after dosing) in pre-weighted plastic containers. Additionally, the last four subjects dosed with 10 mg collected urine for the following periods: 240–264, 408–432, 576–600 and

744–768 h (i.e. days 10, 17, 24 and 31 post-dosing). At the end of these periods additional blood samples for drug-assay were taken. Ivy bleeding time measurements were performed pre-dose and at either 15 minutes (iv route) or 4 h (sc route) as well as 48 h after dose administration (or at discharge) and at the end of each study period.

Assays

Idraparinux concentrations were measured using a validated amidolytic assay based upon anti-factor Xa activity [3]. The assays were performed at the Department of Drug Metabolism and Kinetics of Organon Development GmbH (Waltrop, Germany) (see [2]). Blood samples for APTT and PT assays were drawn at regular time intervals until 336 h relative to the dose of idraparinux. These assays were performed using standard procedures [4]. AT concentration was assessed pre-dose, and at the end of each study occasion. AT was to be assessed using a validated photometric assay with S-2765 as a chromogenic substrate, which is based on the inhibition of factor Xa (Comatic® Antithrombin, Mölndal, Sweden).

Statistical analysis

Post-dose measurements below the reported lower limit of quantification (LOQ) were excluded from the pharmacokinetic analysis. Actual sampling time points were used for calculation of the individual pharmacokinetic parameters using WinNonlin V1.1 (Scientific Consulting, Inc., Apex, NC). The following non-compartmental pharmacokinetic parameters were derived from the individual profiles: the observed peak value (C_{max}) and the associated sampling time (t_{max}), the half-life associated with the terminal elimination phase ($t_{½}$), the Area under the Curve (AUC) calculated using the linear trapezoidal method extrapolated to infinity ($AUC_{0-\infty}$), the plasma clearance and the apparent volume of distribution (Vz). Initially measurements beyond 14 days post-dosing were excluded from the calculations.

Subjects participating in the bioavailability part of the study had non-zero anti-Xa values at the start of the second occasion due to the long half-life of the drug. The AUC attributable to this pre-

value (calculated as pre-value / ($\ln 2 / t_{½}$) was subtracted from the $AUC_{0-\infty}$ resulting in corrected estimates. Absolute subcutaneous bioavailability of 6 mg idraparinux was determined by calculating the ratio $AUC_{0-\infty,sc}$ / $AUC_{0-\infty,iv}$.

Based on the collected urinary concentration data, the cumulative urinary excretion of idraparinux was estimated. The renal clearance was calculated over the first week following dosing where complete urine collection took place. Calculation of the ratio of the first week cumulative urinary excretion over the first week plasma AUC yielded the renal clearance.

Total urinary excretion of idraparinux was estimated using the four subjects in the 10 mg sc group with the extended urine sampling scheme. For each urine collection period, urinary excretion rate was calculated and plotted against mid-collection timepoints This excretion rate profile was integrated using the linear trapezoidal rule and extrapolated using the four final measurements (days 12, 19, 26 and 33). The initial portion of the profile was back extrapolated to time zero. The extrapolated AUC of the profile represents the theoretical urinary excretion of idraparinux.

Additionally, pharmacokinetic estimates were derived from the extended profiles of the 6 mg dose level group obtained by adding the non-zero pre-value observed just before the second dosing occasion to the profile of the first study occasion as if it were a final measurement. Extended profiles were also obtained for the last four subjects of the 10 mg dosing group.

The relationship between body weight and the pharmacokinetic parameters of idraparinux was investigated by calculating correlation coefficients with clearance, elimination half-life, the apparent volume of distribution and dose-normalised C_{max}. The C_{max} and clearance after 10 mg sc idraparinux of this study were compared to previously obtained data in healthy young male volunteers [2] and 90% bioequivalence intervals on these (log-transformed) parameters were calculated.

The base-line corrected Area under the Effect Curve (AUEC) of the APTT-profile was calculated using the linear trapezoidal rule on protocol times and was subsequently divided by the corresponding timespan to result in a weighted average response. The APTT

profile was characterised by the AUEC over the first 6 hours ($AUEC_{0-6}$) and the first 24 hours ($AUEC_{0-24}$). Additionally, the APTT response was characterised using the change from pre-value to the maximum value ($ATTP_{max}$) and the change to the value at 24 hours ($ATTP_{24h}$). PT values were analysed as difference from pre-value at the protocol measurement times. The treatments were compared using one-way ANOVA followed by contrasts on the subcutaneous parameters only. Statistical analysis and calculations were performed using SPSS for Windows (SPSS, Inc., Chicago, Ill.).

RESULTS

No significant adverse events were noted after idraparinux sodium and all subjects completed the study. The most frequently reported drug-related adverse events were haematomas (mainly at puncture sites, i.e. at the site of the sc injection, iv cannulas or bleeding time punctures). Five 5 (2 mg sc), 5 (6 mg sc), 5 (10 mg sc) and 3 (6 mg iv) subjects, respectively, had one or more hematomas. Some, not-dose related, single prolonged bleeding times were observed, and rebleeding at the site of bleeding time assessment was seen once (10 mg sc).

Pharmacokinetics

A summary of the results is given in Figure 1 and Table 1. The data indicate that the maximal plasma levels (C_{max}) and $AUC_{0-\infty}$ increase linearly with the dose. Dose-proportionality was confirmed by one-way analysis of variance. Plasma clearance was independent of dose. The mean absolute bioavailability after sc dosing of 6 mg idraparinux was 94.9 ± 8.0% (range 87.3 – 107.7%). The mean elimination half-life estimate after the 6 and 10 mg doses calculated after the initial study period (i.e. 2 weeks) was 136 ± 27 h. Analysis of the pharmacokinetic parameters for the extended profiles (samples at 4 weeks or beyond also included in the curve fitting) indicate that idraparinux has a very long 'true' half-life (Table 2). After one week of complete urine collection the mean cumulative urinary excretion was 14.5 ± 3.5% after 2 mg, and 17.8 ± 3.1% after 10 mg. The corresponding renal clearances were 0.18 ± 0.06 mL/min after 2 mg, and 0.22 ± 0.04 mL/min after 10 mg

(Table 1). The estimated total urinary excretion for the last 4 subjects of the 10 mg group was 47 ± 4.8%.
The analysis of the relation between body weight and the pharmacokinetic parameters (using Pearson's parametric correlation coefficients) revealed a positive correlation for weight and clearance (r=0.692; p=0.001) and apparent volume of distribution (r=0.548; p=0.019), and a negative correlation with dose-normalized C_{max} (r=-0.643; p=0.004). The 90% bioequivalence intervals on log-transformed C_{max} and clearance, calculated to investigate whether the pharmacokinetics of idraparinux were different for elderly subjects compared to young males (results from [2]) were 0.85 / 1.12 and 0.88 / 1.11, respectively.

Pharmacodynamics
APTT was prolonged after each of the sc dose levels tested in this study (Figure 4). On average, the maximum prolongation was seen 6–12 hours post-dose and it was independent of the dose.
The average (± SD) maximum change from pre-value was 8.3 ± 2.9 seconds for the 10 mg idraparinux dosing group. PT was prolonged at 24 h post-dosing (PT_{24h}) after each of the doses (Figure 4).
The mean maximum increase was 1.18 ± 0.25 seconds after 10 mg.
A dose-related prolongation of the PT_{24h} was apparent across the dose levels; multiple paired t-testing showed significant differences for 10 mg versus 2 mg (p<0.001) and 10 mg versus 6 mg (p=0.002), respectively. For Ivy bleeding time an inconsistent pattern of changes was observed, which was judged not clinically relevant. Post-dose AT concentrations (assessed 12 to 15 days post-dose) did not reveal significant differences compared to baseline values.

Discussion
In many industrialised countries, the elderly constitute the fastest growing subpopulation [5]. Additionally, about 80% of persons of 65 years or older suffer from (chronic) conditions that may require long-term medical treatment [6]. Elderly individuals form a substantial subgroup of the approximately 25% of all non-surgical patients with thromboembolic events, while only limited

data are available on the efficacy and safety of thromboembolic prophylaxis in this specific subgroup of patients [7]. The present study was carried out to investigate the safety, tolerability and pharmacokinetics of idraparinux in subjects over 60 years of age. The compound was well tolerated after single sc doses in the elderly volunteers in this study. Comparable to a previous study in healthy young male subjects, only mild adverse events were noted. The absolute bioavailability of 6 mg sc idraparinux was estimated at 95 ± 8%. A good consistency of pharmacokinetic parameters between the iv and sc route of administration was found. Terminal half-life was 136 ± 27 hours for curves determined over 2 weeks.

After a period of 2 weeks approximately 20% of idraparinux was cleared by the renal route. The ratio of total plasma clearance to renal clearance was 0.21 and 0.27 for the 2 mg and the 10 mg dose, respectively. Furthermore, the estimated total renal excretion was less than 50% after collections up to 31 days after dosing (almost 6 times the calculated elimination half-life). This may indicate that renal excretion is not the only elimination pathway for this pentasaccharide.

Currently, coumarin-derivatives (especially warfarin) are the most widely used anticoagulants for long-term use. The anticoagulant response to warfarin is exaggerated with advancing age [8]. Moreover, the risk of undesired adverse reactions (bleeding complications) for these compounds rises significantly with age [9]. Although this enhanced response in the elderly is considered to be caused by pharmacodynamic factors, a decrease in warfarin-clearance with increasing age has also been reported [10]. In contrast, the bioequivalence intervals for the pharmacokinetic parameters in this study compared to those obtained in healthy young male volunteers were well within the 0.8-1.25 interval. This indicated an absence of age-effects on the pharmacokinetics of idraparinux. Differences in body weight of the subjects in this study explained approximately 50% of the variability observed in clearance, 30% of the variability observed in apparent volume of distribution and approximately 40% of the variability observed in dose-normalised C_{max}. This is probably of minor influence on idraparinux, because,

in general, body composition changes with increasing age are characterised by the concomitant processes of sarcopenia and a steadily decreasing [11] or constant [12] body mass index after the fifth decade.

The effect of a single dose of idraparinux sodium on secondary haemostasis was assessed by APTT and PT measurements. In this study no significant effects were found in these parameters, apart from the PT at 24 h post-dose. The small increases in APTT and PT are unlikely to be of clinical relevance. The finding that APTT and PT are not greatly influenced by the pentasaccharide confirms its very specific action and may also reflect the unsuitability of these markers. Instead, the relationship between anti-Xa activity and the probability of bleeding should be further explored.

Among the requirements the ideal antithrombotic drug should meet, is the possibility of oral administration [13], because a long duration of treatment is necessary for the most important indications (secondary prevention of stroke or myocardial infarction, and possibly primary prevention in high-risk patients). However, dosing of this kind of drugs can be error-prone, especially in the elderly. This is caused by age-related changes occurring in this patient group as comorbidity, polypharmacy, increased possibility of lack of compliance, and increased mental impairment. There are indications that elderly patients are more concerned about effectiveness and safety of medication than convenience [14]. Therefore, the advantage of a single dose once-per-week only drug, without monitoring, may possibly outweigh the discomfort of the sc administration of idraparinux sodium, which may even be self-administered.

To conclude, in elderly people idraparinux is a well-tolerated drug with linear pharmacokinetics, and inducing only minor changes in conventional coagulation assays. Because these data confirm the results of an earlier study with idraparinux in healthy young male volunteers, it is unlikely that age influences the pharmacokinetics of the compound. It can be anticipated that idraparinux may be a useful new drug for long-term prophylactic anti-thrombotic use.

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TABLE 1 **Summary of pharmacokinetic data**
Data up to two weeks.

TREATMENT	C_{MAX} (ng/mL)	$AUC_{0-\infty}$ (mg/L*h)	$t_{1/2}$ (h)	CL (mL/min)	VZ (L)	URINARY EXCRETION (mg)	CL_{RENAL} (mL/min)
SC 2 mg (n=6)	473 (104)	42.7 (12.3)	108 (17)	0.82 (0.2)	7.53 (1.17)	0.29 (0.07)	0.18 (0.06)
SC 6 mg (n=6)	1499 (161)	135.1 (21.9)	132 (19)	0.76 (0.1)	8.39 (1.56)		
SC 10 mg (n=6)	2309 (351)	208.2 (23.6)	132 (30)	0.81 (0.1)	9.37 (2.81)	1.78 (0.31)	0.22 (0.04)
IV 6 mg (n=6)	2223 (482)	142.6 (21.7)	145 (30)	0.71 (0.1)	8.61 (1.94)		

Results are reported as mean (SD); AUC-data corrected for non-zero pre-dose values.

TABLE 2 **Elimination half-life estimates**

	Initial profile			Extended profile		
	FOLLOW-UP (days)	$t_{1/2}$ (h)	N	FOLLOW-UP (days)	$t_{1/2}$ (h)	N
SC 2 mg	11	108 ± 17	6			
SC 6 mg	14	132 ± 19	6	28	259 ± 28	3
SC 10 mg	14	132 ± 30	6	31	397 ± 121	4
IV 6 mg	14	145 ± 30	6	35	284 ± 43	3

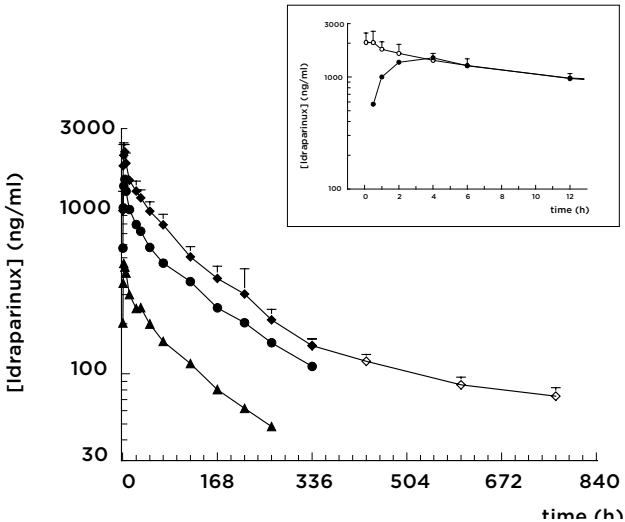

FIGURE 1 **Mean semi-logarithmic plasma profile idraparinux after sc administration.**

The inset shows the differences between the iv and sc administration of 6 mg idraparinux: from the timepoint of 6 hours post-dosing the curves completely overlap. (▲ : sc 2 mg, ●: sc 6 mg, ○: sc 6 mg, ◆: sc 10 mg, ◇: sc 10 mg extended profile [n=4])

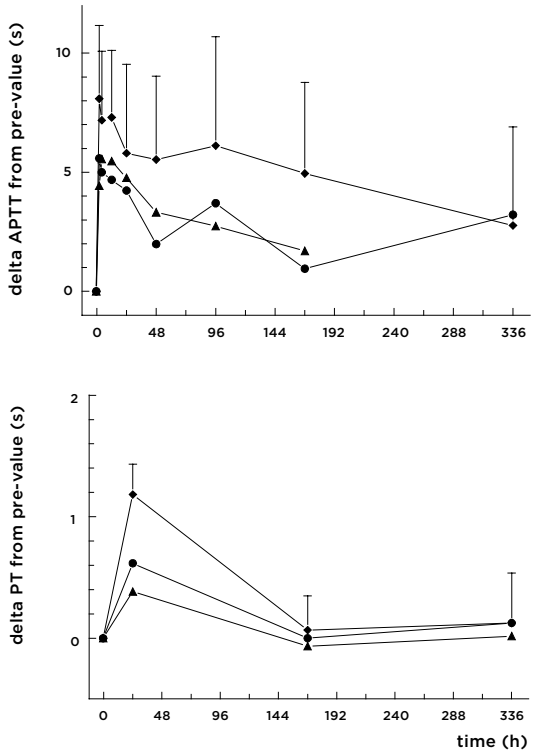

FIGURE 2 Mean change in APTT (upper panel) and PT (lower panel) after sc administration idraparinux (▲ sc 2 mg, ● : sc 6 mg, ◆ : sc 10 mg).

CHAPTER 8

THE MULTIPLE DOSE PHARMACOKINETICS OF THE SYNTHETIC LONG-ACTING PENTASACCHARIDE IDRAPARINUX SODIUM IN VOLUNTEERS WHO RECENTLY COMPLETED TREATED FOR VENOUS THROMBOEMBOLISM

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ABSTRACT

Objectives

To investigate the safety, tolerability and pharmacokinetics of 10 mg subcutaneous (sc) idraparinux, a synthetic pentasaccharide compound with anticoagulant properties and an elimination half-life estimated thus far of about 130 hr, administered four times once-a-week in male and female volunteers, recently discharged from oral anticoagulant treatment.

Methods

Eight subjects (5F/3M) with a history of venous thromboembolism (VTE), available for the first administration of idraparinux within 2 weeks after regular discontinuation of oral anticoagulant treatment, participated in this single center, multiple dose, open label study. Blood sampling and urine collection for anti-Xa activity were done up to 336 hr after the last administration as well as APTT and PT. Individual anti-Xa activity time profiles were analysed using non-compartmental pharmacokinetic techniques with calculation of the area under the curve (AUC), clearance and elimination half- life. Urinary data were used to calculate renal clearance.

Model dependent pharmacokinetics of idraparinux was determined using a two-compartment model with first order absorption utilising the data up to 4 weeks of dosing. Parameters were estimated using non-linear mixed effect modelling with first order conditional estimation.

Results

Idraparinux sodium was well tolerated and no significant adverse events were noted. Steady state drug levels were reached after the third administration. T_{max}-values were comparable to the values found in the single dose studies, while C_{max}, C_{min}, AUC, cumulative urinary excretion as well as the renal clearance were increased compared to the single dose studies. The multiple dose time profile up to one week after dosing can be adequately described using a two-compartmental pharmacokinetic approach. There were no clinically relevant post-dose changes in APTT and PT.

Conclusions
The administration of multiple doses of idraparinux was well tolerated and no significant adverse events were noted. It is anticipated that idraparinux sodium can be a useful new drug for long-term anti-thrombotic use that can be administered once per week.

INTRODUCTION

Idraparinux is a novel synthetic antithrombotic pentasaccharide working by its ability to activate antithrombin (AT). The main activity of AT, a naturally occurring inhibitor of the blood clotting process, is against factor Xa and IIa. In contrast to its animal sourced competitors (unfractionated heparin and low molecular weight heparins (LMWH)), it is manufactured totally by chemical synthesis. The compound is the sodium salt of a sulfated pentasaccharide. Heparin-like drugs accelerate the velocity, but not the extent, of the reaction in which complexes are formed between AT and factor Xa or factor IIa (= thrombin) which dissociate very slowly relative to complexes with the target proteins in the coagulation cascade in the absence of AT. As a result of its limited molecular chain length, idraparinux is selectively active against factor Xa. Hence, the drug binds to AT and thereby specifically potentiates the physiological neutralization of factor Xa and inhibits thrombin formation without direct inactivation of thrombin itself.

In the case of thrombotic risk or complications requiring long-term treatment, use of a safe drug with an extended elimination half-life allowing a low dosing frequency is attractive. Idraparinux is eliminated at a slow rate, with a half-life of approximately 130 h in both young and elderly healthy subjects [1,2]. The extended half-life is due to the high affinity of idraparinux to AT, although binding is not irreversible. Single intravenous doses of the drug were safe up to a dose level of at least 14 mg. Thus, idraparinux sodium could be a promising new drug for long-term anti-thrombotic therapy involving a once weekly dosing regimen. This study was carried out to investigate the safety, tolerability and pharmacokinetics after multiple doses of idraparinux

in subjects recently treated for venous thromboembolism (VTE), available for the first drug administration within 2 weeks after discharge from standard oral anticoagulant treatment.

METHODS

Subjects and Design

The Ethics Committee of the Leiden University Medical Center approved the investigational protocol. This open study was performed according to a single-center, multiple dose design, in which subjects started the treatment with idraparinux sodium within 2 weeks post-discharge from oral anticoagulant treatment. The subjects were recruited from the population under the care of the Dutch Thrombosis Field Monitoring Service (SRTG, The Hague, The Netherlands). Eight subjects (5F/3M; age 35-78 years, body weight 55.7-120.2 kg, normal weight for height) participated in this study after written informed consent was obtained. They all had experienced a single episode of deep venous thrombosis or pulmonary embolism followed by treatment with oral anticoagulant drugs. Otherwise, the subjects were healthy as assessed by a medical screening.

Study Procedures

Subjects were treated with 10 mg idraparinux subcutaneously (sc) four times once per week. Idraparinux was administered in an abdominal skinfold using disposable syringes filled with undiluted drug solution (2.63 mL). Study medication was prepared by the Leiden University Medical Center pharmacy.
In the 1st and 4th study-week pharmacokinetic monitoring took place. Subjects were studied after an overnight fast. Upon arrival at the research unit a short medical history and a physical exam were performed to ensure compliance to the protocol. Then the subjects voided and an iv cannula for blood sampling was inserted in a forearm vein. At zero time idraparinux was administered sc after which the subjects remained at the unit for at least 24 hours. The local monitoring site of the Thrombosis Service administered the second and third dose and performed subsequent blood sampling.

Sampling

No tourniquet was applied when blood was collected during the study period. At the research-unit an iv cannula used for blood sampling was kept patent by intermittent flushing with 0.9% saline. Blood was taken after discarding the contents of the cannula. All other samples were taken by venipuncture. Blood samples for base-line values were taken pre-dose. Before administration of the first dose of idraparinux the Prothrombin Time (PT) value was determined to provide evidence that cessation of oral anticoagulant therapy resulted in normalisation of the patient's coagulation status.

Blood samples for drug-assay, APTT and PT were drawn frequently until 336 h after the 4th administration of idraparinux. The subjects collected urine for measurement of drug concentration in 24-hour periods until 168 h after first dosing and 336 h after fourth dosing in pre-weighted plastic containers. Bleeding time (Ivy method) was measured pre-dose, at 4 and 24 h after the 1st and the 4th (last) administration. In addition, bleeding time was determined at 168 and 336 h after the last drug administration. Blood samples for antithrombin assay were taken pre-dose for all four idraparinux administrations and at the end of the study (i.e. 336 h after the last injection).

Assays

As a direct method for assessing plasma and urine concentrations of idraparinux is not available, these values are derived from the anti-factor Xa activities [3]. The validated assay for anti-Xa activity in plasma and urine was performed with a lower limit of quantification of 8 ng/mL for plasma and 6 ng/mL for urine. APTT and PT assays were performed using standard procedures [4]. Antithrombin concentrations in plasma was to assessed using a photometric assay with S-2765 as a chromogenic substrate and is based on inhibition of factor Xa (Comatic® Antithrombin, Mölndal, Sweden).

Statistical analysis

Non-compartmental pharmacokinetic analysis of two types was performed using the software package WinNonlin V2.1 (Pharsight

Corp., USA). First, overall non-compartmental analysis was determined (over the entire study period) by calculating AUC using the linear trapezoidal method, terminal half life, extrapolated AUC ($AUC_{0-\infty}$) and VZ. For the calculation of terminal half-lives the values starting at 624 hours (26 days) were used in all cases. This decision was based on the values automatically selected by the program, followed by visual inspection. Clearance/F was calculated as total dose (= 40mg)/($AUC_{0-\infty}$).
Subsequently, non-compartmental parameters were determined for week 1 and week 4 ($AUC_{0\text{-}1\ week}$, C_{max}, C_{min}, T_{max}) and compared using paired t-tests after log-transformation (T_{max} untransformed). Weeks 1, 2, 3 and 4 were characterised by the concentration at 4 hours and the concentration just prior to the next dose and these parameters were summarised.
The urinary excretion of idraparinux was determined by calculating cumulative excretion over week 1, over week 4 and over week 4 and 5 combined. The renal clearance for these same periods was determined by dividing the excreted amount by the corresponding plasma idraparinux AUC. Urinary excretion and renal clearance were compared between weeks 1 and 4 using paired t-tests.
Additionally, model dependent pharmacokinetics of idraparinux was determined using a two-compartment model with first order absorption utilising the idraparinux data up to 4 weeks of dosing. Parameters were estimated using non-linear mixed effect modelling as implemented in the NONMEM program (Version V, Nonmem Project Group, University of California, San Francisco, USA) with first order conditional estimation (FOCE), and constant coefficient of variation (CCV) inter-individual and intra-individual variability models. Clearance/F, intercompartmental clearance/F, central volume/F (V_c/F), steady state volume/F (V_{ss}/F) and absorption half-life were estimated. Predicted profiles were generated for the observed four weeks of dosing and if the drug had been administered for an additional eight weeks using the same regimen (10 mg per week).
Area under the Effect Curves (AUECs) for APTT and PT were calculated using BMDP/Dynamic Version 7.0 (Statistical Solutions, Inc., Cork, Ireland). The APTT response was characterised by the

value at 4 hours (close to T_{max}), the value prior to the next dosing (E_{min}), and by dividing the calculated AUECs over weeks 1 and 4 by the corresponding timespan (weighted mean APTT). Values for weeks 1 through 4 were summarised. Values for weeks 1 and 4 were compared using paired t-tests. The PT measurements were summarised and the pre-dose value was compared to the values at 24 hours during week 1 using paired t-tests. Additionally, the week 1 and week 4 values at 24 hours were compared using paired t-tests. Statistical analysis and calculations were performed, using SPSS for Windows (SPSS, Inc., Chicago, Ill., USA).

RESULTS

Idraparinux sodium was well tolerated and no significant adverse events were noted. The most frequently reported adverse events were headache and the development of haematoma at puncture sites, and were considered of a mild intensity. There were no discontinuations.

Pharmacokinetics

A summary of the results is given in Figure 1, Table 1 and Table 2. These data demonstrate that steady state levels with regard to the maximal plasma levels (C_{max}) are already reached after the 2nd administration whereas the steady state levels based on through plasma levels (C_{min}) are reached after the 3rd administration of idraparinux. Comparison of the pharmacokinetic results of week 1 to week 4 showed statistically significant increases in C_{max} (16.9%; 95%CI: 8.1%, 26.5%), C_{min} (60.3%; 95%CI: 46.2%, 75.4%) and $AUC_{0\text{-}168h}$ (30.9%; 95%CI: 20.8%, 41.9%) and that T_{max} values were stable. The results of the analysis of the renal clearance are summarised in Table 3. The mean overall cumulative urinary excretion was 5.81 mg (SD: 0.76) over week 4 and 5, indicating that renal clearance over these two weeks is 0.36mL/min. This is slightly higher than the values known from previous studies with idraparinux sodium [1,2]. Comparison of the results of week 1 and week 4 revealed significant increases in cumulative urinary excretion (mean difference 1.76 mg (95% CI: 1.31, 2.21)), as well as the renal clearance (mean difference 0.11 mL/min (95% CI: 0.07, 0.15)).

Results of the model dependent pharmacokinetics of idraparinux are summarised in Table 4. When data are restricted to 4 weeks, the idraparinux-time profile can be adequately described using a two-compartmental pharmacokinetic approach (Figure 2).

Pharmacodynamics

There were no post-dose changes of clinical relevance in APTT (Table 5). All administrations of idraparinux were followed by a small rise in APTT (approximately 3 seconds, Figure 3). Average weighted mean APTT-values were higher for the fourth week than for the first week of idraparinux administrations: mean difference 2.15 seconds (95% CI: 0.47, 3.84). After reaching a maximum, APTT-values decreased slowly (Figure 3).

No clinically relevant post-dose abnormalities in PT were observed. The 1st administration of idraparinux was followed by a mean increase in PT of 1.57 seconds (95% CI: 1.08, 2.07) comparing the pre-dose values to the values at 24 hours post-dosing. A slight increase in mean PT-values was still present pre-dose to the 4th administration (no measurements of PT were performed in between). The 4th administration was also followed by a small increase in PT-values after which PT-values decreased slowly (Figure 3). The results at 24 hours post-dosing in week 1 and week 4 were not statistically significantly different.

Ivy bleeding times showed a large degree of variation between and within subjects. No statistically significant change in bleeding times was observed comparing the 4 and 24 hour measurements to baseline (median increase at both time points: 4%). Similar, comparison of the data at 4 and 24 hours post dosing between the 4th and 1st dosing did not show significant changes in bleeding time.AT oncentrations (expressed as the ratio of the post-dose over the pre-dose value) increased by 2.4% after the 1st drug administration and by 5.9% after the 4th administration, which was not statistically significant.

DISCUSSION

This phase I study was initiated to extend single dose investigations on the safety and pharmacokinetics of idraparinux in healthy young and elderly volunteers [1,2] to patients to investi-

gate multiple dose regimens in a clinically relevant group. Also, with regard to the safety it was decided to recruit subjects with a history of VTE, because they were already used to anticoagulation while ambulant. Furthermore, it can be envisaged that an additional month of anticoagulant treatment for volunteers with a history of VTE within a month after completion of a 3-months oral treatment might be beneficial to these patients in further reducing or postponing recurrent thromboembolism [5].
Because in previous studies the absolute bioavailability of sc idraparinux was estimated at almost 100%, the study was performed using this route of administration. With regard to the pharmacokinetics, plasma concentrations were determined by using an anti-factor Xa activity assay, since a direct method to measure idraparinux concentrations does not exist. Comparable to the previous studies, the pharmacokinetic behaviour of idraparinux could best be analysed using a non-compartmental approach. Nevertheless, the drug-time profile could be adequately described using a two-compartmental model.
In the earlier studies, within one week after administration, approximately 20-30% of idraparinux was excreted renally. This was also found after the first administration of the compound in the current study. However, a higher renal clearance was found after the fourth dose (Table 3). This might be explained by the following phenomenon: for full expression of the anti-Xa effect of the shorter-acting pentasaccharide fondaparinux, an excess of AT is required [6]. This also is the case for idraparinux [7]. In addition, comparable to unfractionated heparin [8], idraparinux is probably released unchanged from the binding equilibrium of idraparinux with AT if endogenous AT is cleared from the circulation during the natural turnover process, and can possibly bind to newly produced AT. However, it should be considered that idraparinux may also bind to other plasma proteins and that this may limit the amount of free drug available for renal excretion.
This study suggest that a 'steady state situation' with regard to plasma concentrations is reached after 2-3 administrations of idraparinux, because after the 4th administration maximal and minimal concentrations were similar to the preceding administration.

The effects of multiple doses of idraparinux on secondary haemostasis were measured by APTT and PT assessments. The observed small post-dose prolongation in these parameters can be considered not relevant from a clinician's point of view. The small changes suggest that these conventional coagulation assays, though widely used, are not suitable for monitoring idraparinux. No significant effects were noted for changes in bleeding time or AT concentration. At present, coumarin-derivatives are the most widely used anticoagulants for long-term use. Because these have clear drawbacks [9], there is considerable interest in developing new anticoagulants to replace them in long-term out-patient care. Though idraparinux is administered via the subcutaneous route, its predictable pharmacokinetics, circumvention of the need for frequent monitoring, a low dosing frequency, and (to the current knowledge) minimal side effects indicate that this compound could represent an alternative in chronic thrombotic disorders. The mentioned characteristics lead to a low practical inconvenience for the patients, which is also an important item in this matter. In conclusion, the administration of multiple doses of idraparinux was well tolerated, no significant adverse events were noted and only minor changes in APTT and PT. It is anticipated that idraparinux sodium can be a useful new drug for long-term antithrombotic use, administered once per week.

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TABLE 1 Mean (SD) and range of the PK parameters of idraparinux after 4*10mg sc (complete profile) following non-compartmental analysis

	$AUC_{0\text{-last}}$ (mg/L*h)	% extrapolate d*	$AUC_{0\text{-}\infty}$ (mg/L*h)	$t_{1/2}$ (h)	Cl/F (mL/min)	Vz/F (L)
Mean	822 (112)	12.5 (2.6)	941 (135)	216 (25.9)	0.88 (0.12)	16.5 (2.3)
Range	606–953	10.4–16.8	675–1071	177–259	0.75–1.11	12.67–19.8

$AUC_{0\text{-last}}$: area under the curve from timepoint of first administration, until the last measurements (two weeks after the last administration), $AUC_{0\text{-}\infty}$ area under the curve extrapolated to infinity, % extrapolated: percentage of the AUC that had to be extrapolated to get the $AUC_{0\text{-}\infty}$, Cl/F: apparent clearance, Vz/F apparent volume of distribution.

TABLE 2 Comparison non-compartmental PK week 1 and 4

	C_{4H} (mg/L)	C_{MIN} (mg/L)	T_{MAX} (h)	$AUC_{0\text{-}168h}$ (mg/L*h)
week 1	2.16 (0.20)	0.37 (0.06)	3.52 (0.93)	149 (16.4)
week 2	2.49 (0.36)	0.54 (0.10)		
week 3	2.56 (0.43)	0.60 (0.11)		
week 4	2.47 (0.31)	0.59 (0.08)	3.76 (0.71)	192 (24.8)

Results are reported as mean (SD)
C4 h: concentration at 4 h post-administration (close to Cmax-value), Cmin: 'minimum' concentration, just prior to next dosing, Tmax: timepoint of maximum concentration, AUC0-168 h: area under the curve from timepoint of administration until one week after this administration.

TABLE 3 Renal clearance of idraparinux following multiple doses

	WEEK	MEAN (SD)	RANGE
Cumulative excretion over one week	1	1.95 (0.47)	120–2.62
	4	3.68 (0.89)	1.96–4.38
Renal clearance	1	0.22 (0.06)	0.142–0.295
	4	0.324 (0.87)	0.194–0.438

TABLE 4 Summary results model-dependent idraparinux pharmacokinetics

	MEAN	SEM	CV
Clearance/F (ml/hr)	52.6	3.04	14%
% Intercompartmental clearance/F (ml/hr)	678	90.7	31%
V_c/F; central volume of distribution (L)	2.71	0.262	0%
V_{ss}/F; steady state volume of distribution (L)	6.10	0.330	16%
Absorption half-life (hr)	1.06	0.173	26%

Residual variability (constant CV error model): 11%
Mean: population average
SEM: approximate standard error of population average
CV: coefficient of variation of inter-individual variability

TABLE 5 Summary APTT results

	APPT at 4h post dosing ("E_{max}" in seconds)	APPT prior to dosing ("E_{min}" in seconds)	Weighted mean (0–7 days)
week 1	35.4 (4.34)	34.1 (3.41)	34.1 (3.70)
week 2	37.6 (4.85)	35.1 (4.77)	
week 3	38.3 (3.76)	35.1 (4.02)	
week 4	37.5 (5.05)	34.7 (4.74)*	36.2 (5.16)

Results are reported as mean (SD).
Absolute mean pre-dose value (before the first SanOrg34006 dose) was 29.0 s (SD: 4.8 s).
* this is the value one week after the last (4th) dose, as such not followed by a new dose, but this would be the timepoint at which a new dose was to be administered.

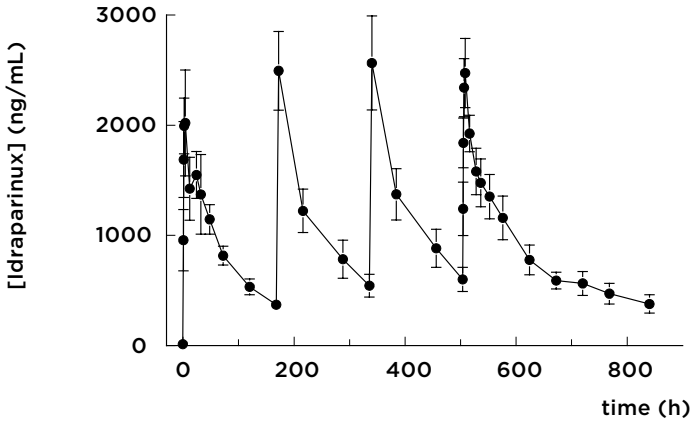

FIGURE 1 **Idraparinux time profile following four sc administrations of 10 mg.**

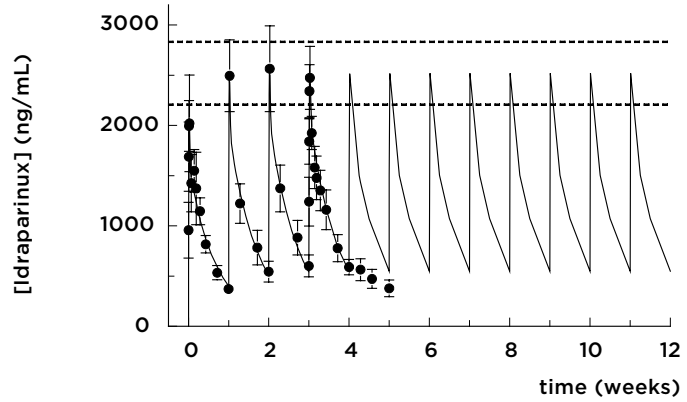

FIGURE 2 **Average idraparinux time profiles with predicted profiles using empirical Bayes estimates and extrapolated profile for 8 additional weeks of administration (using the same dose-regimen).**

The dotted lines indicate the range of predicted average maximum concentration; mean predicted Cmax-value is 2520 ng/mL (range: 2205-2830 ng/mL).

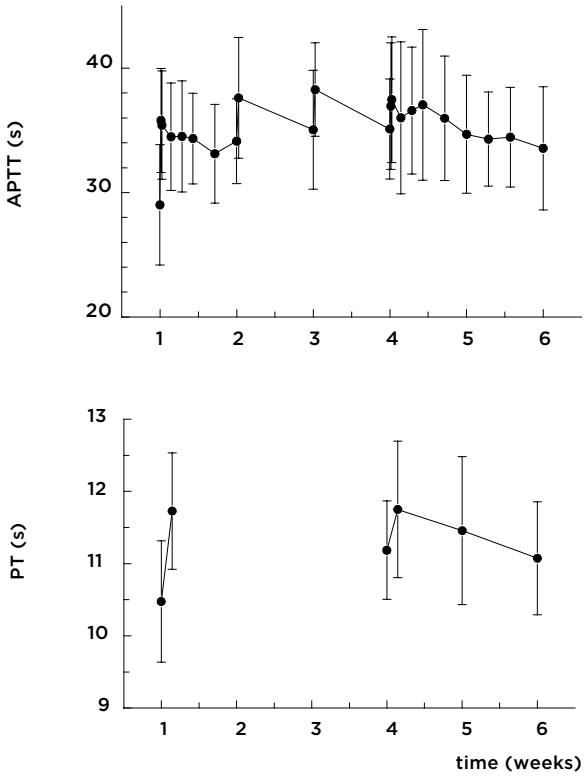

FIGURE 3 Mean time profile of changes in coagulation assays following idraparinux administration at weekly intervals.

THE CLINICAL PHARMACOLOGY OF NOVEL ANTITHROMBOTIC COMPOUNDS

voor Iris

THE CLINICAL PHARMACOLOGY OF NOVEL ANTITHROMBOTIC COMPOUNDS

PROEFSCHRIFT

ter verkrijging van de graad van Doctor
aan de Universiteit Leiden,
op gezag van de Rector Magnificus dr. D.D. Breimer,
hoogleraar in de faculteit der Wiskunde en
Natuurwetenschappen en die der Geneeskunde,
volgens besluit van het College voor Promoties
te verdedigen op woensdag 2 oktober 2002
te klokke 15.15 uur

door

Richard Aloysius Faaij
geboren te Gouda in 1969

PROMOTIECOMMISSIE

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prof dr A. F. Cohen

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Overige leden:
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Prof Dr. M. Danhof
Prof Dr. P. Vermeij
prof dr P. van Brummelen

De uitgave van dit proefschrift werd mede mogelijk mogelijk gemaakt door de Stichting Centre for Human Drug Research, Leiden and NV Organon, Oss.

Design: Andrea Fuchs, Haarlem
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GENERAL INTRODUCTION

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CHAPTER 1

INTRODUCTION

Haemorrhage, the loss of the vital fluid blood, is a potential threat to life. Under normal circumstances, this is averted by the system of blood coagulation. This is a dynamic process occurring to a limited degree physiologically throughout the overall circulation, as evidenced by circulating activation peptides associated with the coagulation proteins. The coagulation system becomes activated with great efficacy in response to vascular damage and involves a large number of plasma proteins and multiple cell-cell and cell-matrix interactions. Owing to the complexity and the biological potential of the blood coagulation system, strict regulation is required in order to avoid uncontrolled clotting or bleeding. Several important control mechanisms have been unravelled. Coagulation and fibrinolysis exist in a balanced equilibrium [1,2]. Despite control mechanisms, disruption of this balance between clot formation and dissolution, in order to achieve haemostasis while preventing either excessive thrombosis or bleeding may develop. This results in a spectrum of diseases, many of which are life threatening and often fatal. On one extreme, if essential components of the coagulation system are deficient or dysfunctional, clot formation is inadequate and (further) bleeding ensues, leading to conditions in which mucous membrane or joint space bleeding is common and dangerous. On the other extreme, in favour of coagulation, lies excessive thrombosis, resulting in blockage of blood vessels and eventual ischaemia of tissues [3]. In between these extremes are patients who have mild disease and develop clotting or bleeding problems only after some inciting event.

COAGULATION

Haemostasis is a physiologic mechanism that maintains blood within the circulation. The body achieves haemostasis by forming a clot when blood vessel injury occurs, stopping propagation of

the clot at the right time, and then dissolving the clot when the vessel has healed. This is due to interplay between a series of biochemical reactions. The constituents of this coagulation cascade are plasma proteins, with the exception of tissue factor, which is an integral membrane protein expressed on non-vascular cells. Physiologically, most of these proteins are enzymes in an inactive form. Once activated, these enzymes are proteases, and hence cleave other specific protein(s) in the cascade. The complex interactions between blood cells, specific soluble plasma proteins and vascular surface maintain the fluidity of blood.
Damage to the endothelial wall exposes tissue underlining to blood components, leading to an explosive reaction. The blood-coagulation cascade has the ability to transduce a small initiating stimulus into a large fibrin clot rapidly terminating blood loss and initiating the healing process. The ultimate extent of the coagulation reaction depends on the amount of activated membrane that can be provided by the damaged tissue and the aggregated platelets that are accumulated in the region of the wound. The coagulation reaction is terminated by a collection of stoichiometric and enzymatic reactions to yield an ultimate system in which procoagulant complexes are destroyed and the residual enzymes inhibited. This occurs by means of a dynamic regulation system. Under physiologic conditions, clot formation will only occur in the region of vascular damage where the membranes are available for procoagulant complex formation.

THE CASCADE

In the past two distinct pathways of blood coagulation were recognised, an intrinsic and an extrinsic pathway, respectively. These pathways were thought to proceed through a series of ordered steps in which inactive zymogens are transformed into active enzymes [4, 5]. As such, the classic cascade has traditionally been taught in textbooks. The intrinsic pathway occurs by physical chemical activation and the extrinsic pathway is activated by tissue factor released from damaged cells. Both pathways are thought to be activated simultaneously to initiate and sustain clot formation (Figure 1).

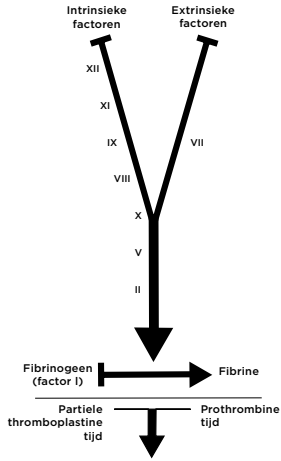


FIGURE 1 **Vascular injury initiates the margination of platelets to the site of injury.**

The coagulation cascade requires the presence of phospholipids, which are provided by platelets, i.e. much of the coagulation cascade occurs on the surface of platelets. Initially, platelets adhere to the damaged endothelial cells via von Willebrand Factor and aggregate to surround the site of vascular damage. In addition, damaged endothelial cells also contribute phospholipids. At this same time, blood is exposed to TF. It is upon this mass of perivascular cell tissue, damaged endothelial cells, and platelet membranes that the coagulation reactions occur and propagate the generation of thrombin; figure from [6].

The so-called Intrinsic Pathway begins with trauma to the blood vessel, exposure of blood to collagen in a damaged vascular wall, or exposure of the blood to a wetable surface such as glass. In the so-called Extrinsic Pathway, the initial step is a traumatised vascular wall or extravascular tissue. Non-vascular tissue cells contain an integral membrane protein called TF. Damage to the vessel wall or extravascular tissue exposes the plasma to TF. Factor VII is a circulating plasma protein that then binds to TF, creating a complex. In doing so, Factor VII is activated to Factor VIIa. This complex, in the presence of Ca^{2+} and phospholipids, activates Factor X to Factor Xa.

In the Combined Pathway (the outcome of both the intrinsic and extrinsic pathway), the inactive molecule prothrombin is converted to the active thrombin by activated Factor X, requiring

the 'prothrombinase complex'. Thrombin cleaves fibrinogen to fibrin, which then polymerises to form fibrin strands.

The current hypothesis is slightly different from the traditional waterfall/cascade hypothesis. The extrinsic pathway is considered the initiator of events. Under normal circumstances, blood and its constituents are not exposed to TF. When either blood vessel or tissue injury occurs, the plasma becomes exposed to TF (only present in non-endothelial cells) initiating coagulation [7]. It is felt that the extrinsic 'pathway' is paramount in generation of enough thrombin to initiate coagulation and that this thrombin then activates the intrinsic 'pathway', which in turn maintains coagulation [8].

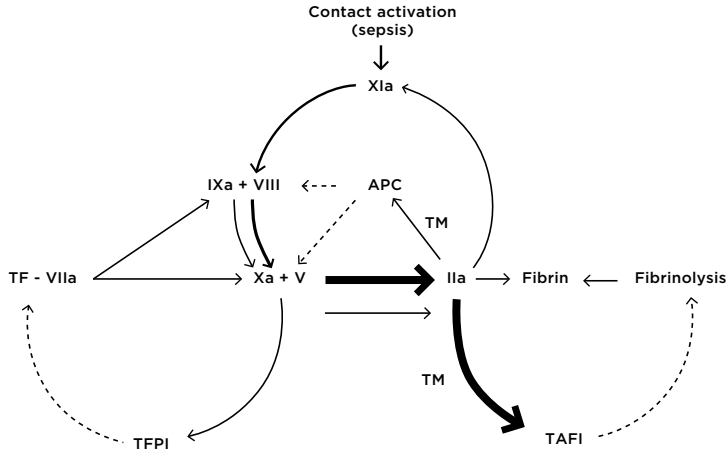


FIGURE 2 **Graphical representation of a revised model for blood coagulation, without the distinction between intrinsic and extrinsic pathways; figure from [8].**

Regulation

The regulatory mechanisms of the coagulation cascade serve two main functions. Limit the amount of fibrin clot formed to avoid ischaemia of tissues. Localise clot formation to the site of tissue or vessel injury, thereby preventing widespread thrombosis.

Physiologically, coagulation is a continuous process in which the anticoagulants are in excess to the procoagulants in blood. The improper functioning of these coagulation regulators can cause thromboembolic disease. The main regulators are Tissue Factor Pathway Inhibitor (TFPI), Antithrombin (AT, formerly known as AT-III), activated Protein C (APC) and Protein S, Thrombomodulin (TM) and the fibrinolytic system (Figure 2). To summarise, the net effect of all the coagulation reactions is that the wound site is plugged with a platelet-fibrin clot and the overall reaction is terminated. The clot represents a temporary barrier to blood flow and must be replaced by reconstitution of the vascular architecture via cellular proliferation and regeneration of the connective tissue of the intima.

THROMBOSIS

Venous thromboembolism (VTE), which includes deep vein thrombosis (DVT) and pulmonary embolism (PE), is a common clinical problem. It is the result of a delicate interplay between three pro-coagulative factors: the so-called Virchowís triad [9]. Alterations in the elements of this triad are major contributors to (venous) thrombosis, depending on specific risk factors that are present in a given patient [10]. Thrombosis is a multicausal disease; hypercoagulable states (congenital and acquired) arise when there is an imbalance between the anticoagulant and prothrombotic activities of plasma in which the prothrombotic activities predominate. These factors may act independently of each other or in concert [11,12].

Antithrombotic Compounds

The primary aim of VTE-treatment is to prevent recurrent thrombotic events at a minimal risk of bleeding [13]. All currently available antithrombotic therapies with either anticoagulants or platelet-active drugs are prophylactic, since these agents interrupt progression of the thrombotic process, but (unlike thrombolytic agents) do not actively resolve it [14,15].
Until recently, unfractionated heparin, vitamin K-antagonists (i.e. coumarins) and aspirin were the only anticoagulant agents

in wide use. The antithrombotic properties of these drugs were identified before their mechanisms of actions were fully defined [16,17,18] and the indications, optimal dose and duration of treatment for these agents were uncertain. The principal reason for this uncertainty was the lack of evidence-based data, because these antithrombotic agents were introduced before the well-designed clinical trial became the accepted standard for therapeutic decision making [19,16]. For instance, only in the 1990s it became clear that that the use of UFH in the initial treatment of venous thromboembolism (VTE) is necessary to prevent pulmonary embolism (PE) and recurrent thrombosis [20]. Nevertheless, in general, clinicians were comfortable with the use of these compounds.

Heparin

More than 80 years after its discovery the sulphated polysaccharide heparin remains an important tool in clinical practice, and has been the most widely used anticoagulant drug over the past 40-50 years. It functions in life as a component of the heparans that line the inner walls of the microvascular system, but unfractionated heparin (UFH), as a drug is a heterogeneous extract, from either porcine or bovine sources, administered by injection to circulate in the bloodstream.
The anticoagulant effect of UFH is mediated largely through its interaction with the plasma proteinase inhibitor AT. Binding and activation of AT by heparin-like GAGs depends on a specific pentasaccharide fragment [21]. For the inhibition of thrombin, UFH must form a bridge between thrombin and AT. It has been shown that molecules of UFH with fewer than 18 saccharide-units are unable to bind thrombin and AT simultaneously and, as a result cannot catalyse thrombin inhibition. For the inhibition of factor Xa this bridging is not necessary and UFH fragments with smaller numbers of saccharide units are capable of catalysing the inhibition of factor Xa by AT, providing the high-affinity pentasaccharide sequence is present [22].

ANTITHROMBIN

Antithrombin (AT, formerly known as AT-III) is a protein synthesised by liver and endothelial cells. It is a member of the serpin (serine proteinase inhibitor) superfamily of proteins. It is a single chain glycoprotein with a molecular weight of approximately 58,000 Da. The normal human plasma level is about 2-3 μM. The half-life of the AT in plasma is about 70 hours. [23]. Two isoforms of AT exist: AT-α has four glycosylation sites, whereas AT-ß, which constitutes 10% of plasma AT, lacks one of these glycosylations sites, resulting in the (-isoform having a naturally higher affinity for heparin [24]. AT is an important physiological inhibitor of coagulation factors in plasma [25,26]. The uncatalysed reaction between the serine proteases and AT is relatively slow. The serine proteases still have time to generate thrombin and fibrin before becoming inactivated. However, in the presence of particular sulphated heparin-like GAGs, the reaction between AT and the serine proteases is virtually instantaneous [27]. For instance, the binding of UFH to AT causes a conformational change in AT that accelerates its ability to inactivate the coagulation enzymes thrombin and factor Xa by about 1000 times [28].

TABLE 1 Serine proteases inhibited by AT*

COAGULATION	FIBRINOLYSIS	KININ SYSTEM
Factor XIIa	Plasmin	Kallikrein
Factor Xia		
Factor Ixa		
Factor VIIa		
Factor Xa		
Thrombin		

*AT also inhibits activated enzymes involved in fibrinolysis and the kinin system

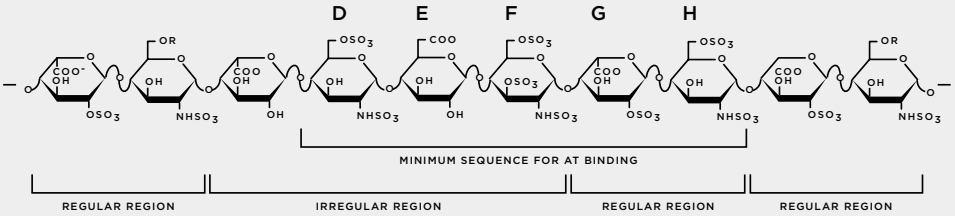


FIGURE 3 **Especially the sulphate groups in the heparin pentasaccharide are critical for tight binding to AT.**
It is the pentasaccharide sequence that confers the molecular affinity for AT [29]. Whereas the AT conformational change is necessary and sufficient for the inhibitor to accelerate the inactivation of factor Xa, it is not sufficient for accelerated thrombin inhibition. The latter acceleration additionally requires a longer heparin chain to bridge the proteinase and the inhibitor in a ternary complex.

Next to its mode of action via AT, heparin can inactivate thrombin by binding to HC-II, although much higher doses of UFH are required. And it releases TFPI from endothelium, which also contribute to its antithrombotic effect. It is believed that heparin primarily acts through interactions with these endogenous cofactors, however, many additional factors may be responsible for the anticoagulant and antithrombotic effects of this compound. Much remains uncertain about UFH: particularly the non-anticoagulant properties are poorly understood [30,31]. Heparin has pharmacokinetical, biophysical and biological limitations [32,33]. This has the following implications for its clinical use: UFH has a poor subcutaneous bioavailability (especially at low doses), an unpredictable dose response, a relatively short plasma half-life, a need for close laboratory monitoring, and a narrow benefit/risk ratio [34]. Therefore, continuous administration of UFH by the intravenous route is the current standard practice. And treatment or prophylactic administration of UFH requires hospitalisation, which limits the mobility of the patients, exposes them to the risks of hospital-acquired infections and increases management costs [35,36]

HEPARINS

The anticoagulant response to a standard dose of UFH varies widely between patients, because UFH is heterogeneous with respect to molecular size, anticoagulant activity, and pharmacokinetic properties.

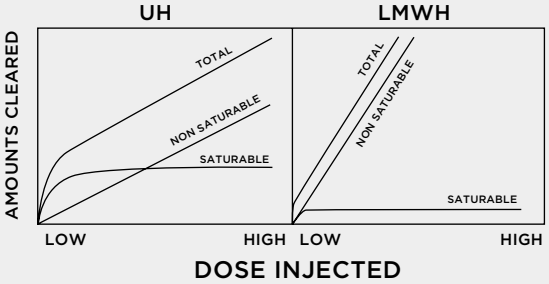
Heparin represents a heterogeneous mixture of polysaccharides and oligosaccharides with a molecular weight range of 1200 to 40,000 D (mean molecular weight of 15,000 D, approximately 50 monosaccharide units). Depending upon the source and method of manufacturing, 10 to 30 molecular species may be present in a given heparin preparation. The heterogeneity in a molecular structure of heparin presents a complex problem because conventional pharmacologic concepts are not applicable to the study of heparin. For instance, radiolabelling procedures typically used for many other drugs are very difficult, making that the pharmacokinetics of UFH are mostly expressed in terms of its pharmacodynamic activity [37].

The pharmacokinetics of UFH are rather complex:

- The clearance of UFH chains is influenced independently by two properties: affinity towards AT and length of the polysaccharide chains. High-affinity material has a slower clearance than low-affinity material and higher-molecular-weight species (the longer chains) are cleared from the circulation more rapidly than the lower-molecular-weight species. After parenteral administration, the UFH composition is progressively changed: there is a progressive increase in the anti Xa/anti-IIa ratio after the injection, resulting from the faster clearance of the anti-IIa activity [38].
- UFH is metabolised by the combination of two mechanisms, which operate in conjunction with each other of a non-dose-related saturable cellular mechanism (rapid zero-order clearance), followed by a slower dose-related renal mechanism (first-order clearance). The saturable mechanism of UFH-clearance represents the endothelial and reticuloendothelial cells (mainly reflecting hepatic uptake). The non-saturable and linear removal mechanism of UFH is represented by renal

elimination. The relative contribution of the two mechanisms to the clearance of UFH varies with the dose and the molecular weight of the UFH-preparation injected. The amounts of UFH cleared per time unit via the saturable mechanism initially increase rapidly with the dose delivered but tend to plateau at higher doses. In contrast, the amounts of UFH cleared via the non-saturable mechanism remain linearly correlated to the dose. Low doses of UFH are mainly removed by the highly efficient saturable mechanism while, at higher doses, the relative contribution of the non-saturable mechanism becomes pre-eminent (see Figure) [39,40].

- Existing non-specific binding to plasma proteins (see later).



Following properties also play an important role in the heterogeneous, unpredictable anticoagulant response of UFH:

- Only one-third of the UFH molecules administered to patients have AT mediated activity. The remaining two thirds of UFH has minimal anticoagulant activity at therapeutic concentrations that are used clinically.
- The chain length of the molecules influences the anticoagulant profile of UFH.
- Non-specific binding of UFH to plasma proteins reduces its anticoagulant activity, because less is available to interact with AT; there is a wide variability in plasma concentrations of heparin-binding proteins, even more complicated is the fact that some of these proteins are acute-phase reactants, whereas others are released during the clotting process [22].

There are important pharmacokinetic differences between UFH and LMWHs. The contribution of the saturable mechanism to the clearance of LMWHs is negligible and elimination of LMWHs is primarily achieved via renal filtration (see Figure). This explains the dose independence of the pharmacokinetic-parameters of LMWHs, the excellent sc bioavailability at any dose, and the much more prolonged duration of biological activity in patients with renal insufficiency [41].
However, because LMWHs still carry some risk of bleeding and HIT, it has been suggested that agents with an even higher anti-Xa/anti-IIa ratio may exhibit an even more favourable benefit/risk ratio (e.g. leading to the investigation of the pentasaccharides).

In addition, some patients develop relative UFH resistance and require a large dose of UFH to achieve a response in the APTT. And a rebound thrombin generation phenomenon was reported when UFH is abruptly stopped [42,43].

The rules for optimal utilisation of UFH have been established empirically and confirmed relatively recently using animal models. Unfortunately, due to its heterogeneity, there is no proportionality between the dose of UFH injected and the anti-coagulant effect. Unless a prescriptive heparin nomogram is used, many patients receive inadequate UFH in the initial 24–48 h of treatment [44]. This inadequate therapy has been shown to increase the incidence of venous thromboembolism during follow up. Thus, frequent monitoring of the dose is required and the dose must be adapted for each patient. Monitoring is usually done by the APTT, a test that is sensitive to the inhibitory effects of UFH on thrombin, factor Xa, and factor IXa. However, there is only a moderate correlation between APTT-levels and UFH-concentrations and a diurnal variation in the APTT response in patients on a constant infusion of intravenous UFH [45,46].

LMWHs

It was discovered that UFH was composed of different fractions with anticoagulant activity fractions with high affinity and low affinity to AT; only about one third of the UFH-molecules contains the high-affinity pentasaccharide and its distribution among the UFH-molecules seems to be random. In addition, it was observed that low molecular weight components of UFH, progressively lose their potency to prolong APTT while retaining their ability to inhibit activated factor X, and that LMWHs induce less bleeding in animal models than UFH [47,48]. Hence the attempts to obtain fractions of UFH with more favourable properties.

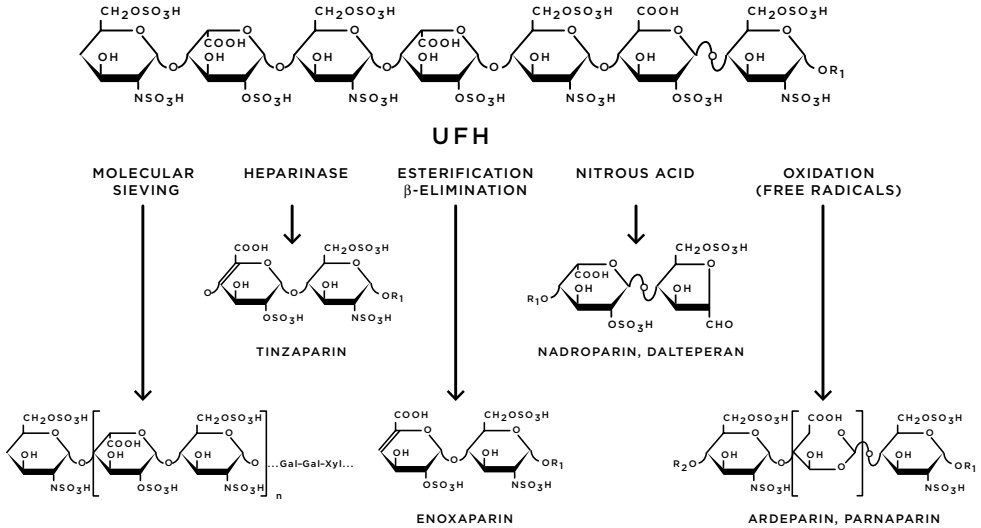


FIGURE 4 **LMWHs are fragments of UFH are prepared by different techniques (controlled processes).**

Currently, preparations of LMWHs in general use have a variable molecular weight distribution (mean molecular weight of about 5000) and, therefore, are likely to have different pharmacokinetic properties. Like UFH the LMWHs are polysulphated uronic acid-amino sugar derivatives and, as such, polyanionic electrolytes. From [49].

It is about 20 years since the first LMWH preparation was reported and, since then, several products have been introduced. Although with a narrower range than UFH, LMWHs still present a certain degree of structural and functional heterogeneity. But it is a class of anticoagulants that has pharmacokinetic and biological advantages over UFH. Comparable to UFH, LMWHs exert their anticoagulant activity by activating AT. But as most of the saccharide chains are composed of less than 18 saccharides, they have a reduced ability to catalyse thrombin inhibition relative to their ability to inhibit factor Xa [38]. The LMWHs have a predictable lower clearance (enabling once- or twice-daily injection), a prolonged half-life, a sc bioavailability close to 100%, and a predictable antithrombotic response based on body weight permitting treatment without laboratory monitoring. These advantages are translated clinically into (1) greater convenience afforded by the ability to administer LMWHs by sc injection and the associated cost reduction resulting from reduced hospital stay and (2) a lower incidence of HIT and possibly of osteoporosis.

The LMWHs have shown to be at least as effective as UFH in the management of patients with VTE [50,33]. And currently, due to the advantages over UFH combined with the increased potential for outpatient treatment [51,52], the LMWHs have established their niche as an important class of antithrombotic compounds licensed all over the world [22]. Because LMWHs are more convenient for the patient and the nursing staff they are becoming more and more widely used in clinical practice [48]. This has also prompted studies exploring the safety and efficacy in other settings than VTE (e.g. patients with acute coronary syndromes and after percutaneous coronary interventions) [53].

A greater understanding of structure activity relationships has led to further modifications in the LMWH-manufacturing process. This resulted in so-called second generation LMWHs with a lower mean molecular weight and a more precisely defined composition of polysaccharide chains, leading to compounds with a optimised and balanced molecular and biochemical profile [54].

In meta-analysis, no convincing difference was found between LMWH and UFH in terms of recurrent VTE, major bleeding, or associated thrombocytopenia [50]. However, it was found that fewer patients who were treated with LMWH died during follow-up. The validity of this mortality is uncertain. Because this survival advantage seems to be greatest in patients with an underlying malignancy, an anti-cancer effect of LMWH has been proposed [55]. Other unresolved issues related to the use of LMWHs are the need for monitoring in certain patient subgroups, the cost-benefit relative to UFH, the interchangeability of the different LMWH preparations and the reversal of the anti-coagulant effect (an antidote) [53].

SEARCH FOR NEW ANTITHROMBOTICS

Rationale

Despite an enormously increased knowledge of the blood coagulation mechanism and many advances in therapy, treatment of VTE is still far from optimal and hampered by the disadvantages of the current antithrombotic therapies compromising safety and efficacy [56].

CURRENT STATUS OF TREATMENT

In recent years, great strides have been made in defining the optimal intensity and optimal duration of anticoagulant therapy. The need for initial treatment with heparin was demonstrated [20]. It became also clear that a 4- to 5-day course of heparin was as effective as a 9- to 10-day course of heparin [57,58]. The latter is important because it allows patients to be discharged from the hospital earlier and reduces the risk of adverse effects.

After initial treatment with heparins, there is a need for long-term anticoagulant therapy to prevent recurrent VTE. Oral anticoagulants can be started within 24 hours of initiating UFH or LMWHs at doses sufficient to achieve moderate intensity anticoagulation (INR of 2.0 to 3.0), which is as effective in preventing recurrent VTE and produces less bleeding than an

INR of 3.0 to 4.5. [59,60]. All patients with VTE should receive at least 3 months of secondary prophylaxis with vitamin K antagonists. In certain patients, this might be prolonged to 6 months or even to 2 years. These optimal durations are likely to be even longer in patients with PE or thrombophilia [13]. Long-term therapy is only indicated for patients with multiple episodes of VTE, certain hypercoagulable states and those with continuing risk factors for recurrence (hereditary thrombophilia, or active cancer) [61].
Additionally, elastic compression stockings should be considered as a treatment for patients with DVT to prevent the development of the post-thrombotic syndrome. If vitamin K antagonists are contraindicated, therapeutic doses of UFH or LMWHs should be considered. Currently, the use of caval vein filters can only be defended in very specific patients [13].

Although LMWHs overcome the pharmacokinetic and some of the biological limitations, both these classes of heparin share the same biophysical limitations [33]. The need for improvement of the current treatments has prompted ongoing research into developing novel agents) [29]. These should differ from established therapies in their biochemical actions, conferring a greater risk/benefit ratio due to inducement of a stable and predictable anticoagulated state (periodic episodes of excessive or inadequate anticoagulation can be avoided. Convenience and ease of dosing are also key requirements. In addition, a rapid achievement of a therapeutic intensity is necessary for maximum efficacy.

Because of the increased demand for these compounds, the increase in bovine spongiform encephalopathy (BSE) and stricter agriculture controls, it can be anticipated that the supply of animal source tissue will reach a limitation. Both UFH and LMWHs are obtained from mammalian tissues. The availability of synthetic antithrombotic agents will provide a timely source to fill the gap. Chemically synthesised compounds are free of viral

contaminants, they can be used universally, and quarantine regulations may not be applicable to the production and international transport of these agents. Furthermore, due to the defined chemical and biological properties, the biological/pharmacological differences as seen amongst the heparins will not be seen with these compounds.

HAEMOSTATIC BALANCE – FOCUS ON THROMBIN

Considerable information has been gleaned since the 1950s about the various components of both the coagulation and fibrinolytic systems and evidence has accumulated to support mechanistically the global concept of a haemostatic balance [1,2]. It appears procoagulants have the capacity to act as anticoagulants under certain circumstances. The most notable example of these activities, which seem to alternate between being procoagulant and anticoagulant is the terminal expression of the coagulation system: thrombin.

Once generated, thrombin is a powerful procoagulant. It catalyses the further conversion of factors V and VIII to their activated forms through a positive feedback mechanism and converts more prothrombin to thrombin (see Figure 2). In this manner, thrombin is able to accelerate the entire cascade once generated, resulting in the formation of large amounts of fibrin. Thrombin generation is much like an explosion: when the cascade is activated, the amount of product formed in the individual reactions increases logarithmically as the cascade progresses. On the other hand, thrombin also activates protein C, which attacks components of the clotting system (factors Va and VIIIa) reducing the amount of thrombin formed. In addition, thrombin is an agonist for the vascular secretion of t-PA and urokinase, thus positively influencing plasminogen activation and fibrinolysis. The efficiency of dissolution of the fibrin clot is enhanced by the binding of plasmin to lysine-binding sites that are generated during the fibrinolysis process. In contrast, thrombin also behaves as a fibrinolysis antagonist by its activation of TAFIa.

TAFIa is derived from the inactive precursor TAFI by thrombin bound to TM [62]. In addition to these roles in haemostasis, thrombin generation is also important in other contexts. It is capable of affecting a variety of cells and e.g. enhances endotoxin-induced cytokine release, which contributes to the mediation of septic shock [63]. The exploration of the complex biological role of thrombin as multifunctional enzyme still reveals more roles [64].

To summarise, thrombin is required for clot generation, clot stabilisation, clotting inhibition, and both fibrinolysis enhancement and inhibition, which makes the choreography of thrombin expression is a complex and highly regulated process [65]. Nevertheless, once a tiny amount of thrombin is produced, massive thrombin generation erupts during coagulation. Thrombin generation resulting in platelet activation and fibrin formation is an important pathogenetic mechanism involved in the development of thrombosis.

Directions

The central role of thrombin has prompted newer antithrombotic strategies to inhibit thrombogenesis by focusing on the inhibition of thrombin or preventing thrombin generation [66,67]. With the assumption that a safe and effective level of anticoagulation could be achieved without excessive bleeding, thrombin-specific inhibitors were developed as anticoagulants. Results form early clinical trials have shown that the therapeutic range of thrombin inhibitors may be limited by bleeding complications [68,69,70]. Furthermore, thrombin inhibitors will not by themselves prevent the continuing conversion of prothrombin to thrombin as long as the activity of factor Xa in the prothrombinase complex is uninhibited.

Because intervention at early stages of the coagulation cascade will already prevent the generation of thrombin, factor Xa has become increasingly of interest as an alternative target for the development of new anticoagulant/antithrombotic drugs [66,67]. To control the activity of this activated coagulation factor

eliminates the continued production of thrombin by either extrinsic or intrinsic pathways without interfering with a basal level of thrombin activity necessary for normal haemostasis. This would provide a slower more regulated control with minimal bleeding risk because some clot formation is still possible under treatment. [71,72]. In addition, the thrombin catalysed activation of the protein C-pathway is still possible. And indeed, inhibitors of factor Xa showed a more favourable antithrombotic/bleeding ratio in experimental models [66,67]. This has led to the development of numerous compounds that cause an inactivation of factor Xa, either indirectly via the potentiation of endogenous anticoagulant mechanisms or directly.

ANTI-XA COMPOUNDS

Heparin successors – indirect inhibitors

UFH is the prototypical glycosaminoglycan (GAG) antithrombotic with multiple biological actions, which contribute to both its therapeutic and adverse effects. Research efforts into indirect thrombin inhibition have been directed at developing agents that exhibit the favourable attributes of UFH while eliminating actions that jeopardise clinical safety.

Inhibition of the cascade at the earliest stage possible without altering normal haemostasis is a highly rational approach to thrombosis prevention. Standard UFH inhibits factor Xa and factor IIa to the same degree, but because low doses of UFH can prevent thrombosis with only little effect on coagulation, it was suggested that factor Xa inhibition may be more relevant than factor IIa inhibition in therapeutic anticoagulation. In addition, since the ability of heparin fragments to reinforce AT-mediated factor Xa inhibition appeared to be independent of their size; it was logical to look for the smallest fragments able to catalyse AT-mediated factor Xa inhibition. Initially, the study of the structure-activity relationship of heparin has brought about the development of heparin derivatives (LMWHs or non-heparin GAGs) with an increased anti-Xa/anti-IIa ratio. The most unique heparin derivative that has been identified is a pentasaccharide that binds to AT to elicit a factor Xa inhibitory response.

The original pentasaccharide sequence was identified from natural heparin by fractionation procedures. A specific pentasaccharide of a predetermined sequence based of these findings was subsequently synthesised by glycosaminoglycan synthesis. Later the α-methyl pentasaccharide (Org31540 / SR90107A) was synthesised with identical biological properties [73,74,75].

TABLE 1 Comparison UFH, LMWH and pentasaccharide

DETERMINANT	UFH	LMWH	PENTASACCHARIDE
Mean molecular weight	12000 – 15000	4000 – 6500	1700
Saccharide units	40 – 50	13 – 22	5
Anti Xa:anti IIa activity	1:1	2:1 – 4:1	Specific anti-Xa act ≈ 1000 U/mg; no anti-IIa act.
SC bioavailability	Low	High	100%
Dose-dependent clearance	+	-	-
Inhibited by Platelet Factor 4	+++	+	-
Inhibits platelet function	++++	+	-
Increases vascular permeability	+	-	-

Direct inhibitors

Another approach for more effective antithrombotic agents has focused attention on the potential limitations of indirect thrombin inhibition. The antithrombotic effects of heparins/heparinoids depend on the presence of endogenous cofactors. It also requires a sufficient proportion of molecules with the pentasaccharide moiety to bind to AT and inhibit factor Xa, or a sufficiently high concentration of larger molecules to inhibit factor IIa effectively. The heparins may be inactivated by heparinase and PF4, and bound by plasma proteins, limiting its availability for therapeutic action. Even more significant is the inability of the UFH-AT complex to inhibit clot-bound thrombin, which may therefore act as a protected, ongoing source of thrombogenesis at sites of pathological thrombus formation. In contrast, specific direct antithrombin agents are able to

inactivate clot-bound thrombin as well as free thrombin [76]. So there has been keen interest in the development of specific, direct inhibitors of factor Xa. When engaged in the prothrombinase complex, this factor proteolytically cleaves prothrombin to generate thrombin, whereas thrombin cleaves a variety of substrates that have key roles in haemostasis and thrombosis. Targeted inhibition of coagulation by selective factor Xa may yield superior antithrombotic efficacy, safety, or both, compared with the less selective conventional antithrombotic agents [56].

Summary

The group of synthetic Xa-inhibitors represents an emerging new class of drugs, which are derived either from natural sources or are synthesised chemically and are chemical and functional very heterogeneous [56]. All have a low molecular mass and some may be available for oral administration. In preclinical studies, the efficacy-safety ratios with these agents were better than those of UFH and antithrombin agents. Compared with direct antithrombin agents, factor Xa-inhibiting agents seem safer and may not induce bleeding and a fibrinolytic deficit.
However, a clear clinical developmental approach is not evident, which hampers a direct comparison of the compounds.
The pentasaccharides were developed for the prophylaxis of deep venous thrombosis with orthopaedic surgery. Most advanced in clinical development is the 'natural' pentasaccharide, Org31540 / SR90107A [73], which is recently registered (Arixtra®, fondaparinux) [77]. Because of its prolonged duration of action, the methylated derivative of this pentasaccharide is being considered for extended prophylaxis of thromboembolism in various conditions [78]. Most of the non-heparinomimetic anti-Xa drugs are in development for cerebrovascular and cardiovascular indications such as acute coronary syndromes and thrombotic / ischemic stroke. However, only limited data are available on the use of these agents in specific indications. Preclinical data must be validated to justify their use in specific thrombotic disorders.

Major differences can be expected between the heparinomimetic and non-heparinomimetic anti-Xa agents (in terms of pharmacodynamics and toxicology). The synthetic heparinomimetics factor Xa inhibitors, like UFH and LMWHs, produce their antithrombotic actions indirectly by binding with AT and exhibit sustained anti-Xa effects. An equimolar amount of AT is required for full expression of the antifactor Xa effects of these compounds. At plasma concentrations higher than 3mg/mL (upper limit of 'normal' for AT in plasma) no additional anticoagulant effect can be obtained. Once bound to AT, a macromolecular complex is generated that cannot penetrate the formed thrombus and endovascular lesions.
In contrast, the direct-acting anti-Xa agents produce a short-duration effect, but these small agents can penetrate the clot and significantly inhibit clot-bound Xa. On the other hand, these peptidomimetics do not activate endogenous vascular-bound AT. As yet, the clinical implications of these differences is unknown. Within each subclass, individual drugs might also have quite different characteristics, just like the LMWHs. Each drug therefore requires individual dose-finding studies for each indication [79].

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CHAPTER 2

OUTLINE OF THESIS

Some major developments in the area of antithrombotic therapy have occurred during the past decades as an increased understanding of the molecular mechanisms underlying thrombogenesis, combined with the biotechnology revolution of the 1980s, has resulted in new anticoagulants appearing at a breath-taking pace [1,2]. The current campaign to identify novel direct inhibitors of thrombin and factor Xa is largely fuelled by the perceived limitations and shortcomings of the only orally applicable group of antithrombotics, the coumarins. Despite some major advances in the use of vitamin K-antagonists during the 1980s, their control can be difficult. In particular, the intensity of the coumarin-induced anticoagulant state is affected by numerous food and drug interactions. Consequently, patients must be monitored in order to avoid periods of excessive or inadequate anticoagulation. This poses a major inconvenience to the patient and contributes to the costs of treatment. Furthermore, there is biochemical evidence suggestive of a rebound hypercoagulable state after therapy with oral anti-coagulants is stopped [3], and oral anticoagulants are one of the few medication classes for which an age-related increase in sensitivity has been demonstrated [4].

Outline

Circumventing the need for frequent patient monitoring by using inhibitors of thrombin and factor Xa, would represent a welcome advance in the treatment of chronic thrombotic disorders [5]. This thesis takes you by the hand and guides you through a part of the search for such compounds. The thesis is divided in five different sections.

In section I, **CHAPTER 1** is a general introduction and covers the current hypothesis of coagulation. Thereafter, an introduction is given about the currently available anticoagulant drugs with

a focus on the heparin-like compounds. Based on the shortcomings of these compounds, the rationale is given why there is a tremendous search for newer compounds. At this point the attention turns to factor Xa, together with thrombin one of the key enzymes in coagulation. The current chapter, CHAPTER 2, begins with a introduction, which clarifies the need for more convenient routes of administration (other than iv/sc). Thereafter the general outline of this thesis is described.
Section II describes the findings of several Phase I studies with new antithrombotic compounds. This section starts with the 'natural pentasaccharide' fondaparinux and contains three studies. This pentasaccharide is mainly cleared by the renal route, therefore a study was conducted in subjects with various degrees of renal function impairment (chapter 3). The next two studies are interaction studies, because it could be anticipated that simultaneous administration with fondaparinux could occur in clinical practice: one study was performed with the oral anticoagulant warfarin, and one with the non-steroidal anti-inflammatory drug (NSAID) piroxicam (CHAPTERS 4 and 5).
It is more and more recognised that for certain indications people need to be treated for longer periods of time. The use of a safe drug with an extended elimination half-life allowing a low dosing frequency would be attractive. Therefore, a longer-acting pentasaccharide was developed, the methylated derivative of fondaparinux. Four phase I studies are described in this section with this compound, SanOrg34006. The first entry-into-man study was a single rising dose study in healthy young male volunteers (CHAPTER 6). However, as most of the target population will be of older age, this study was more or less repeated in a population of healthy subject over 60 years of age (using three selected doses of the compound; CHAPTER 7).
In both these studies, investigation of the subcutaneous (sc) bioavailability was implemented in the study design. Based on the findings of these studies, the third study was conducted, in which the pharmacokinetics and pharmacodynamics of sc multiple-doses in subjects, recently treated for VTE, were investigated (CHAPTER 8). The fourth study was a study to

investigate the interaction between this compound and warfarin (CHAPTER 9).

Whereas these pentasaccharides selectively inhibit coagulation factor Xa, a compound obtained by full chemical synthesis, combining both modes of action of UFH (anti IIa, as well as anti-Xa activity) might be advantageous, especially in arterial thromboembolic diseases. The glycoconjugate Org 36764 consists of two carbohydrate domains interconnected via an inert polyethylene glycol spacer. By means of its two domains it mimics heparin in selective acceleration of the inhibition of Xa, as well as providing the template required for bringing AT and thrombin together, resulting in the interactions that lead to thrombin inhibition. In CHAPTER 10, a first entry-into-man study is described with this compound. As described in section I, thrombin is the final key enzyme in the coagulation cascade, on the crossroad of extrinsic and intrinsic coagulation; therefore, it is an attractive target for an antithrombotic compound. The final chapter in this section covers an interaction study of the direct-acting thrombin inhibitor napsagatran (formerly known as Ro 46-6420) and warfarin (CHAPTER 11).

Development of an orally active anticoagulant with minimal bleeding side effects, and sufficient duration of action to allow once or twice daily dosing, would be essential if a compound is to compete favourably with the coumarins. Such a compound could provide benefits in various indications (e.g. the treatment and prevention of DVT, acute and chronic restenosis following angioplasty, endotoxin-induced DIC, and in long-term out-patient care) [6]. However, as yet a suitable replacement for the coumarins has not been found, although orally active thrombin and factor Xa inhibitors are under development [1].

Section III describes studies performed to investigate the possibilities of other routes of administration of anticoagulant drugs than the conventional iv or sc injection. Currently, the Low-Molecular-Weight heparins (LMWHs) are emerging as the antithrombotic agents of choice instead of unfractionated heparin (UFH) in clinical practice. This section therefore starts with a study performed with pentosan polysulphate, which is a

semi-synthetic LMWH that was supposed to be orally applicable (CHAPTER 12). Because the longer-acting pentasaccharide SanOrg34006 had shown SL bioavailability in dogs, this route of administration was investigated in a group of healthy young male volunteers (CHAPTER 13). YM 466 is a synthetic compound acting directly against activated coagulation factor X (Xa). The next chapter in this section describes the first Phase I experiences with this compound and covers the first entry-into-man study with single rising oral doses, an investigation of the absolute oral bioavailability, as well as investigation of the influence of food on this oral bioavailability (CHAPTER 14).

Despite an enormously increased knowledge of the blood coagulation mechanism and many advances in therapy, treatment of VTE is still far from optimal and hampered by the disadvantages of the present antithrombotic therapies compromising safety and efficacy. The group of newly developed antithrombotic drugs represents a marked structural and functional heterogeneous group of agents that are targeted to modulate different biochemical pathways leading to thrombosis. Additionally, the assessment of the efficacy of these new anticoagulant treatments in patients with symptomatic thrombosis is impeded by the low incidence of the outcome measures of choice (symptomatic venous thromboembolic complications). This has provided incentives to develop approaches for monitoring the alterations of haemostatic mechanisms induced by new agents. Therefore, there is a scope in finding models that can serve as alternative efficacy outcome to relate the pharmacokinetics to the pharmacodynamics in order to evaluate the safety and efficacy in early phase thrombo-prophylactic studies.
In section IV it is tried to evaluate these items based on characteristics of the earlier described compounds in Phase I-studies. In order to get an early idea on the compounds, these were compared based on their selectivity towards coagulation factor Xa (CHAPTER 15), based on their effects on the currently used conventional coagulation assays: the activated partial thromboplastin time (APTT) and prothrombin time (PT)

(CHAPTER 16). During the Phase I studies with anticoagulant drugs with various mechanisms of action on the coagulation cascade, a bedside monitor (Coagucheck Plus® – formerly called Biotrack 512®) coagulation monitor (Roche Diagnostics, Mannheim, Germany) was used to determine the APTT and PT as initial safety measure. As it is essential to know the reliability of the results obtained by such a bedside monitor, these were compared with the 'golden' standard laboratory assays for APTT and PT (CHAPTER 17).

The last section of this thesis, section V, summarises the findings described in earlier chapters and is an overall discussion with regard to these results.

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SECTION II

NEW COMPOUNDS IN HEALTHY VOLUNTEERS

CHAPTER 3–11

CHAPTER 3

THE INFLUENCE OF RENAL FUNCTION ON THE PHARMACOKINETICS AND PHARMACODYNAMICS OF THE NOVEL ANTITHROMBOTIC ORG31540/SR90107A

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submitted

ABSTRACT

Aims

To assess the safety/tolerability and the pharmacokinetics/pharmacodynamics of fondaparinux administered as a single intravenous bolus injection to four groups of subjects with varying degrees of renal impairment.

Methods

Twenty subjects (12 females / 8 males) with varying degrees of renal failure were administered fondaparinux 4 mg intravenously. The pharmacokinetics of the drug were evaluated using plasma and urine concentrations. The relationship between the pharmacokinetic parameters and creatinine clearance (calculated using Cockroft & Gault) was investigated using linear regression analysis.

Results

No clinically relevant differences in vital signs, APTT and bleeding time were noted. C_{max} and V_{ss} of fondaparinux were independent of creatinine clearance (used as measure for renal function). Clearance of fondaparinux was decreased and its terminal elimination half-life was significantly prolonged in subjects with renal function impairment.
The clearance of fondaparinux showed a proportional increase with the creatinine-clearance ($r^2 = 0.90$).

Conclusion

The clearance of fondaparinux is highly correlated with the creatinine clearance, calculated from a single plasma creatinine assessment. This allows simple dose regimen adaptation.

INTRODUCTION

Fondaparinux is a fully chemically synthesised pentasaccharide, identical to the antithrombin (AT, formerly called AT-III) binding sequence in heparin. Fondaparinux inhibits factor Xa selectively, while the compound is devoid of anti-thrombin activity and does not affect platelet function. These properties of the drug are

reflected in very low bleeding enhancement demonstrated in *in vitro* and *in vivo* experiments [1,2,3]. In clinical practice, subcutaneous administered fondaparinux is intended to substitute (LMW-)heparins used concurrently with vitamin K-antagonists in the treatment and prevention of deep vein thrombosis and pulmonary embolism, and probably the prevention of clotting (or coagulation) occurring at thrombogenic surfaces as in haemodialysis or other forms of extra-corporal circulation. It is therefore likely that this compound will be administered to subjects with reduced renal function, as a result of the composition of the target subject population or when the drug is used during haemodialysis. In both cases, knowledge of the pharmacokinetics and pharmacodynamics in relation to renal function is essential.
Therefore, a study was performed to assess the safety, tolerability and the pharmacokinetics and pharmacodynamics of a single intravenous (iv) dose of fondaparinux administered to subjects with different degrees of renal function, varying from normal to severely impaired.

METHODS

Subjects and study-design

The study had an open design. Twenty subjects participated in the study. These subjects were categorised in one of four groups on the basis of their actual renal function as reflected by a creatinine clearance (CL_{CR}). Fifteen subjects had an impaired renal function as follows: group I, severe renal impairment (CL_{CR} 10-30 mL/min); group II, moderate renal impairment (CL_{CR} ≥30-60 mL/min); and group III, mild renal impairment (CL_{CR} ≥60-90 mL/min). Five subjects had a normal renal function. This group of young healthy volunteers (group IV) served as a control group. The creatinine clearance was calculated from the plasma creatinine by applying the formula of Cockroft and Gault [4].
The subjects with renal function impairment were recruited from the outpatient clinic of the department of Nephrology of Leiden University Medical Center. These subjects had stable renal

function as reflected by fluctuations in serum creatinine level of less than 30% over the last 6 months before study entry (measured regularly at the outpatient clinic). The control subjects were recruited from the Leiden student population. The control-subjects were younger than the patient-subjects but previous studies demonstrated no obvious effect of age on pharmacokinetics [5]. The study was conducted according to the principles of the 'Declaration of Helsinki'. Approval was obtained from the Ethics Committee of the Leiden University Medical Center and all subjects gave written informed consent prior to inclusion in the study.

Treatments

Subjects were admitted to the research unit after an overnight fast. Following arrival, a light breakfast was provided. After iv cannulation (one cannula in each arm) baseline blood samples were taken and vital signs were measured. Each subject was administered a single dose of 4 mg fondaparinux as a slow iv bolus injection over 30 seconds using a calibrated infusion-pump. Blood sampling, measurements and urine collection took place at regular time intervals. Adequate urine flow was maintained by administration of oral fluids in relation to the urine volume passed. Lunch and dinner were provided at approximately 5 and 10 hours after drug administration. After 24 hours the subjects were discharged from the research-unit but returned for sampling at 36, 48 and 72 hours. Additional blood sampling was done at 120, 144 and 168 hours for patients in the two groups with the poorest renal function.

Sample collection

No tourniquet was applied when blood was collected. The iv cannula used for blood sampling was kept patent by intermittent flushing with of 0.9% saline. The blood was taken after discarding the contents of the cannula. Blood samples for fondaparinux-quantification were drawn pre-dose and at 5, 10, 15, 30, 45 minutes, and 1, 2, 3, 4, 6, 9, 12, 18, 24, 36, 48, 72, 120, 144 and 168 hours relative to drug administration. Blood samples for the APTT

assay were drawn pre-dose and at 15 minutes and 24 hours. Urine was collected over the 0–6, 6–12, 12–24 and 24–48 hour intervals. Concentrations in plasma and urine were determined using a validated, anti-Xa based, amidolytic assay with S2222 as substrate at the department of Drug Metabolism and Kinetics of NV Organon (Oss, The Netherlands). APTT was assessed by standard procedure [6] on a STA coagulation analyser (Boehringer Mannheim, Diagnostica Stago) with the STA APTT-reagent (Boehringer Mannheim).

Safety Analysis

Blood samples for routine haematology and serum biochemistry were collected pre-dose and at 72h after drug administration. A general physical examination was performed at 72 hours after dose administration. Blood pressure and heart rate were measured using an automated blood pressure monitor (Nihon Kohden MPV-7201, Tokyo, Japan) pre-dose and at 15 min, 2, 12, 24, 48 and 72 hours after dose administration. A twelve-lead electrocardiogram was made pre-dose, 1 and 24 hours after dose administration using an electrocardiograph (CardiofaxV ECAPS12, Nihon Kohden, Tokyo, Japan).

Statistics

The drug-concentration time profiles were analysed using non-compartmental. The following kinetic parameters were derived: the peak concentration (C_{max}), the elimination half-life associated with the terminal elimination phase ($t_{½}$), the AUC extrapolated to infinity using the linear trapezoidal rule ($AUC_{0-\infty}$), and the clearance (CL) calculated using $AUC_{0-\infty}$. In addition, the steady-state volume of distribution (V_{ss}) was estimated using a two-compartment open model with weighting equal to 1/(predicted value)^2. Renal clearance of fondaparinux was calculated as the cumulative urinary excretion of the compound over the first 48 hours of urine collections divided by the corresponding plasma AUC. Calculations were performed, using WinNonlin V1.1 software (Scientific Consulting, Inc., Apex, NC).

The relationship between the creatinine clearance and drug

clearance and the relationship between plasma clearance and renal clearance were investigated using linear regression. In order to investigate the possible influence of fondaparinux on renal function, the creatinine clearance values were compared pre-dose and post-dose, using paired samples t-tests. Creatinine clearance was calculated according to Cockroft & Gault [4] from the serum biochemistry samples taken approximately 60 minutes before and 72 hours after fondaparinux administration. Statistical analysis was performed, using SPSS for Windows V6.1.2 (SPSS, Inc., Chicago, IL). With regard to the pharmacodynamic parameters assessed during the study, no formal statistical analysis was performed.

RESULTS

A summary of the demographics is giving in Table 1. Except for the abnormalities related to renal impairment, none of the subjects had any significant abnormalities in medical history, physical examination and routine laboratory tests, including coagulation screen. Controlled hypertension was present and treated by their specialists in eight subjects. One subject occasionally suffered from migraine. All subjects completed the study without serious adverse events. No changes were seen in renal function during the study as the creatinine clearance showed no significant difference comparing pre-dose with 72 hours after drug administration (paired t-test, $p=0.92$). Minor haematomas around venipuncture sites were reported for 2 subjects and one subject noted a trace of blood after sneezing. One subject reported a mild headache and three subjects reported light-headedness. Two subjects reported minor gastrointestinal complaints. All adverse events were mild and of single occasion and no actions were taken, except for treatment of headache in one subject.

PHARMACODYNAMICS

The APTT value of each subject was in the normal range and no obvious changes due to drug administration were detected. No obvious changes were detected in the post-dose samples for the routine laboratory parameters compared to the pre-dose values.

PHARMACOKINETICS

The mean plasma concentration-time curves for the four groups following the iv-bolus injection are shown in Figure 1. A summary of the pharmacokinetic analysis results is given in Table 2. These data indicate that the maximal plasma levels and volume of distribution did not differ between the groups. However, the parameters reflecting drug elimination were influenced by the degree of renal dysfunction. The individual estimates of drug clearance were linearly related to the assessed creatinine clearance and correlated well ($r^2 = 0.898$; Figure 2). This was also reflected by the urinary excretion of fondaparinux over the 48-h period, which decreased with the degree of renal function (Table 2).

The renal clearance of the compound was greatest in healthy subjects and declined in subjects with impaired renal function. The relationship between total clearance and renal clearance can be described as CL_{total} (in mL/min) = 0.73 + 1.24 * CL_{renal} ($r^2 = 0.966$). Since the intercept of this relationship was significantly different from zero, the compound is partly cleared by other routes.

DISCUSSION

The main objective of this study was to assess the pharmacokinetics of fondaparinux after administration of a single intravenous bolus injection to subjects with normal renal function and different degrees of renal failure. The peak concentration was similar in all four groups of subjects, as was the volume of distribution. However, substantial differences between the groups were found for terminal elimination half-life, AUC and clearance of fondaparinux. The plasma fondaparinux clearance was linearly related to the creatinine clearance (as a measure for renal function) estimated with the formula of Cockroft and Gault [4]. This confirms that the compound is cleared almost entirely through the kidney.

The other objective was to assess the safety and tolerability of fondaparinux in these groups of subjects. It was shown that the drug had no significant effects on the vital signs, ECG, and

routine laboratory parameters. These observations confirm the results of pre-clinical studies and the good safety/tolerability-profile observed in studies involving healthy subjects.
The effects of a single dose of fondaparinux on secondary haemostasis as measured by APTT in relation to renal function were also assessed. The APTT did not change following drug administration. However, some caution should be taken to make this finding synonymous with the conclusion that secondary haemostasis is not influenced by administration of fondaparinux. The finding that APTT is not greatly influenced by the very specific action of fondaparinux may also reflect unsuitability of this marker to monitor the effect of the drug. Although the relation between the anti-Xa activity and the probability of bleeding is unknown, this measure is a better predictor for the amount of fondaparinux in the circulation. The iv administration of a single dose of 4 mg fondaparinux was well tolerated and no significant adverse events were seen in this study-population consisting of young healthy volunteers and elderly subjects with different degrees of renal dysfunction.
It can be concluded that the pharmacokinetics of fondaparinux are strongly dependent on renal function. The drug clearance correlated linearly with the creatinine clearance allowing an easy calculation to adapt the dose-regimen of fondaparinux in accordance with the renal function in patients with renal dysfunction.

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LEGENDS TO TABLES

TABLE 1 Subject Characteristics

	Renal Function			
VARIABLE	GROUP I (10–30 mL/min)	GROUP II (≥30–60 mL/min)	GROUP III (≥60–90 mL/min)	GROUP IV (≥90–140 mL/min)
female/male	2/3	4/1	4/1	2/3
age (yrs)	54.8 (51–61)	53.6 (48–59)	47.0 (34–53)	23.2 (21–26)
body-weight (kg)	75.6 (63.5–92.0)	64.9 (63.5–76.0)	72.9 (59.6–81.0)	70.2 (50.0–88.1)
height (cm)	168.6 (158–178)	164.0 (158–175)	164.4 (157–175)	178.6 (170-186)

Reported as mean (ranges)

TABLE 2 Mean (SD) pharmacokinetic parameters derived from Non-Compartmental analysis) for fondaparinux

GROUP	CL_{CR} (mL/min)	C_{MAX} (ng/mL)	$AUC_{0-\infty}$ (mg*hr/L)	$t_{1/2}$ (h)	CL (mL/min)	V_{SS} (L)	EXCRETION (mg)	CL_{RENAL} (mL/min)
I	20.8 (7.9)	1160 (200)	50.2 (10.0)	71.5 (11.7)	1.37 (0.29)	6.78 (0.98)	0.68 (0.30)	0.54 (0.27)
II	46.6 (10.3)	1210 (205)	21.0 (5.4)	28.7 (7.5)	3.35 (0.85)	7.02 (0.69)	1.87 (0.22)	2.26 (0.59)
III	82.2 (5.5)	1220 (275)	13.2 (2.2)	17.9 (0.9)	5.22 (1.15)	7.06 (1.31)	2.45 (0.29)	3.77 (1.25)
IV	126.3 (33.7)	1050 (237)	8.7 (1.3)	13.1 (3.6)	7.82 (1.21)	8.16 (2.44)	2.65 (0.24)	5.51 (0.54)

$AUC_{0-\infty}$ = Area under the concentration-time curve extrapolated to infinity
CL_{CR} = Creatinine Clearance calculated predose (according to Cockroft & Gault)[4]
CL = Clearance
CL_{renal} = Renal clearance
C_{max} = Peak concentration
Excretion = Cumulative urinary excretion over sampling period (48 hours)
$t_{½}$ = Elimination half-life
V_{ss} = Steady state volume of distribution (calculated using model-dependent analysis)

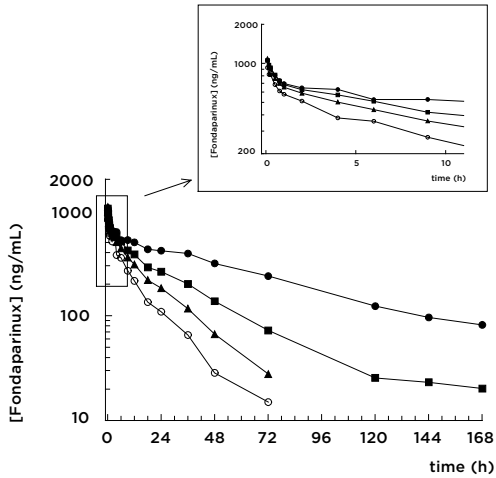

FIGURE 1 **Average plasma concentration-time curves per group; ● group I; ■ group II; ▲ group III; ○ group IV**

(Group I: severe renal impairment (CLCR 10-30 mL/min); group II: moderate renal impairment (CLCR≥30-60 mL/min); group III: mild renal impairment (CLCR≥60-90 mL/min); group IV: young healthy volunteers (CLCR≥90-140 mL/min)).

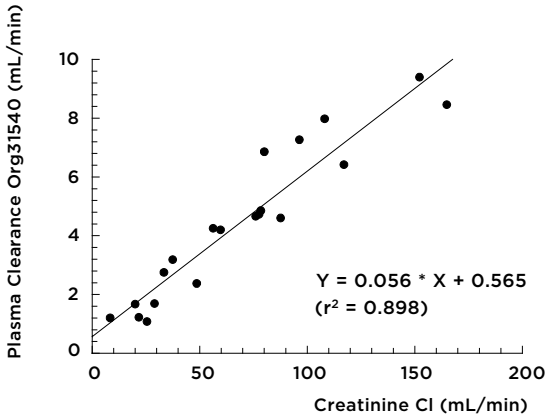

FIGURE 2 **Relationship between plasma clearance of fondaparinux and creatinine clearance.**

CHAPTER 4

ABSENCE OF AN INTERACTION BETWEEN THE SYNTHETIC PENTASACCHARIDE FONDAPARINUX AND ORAL WARFARIN

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ABSTRACT

Aim

To investigate the interaction between the antithrombotic pentasaccharide fondaparinux (subcatneously) and oral warfarin in healthy subjects.

Methods

This study was performed using a randomised, 3-way cross-over, placebo-controlled, double-blind design in 12 male subjects. The treatment consisted of five subcutaneous (sc) injections of fondaparinux (4mg) or placebo at 24 h intervals. Oral warfarin or placebo was administered at the time of the fourth (15mg) and fifth (10mg) sc injection. Blood samples for pentasaccharide assay, prothrombin time (PT) and activated partial thromboplastin time (APTT) were drawn before the first sc dose of pentasaccharide and over a 6-day period thereafter.

Results

Fondaparinux administered alone or in combination with oral warfarin was well tolerated and no serious adverse events were observed. No differences were found in the AUC (43 vs. 44 mg/l*h), C_{max}(645 vs. 678 ng/mL) or elimination half-life (13.8 vs. 14.1hr) of fondaparinux administered as single drug or in combination with warfarin. The effect of warfarin on PT (mean maximal increase: 8.2 sec.) was not influenced by the presence of fondaparinux (mean maximal increase in PT: 9.1 sec.). After all treatments a small insignificant rise in APTT occurred. No further differences could be detected in the effects after the three treatments.

Conclusions

The co-administration of warfarin did not influence the pharmacokinetics of fondaparinux. If these findings can be extrapolated to patients, PT can still be used to monitor the effect of oral anticoagulants during the switch from antithrombotic treatment with pentasaccharide to full oral anticoagulant therapy.

INTRODUCTION

The pentasaccharide fondaparinux (formerly known as Org31540 / SR90107A) is a fully synthetic antithrombotic. The chemical structure of the compound is identical to that of the antithrombin (AT) binding domain of heparin. The drug selectively inhibits factor Xa, is devoid of anti-thrombin activity and does not affect platelet function. As a consequence the drug exhibits a low bleeding tendency, as demonstrated *in vitro* and *in vivo* [1–3]. Assessment of the safety and efficacy of fondaparinux in the prophylaxis and treatment of deep venous thrombosis is an integral part of the clinical development of the compound [4]. Simultaneous administration of subcutaneously (sc) administered pentasaccharide and oral anticoagulants can be anticipated in clinical practice. The change from pentasaccharide to oral anticoagulants would normally occur using a loading dose of oral anticoagulants followed by regular prothrombin time (PT) assessments after 48–72 hours. Combination treatment could potentially effect this measurement and lead to erroneous dose adjustment of the warfarin.

The objectives of this study were to evaluate the possible pharmacokinetic and pharmacodynamic interaction between sc administered fondaparinux and orally administered warfarin.

METHODS

Subjects and Design

The Ethics Committee of Leiden University Medical Center approved the protocol. The study was conducted according to a randomised, 3-way cross-over, placebo-controlled double-blind study design. Wash-out between the study periods was 2 weeks. Twelve healthy males with a normal coagulation screen (age: 19–27 yr., body weight: 61–87 kg) participated in this study after written informed consent was obtained.

Treatments

Subjects received the following treatments during the study: pentasaccharide (Penta-only), oral warfarin (Warf-only) or the combination (Penta+Warf). The Penta-only treatment consisted

of five 4mg sc injections of fondaparinux (Arixtra®) at 24 hr intervals and oral placebo tablets concomitant with the 4th and 5th injection. The Warf-only treatment consisted of five sc placebo (0.9% saline) injections at 24 hrs. At the time of the 4th sc dose, 15 mg of oral warfarin sodium (Coumadin®) was administered followed by 10 mg of warfarin at the 5th injection. During combination treatment the subjects received 5 sc injections of the pentasaccharide and oral warfarin as indicated for the single drug treatment. All subjects received a single oral dose of 10mg of vitamin K (Konakion®) at 132h after the first sc injection, to reverse any residual effect of warfarin (see figure 2 for a schematic view of the study design). Subjects were studied after an overnight fast. They were admitted to the research unit on the evening before first administration of the pentasaccharide and remained there for 12h after each sc injection. The subjects stayed in the unit from the fourth sc injection and first oral administration until at least 36h after the fifth sc injection.

Blood Sampling

Blood was collected without the use of a tourniquet from an iv cannula which was kept patent by intermittent flushing with of 0.9% saline. Blood samples for baseline values were taken before dosing. Blood samples for drug analysis, PT and activated partial thromboplastin time (APTT) were drawn at regular time-intervals until 144h after the first sc dose of the pentasaccharide. Blood samples for routine haematology and serum biochemistry were taken prior to dosing and at the end of each study period.

Laboratory Tests

Anti-factor Xa activity in plasma (reflecting the pentasaccharide concentration) was assessed using a validated amidolytic photometric assay at the Department of Drug Metabolism and Kinetics of NV Organon. Briefly, a calibration curve for the relationship between penatsaccharide concentration and anti-Xa activity is prepared allowing expressing the measured anti-Xa activity in concentration units of the pentasaccharide.
The detection limit of the assay is 2.4 ng/ml, accuracy varied

between 94–104% and the precision ranged between 3.1–6.7%. The PT and APTT assays were performed following standard procedures with a STA coagulation analyser (Boehringer Mannheim, Diagnostica Stago) using the reagents provided by the manufacturer.

Statistical analysis

The drug-concentration time profiles were analysed using non-compartmental pharmacokinetic techniques with the WinNonlin program (Version 1.1, Scientific Consulting, Inc.). Points included for calculation of terminal half-life were automatically determined by the program and visually checked for adequacy. The following parameters were derived: AUC up to the last measurable concentration using the linear trapezoidal rule ($AUC_{0\text{-last}}$), AUC extrapolated to infinity ($AUC_{0\text{-}\infty}$), terminal half-life ($t_{½}$), clearance/F (calculated using $AUC_{0\text{-}\infty}$ and assuming a total dose of 20mg fondaparinux), AUC from 72h (from the first administration of warfarin) up to the last measurable concentration ($AUC_{72h\text{-last}}$), peak concentration (C_{max}) and time to reach peak concentration (T_{max}). The last two parameters were calculated relative to the final sc administration.

APTT and PT were analysed using the uncorrected area under the effect curve (AUEC) and on baseline-corrected data (AUEC above average pre-value) divided by the corresponding time span. This pre-value correction results in a weighted average increase above baseline for which 95% confidence intervals were calculated to test whether a significant increase from baseline occurred. Maximal effect (E_{max}) and the time to reach this maximal effect (T_{max}) on the basis of observed data were calculated for APTT and PT during the Warf-only and Penta+Warf treatments. All contrasts were calculated using paired t-tests on untransformed measures except for AUC and C_{max} parameters that were log-transformed. Log-transformed contrasts were back-transformed, resulting in parameters that can be interpreted as percentage increase due to the addition of warfarin along with 95% confidence intervals. Statistical analysis and calculations were performed using SPSS for Windows (SPSS, Inc., Chicago, IL).

RESULTS

All subjects completed the study without serious adverse events. Two subjects reported mild gastrointestinal complaints after intake of placebo-tablets. Mild headaches were reported by 4 subjects (a total of six episodes) and three subjects reported an episode of malaise. Minor haematomas around venipuncture sites were reported for 4 subjects. One subject reported a bleeding episode of approximately 10 minutes, after he cut himself while shaving and one subject had a spontaneous epistaxis between two study periods.

Pharmacokinetics

The mean plasma concentration-time curves for the treatments are shown in figure 2. No statistically significant difference between Penta-only and the combination treatment was found. Co-administration of warfarin led to a non-significant increase in C_{max} of 5.1% (95% CI: -1.1, +11.7%), $AUC_{0-\infty}$ of 2.5% (-1.1, +6.2%) and $AUC_{72h\text{-}last}$ of 2.8% (-2.8, +8.7%).

Pharmacodynamics

The mean PT-time curves for the different treatments are shown in figure 2. The treatment with Warf-only as well as Penta+Warf resulted in a statistically significant increase in the time-corrected AUEC and E_{max} compared to the Penta-only treatment. The difference was 2.6 seconds (95% CI: +1.9, +3.3s) and 2.9 seconds (+1.8, +3.9s), respectively for the time-corrected AUECs and 8.2 seconds (+3.6, +12.7s) and 9.1 seconds (+4.0, +14.2s) for E_{max}. No statistically significant difference was found between the Warf-only treatment and the combination treatment for the time-corrected AUEC (both with and without pre-value correction), E_{max} and T_{max}. The APTT increased above baseline after Penta-only (mean: 3.1 seconds; 95% CI: +0.7, +5.5s) and after Warf-only (3.2 seconds; 95% CI: +0.3, +6.2s). The combination treatment resulted in a greater increase (5.0 seconds; 95% CI: 4.0, 5.9), which however was not significantly higher than the summed increase after single drug treatment (figure 5). This suggests a possible additive effect of both drugs on APTT.

Discussion

The objective of this study was to investigate the possible pharmacokinetic and pharmacodynamic interaction of sc administered pentasaccharide fondaparinux and oral warfarin in healthy male volunteers. For warfarin a loading dose regimen was chosen to avoid the cumbersome procedure of attaining stable oral anticoagulant concentrations in healthy volunteers.

It is recognised that with this dose-regimen a full suppression of all vitamin K-dependent clotting factors is not reached. However, it provides answers for the clinical situation during which the pentasaccharide and oral anticoagulants are administered concomitantly. This is of particular importance for the effects of the combination of the two drugs on the PT at the time the switch from pentasaccharide treatment to oral anticoagulants occurs clinically. In addition, previous studies using a single loading dose of 25 mg of warfarin have been proven to provide useful information on this relevant issue [5–8]. The pharmacokinetic profile of pentasaccharide given in combination with warfarin was identical to that of pentasaccharide alone. Hence, no pharmacokinetic interaction was detected.

PT is the most commonly used test to monitor orally administered anticoagulants [9]. In this study PT did not change from baseline with the pentasaccharide-only treatment. Treatment with warfarin alone or in combination with the pentasaccharide prolonged PT as expected. However, the presence of the pentasaccharide did not influence the effect of warfarin on the PT. It can thus be concluded that PT can remain to be used to monitor the effect of oral anticoagulants during the switch from anticoagulant treatment with pentasaccharide to oral anticoagulant therapy.

The administration of pentasaccharide or warfarin alone resulted in a small (approximately 3 second) rise in APTT. The increase in APTT after the combination treatment was only slightly more than that after the monotherapy.

In conclusion, concomitant oral administration of warfarin does not affect the pharmacokinetics of the novel pentasaccharide fondaparinux in healthy volunteers. In addition, warfarin-

induced increase in PT (INR) was not influenced by concomitant subcutaneous pentasaccharide treatment. Accordingly, if this finding can be extrapolated to patients, PT can safely be used to monitor the effect of warfarin during the switch from pentasaccharide treatment to warfarin.

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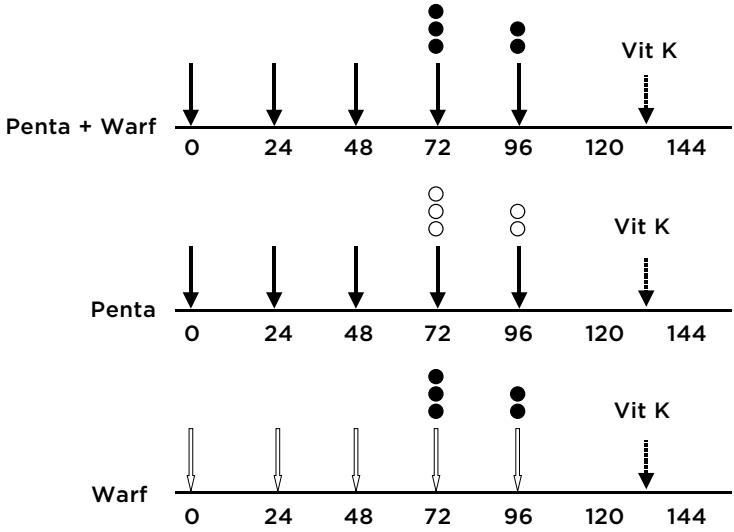

FIGURE 1 **Schematic view of the study design.**
Closed symbols indicate active treatment with pentasaccharide (arrows) or warfarin (circles), and open symbols indicate corresponding placebo treatment. K indicates vitamin K administration.

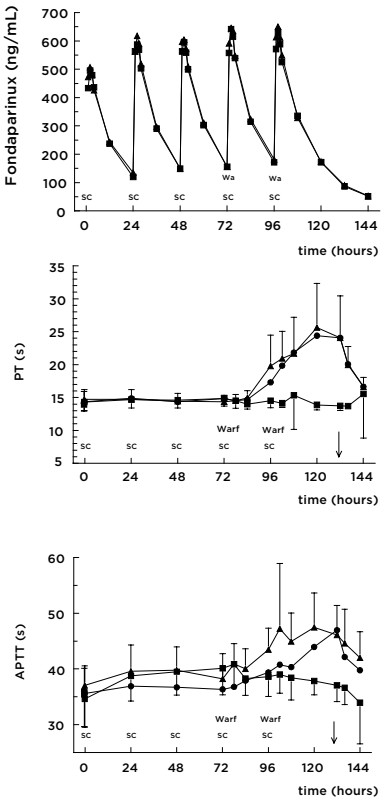

FIGURE 2 **Average pentasaccharide plasma concentration-time profile (upper panel), average PT-values (middle panel), and average APTT-values (lower panel) per treatment ■: pentasaccharide; ▲: pentasaccharide plus warfarin; ●: warfarin).**

The sc pentasaccharide injections and oral warfarin administrations are indicated. The arrow indicates the timepoint at which vitamin K was administered.

CHAPTER 5

ORAL PIROXICAM DOES NOT INTERACT WITH THE NOVEL SYNTHETIC ANTITHROMBOTIC PENTASACCHARIDE FONDAPARINUX

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ABSTRACT

Objective

To investigate possible pharmacokinetic and pharmacodynamic interactions between the subcutaneously (sc) administered novel pentasaccharide fondaparinux and oral piroxicam as a representative of the class of non-steroidal anti-inflammatory drugs (NSAIDs).

Methods

The study was performed in 13 healthy subjects in a 3-way crossover, randomised, double-blind study with a three-week washout period between occasions. After pre-treatment with 6 oral doses of piroxicam (20 mg at 24-h intervals), the subjects received sc injections of 10 mg pentasaccharide at 0, 24, 48 and 72 hours while piroxicam was continued. Blood samples for drug assay for pentasaccharide, collagen induced platelet aggregation (CIPA), and APTT were drawn pre-dose and over a 5-day period after the first dose of the pentasaccharide. Gastrointestinal blood loss was estimated by fecal porphyrin content. Comparisons were made using paired t-tests and are reported as mean difference and 95% confidence intervals.

Results

The combination treatment was well tolerated. Over the period that the chance for an interaction was maximal (from the 4th sc injection onwards) no differences were noted in the pharmacokinetics of the pentasaccharide with or without co-administration of piroxicam, as indicated by the absence of significant differences between the treatments for the main parameters for the pentasaccharide (C_{max} (11; CI: -92,+115 ng/mL), elimination half-life (0.3; CI: -0.44, +1.1 hr) and $AUC_{72-\infty}$ (0.59; CI: -0.87, +2.06 mg*h/L). Compared to the treatment with the pentasaccharide alone, piroxicam decreased CIPA in a similar fashion when given alone (5.5Ω; +0.3,+10.7Ω) or in combination with the pentasaccharide (5.0Ω+1,6,+8.4Ω). Also, no differences were observed in gastrointestinal blood loss.

Conclusion

This study has shown that no pharmacokinetic or pharmacodynamic interactions occur during concomitant use of the pentasaccharide fondaparinux and the NSAID piroxicam.

INTRODUCTION

The antithrombotic pentasaccharide fondaparinux (formerly called fondaparinux) is fully synthetically prepared and potentiates the anti-factor Xa activity of antithrombin. The compound is devoid of anti-factor IIa activity and does not affect platelet function, which is reflected in a low bleeding enhancement. The drug is safe and well tolerated after single subcutaneous (sc) doses up to 30mg in young subjects and 18mg in elderly healthy subjects. The drug shows linear kinetics and is renally cleared. Following multiple dosing, steady state is reached after 3 days [1]. Recently, data have became available that fondaparinux has the potential to improve significantly the risk-benefit ratio for the prevention of venous thromboembolism (VTE), as compared with low-molecular-weight heparin in patients who underwent total hip replacement [2].

The selectivity and good tolerability of the pentasaccharide appears to represent an improvement over the presently available drugs, although this is currently based on limited information currently in the public domain from a large phase III program in prevention of VTE in orthopedic surgery [3-6].

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most widely used classes of drugs [7]. This is particularly true for patients with chronic joint disorders as rheumatoid arthritis or arthrosis. Patients with these conditions are frequently candidates for joint-replacement surgery, a condition known to be associated with a high incidence of VTE. As pentasaccharide may become a new therapy in the prevention of post-surgery venous thrombosis, it is highly likely that the combination of this drug and NSAIDs will be encountered frequently during clinical care. Among the NSAIDs, piroxicam is the most frequently prescribed, probably because its relatively long elimination half-life (approximately 50 hours) and its relatively low ulcerogenic

potential compared to other NSAIDs [8]. This makes the drug attractive for use in chronic conditions as rheumatoid arthritis and related disorders.
Therefore a study was undertaken to investigate the possible pharmacokinetic and pharmacodynamic interactions between fondaparinux and piroxicam as a model NSAID.

METHODS

The study was performed in 13 healthy male volunteers (aged: 19–27 yr., normal weight for height), using a randomised, 3-way crossover, placebo-controlled, double blind design. The washout period between treatments was 3 weeks. The experiment was conducted according to the principles of the 'Declaration of Helsinki' and in accordance with the Guideline for Good Clinical Practice. The Medical Ethics Committee of Leiden University Medical Center (LUMC) approved the study protocol and all subjects gave written informed consent.

Treatments

For this study a dose-regimen of four 10mg of fondaparinux to be administered sc in the abdominal skin region, every 24 hours during 4 days was selected. The pentasaccharide was supplied by NV Organon as pre-filled syringes containing 1.0mL in which the drug was dissolved. Placebo sc injection was a similar volume of 0.9% saline. Pre-treatment with oral piroxicam (20 mg) started 6 days before the first dose of pentasaccharide and was continued during pentasaccharide administration. Thus, piroxicam and matching placebo (obtained via the hospital pharmacy of LUMC) were administered at 24 hr intervals for 10 days. As TxA_2-production is almost completely (but reversibly) blocked after single dose piroxicam above 20mg [9], the study design was such that that the concentrations of piroxicam and pentasaccharide (even for possible arterial indications) were in a clinically relevant window and complete blockade of TxA_2-production over the entire duration of the experiment was achieved.

Study Periods

During the pre-treatment period subjects reported to the research-unit every morning. After a check for adverse events and compliance to the study protocol restrictions, piroxicam was administered and subjects could leave. The evening before the first sc drug administration, the subjects were admitted to the unit. At arrival a brief medical history was taken and a physical examination took place. The next morning, prior to the zero time point, an intravenous cannula was inserted and bleeding time measurement (Ivy method) took place. Then blood samples were drawn for drug assay, collagen-induced platelet aggregation (CIPA) and activated partial thromboplastin time (APTT). Subsequently, the subjects were administered sc fondaparinux and oral piroxicam. Drug administration was repeated at 24, 48 and 72 hours. During the stay at the unit subjects collected a random sample from every portion of fresh feces. In addition, routine hematology, biochemistry and urinalysis was performed prior to and upon completion of each treatment period, and bleeding time (Ivy) was measured at 2 and 48 hrs after the last dose of the pentasaccharide. The subjects remained in the unit up to 48h after the last sc injection.

Sampling and assays

The intravenous cannula used for blood sampling was kept patent by intermittent injection of 0.9% saline and blood was taken without using a tourniquet. After the first sc injection, blood samples for Pentasaccharide concentration, CIPA and APTT were taken at regular time-intervals. Blood samples for drug assay and APTT were centrifuged and the separated plasma was stored at –40° until analysis. The pentasaccharide plasma concentrations were assessed using a validated, anti-Xa based, amidolytic assay with the substrate S2222. The detection limit of the assay was 2.4 ng/ml, accuracy varied between 94–104% and the precision ranged between 3.1–6.7%. APTT assay was performed, using standard procedures with a STA coagulation analyser (Boehringer Mannheim, Diagnostica Stago) with the STA APTT reagent (Boehringer Mannheim).

CIPA was measured in 5mL of free flowing blood collected in citrate containing tubes (1 part citrate: 9 parts venous blood) immediately after blood collection with an impedance method. Briefly, a very small electric current is passed between electrodes that are put into the blood sample. During the initial contact of the blood, the electrodes become coated with a monolayer of platelets. When collagen is added, platelets aggregate on the monolayer thereby increasing the impedance (expressed in Ω). The assays were performed using collagen 1 µg/ml as aggregating agent with a Chronolog Aggregometer (model 590), according to the manufacturer s instructions.
Gastrointestinal blood loss was quantified by measuring the cumulative porphyrin content of the collected fecal samples using HPLC with spectrofluorometric detection using coproporphyrin as internal standard [10]. This methodology allows to calculate the ratio of the sum of deuteroporphyrin and pemptoporphyrin over coproporphyrin (ratio I), which is a biomarker for the total amount of hemoglobin lost in the gastrointestinal tract. In addition, the ratio of chemproporphyrin minus protoporphyrin over coproporphyrin (ratio II) can be calculated, which is a measure of intact heme, which can be present in the case of extensive blood loss in the gastrointestinal tract [11-13]

Pharmacokinetic analysis
Non-compartmental pharmacokinetic analysis was performed on the pentasaccharide concentration-time profiles using standard techniques and commercially available software (WinNonlin, version 3.1, Pharsight Corp., USA). Most parameters were calculated across the four doses of the compound. The peak concentration (C_{max}) and the time to reach these peak concentrations (T_{max}) were determined. From the time course profiles the following parameters were derived: the area under the concentration-time curve for the intervals from zero time point up to the last measurable concentration (C_{last}) using the trapezoidal rule ($AUC_{0\text{-}last}$), the half-life associated with the terminal elimination phase ($t_{½}$), the AUC extrapolated to infinity ($AUC_{0\text{-}\infty}$), and the apparent volume of distribution.

The C_{max} and T_{max}, and AUCs were also determined for the last administration of pentasaccharide ($AUC_{72h\text{-}last}$ and $AUC_{72\text{-}\infty}$). The terminal half-life was calculated using log-linear regression on the terminal part of the curve. Points for inclusion in the regression equation were automatically determined by the program, and subsequently visually checked for adequacy. The apparent clearance of the pentasaccharide was calculated as total dose administered divided by the $AUC_{0\text{-}\infty}$. This provides a measure of the average clearance over the entire study period. The differences in pharmacokinetic parameters between the combined treatment of fondaparinux + piroxicam and fondaparinux-only were compared.

Pharmacodynamic analysis

For each subject-occasion combination the area under the effect curve (AUEC) for the amplitude in the aggregometry test (CIPA) and APTT was calculated using the linear-trapezoidal rule for the period from zero time up to the last measurement and from 72 hours up to the last measurement. These AUECs were analysed with and without correction for baseline values. Contrasts were calculated for the AUEC corrected for baseline of the CIPA and APTT for the combination treatment versus the two single drug treatments, and versus the summation of the effects of the single drug treatments. Without baseline correction the AUECs for these parameters were compared between the three different treatments. Fecal blood loss was analysed by calculating contrasts for ratio I and II for the combination treatment versus the pentasaccharide and versus piroxicam. Bleeding time values were analysed by comparing the observed values (with and without pre-value correction) between treatment groups. For all parameters, contrasts were evaluated using paired Student t-tests and presented with 95% confidence intervals. However, the parameters used for fecal blood loss were not normally distributed and hence analysed non-parametrically using Wilcoxon's signed ranks test. Statistical analysis and calculations were performed with SPSS for Windows V9.0.1 (SPSS, Inc., Chicago, IL).

RESULTS

General

Due to a dispensing error at the pharmacy, one subject received sc placebo-injections instead of active pentasaccharide during one study-period. This only came to light after he had completed all his study-periods, and therefore, he was replaced. As such, this study was carried out with 13 subjects. The results of the replaced subject were used as much as possible.

None of the changes in assessed safety indices was of clinical relevance. All adverse experiences were mild in severity and none required medical intervention. The most frequently reported adverse event was development of hematoma(s) at puncture sites; the incidence was highest following the combination of pentasaccharide and piroxicam (11 times) compared to 5 times during the mono-treatments.

Pharmacokinetics

Mean plasma concentration time profiles are presented in Figure 1. The pharmacokinetic parameters for fondaparinux during both treatments are summarized in Table 1. Piroxicam did not influence the pharmacokinetics of the pentasaccharide. A small, but significant difference was found in the maximum drug plasma concentration after the first administration. Furthermore, none of the other pharmacokinetic parameters was statistically significant between the treatments.

Pharmacodynamics

Administration of piroxicam (either alone or in the combination treatment) resulted in lower impedance in CIPA test (figure 2). The mean decrease was 5.5Ω (95%CI: 0.3-10.7Ω) between pentasaccharide and piroxicam monotherapy, and 5.0Ω (1.6-8.4Ω) between pentasaccharide monotherapy and the combination therapy. No significant differences between treatments were found for the fecal porphyrin excretion rates over the study period of 5 days (table 2).

Following both treatments with the pentasaccharide a small, but significant increase was found in the average APTT. This increase

was comparable for both treatments, because no significant difference was found in the average APTT between the combination treatment and the pentasaccharide mono-treatment. This was confirmed by t-tests; the mean difference for the combination treatment vs. piroxicam was 5.5 seconds (95%CI: 3.6-7.4), and the mean difference between pentasaccharide monotreatment and piroxicam was 5.2 seconds (95%CI: 2.6-7.7). For the pharmacodynamic measures (APTT and CIPA), the analysis for the period after the last administration ($AUEC_{72h\text{-}last}$ and $AUEC_{72\text{-}\infty}$) gave similar results as found for the entire study period. For both parameters, the paired samples test after correction for pre-value confirmed the results for the uncorrected (observed) data. Moreover, no difference was found between the combination treatment and the summation of effects of the mono-treatments, indicating absence of a synergistic interaction between the pentasaccharide and the NSAID on these parameters. The average (SD) pre-dose bleeding time was 183 (76), 179 (41), 164 (47) seconds for the pentasaccharide only, piroxicam only, and for the combination treatment respectively. At 2 hours after the first administration of pentasaccharide non-significant increases were noted for the respective treatments. After the last pentasaccharide administration the bleeding times were at the same level as the pre-treatment values, hence no prolongation of bleeding time was noted in this study.

DISCUSSION

Pentasaccharide administered subcutaneously to healthy male volunteers at the dose 10 mg in combination with the oral NSAID piroxicam was well tolerated and no serious adverse events were observed. The dose of pentasaccharide studied was in the high range of the therapeutic doses (comprised between 2.5 mg and 12 mg considering all phases II and III studies both in venous and arterial thrombotic disorders including prevention and curative treatments). This pentasaccharide alone, or in combination with piroxicam, had no obvious effects on vital signs and routine laboratory parameters in blood. These observations confirm results from pre-clinical and prior clinical studies. The occurrence

of hematoma at puncture sites was highest following the combined treatment, but all adverse events were mild in severity and none required medical intervention.

The co-administration of piroxicam did not influence the pharmacokinetics of fondaparinux. A statistically significant effect on C_{max} after the first dose of the compound was observed, however, taken into account the effect size (70 ng/mL, approximately 6%) this can be considered not clinically relevant. Platelet aggregation was not influenced by the single administration of the pentasaccharide. The administration of the pentasaccharide in combination with piroxicam was followed by a small decrease in platelet aggregation similar to the CIPA-decrease after the single administration of the NSAID. As the main mode of action of all NSAIDs is by interfering with the prostaglandin synthesis, it is likely that concomitant use of 10 mg of pentasaccharide and NSAIDs does not result in any relevant interaction on platelet aggregation. This was further confirmed by the absence of an interaction on bleeding time and gastrointestinal blood loss. As orally administered NSAIDs may affect the gastroduodenal mucosa, [14], all subjects underwent a fecal occult blood test at screening, commonly used for diagnostic purposes. This confirmed the absence of gastro-intestinal blood loss before the study. No differences between treatments in this study were found with regard to fecal porphyrin excretion (used as measure for gastrointestinal blood loss). It can be concluded that the co-administration of piroxicam and fondaparinux does not result in an increase in fecal porphyrin excretion in comparison to piroxicam alone.

The administration of fondaparinux alone, or in combination with piroxicam was followed by only a small rise in APTT, which confirms the high selectivity of the anti-Xa pentasaccharide.

In conclusion, sc administered fondaparinux in combination with oral piroxicam well tolerated and no pharmacokinetic or pharmacodynamic interactions were found between the pentasaccharide and piroxicam used as a representative of the group of NSAIDs.

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TABLE 1 Mean (SD) pharmacokinetic parameters of the pentasaccharide fondaparinux in the absence or presence of piroxicam.

Parameter	PENTASACCHARIDE + PIROXICAM N=13) Mean (SD)	PENTASACCHARIDE (N=12) Mean (SD)	PAIRED DIFFERENCES* Mean (95%CI)
$AUC_{0\text{-last}}$	74.1 (10.5)	75.7 (10.7)	0.82 (-3.35 , 1.72)
$t_{½}$	13.9 (1.9)	13.6 (1.5)	0.31 (-0.44 , 1.07)
$AUC_{0\text{-}\infty}$	76.8 (11.5)	78.3 (11.5)	-0.66 (-3.39 , 2.08)
V_z/F	9.2 (0.9)	8.8 (1.0)	0.22 (-0.20 , 0.64)
Cl/F	464 (72)	455 (68)	4.5 (-12.9 , 22.0)
C_{max} 1st dose	1068 (134)	1139 (144)	-70 (-114 , -26)
T_{max} 1st dose	2.27 (0.60)	2.54 (0.49)	-0.26 (-0.65 , 0.13)
$AUC_{72\text{-last}}$	24.3 (4.3)	24.3 (4.2)	0.43 (-0.89 , 1.76)
$AUC_{72\text{-}\infty}$	27.0 (5.38)	26.8 (5.07)	0.59 (-0.87 , 2.06)
C_{max} 4th dose	1453 (214)	1459 (292)	11 (-92 , 115)
T_{max} 4th dose	2.01 (0.41)	2.25 (1.43)	-0.24 (-1.02 , 0.54)

AUCs in mg*h/L; $t_{½}$ in h; Cl/F in mL/h; C_{max} in ng/mL; CT_{max} in h; V_z/F = apparent volume of distribution (in L). * Paired Samples Test (degrees of freedom=11).

TABLE 2 **Mean fecal porphyrin excretion rate.**
Ratio I(the sum of deuteroporphyrin and pemptoporphyrin over coproporphyrin) and ratio II (ratio of chemproporphyrin minus protoporphyrin over coproporphyrin)

RATIO	TREATMENT	MEAN (SD)	MEDIAN	RANGE	INTERQUARTILE RANGE
I	Fondaparinux	6.78 (5.78)	4.22	0.72 - 18.32	10.52
	Piroxicam	6.10 (5.32)	4.26	1.23 - 20.30	3.07
	Fondaparinux + piroxicam	6.80 (5.63)	6.03	1.48 - 17.89	6.25
II	Fondaparinux	33.06 (43.70)	22.53	0.70 - 148.73	26.00
	Piroxicam	31.85 (33.82)	21.69	1.46 - 112.47	25.39
	Fondaparinux +piroxicam	18.96 (15.07)	16.84	1.01 - 44.51	26.24

Reference values: ratio I < ratio II, and ratio II < 32.

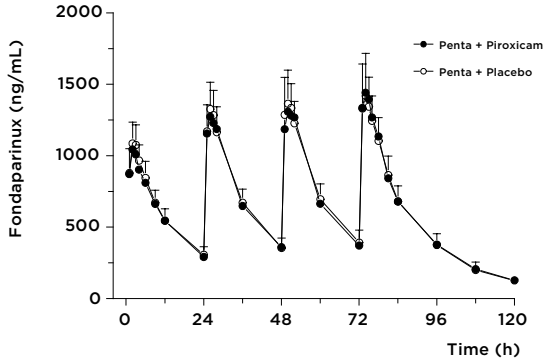

FIGURE 1 **Mean (SD) concentration time profiles after four sc doses of pentasaccharide with or without co-administration of piroxicam.**

Doses of the pentasaccharide were given at 0, 24, 48 and 72 hours. Piroxicam was administered at 24-hr intervals for 10 days starting 6 days before pentasaccharide dosing.

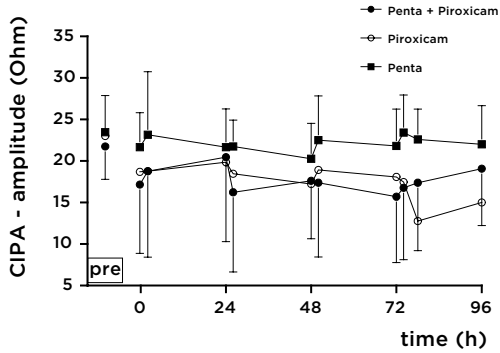

FIGURE 2 **Mean (SD) profiles of the amplitude in the collagen-induced platelet aggregation test for each treatment period.**

Doses of the pentasaccharide were given at 0, 24, 48 and 72 hours. Piroxicam was administered at 24-hr intervals for 10 days starting 6 days before pentasaccharide dosing. Pre: indicates value before piroxicam administration.

CHAPTER 6

A PHASE I SINGLE RISING DOSE STUDY TO INVESTIGATE THE SAFETY, TOLERABILITY AND PHARMACOKINETICS OF IDRAPARINUX SODIUM IN HEALTHY YOUNG MALE VOLUNTEERS

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ABSTRACT

Objectives

Idraparinux is a selective coagulation protein Xa inhibitor, designed to be long acting. Objective of the current study was to investigate the pharmacokinetics and tolerability and safety of consecutive rising doses of intravenously (iv) administered idraparinux in healthy young volunteers and to estimate the absolute bioavailability of subcutaneous (sc) idraparinux at two doses.

Methods

The study had a double-blind single rising iv dose design with an included sc bioavailability protocol. Five subjects received idraparinux and one subject placebo per dose level. For the sc bioavailability protocol six subjects received idraparinux and one placebo. The dose-increments were 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12 and 14 mg as twenty seconds iv bolus injections. In the sc study 2 or 10 mg was given. Blood sampling was done up to 336 h for pharmacokinetics and pharmacodynamics (APTT/PT, AT, anti-IIa activity). Urine was collected up to 168 h.

Results

Idraparinux sodium was well tolerated and no significant adverse events were noted. AUC and Cmax increased in a dose-proportional manner, indication linear pharmacokinetics. Total plasma clearance was independent of dose, whereas terminal half-life estimates reached a plateau of about 120 hours (following doses of 6 mg or higher; range of means: 116–127 h). Indications of an even longer elimination half-life were found. Idraparinux sodium plasma clearance correlated with body weight (r=0.503; p=0.003). The sc bioavailability was 93% after 2 mg idraparinux sc and 99.8% after 10 mg sc with good consistency of the pharmacokinetic parameters between the sc and iv route of administration. Following one week of urine collections, almost 20% of idraparinux was cleared by the renal route (range: 14.3-23.3% for dosages of 1 mg or higher). Renal clearance and mean urinary excretion rate increased with dose. A small, clinically insignificant effect in APTT and PT was noted.

Conclusions
Idraparinux sodium is an antithrombotic compound with linear pharmacokinetics and an absolute bioavailability after sc administration of almost 100% and a terminal half-life of about 5 days. It is therefore anticipated that idraparinux may be a useful new drug for long-term anti-thrombotic use.

INTRODUCTION

Idraparinux sodium (Figure 1) is a novel synthetic pentasaccharide compound with anticoagulant properties *in vitro* and *in vivo*. Preclinical studies indicated that it works by activation of antithrombin (AT), the naturally occurring inhibitor of the blood clotting process. The main activity of AT is against factor Xa and IIa (thrombin). Heparin-like drugs accelerate the reaction that forms complexes between AT and factor Xa or factor IIa. These complexes dissociate very slowly relative to their target protein in the coagulation cascade. Recently, synthetic analogues of the heparin pentasaccharide sequence that binds to AT have become available, which selectively inhibit factor Xa. One of these compunds (fondaparinux) has been registered. Most anticoagulants presently available need to be administered at least once daily. However, it is increasingly recognised that people at risk of thrombotic disorders need to be treated over an extensive period of time (months, years, or even life-long). Prolonged antithrombotic treatment ideally requires a dose regimen with a drug with an extended elimination half-life, enabling a low dosing frequency. On the basis of the slow elimination rate of idraparinux compared to fondaparinux observed in pre-clinical studies, it was anticipated that idraparinux sodium could be a useful new drug for prolonged antithrombotic treatment. Therefore this study was performed to investigate the safety, tolerability and pharmacokinetics of intravenously (iv) administered idraparinux. As it can be envisaged that for clinical use this compound will be administered subcutaneously (sc), the absolute bioavailability of sc idraparinux was investigated in the same experiment. Since animal data suggested that the compound was partly renally cleared, investigation of its renal clearance was also part of the analysis.

METHODS

Subjects and Design

This study was conducted according to a single rising dose, randomised placebo-controlled design. Parallel dose-level groups started consecutively with at least one-week intervals, double blind within each dose level. Subjects were allowed to participate more than once, provided a washout period of at least 4 weeks was observed. The bioavailability part of the study was also double blind regarding placebo administration. Iv and sc administration of idraparinux within relevant dose levels was not blinded; no dummy treatments were used, but the order of administration was randomised.

All rising dose groups consisted of six subjects, with dose levels 0.25, 0.5, 1, 4, 6, 8, 12 and 14 mg idraparinux. Five subjects were randomised to active treatment and one subject to placebo. All iv doses were administered in an identical volume of 5 mL as a 20 seconds bolus injection, and the sc doses consisted of a volume 0.53 and 2.63 mL for administration of the 2 and 10 mg doses. Syringes for iv or sc injection used as placebo, contained an equal volume of 0.9% saline solution. All study medication was prepared by the Leiden University Medical Center pharmacy.

The bioavailability part of the study included six subjects receiving active treatment plus one receiving placebo for the dose levels involved (2 and 10 mg). Thus, in total fourteen subjects participated in this part. First subjects were randomised to receive idraparinux iv or sc and either idraparinux or placebo. The order was reversed after a washout period of at least 4 weeks. Subjects receiving placebo the first time also received placebo the second time.

Procedures

All subjects participated in this study after written informed consent was obtained. The Ethics Committee of Leiden University Medical Center approved the investigational protocol. The subjects were studied after an overnight fast. After arrival at the CHDR unit a short medical history and a physical exam were performed to ensure compliance to the protocol restrictions. Subjects were exposed to no more than four single injections.

Sampling

No tourniquet was applied when blood was collected during the study days. An iv cannula used for blood sampling was kept patent by intermittent flushing with 0.9% saline. Blood was taken after discarding the contents of the cannula. Blood samples for baseline values were taken pre-dose.

Pharmacokinetic Assessments

Blood samples for drug-assay were drawn frequently until 336 h after administration of idraparinux. Urine was collected for measurement of drug concentration over the following time-intervals: 0-4, 4-12, 12-24 h, and subsequently in 24-hour periods until maximally 168 h (1 week) after dosing in pre-weighted plastic containers. In view of the fact that a physicochemical method for assessing plasma and urine concentrations of idraparinux is not available, a detection method based on the anti-factor Xa activity of the idraparinux-AT complex was used. Since there is no evidence of the formation of active metabolites of the drug in vivo (data on file) the specificity of this assay can be assumed. The validated assay in plasma and urine was performed at the Department of Drug Metabolism and Kinetics of Organon Development GmbH (Waltrop, Germany). In the sample preparation the presence of sufficient AT is secured either by dilution of samples with plasma or the addition of AT (in urine samples). Idraparinux sodium binds to AT to form a complex which inactivates factor Xa by irreversible binding. Subsequently, factor Xa is added in excess and the remaining free factor Xa catalyses the generation of paranitroalanine (pNA) by the hydrolysis of the peptide pNA conjugate. The liberated pNA has a yellow color that can be measured spectrophotometrically at 405 nm. The measured color is inversely proportional to the idraparinux concentration in the sample. Lower limits of quantification were 16 ng/mL in plasma and 6 ng/mL in urine.

Pharmacodynamic Assessments

Blood samples for APTT/PT, AT and anti-factor IIa-activity assays were drawn at regular time intervals until 336 h relative to the

dose of idraparinux. Bleeding time (Ivy method) was done at screening (baseline value) and at 15 min and 48 hrs after dosing.

Laboratory Parameters

APTT and PT assays were performed using standard procedures [1]. AT in plasma was assessed using a photometric assay with S-2765 as a chromogenic substrate and is based on the inhibition of factor Xa (Comatic© Antithrombin, Mölndal, Sweden). The heparin anti-IIa activity was determined using a photometric assay with thrombin substrate H-D-HHT-L-Ala-L-Arg-pNA.AcOH (Spectrolyse® Heparin anti-IIa, Biopool, Umea, Sweden). An aliquot of the corresponding idraparinux dosing formulation was used as calibrator.

Statistical analysis

Non-compartmental pharmacokinetic analysis was performed using WinNonlin V1.1 (Scientific Consulting, Inc., Apex, NC). The following parameters were derived from the time course profiles: the peak concentration (C_{max}) and the time to reach these peak concentrations (t_{max}), the AUC from the time intervals from zero time point up to the last measured concentration using the lineair trapezoidal rule ($AUC_{0\text{-last}}$), the elimination rate constant associated with the terminal elimination phase (λ_z) and its associated half-life ($t_{½}$) the AUC extrapolated to infinity ($AUC_{0\text{-}\infty}$), the plasma clearance (CL) and apparent volume of distribution (Vz). The number of points used for λ_z calculation were automatically determined by the pharmacokinetic-program and were visually checked for adequacy. The sc bioavailability was calculated by dividing the $AUC_{0\text{-}\infty}$for the sc administration by the $AUC_{0\text{-}\infty}$ for the iv administration. Non-zero pre-values were dealt with by subtracting from the total AUC, the AUC attributable to these non-zero pre-values (calculated as pre-value/λ_z, where λ_z is the estimated elimination rate constant for that particular curve). The pharmacokinetic parameters resulting from iv administration were compared between doses using one-way analysis of variance with factor dose; AUC and C_{max} were dose-normalised. Between-dose variability was further investigated by estimating

a linear trend over dose. Primary analysis was performed for the non-extended profiles (see further).
The urinary excretion of idraparinux was calculated using cumulative excretion and percentage of dose excreted. For each collection interval, the excretion rate was calculated by dividing the amount excreted over the interval by the corresponding time period. The renal clearance was calculated by dividing the cumulative excretion by the plasma AUC with endpoint closest to the end of the urine collection period ($AUC_{0\text{-}t}$).For repeatedly measured pharmacodynamic variables (APTT/PT, AT and anti-IIa activity), the Areas Under the Effect Curves (AUECs) using the linear trapezoidal rule on protocol times were calculated after subtraction of pre-values, both for the periods 0–6 h as well as 0–24 h post-dosing using BMDP/Dynamic Version 7.0 (BMDP Statistical Software, Inc., Los Angeles, CA). Subsequently, these areas were divided by the corresponding time span to result in a weighted average response (AUEC/time). Additionally, the change from pre-value to E_{max}(delta E_{max}) and the change from pre-value to t=24 h post-dosing (delta 24h) were calculated. Pharmacodynamic parameters (iv administration) were compared between doses using one-way analysis of variance with factor dose. Between-dose variability was further investigated by estimating a linear trend over dose.
Statistical analysis and calculations were performed using SPSS for Windows V8.0.1 (SPSS, Inc., Chicago, IL).

RESULTS

The study was performed in 40 volunteers. Nine received placebo treatment during at least one occasion. Two subjects, treated with placebo were given idraparinux on a second occasion. Thus, a total number of 33 subjects were treated with at least one dose of idraparinux sodium. The compound was well tolerated and no significant adverse events were noted. Most of the reported adverse events were local hematoma at the injection or sampling site. There was no apparent dose-relationship in the incidence of adverse events and there was no difference between idraparinux and placebo concerning safety. There were no discontinuations.

It was decided to introduce bedside APTT/PT-measurements with the Biotrack(512 Coagulation Monitor of Ciba Corning® (Coagu-Chek® *Plus*). This monitor provides an integrated system for immediate measurement of PT and APTT in one drop of whole blood [2]. This technique was used for initial safety measurements only. Measurements were within normal ranges for the subjects at all timepoints. However, since effects on bedside measurements were seen at dosages of 10 mg, the number of timepoints at which these measurements were taken was increased. For instance at the 14 mg-group a consistent and distinct increase of the bedside-APTT of 13 sec (approximately 165% of baseline values) was found; Figure 2. This and the fact that it appeared that the drug showed a very long elimination half-life, and it could be expected that the chance on drug-related AEs would increase at higher doses, made that it was decided to abandon dosing beyond the 14 mg dose.

Pharmacokinetics

Terminal half-life, $AUC_{0-\infty}$, clearance and Vz are not reported for doses below 1 mg because of poor definition of the terminal phase. All non-zero pre-dose concentrations were obtained when previous drug-administration had occurred. The percentage of the total AUC attributable to these non-zero pre-values was about 4% maximally (for the iv 10 mg dosage-group, less for the other groups).

A summary of the results is given in Figure 3 and Table 1. The data indicate that the maximal plasma levels (C_{max}) and $AUC_{0-\infty}$ increased linearly with the dose (Figure 4). Dose-linearity was confirmed by one-way analysis of variance. Plasma clearance was independent of dose. Terminal half-life increased with dose ($p=0.001$), but reached a plateau of about 120 hours based on 2 week profile for dosages 6 mg iv and higher. Mean sc bioavailability was 93.0% (SD: 8.36%; range: 81.8-105.2%) after 2 mg idraparinux sc and 99.8% (SD: 5.85%; range: 89.2-104.6%) after 10 mg sc. Additionally, pharmacokinetic parameters were determined for those occasions where non-zero pre-values of the next occasion were added as if they were a final measurement. Samples at 4 weeks post-dosing or beyond were also included in the curve

fitting. Results of this analysis indicated that idraparinux has a very long terminal half-life of approximately 300 hours, when sampling is continued for a sufficiently long time.
The urinary excretion increased with dose. Mean cumulative urinary excretion was about 20% of the administered dose (range of means: 14.3–23.3% for dosages of 1 mg or higher). At higher plasma concentrations a greater part was cleared by the renal route ($p<0.001$); average renal clearance ranged from 0.17 mL/min for the 0.5 mg dose group to 0.30 mL/min for the 14 mg dose group (Table 1). The dose-corrected renal excretion rate calculated for the first urine-collection portion (0–4 h post-dosing) increased with dose (Figure 5).

Pharmacodynamics
APTT and PT AUEC versus dose plots are presented in Figure 6. Maximum individual increase observed in this study was 17.3 seconds for the APTT and 2.7 seconds for the PT, both observed in subjects after 14mg idraparinux iv. The mean increase in this dose-group was 9.1 seconds for the APTT and 1.9 seconds for the PT, respectively.
With regard to the analysis concerning the between-dose variability for the APTT the p values for linear trend with dose were just above 0.05 for all measures. There was no statistically significant increase with dose for APTT (Figure 6). The slope of the regression-line calculated for the $AUEC_{0\text{-}6h}$ for APTT is 0.436 s/mg (95%CI: 0.17-0.70). For the PT a significant increase with increasing dose was observed for the weighted average response: $AUEC/time_{0\text{-}6h}$ and $AUEC/time_{0\text{-}24h}$ (p values 0.002 and 0.021, respectively).
For PT the slope of the regression-line for the $AUEC_{0\text{-}6h}$ was 0.094 s/mg (95%CI: 0.05, 0.14) (Figure 6). For AT the relationship from the regression of the $AUEC_{0\text{-}6h}$ on dose can be described as AT $AUEC_{0\text{-}6h}$ = –2.24 + 0.533*dose (95%CI for the slope: 0.05, 1.02).
Anti-IIa activity remained virtually unchanged after dosing idraparinux at the dose-levels tested in this study and none of the response measures reached statistical significance.
Bleeding times remained within the clinically acceptable range for all doses. There was a weak dose-response relationship (increase of approximately 2% per mg idraparinux at the t=15 min assessment).

Additional Analysis

The relationship between body weight and body mass index (BMI) and the pharmacokinetic parameters of the compound idraparinux was investigated by calculating correlation coefficients with C_{max}, elimination half-life, clearance, and the apparent volume of distribution. Only parameters for iv administered doses higher than 0.5 mg were used. For subjects participating on multiple occasions, the average parameter for the occasions was used. Clearance was significantly correlated with bodyweight (Pearson's (parametric) correlation coefficient r=0.503; p=0.003).

DISCUSSION

Because pre-clinical studies had shown that idraparinux sodium, a novel synthetic pentasaccharide compound selectively inhibiting factor Xa, was eliminated at a slow rate, it was anticipated that idraparinux could be a potential new drug for long-term antithrombotic use. This study confirms the favorable safety/tolerability-profile found in preclinical studies and studies conducted with another pentasaccharide (the shorter acting fondaparinux sodium) [3]. Comparable to data found in idraparinux-treated animals and studies conducted with fondaparinux an almost complete absolute SC bioavailability was found, with a good consistency of pharmacokinetic parameters between the IV and SC route of administration. With regard to the pharmacokinetics, plasma concentrations were determined by using an anti-Xa activity assay, since a direct method to measure idraparinux levels does not exist.

The peak concentration and AUC linearly increased with the dose. Elimination half-life estimates increased with dose, but reached a plateau of about 120 hours based on a 2 week profile for dosages higher than 6 mg iv. This is most likely caused by the extended measurement period at higher doses, and thus a poorer definition of the terminal elimination phase at lower doses. As it is known that low endogenous anti-Xa levels can be measured (albeit with substantial variability), this may have further complicated the assessment of the elimination phase of the lowest doses.

After a washout period of at least 4 weeks some of the subjects

still showed detectable plasma concentrations of idraparinux sodium prior to a next administration. Non-compartmental pharmacokinetic parameters were derived from extended profiles obtained by adding these non-zero values observed just before the next dosing occasion to the profile associated with the previous dosing occasion. Comparing plasma concentrations of idraparinux sodium at timepoints up to 2 weeks and up to 4 weeks (or beyond) it can be concluded that the elimination half-life estimate increased from 120 to 300 hours. This half-life was associated with low concentration levels in the extended portion of the idraparinux sodium plasma profiles. Supported by the concept that unfractionated heparin disappears as free heparin from the equilibrium heparin-AT [6], idraparinux sodium released as a result of endogenous AT turn-over can possibly bind to newly produced AT, and the half-life of idraparinux sodium can be greater than the elimination half-life of AT (which is about 70 h). Therefore, it is conceivable that the prolonged half-life is associated with redistribution of idraparinux sodium.
The clinical relevance of this prolonged half-life and its relevance in terms of accumulation during repeated dosing is yet to be assessed.
In an *in vitro study*, undertaken to determine if the plasma concentrations of AT would be the rate limiting factor for the potency of this group of pentasaccharides, it was determined that an equimolar amount of AT was required for full expression of the anti-Xa effect of fondaparinux [see 4]. In this study approximately 20% of idraparinux was cleared within a week by the renal system with a tendency towards a higher renal clearance rate at higher plasma concentrations (i.e. following dosages exceeding 6 mg). This phenomenon can best be explained by concentration-dependent renal clearance of the pentasaccharide. Even though the mean plasma AT concentration in healthy human subject is approximately 200 µg/mL [5], it seems after administration of higher dosages of the compound the capacity for idraparinux binding by AT is exceeded, resulting in a higher free fraction which is renally excreted. Nevertheless, after one week of complete urine-collections, mean maximum percentage of the

dose excreted in the urine was already 23.3 % (SD: 2.46)and it can be anticpated that over longer collection periods this fraction may increase. Nevertheless, it seems that the total plasma clearance remains stable at approximately 0.9 mL/min for doses above 2 mg. The effects of a single dose of idraparinux on secondary haemostasis as measured by APTT and PT were also assessed. The bedside assessment in whole blood seemed to overestimate the effects. For the more reliable laboratory assessments, small increased in mean APTT and PT were noted. This finding confirms that the conventional coagulation assays are not greatly influenced by the very specific action of this pentasaccharide. In addition, the changes in APTT/PT seem to be clinically unimportant. Though the relation between the anti-Xa activity and the probability of bleeding is unknown, this parameter is a better predictor for the effect of idraparinux in the circulation.
In conclusion, idraparinux sodium is at least partly renally cleared. Its long elimination half-life is expected to allow once per week dosing in the prophylactic treatment of thromboembolic disorders.

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TABLE 1 **Summary of pharmacokinetic results**
(data points included up to two weeks)

TREATMENT	C_{MAX} (ng/mL)	$AUC_{0-\infty}$ (mg/L*h)	$t_{1/2}$ (h)	CL (mL/min)	VZ (L)	CL_{RENAL} (mL/min)	FRACTION renal
IV 0.25mg (n=5)	84.3 (5.2)	*	*	*	4.93 (1.7)		
IV 0.5mg (n=5)	147 (27)	*	*	*	7.72 (1.9)	0.17 (0.10)	
IV 1mg (n=5)	309 (42)	16.7 (1.5)	82.4 (16.8)	1.00 (0.09)	7.08 (1.1)	0.19 (0.06)	0.20 (0.06)
IV 2mg (n=6)	582 (64)	37.7 (5.6)	102.2 (17.0)	0.90 (0.13)	7.91 (1.4)	0.20 (0.03)	0.22 (0.03)
IV 4mg (n=5)	1320 (120)	78.7 (7.3)	99.6 (6.1)	0.85 (0.08)	7.32 (0.04)	0.20 (0.04)	0.23 (0.04)
IV 6mg (n=8)	1860 (230)	117.3 (7.7)	116.3 (27.1)	0.86 (0.06)	8.64 (2.2)	0.19 (0.05)	0.23 (0.06)
IV 8mg (n=5)	2560 (240)	149.1 (22.0)	122.0 (28.8)	0.91 (0.13)	9.48 (2.1)	0.23 (0.09)	0.25 (0.09)
IV 10mg (n=6)	2850 (270)	205.1 (13.0)	127.3 (15.7)	0.82 (0.05)	8.69 (1.3)	0.29 (0.05)	0.35 (0.04)
IV 12mg (n=5)	3430 (720)	223.9 (30.9)	122.9 (22.7)	0.91 (0.12)	9.54 (2.0)	0.25 (0.04)	0.28 (0.04)
IV 14mg (n=5)	3840 (360)	261.5 (13.6)	119.6 (22.1)	0.89 (0.05)	9.13 (1.2)	0.30 (0.07)	0.33 (0.07)
SC 2mg (n=6)	426 (43)	34.8 (4.3)	95.9 (10.8)	0.97 (0.12)	8.09 (1.6)	0.20 (0.04)	0.21 (0.04)
SC 10mg (n=6)	2180 (132)	204.4 (12.6)	134.3 (28.2)	0.82 (0.05)	9.14 (1.8)	0.29 (0.06)	0.35 (0.07)

Results are from non-compartmental analysis, corrected for non-zero pre-values and reported as mean (SD). *Terminal $t_{½}$, $AUC_{0-\infty}$ and clearance are not reported for doses below 1 mg because of poor definition of the terminal phase.

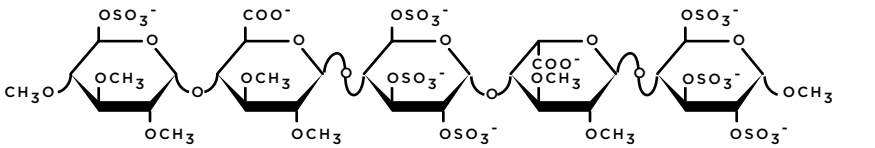

FIGURE 1 Chemical Structure idraparinux

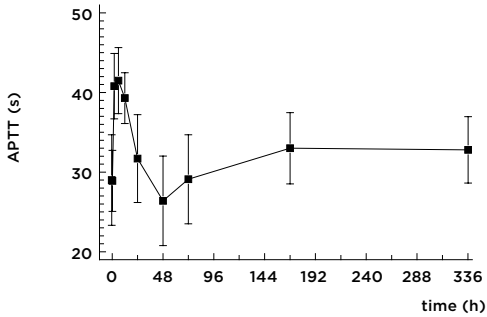

FIGURE 2 Mean 'bedside' APTT-results following administration of 14mg idraparinux iv.

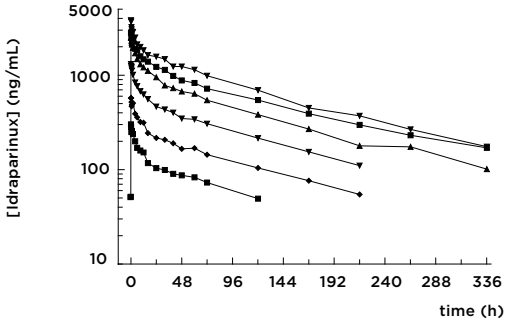

FIGURE 3 Average concentration-time curves per group following intravenous administration

(logarithmic scale; ■ 1mg, ◆ 2mg, ▼ 4mg, ▲ 8mg, ■ 10mg, ▼ 14mg are the administered dosages group).

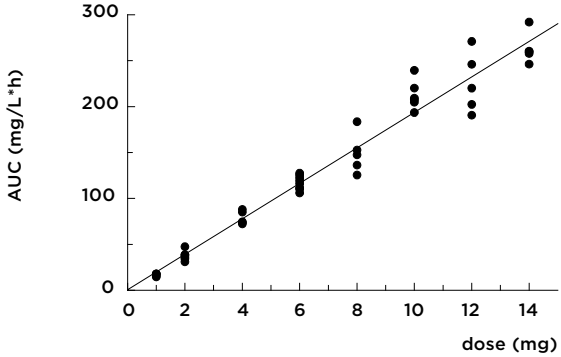

FIGURE 4 AUC extrapolated to infinity versus dose

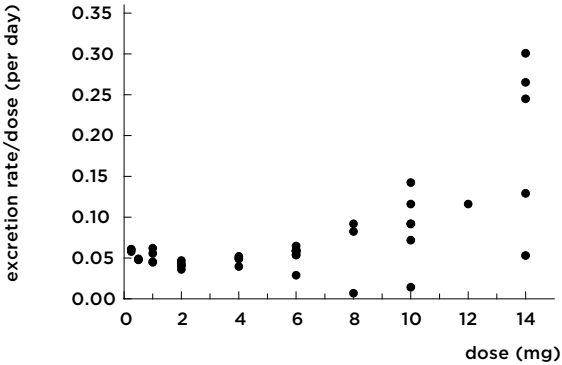

FIGURE 5 Urinary excretion rate/dose versus dose calculated for the first urine-sampling period: 0–4 hours post-dosing.

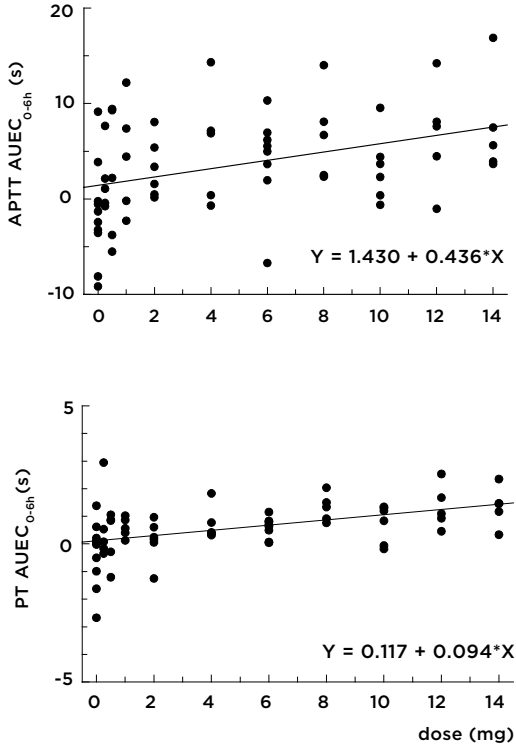

FIGURE 6 **APTT and PT AUEC above baseline (calculated for the period 0–6 hours post-dosing).**
Predicted increase after 14mg idraparinux iv is 7.53 seconds for the APTT and 1.44 seconds for the PT.

CHAPTER 7

THE EFFECTS OF AGE ON THE PHARMACOKINETICS AND EFFECTS OF THE LONG-ACTING SYNTHETIC PENTASACCHARIDE IDRAPARINUX SODIUM

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ABSTRACT

Objectives

To investigate the safety, tolerability and pharmacokinetics and absolute bioavailability of subcutaneously (sc) administered idraparinux in elderly volunteers.

Methods

Eighteen volunteers (8F/10M; 3 groups of six subjects) were included in an open label study. The first group received 2 mg idraparinux sc. The second group received 6 mg sc and intravenously (iv) in a randomised crossover fashion. The third group received 10 mg sc. Blood sampling was done up to 336 hrs for pharmacokinetics and pharmacodynamics (APTT/PT). Urine was collected up to 168 hrs. Individual plasma concentration-time profiles were analysed model-independently. Urinary data were used to calculate the renal clearance. The pharmacodynamic response was analysed by maximal effect values and the time-integrated AUEC responses.

Results

Idraparinux sodium was well tolerated and no significant adverse events were noted. Plasma clearance was independent of dose and correlated with body weight. The drug demonstrated linear pharmacokinetics, and the sc bioavailability was almost 100%. The extrapolated theoretical urinary excretion of idraparinux was almost 50%. The mean (SD) terminal half-life was 136 ± 27) hr. The drug induced a maximal (SD) increase in APTT of 8.3 (2.9) sec and in PT of 1.2 (0.3) sec.

Conclusions

In elderly subjects idraparinux is almost 100% bioavailable after sc administration, shows linear pharmacokinetics and elicits only minor changes in APTT/PT . The data from this study are comparable to data in young subjects, indicating absence of influence of age on the pharmacokinetics of this highly selective anti-Xa compound. It is anticipated that idraparinux sodium can be a useful new drug for long-term anti-thrombotic use.

Introduction

Idraparinux is a novel synthetic pentasaccharide compound with anticoagulant properties *in vitro* and *in vivo*. Pre-clinical studies indicate that it works by activation of antithrombin (AT), the naturally occurring inhibitor of the clotting process. The main activity of AT is against factor Xa and thrombin. Heparin-like drugs accelerate the reaction that forms complexes between AT and factor Xa or factor IIa. These complexes dissociate very slowly relative to the complexes with the target protein in the coagulation cascade. Recently, synthetic analogues of heparin pentasaccharide sequence that binds to AT have become available, which selectively inhibit factor Xa. One of these compunds (fondaparinux) has been registered. Unlike heparin, the pentasaacharides are selectively active against factor Xa and idraparinux has a specific activity of about 1400 anti-Xa units/mg. Previous experience with idraparinux sodium and other pentasaccharides suggests that a highly selective anti-thrombotic effect can be elicited with this group of compounds [1,2]. If thrombotic risk or complications require prolonged treatment, a safe drug with an extended elimination half-life allowing a low dosing frequency would be preferable. Idraparinux has an elimination half-life of approximately 130 h in young subjects and a subcutaneous (sc) bioavailability of almost 100%.

The present study was carried out to investigate the safety, the tolerability and pharmacokinetics in healthy elderly subjects. As it can be envisaged that for clinical use idraparinux sodium will be administered sc in this target population, three sc doses were employed in this study. The absolute bioavailability of the compound was also estimated for one dose in this population.

METHODS

Subjects and Design

Eighteen healthy subjects (8F/10M; age: 60-71 years; body weight 56.5-91.8 kg) with a normal coagulation screen participated in this study after written informed consent was obtained. The Ethics Committee of Leiden University Medical Center approved the investigational protocol.

This open label study was conducted according to a single rising dose design. Three groups (six subjects each) were dosed with 2, 6 or 10 mg idraparinux sodium sc. The 2 and 10 mg dose were only sc administered. The 6 mg dose was administered twice (once intravenously (iv) and once sc) in a randomised crossover fashion. For this group the washout period between study-occasions was at least 4 weeks.
For the 6 mg IV dose, disposable syringes were filled as close as possible to 5 mL with diluted drug solution (diluted with 0.9% saline solution). For the sc doses, disposable syringes were filled with undiluted medication solution (as provided by the sponsor) as close as possible to the minimum required volume (i.e. 0.53, 1.58, and 2.63 mL for the 2, 6 and 10 mg doses, respectively).
All drug preparations were done by Leiden University Medical Center pharmacy.

Study Days
Subjects were studied after an overnight fast. Upon arrival at the CHDR unit a short medical history and a physical exam were performed to ensure compliance to the protocol restrictions. Then the subjects voided and an iv cannula for blood sampling was inserted in a forearm vein. During the iv study days an additional cannula was inserted in the other arm. Idraparinux was administered sc in an abdominal skinfold or as a slow iv bolus injection.

Sampling
Free flowing blood from an iv cannula that was kept patent by intermittent flushing with 0.9% saline was sampled. Blood was taken after discarding the contents of the cannula. Blood samples for base-line values were taken pre-dose. Blood samples for drug-assay were drawn frequently until 336 h after drug administration (264 h for sc 2 mg). The subjects participating in the 2 and 10 mg group collected urine for measurement of drug excretion (time intervals: 0-12, 12-24 h and subsequently in 24-hour periods until 168 h after dosing) in pre-weighted plastic containers. Additionally, the last four subjects dosed with 10 mg collected urine for the following periods: 240–264, 408–432, 576–600 and

744–768 h (i.e. days 10, 17, 24 and 31 post-dosing). At the end of these periods additional blood samples for drug-assay were taken. Ivy bleeding time measurements were performed pre-dose and at either 15 minutes (iv route) or 4 h (sc route) as well as 48 h after dose administration (or at discharge) and at the end of each study period.

Assays

Idraparinux concentrations were measured using a validated amidolytic assay based upon anti-factor Xa activity [3]. The assays were performed at the Department of Drug Metabolism and Kinetics of Organon Development GmbH (Waltrop, Germany) (see [2]). Blood samples for APTT and PT assays were drawn at regular time intervals until 336 h relative to the dose of idraparinux. These assays were performed using standard procedures [4]. AT concentration was assessed pre-dose, and at the end of each study occasion. AT was to be assessed using a validated photometric assay with S-2765 as a chromogenic substrate, which is based on the inhibition of factor Xa (Comatic® Antithrombin, Mölndal, Sweden).

Statistical analysis

Post-dose measurements below the reported lower limit of quantification (LOQ) were excluded from the pharmacokinetic analysis. Actual sampling time points were used for calculation of the individual pharmacokinetic parameters using WinNonlin V1.1 (Scientific Consulting, Inc., Apex, NC). The following non-compartmental pharmacokinetic parameters were derived from the individual profiles: the observed peak value (C_{max}) and the associated sampling time (t_{max}), the half-life associated with the terminal elimination phase ($t_{½}$), the Area under the Curve (AUC) calculated using the linear trapezoidal method extrapolated to infinity ($AUC_{0-\infty}$), the plasma clearance and the apparent volume of distribution (Vz). Initially measurements beyond 14 days post-dosing were excluded from the calculations.

Subjects participating in the bioavailability part of the study had non-zero anti-Xa values at the start of the second occasion due to the long half-life of the drug. The AUC attributable to this pre-

value (calculated as pre-value / ($\ln 2 / t_{½}$) was subtracted from the $AUC_{0-\infty}$ resulting in corrected estimates. Absolute subcutaneous bioavailability of 6 mg idraparinux was determined by calculating the ratio $AUC_{0-\infty,sc}$ / $AUC_{0-\infty,iv}$.

Based on the collected urinary concentration data, the cumulative urinary excretion of idraparinux was estimated. The renal clearance was calculated over the first week following dosing where complete urine collection took place. Calculation of the ratio of the first week cumulative urinary excretion over the first week plasma AUC yielded the renal clearance.

Total urinary excretion of idraparinux was estimated using the four subjects in the 10 mg sc group with the extended urine sampling scheme. For each urine collection period, urinary excretion rate was calculated and plotted against mid-collection timepoints This excretion rate profile was integrated using the linear trapezoidal rule and extrapolated using the four final measurements (days 12, 19, 26 and 33). The initial portion of the profile was back extrapolated to time zero. The extrapolated AUC of the profile represents the theoretical urinary excretion of idraparinux.

Additionally, pharmacokinetic estimates were derived from the extended profiles of the 6 mg dose level group obtained by adding the non-zero pre-value observed just before the second dosing occasion to the profile of the first study occasion as if it were a final measurement. Extended profiles were also obtained for the last four subjects of the 10 mg dosing group.

The relationship between body weight and the pharmacokinetic parameters of idraparinux was investigated by calculating correlation coefficients with clearance, elimination half-life, the apparent volume of distribution and dose-normalised C_{max}. The C_{max} and clearance after 10 mg sc idraparinux of this study were compared to previously obtained data in healthy young male volunteers [2] and 90% bioequivalence intervals on these (log-transformed) parameters were calculated.

The base-line corrected Area under the Effect Curve (AUEC) of the APTT-profile was calculated using the linear trapezoidal rule on protocol times and was subsequently divided by the corresponding timespan to result in a weighted average response. The APTT

profile was characterised by the AUEC over the first 6 hours ($AUEC_{0-6}$) and the first 24 hours ($AUEC_{0-24}$). Additionally, the APTT response was characterised using the change from pre-value to the maximum value ($ATTP_{max}$) and the change to the value at 24 hours ($ATTP_{24h}$). PT values were analysed as difference from pre-value at the protocol measurement times. The treatments were compared using one-way ANOVA followed by contrasts on the subcutaneous parameters only. Statistical analysis and calculations were performed using SPSS for Windows (SPSS, Inc., Chicago, Ill.).

Results

No significant adverse events were noted after idraparinux sodium and all subjects completed the study. The most frequently reported drug-related adverse events were haematomas (mainly at puncture sites, i.e. at the site of the sc injection, iv cannulas or bleeding time punctures). Five 5 (2 mg sc), 5 (6 mg sc), 5 (10 mg sc) and 3 (6 mg iv) subjects, respectively, had one or more hematomas. Some, not-dose related, single prolonged bleeding times were observed, and rebleeding at the site of bleeding time assessment was seen once (10 mg sc).

Pharmacokinetics

A summary of the results is given in Figure 1 and Table 1. The data indicate that the maximal plasma levels (C_{max}) and $AUC_{0-\infty}$ increase linearly with the dose. Dose-proportionality was confirmed by one-way analysis of variance. Plasma clearance was independent of dose. The mean absolute bioavailability after sc dosing of 6 mg idraparinux was 94.9 ± 8.0% (range 87.3 – 107.7%). The mean elimination half-life estimate after the 6 and 10 mg doses calculated after the initial study period (i.e. 2 weeks) was 136 ± 27 h. Analysis of the pharmacokinetic parameters for the extended profiles (samples at 4 weeks or beyond also included in the curve fitting) indicate that idraparinux has a very long 'true' half-life (Table 2). After one week of complete urine collection the mean cumulative urinary excretion was 14.5 ± 3.5% after 2 mg, and 17.8 ± 3.1% after 10 mg. The corresponding renal clearances were 0.18 ± 0.06 mL/min after 2 mg, and 0.22 ± 0.04 mL/min after 10 mg

(Table 1). The estimated total urinary excretion for the last 4 subjects of the 10 mg group was 47 ± 4.8%. The analysis of the relation between body weight and the pharmacokinetic parameters (using Pearson's parametric correlation coefficients) revealed a positive correlation for weight and clearance (r=0.692; p=0.001) and apparent volume of distribution (r=0.548; p=0.019), and a negative correlation with dose-normalized C_{max} (r=-0.643; p=0.004).
The 90% bioequivalence intervals on log-transformed C_{max} and clearance, calculated to investigate whether the pharmacokinetics of idraparinux were different for elderly subjects compared to young males (results from [2]) were 0.85 / 1.12 and 0.88 / 1.11, respectively.

Pharmacodynamics

APTT was prolonged after each of the sc dose levels tested in this study (Figure 4). On average, the maximum prolongation was seen 6–12 hours post-dose and it was independent of the dose.
The average (± SD) maximum change from pre-value was 8.3 ± 2.9 seconds for the 10 mg idraparinux dosing group. PT was prolonged at 24 h post-dosing (PT_{24h}) after each of the doses (Figure 4).
The mean maximum increase was 1.18 ± 0.25 seconds after 10 mg.
A dose-related prolongation of the PT_{24h} was apparent across the dose levels; multiple paired t-testing showed significant differences for 10 mg versus 2 mg ($p<0.001$) and 10 mg versus 6 mg (p=0.002), respectively. For Ivy bleeding time an inconsistent pattern of changes was observed, which was judged not clinically relevant. Post-dose AT concentrations (assessed 12 to 15 days post-dose) did not reveal significant differences compared to baseline values.

Discussion

In many industrialised countries, the elderly constitute the fastest growing subpopulation [5]. Additionally, about 80% of persons of 65 years or older suffer from (chronic) conditions that may require long-term medical treatment [6]. Elderly individuals form a substantial subgroup of the approximately 25% of all non-surgical patients with thromboembolic events, while only limited

data are available on the efficacy and safety of thromboembolic prophylaxis in this specific subgroup of patients [7]. The present study was carried out to investigate the safety, tolerability and pharmacokinetics of idraparinux in subjects over 60 years of age. The compound was well tolerated after single sc doses in the elderly volunteers in this study. Comparable to a previous study in healthy young male subjects, only mild adverse events were noted. The absolute bioavailability of 6 mg sc idraparinux was estimated at 95 (8%. A good consistency of pharmacokinetic parameters between the iv and sc route of administration was found. Terminal half-life was 136 (27 hours for curves determined over 2 weeks.

After a period of 2 weeks approximately 20% of idraparinux was cleared by the renal route. The ratio of total plasma clearance to renal clearance was 0.21 and 0.27 for the 2 mg and the 10 mg dose, respectively. Furthermore, the estimated total renal excretion was less than 50% after collections up to 31 days after dosing (almost 6 times the calculated elimination half-life). This may indicate that renal excretion is not the only elimination pathway for this pentasaccharide.

Currently, coumarin-derivatives (especially warfarin) are the most widely used anticoagulants for long-term use. The anticoagulant response to warfarin is exaggerated with advancing age [8]. Moreover, the risk of undesired adverse reactions (bleeding complications) for these compounds rises significantly with age [9]. Although this enhanced response in the elderly is considered to be caused by pharmacodynamic factors, a decrease in warfarin-clearance with increasing age has also been reported [10]. In contrast, the bioequivalence intervals for the pharmacokinetic parameters in this study compared to those obtained in healthy young male volunteers were well within the 0.8-1.25 interval. This indicated an absence of age-effects on the pharmacokinetics of idraparinux. Differences in body weight of the subjects in this study explained approximately 50% of the variability observed in clearance, 30% of the variability observed in apparent volume of distribution and approximately 40% of the variability observed in dose-normalised C_{max}. This is probably of minor influence on idraparinux, because,

in general, body composition changes with increasing age are characterised by the concomitant processes of sarcopenia and a steadily decreasing [11] or constant [12] body mass index after the fifth decade.

The effect of a single dose of idraparinux sodium on secondary haemostasis was assessed by APTT and PT measurements. In this study no significant effects were found in these parameters, apart from the PT at 24 h post-dose. The small increases in APTT and PT are unlikely to be of clinical relevance. The finding that APTT and PT are not greatly influenced by the pentasaccharide confirms its very specific action and may also reflect the unsuitability of these markers. Instead, the relationship between anti-Xa activity and the probability of bleeding should be further explored.

Among the requirements the ideal antithrombotic drug should meet, is the possibility of oral administration [13], because a long duration of treatment is necessary for the most important indications (secondary prevention of stroke or myocardial infarction, and possibly primary prevention in high-risk patients). However, dosing of this kind of drugs can be error-prone, especially in the elderly. This is caused by age-related changes occurring in this patient group as comorbidity, polypharmacy, increased possibility of lack of compliance, and increased mental impairment. There are indications that elderly patients are more concerned about effectiveness and safety of medication than convenience [14]. Therefore, the advantage of a single dose once-per-week only drug, without monitoring, may possibly outweigh the discomfort of the sc administration of idraparinux sodium, which may even be self-administered.

To conclude, in elderly people idraparinux is a well-tolerated drug with linear pharmacokinetics, and inducing only minor changes in conventional coagulation assays. Because these data confirm the results of an earlier study with idraparinux in healthy young male volunteers, it is unlikely that age influences the pharmacokinetics of the compound. It can be anticipated that idraparinux may be a useful new drug for long-term prophylactic anti-thrombotic use.

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TABLE 1 **Summary of pharmacokinetic data**

Data up to two weeks.

TREATMENT	C_{MAX} (ng/mL)	$AUC_{0-\infty}$ (mg/L*h)	$t_{1/2}$ (h)	CL (mL/min)	VZ (L)	URINARY EXCRETION (mg)	CL_{RENAL} (mL/min)
SC 2 mg (n=6)	473 (104)	42.7 (12.3)	108 (17)	0.82 (0.2)	7.53 (1.17)	0.29 (0.07)	0.18 (0.06)
SC 6 mg (n=6)	1499 (161)	135.1 (21.9)	132 (19)	0.76 (0.1)	8.39 (1.56)		
SC 10 mg (n=6)	2309 (351)	208.2 (23.6)	132 (30)	0.81 (0.1)	9.37 (2.81)	1.78 (0.31)	0.22 (0.04)
IV 6 mg (n=6)	2223 (482)	142.6 (21.7)	145 (30)	0.71 (0.1)	8.61 (1.94)		

Results are reported as mean (SD); AUC-data corrected for non-zero pre-dose values.

TABLE 2 **Elimination half-life estimates**

	Initial profile			Extended profile		
	FOLLOW-UP (days)	$t_{1/2}$ (h)	N	FOLLOW-UP (days)	$t_{1/2}$ (h)	N
SC 2 mg	11	108 ± 17	6			
SC 6 mg	14	132 ± 19	6	28	259 ± 28	3
SC 10 mg	14	132 ± 30	6	31	397 ± 121	4
IV 6 mg	14	145 ± 30	6	35	284 ± 43	3

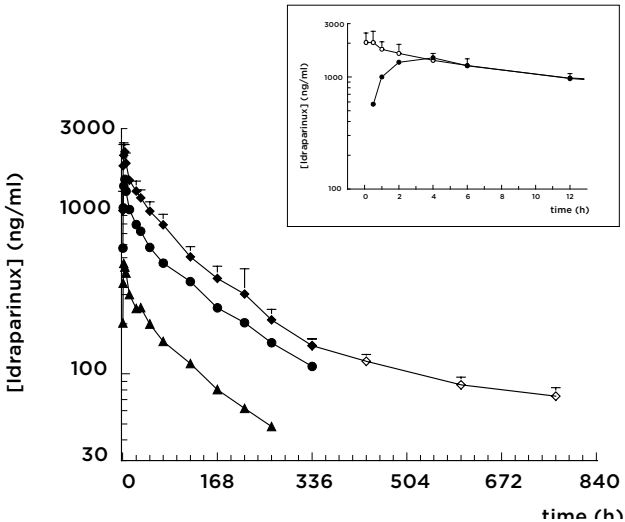

FIGURE 1 **Mean semi-logarithmic plasma profile idraparinux after sc administration.**

The inset shows the differences between the iv and sc administration of 6 mg idraparinux: from the timepoint of 6 hours post-dosing the curves completely overlap. (▲: sc 2 mg, ●: sc 6 mg, ○: sc 6 mg, ◆: sc 10 mg, ◇: sc 10 mg extended profile [n=4])

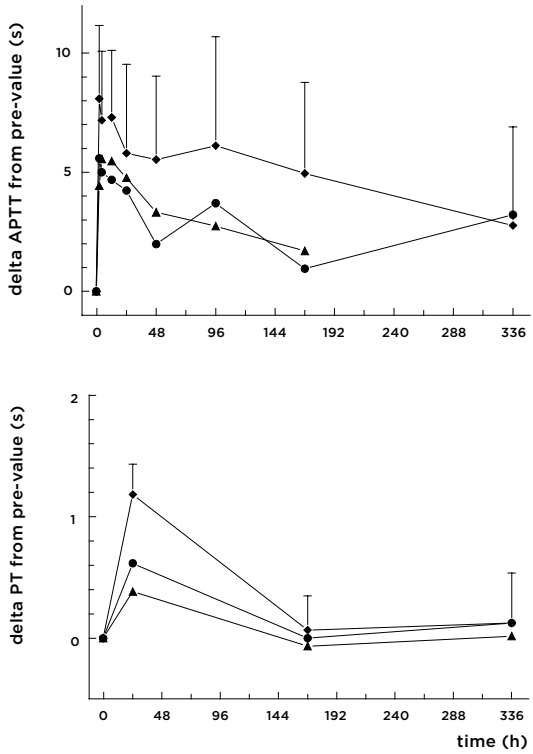

FIGURE 2 Mean change in APTT (upper panel) and PT (lower panel) after sc administration idraparinux (▲ sc 2 mg, ● : sc 6 mg, ◆ : sc 10 mg).

CHAPTER 8

THE MULTIPLE DOSE PHARMACOKINETICS OF THE SYNTHETIC LONG-ACTING PENTASACCHARIDE IDRAPARINUX SODIUM IN VOLUNTEERS WHO RECENTLY COMPLETED TREATED FOR VENOUS THROMBOEMBOLISM

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ABSTRACT

Objectives

To investigate the safety, tolerability and pharmacokinetics of 10 mg subcutaneous (sc) idraparinux, a synthetic pentasaccharide compound with anticoagulant properties and an elimination half-life estimated thus far of about 130 hr, administered four times once-a-week in male and female volunteers, recently discharged from oral anticoagulant treatment.

Methods

Eight subjects (5F/3M) with a history of venous thromboembolism (VTE), available for the first administration of idraparinux within 2 weeks after regular discontinuation of oral anticoagulant treatment, participated in this single center, multiple dose, open label study. Blood sampling and urine collection for anti-Xa activity were done up to 336 hr after the last administration as well as APTT and PT. Individual anti-Xa activity time profiles were analysed using non-compartmental pharmacokinetic techniques with calculation of the area under the curve (AUC), clearance and elimination half- life. Urinary data were used to calculate renal clearance.

Model dependent pharmacokinetics of idraparinux was determined using a two-compartment model with first order absorption utilising the data up to 4 weeks of dosing. Parameters were estimated using non-linear mixed effect modelling with first order conditional estimation.

Results

Idraparinux sodium was well tolerated and no significant adverse events were noted. Steady state drug levels were reached after the third administration. T_{max}-values were comparable to the values found in the single dose studies, while C_{max}, C_{min}, AUC, cumulative urinary excretion as well as the renal clearance were increased compared to the single dose studies. The multiple dose time profile up to one week after dosing can be adequately described using a two-compartmental pharmacokinetic approach. There were no clinically relevant post-dose changes in APTT and PT.

Conclusions
The administration of multiple doses of idraparinux was well tolerated and no significant adverse events were noted. It is anticipated that idraparinux sodium can be a useful new drug for long-term anti-thrombotic use that can be administered once per week.

INTRODUCTION

Idraparinux is a novel synthetic antithrombotic pentasaccharide working by its ability to activate antithrombin (AT). The main activity of AT, a naturally occurring inhibitor of the blood clotting process, is against factor Xa and IIa. In contrast to its animal sourced competitors (unfractionated heparin and low molecular weight heparins (LMWH)), it is manufactured totally by chemical synthesis. The compound is the sodium salt of a sulfated pentasaccharide. Heparin-like drugs accelerate the velocity, but not the extent, of the reaction in which complexes are formed between AT and factor Xa or factor IIa (= thrombin) which dissociate very slowly relative to complexes with the target proteins in the coagulation cascade in the absence of AT. As a result of its limited molecular chain length, idraparinux is selectively active against factor Xa. Hence, the drug binds to AT and thereby specifically potentiates the physiological neutralization of factor Xa and inhibits thrombin formation without direct inactivation of thrombin itself.

In the case of thrombotic risk or complications requiring long-term treatment, use of a safe drug with an extended elimination half-life allowing a low dosing frequency is attractive. Idraparinux is eliminated at a slow rate, with a half-life of approximately 130 h in both young and elderly healthy subjects [1,2]. The extended half-life is due to the high affinity of idraparinux to AT, although binding is not irreversible. Single intravenous doses of the drug were safe up to a dose level of at least 14 mg. Thus, idraparinux sodium could be a promising new drug for long-term anti-thrombotic therapy involving a once weekly dosing regimen. This study was carried out to investigate the safety, tolerability and pharmacokinetics after multiple doses of idraparinux

in subjects recently treated for venous thromboembolism (VTE), available for the first drug administration within 2 weeks after discharge from standard oral anticoagulant treatment.

METHODS

Subjects and Design

The Ethics Committee of the Leiden University Medical Center approved the investigational protocol. This open study was performed according to a single-center, multiple dose design, in which subjects started the treatment with idraparinux sodium within 2 weeks post-discharge from oral anticoagulant treatment. The subjects were recruited from the population under the care of the Dutch Thrombosis Field Monitoring Service (SRTG, The Hague, The Netherlands). Eight subjects (5F/3M; age 35-78 years, body weight 55.7-120.2 kg, normal weight for height) participated in this study after written informed consent was obtained. They all had experienced a single episode of deep venous thrombosis or pulmonary embolism followed by treatment with oral anticoagulant drugs. Otherwise, the subjects were healthy as assessed by a medical screening.

Study Procedures

Subjects were treated with 10 mg idraparinux subcutaneously (sc) four times once per week. Idraparinux was administered in an abdominal skinfold using disposable syringes filled with undiluted drug solution (2.63 mL). Study medication was prepared by the Leiden University Medical Center pharmacy.
In the 1st and 4th study-week pharmacokinetic monitoring took place. Subjects were studied after an overnight fast. Upon arrival at the research unit a short medical history and a physical exam were performed to ensure compliance to the protocol. Then the subjects voided and an iv cannula for blood sampling was inserted in a forearm vein. At zero time idraparinux was administered sc after which the subjects remained at the unit for at least 24 hours. The local monitoring site of the Thrombosis Service administered the second and third dose and performed subsequent blood sampling.

Sampling

No tourniquet was applied when blood was collected during the study period. At the research-unit an iv cannula used for blood sampling was kept patent by intermittent flushing with 0.9% saline. Blood was taken after discarding the contents of the cannula. All other samples were taken by venipuncture. Blood samples for base-line values were taken pre-dose. Before administration of the first dose of idraparinux the Prothrombin Time (PT) value was determined to provide evidence that cessation of oral anticoagulant therapy resulted in normalisation of the patient's coagulation status.

Blood samples for drug-assay, APTT and PT were drawn frequently until 336 h after the 4th administration of idraparinux. The subjects collected urine for measurement of drug concentration in 24-hour periods until 168 h after first dosing and 336 h after fourth dosing in pre-weighted plastic containers. Bleeding time (Ivy method) was measured pre-dose, at 4 and 24 h after the 1st and the 4th (last) administration. In addition, bleeding time was determined at 168 and 336 h after the last drug administration. Blood samples for antithrombin assay were taken pre-dose for all four SanOrg34006 administrations and at the end of the study (i.e. 336 h after the last injection).

Statistical analysis

Non-compartmental pharmacokinetic analysis of two types was performed using the software package WinNonlin V2.1 (Pharsight Corp., USA). First, overall non-compartmental analysis was determined (over the entire study period) by calculating AUC using the linear trapezoidal method, terminal half life, extrapolated AUC ($AUC_{0-\infty}$) and VZ. For the calculation of terminal half-lives the values starting at 624 hours (26 days) were used in all cases. This decision was based on the values automatically selected by the program, followed by visual inspection. Clearance/F was calculated as total dose (= 40mg)/($AUC_{0-\infty}$).

Subsequently, non-compartmental parameters were determined for week 1 and week 4 ($AUC_{0\text{-}1\ week}$, C_{max}, C_{min}, T_{max}) and compared using paired t-tests after log-transformation (T_{max} untransformed).

Weeks 1, 2, 3 and 4 were characterised by the concentration at 4 hours and the concentration just prior to the next dose and these parameters were summarised. The urinary excretion of idraparinux was determined by calculating cumulative excretion over week 1, over week 4 and over week 4 and 5 combined.

The renal clearance for these same periods was determined by dividing the excreted amount by the corresponding plasma idraparinux AUC. Urinary excretion and renal clearance were compared between weeks 1 and 4 using paired t-tests.

Additionally, model dependent pharmacokinetics of idraparinux was determined using a two-compartment model with first order absorption utilising the idraparinux data up to 4 weeks of dosing. Parameters were estimated using non-linear mixed effect modelling as implemented in the NONMEM program (Version V, Nonmem Project Group, University of California, San Francisco, USA) with first order conditional estimation (FOCE), and constant coefficient of variation (CCV) inter-individual and intra-individual variability models. Clearance/F, intercompartmental clearance/F, central volume/F (V_c/F), steady state volume/F (V_{ss}/F) and absorption half-life were estimated. Predicted profiles were generated for the observed four weeks of dosing and if the drug had been administered for an additional eight weeks using the same regimen (10 mg per week).

Area under the Effect Curves (AUECs) for APTT and PT were calculated using BMDP/Dynamic Version 7.0 (Statistical Solutions, Inc., Cork, Ireland). The APTT response was characterised by the value at 4 hours (close to T_{max}), the value prior to the next dosing (E_{min}), and by dividing the calculated AUECs over weeks 1 and 4 by the corresponding timespan (weighted mean APTT). Values for weeks 1 through 4 were summarised. Values for weeks 1 and 4 were compared using paired t-tests. The PT measurements were summarised and the pre-dose value was compared to the values at 24 hours during week 1 using paired t-tests. Additionally, the week 1 and week 4 values at 24 hours were compared using paired t-tests. Statistical analysis and calculations were performed, using SPSS for Windows (SPSS, Inc., Chicago, Ill., USA).

Results

Idraparinux sodium was well tolerated and no significant adverse events were noted. The most frequently reported adverse events were headache and the development of haematoma at puncture sites, and were considered of a mild intensity. There were no discontinuations.

Pharmacokinetics

A summary of the results is given in Figure 1, Table 1 and Table 2. These data demonstrate that steady state levels with regard to the maximal plasma levels (C_{max}) are already reached after the 2nd administration whereas the steady state levels based on through plasma levels (C_{min}) are reached after the 3rd administration of idraparinux. Comparison of the pharmacokinetic results of week 1 to week 4 showed statistically significant increases in C_{max} (16.9%; 95%CI: 8.1%, 26.5%), C_{min} (60.3%; 95%CI: 46.2%, 75.4%) and $AUC_{0\text{-}168h}$ (30.9%; 95%CI: 20.8%, 41.9%) and that T_{max} values were stable. The results of the analysis of the renal clearance are summarised in Table 3. The mean overall cumulative urinary excretion was 5.81 mg (SD: 0.76) over week 4 and 5, indicating that renal clearance over these two weeks is 0.36mL/min. This is slightly higher than the values known from previous studies with idraparinux sodium [1,2]. Comparison of the results of week 1 and week 4 revealed significant increases in cumulative urinary excretion (mean difference 1.76 mg (95% CI: 1.31, 2.21)), as well as the renal clearance (mean difference 0.11 mL/min (95% CI: 0.07, 0.15)). Results of the model dependent pharmacokinetics of idraparinux are summarised in Table 4. When data are restricted to 4 weeks, the idraparinux-time profile can be adequately described using a two-compartmental pharmacokinetic approach (Figure 2).

Pharmacodynamics

There were no post-dose changes of clinical relevance in APTT (Table 5). All administrations of idraparinux were followed by a small rise in APTT (approximately 3 seconds, Figure 3). Average weighted mean APTT-values were higher for the fourth week than for the first week of idraparinux administrations: mean differen-

ce 2.15 seconds (95% CI: 0.47, 3.84). After reaching a maximum, APTT-values decreased slowly (Figure 3).
No clinically relevant post-dose abnormalities in PT were observed. The 1st administration of idraparinux was followed by a mean increase in PT of 1.57 seconds (95% CI: 1.08, 2.07) comparing the pre-dose values to the values at 24 hours post-dosing. A slight increase in mean PT-values was still present pre-dose to the 4th administration (no measurements of PT were performed in between). The 4th administration was also followed by a small increase in PT-values after which PT-values decreased slowly (Figure 3). The results at 24 hours post-dosing in week 1 and week 4 were not statistically significantly different.
Ivy bleeding times showed a large degree of variation between and within subjects. No statistically significant change in bleeding times was observed comparing the 4 and 24 hour measurements to baseline (median increase at both time points: 4%). Similar, comparison of the data at 4 and 24 hours post dosing between the 4th and 1st dosing did not show significant changes in bleeding time.
AT concentrations (expressed as the ratio of the post-dose over the pre-dose value) increased by 2.4% after the 1st drug administration and by 5.9% after the 4th administration, which was not statistically significant.

DISCUSSION

This phase I study was initiated to extend single dose investigations on the safety and pharmacokinetics of idraparinux in healthy young and elderly volunteers [1,2] to patients to investigate multiple dose regimens in a clinically relevant group. Also, with regard to the safety it was decided to recruit subjects with a history of VTE, because they were already used to anticoagulation while ambulant. Furthermore, it can be envisaged that an additional month of anticoagulant treatment for volunteers with a history of VTE within a month after completion of a 3-months oral treatment might be beneficial to these patients in further reducing or postponing recurrent thromboembolism [5].
Because in previous studies the absolute bioavailability of sc idraparinux was estimated at almost 100%, the study was

performed using this route of administration. With regard to the pharmacokinetics, plasma concentrations were determined by using an anti-factor Xa activity assay, since a direct method to measure idraparinux concentrations does not exist. Comparable to the previous studies, the pharmacokinetic behaviour of idraparinux could best be analysed using a non-compartmental approach. Nevertheless, the drug-time profile could be adequately described using a two-compartmental model.

In the earlier studies, within one week after administration, approximately 20-30% of idraparinux was excreted renally. This was also found after the first administration of the compound in the current study. However, a higher renal clearance was found after the fourth dose (Table 3). This might be explained by the following phenomenon: for full expression of the anti-Xa effect of the shorter-acting pentasaccharide fondaparinux, an excess of AT is required [6]. This also is the case for idraparinux [7]. In addition, comparable to unfractionated heparin [8], idraparinux is probably released unchanged from the binding equilibrium of idraparinux with AT if endogenous AT is cleared from the circulation during the natural turnover process, and can possibly bind to newly produced AT. However, it should be considered that idraparinux may also bind to other plasma proteins and that this may limit the amount of free drug available for renal excretion. This study suggest that a 'steady state situation' with regard to plasma concentrations is reached after 2-3 administrations of idraparinux, because after the 4th administration maximal and minimal concentrations were similar to the preceding administration.

The effects of multiple doses of idraparinux on secondary haemostasis were measured by APTT and PT assessments. The observed small post-dose prolongation in these parameters can be considered not relevant from a clinician's point of view. The small changes suggest that these conventional coagulation assays, though widely used, are not suitable for monitoring idraparinux. No significant effects were noted for changes in bleeding time or AT concentration.

At present, coumarin-derivatives are the most widely used anticoagulants for long-term use. Because these have clear drawbacks

[9], there is considerable interest in developing new anticoagulants to replace them in long-term out-patient care. Though idraparinux is administered via the subcutaneous route, its predictable pharmacokinetics, circumvention of the need for frequent monitoring, a low dosing frequency, and (to the current knowledge) minimal side effects indicate that this compound could represent an alternative in chronic thrombotic disorders. The mentioned characteristics lead to a low practical inconvenience for the patients, which is also an important item in this matter.

In conclusion, the administration of multiple doses of idraparinux was well tolerated, no significant adverse events were noted and only minor changes in APTT and PT. It is anticipated that idraparinux sodium can be a useful new drug for long-term antithrombotic use, administered once per week.

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TABLE 1 Mean (SD) and range of the PK parameters of idraparinux after 4*10mg sc (complete profile) following non-compartmental analysis

	$AUC_{0\text{-last}}$ (mg/L*h)	% extrapolate d*	$AUC_{0\text{-}\infty}$ (mg/L*h)	$t_{1/2}$ (h)	Cl/F (mL/min)	Vz/F (L)
Mean	822 (112)	12.5 (2.6)	941 (135)	216 (25.9)	0.88 (0.12)	16.5 (2.3)
Range	606–953	10.4–16.8	675–1071	177–259	0.75–1.11	12.67–19.8

$AUC_{0\text{-last}}$: area under the curve from timepoint of first administration, until the last measurements (two weeks after the last administration), $AUC_{0\text{-}\infty}$ area under the curve extrapolated to infinity, % extrapolated: percentage of the AUC that had to be extrapolated to get the $AUC_{0\text{-}\infty}$, Cl/F: apparent clearance, Vz/F apparent volume of distribution.

TABLE 2 Comparison non-compartmental PK week 1 and 4

	C_{4H} (mg/L)	C_{MIN} (mg/L)	T_{MAX} (h)	$AUC_{0\text{-}168h}$ (mg/L*h)
week 1	2.16 (0.20)	0.37 (0.06)	3.25 (0.93)	149 (16.4)
week 2	2.49 (0.36)	0.54 (0.10)		
week 3	2.56 (0.43)	0.60 (0.11)		
week 4	2.47 (0.31)	0.59 (0.08)	3.76 (0.71)	192 (24.8)

Results are reported as mean (SD)
C4 h: concentration at 4 h post-administration (close to Cmax-value), Cmin: 'minimum' concentration, just prior to next dosing, Tmax: timepoint of maximum concentration, AUC0-168 h: area under the curve from timepoint of administration until one week after this administration.

TABLE 3 Renal clearance of idraparinux following multiple doses

	WEEK	MEAN (SD)	RANGE
Cumulative excretion over one week	1	1.95 (0.47)	120–2.62
	4	3.68 (0.89)	1.96–4.38
Renal clearance	1	0.22 (0.06)	0.142–0.295
	4	0.324 (0.87)	0.194–0.438

TABLE 4 Summary results model-dependent idraparinux pharmacokinetics

	MEAN	SEM	CV
Clearance/F (ml/hr)	52.6	3.04	14%
% Intercompartmental clearance/F (ml/hr)	678	90.7	31%
V_c/F; central volume of distribution (L)	2.71	0.262	0%
V_{ss}/F; steady state volume of distribution (L)	6.10	0.330	16%
Absorption half-life (hr)	1.06	0.173	26%

Residual variability (constant CV error model): 11%
Mean: population average
SEM: approximate standard error of population average
CV: coefficient of variation of inter-individual variability

TABLE 5 Summary APTT results

	APPT at 4h post dosing ("E_{max}" in seconds)	APPT prior to dosing ("E_{min}" in seconds)	Weighted mean (0–7 days)
week 1	35.4 (4.34)	34.1 (3.41)	34.1 (3.70)
week 2	37.6 (4.85)	35.1 (4.77)	
week 3	38.3 (3.76)	35.1 (4.02)	
week 4	37.5 (5.05)	34.7 (4.74)*	36.2 (5.16)

Results are reported as mean (SD).
Absolute mean pre-dose value (before the first SanOrg34006 dose) was 29.0 s (SD: 4.8 s).
* this is the value one week after the last (4th) dose, as such not followed by a new dose, but this would be the timepoint at which a new dose was to be administered.

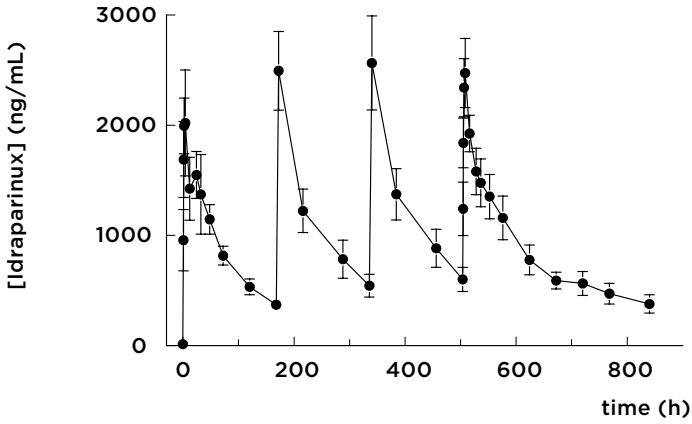

FIGURE 1 **Idraparinux time profile following four sc administrations of 10 mg.**

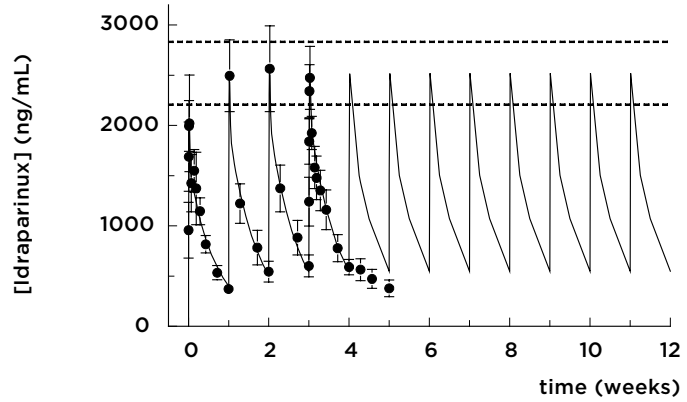

FIGURE 2 **Average idraparinux time profiles with predicted profiles using empirical Bayes estimates and extrapolated profile for 8 additional weeks of administration (using the same dose-regimen).**

The dotted lines indicate the range of predicted average maximum concentration; mean predicted Cmax-value is 2520 ng/mL (range: 2205-2830 ng/mL).

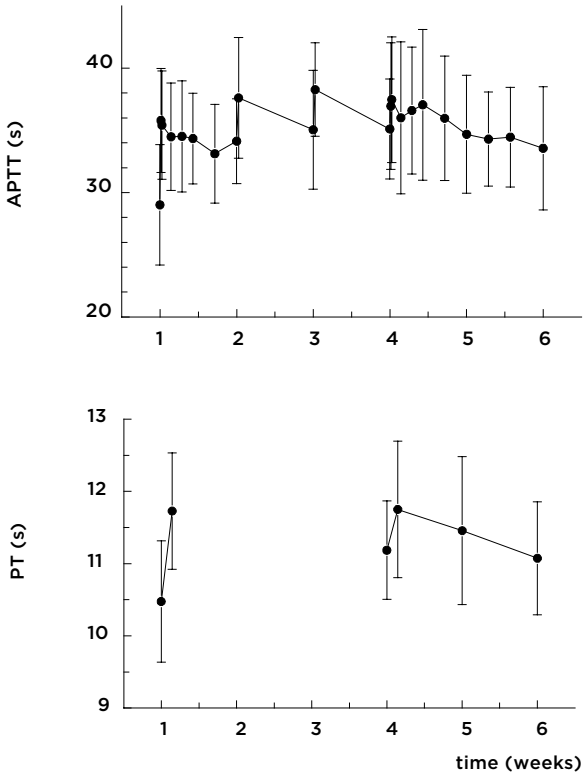

FIGURE 3 Mean time profile of changes in coagulation assays following idraparinux administration at weekly intervals.

CHAPTER 9

AN INTERACTION STUDY WITH SUBCUTANEOUSLY ADMINISTERED IDRAPARINUX SODIUM AND ORAL WARFARIN IN HEALTHY YOUNG MALE VOLUNTEERS

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ABSTRACT

Objective

To investigate the interaction of a single subcutaneous dose idraparinux sodium and oral warfarin regarding their tolerability, pharmacokinetics and pharmacodynamics

Methods

The study was performed using a randomised, double-blind study-design, with two parallel groups of 8 subjects. The subjects received a single subcutaneous (sc) 10 mg dose idraparinux or placebo and oral warfarin. Warfarin was administered at 24 h (15 mg) and 48 h (10 mg) after administration of idraparinux. Blood samples for drug-assay, PT and APTT were drawn regularly until 432 h after drug administration.

Non-compartmental idraparinux pharmacokinetic analysis was performed. For the influence of warfarin on idraparinux pharmacokinetics, the comparison was made using historical data. The pharmacodynamic response was characterised by the time-integrated and maximal response of the PT and APTT.

Results

This study showed no influence of oral warfarin on the pharmacokinetics of a single sc dose of idraparinux. The co-administration of this pentasaccharide did not influence the effect of warfarin on the PT (INR). Although idraparinux increased APTT compared to placebo, the placebo plus warfarin treatment resulted in a greater increase in APTT than the idraparinux plus warfarin treatment.

Conclusion

The co-administration of warfarin did not influence the pharmacokinetics of idraparinux. Idraparinux does not have a clinically relevant interaction on the effect of warfarin on APTT. The PT remains a suitable parameter to assess the effect of oral warfarin during the first 48 hours when idraparinux is concomitantly administered.

INTRODUCTION

Idraparinux is a novel antithrombotic characterised as a sulphated pentasaccharide that is manufactured totally by chemical synthesis. It shows great similarity to the pentasaccharide sequence of heparin. It has been shown that idraparinux, which is administered as the sodium salt, activates antithrombin (AT), thereby accelerating the velocity of the reaction in which complexes are formed between AT and factor Xa or factor IIa (= thrombin). As a result of its limited molecular chain length, idraparinux is selectively active against factor Xa. Hence, the drug binds to AT and thereby specifically potentiates the physiological neutralization of factor Xa and inhibits thrombin formation without direct inactivation of thrombin itself.

After venous thromboembolism (VTE) treatment, patients are prophylactically treated to prevent recurrence of thromboembolic events. Such secondary prophylaxis ideally requires a drug with an extended elimination half-life, enabling a low dosing frequency. Idraparinux is potentially such a compound, because of its slow elimination half-life of approximately 130 hours allowing a once weekly dosing regimen [1,2]. The extended half-life is due to tight binding to AT [3], although this bond is not irreversible.

A combination of subcutaneously (sc) administered idraparinux sodium and oral anticoagulants might occur in clinical practice, during the switch from idraparinux sodium to oral anticoagulants. This would normally occur using a loading dose of oral anticoagulants followed by a PT-check after 48-72 hours.

The current interaction study was performed to evaluate possible interaction(s) between idraparinux sodium and the oral anticoagulant warfarin.

METHODS

Subjects and Design

Sixteen healthy males with a normal coagulation screen (age: 18-35 yr., body weight: 57-101 kg) participated after written informed consent. The Ethics Committee of Leiden University Medical Center approved the study protocol. The study was carried out according to a randomised, double blind study design, in which

parallel groups of eight subjects received a single dose sc idraparinux sodium or placebo and oral warfarin.
Idraparinux was administered in an abdominal skinfold using disposable syringes filled with undiluted drug solution (2.63 mL). Placebo sc injections consisted of an equal volume 0.9% saline solution. Sodium-warfarin (Coumadin() was administered divided in two doses: 15 mg 24 h after idraparinux sodium administration and another 10 mg 24 hours later. All study medication was prepared by the pharmacy of Leiden University Medical Center.

Study Procedures
Subjects arrived at the research unit the evening before test drug administration. Upon arrival a short medical history was taken and a physical examination was done to ensure compliance to the study protocol restrictions. The subjects were studied after an overnight fast. The next morning, the subjects voided and an iv cannula for blood sampling was inserted in a forearm vein. At zero time idraparinux or matching placebo was administered sc. At 24 hrs after the injection the subjects received 15 mg of warfarin and at 48 hrs after the injection another 10 mg warfarin was taken orally. The subjects remained in the unit for at least 96 hours post-dosing. Then 10 mg vitamin K (Konakion®) was administered to all subjects to reverse any residual warfarin effects.

Sampling and assays
Free-flowing blood was collected during the study period. At the research-unit an iv cannula used for blood sampling was kept patent by intermittent flushing with 0.9% saline. Blood was taken after discarding the contents of the cannula. Blood samples for drug-assay were drawn frequently until 432 h after administration of idraparinux.
Plasma drug concentrations were assessed using a validated photometric assay. The method is based on the inhibition of factor Xa by the AT-idraparinux complex in the presence of an excess of AT. To screen the performance of the assay, each series of analyses was checked using quality control samples. The limit of quantifi-

cation was 8.0 ng/mL in plasma. The assay was performed at the Department of Drug Metabolism and Kinetics of Organon Development GmbH (Waltrop, Germany), as described earlier [1].
Blood samples for prothrombin time (PT) and activated partial thromboplastin time (APTT) assays were drawn at regular time intervals until 432 h relative to dose of idraparinux. These assays were performed using standard procedures [4]. Blood samples for factor VII activity assay were pre-dose up to 120 h after idraparinux administration. The assay was performed using a two-stage photometric assay with S-2765 as a chromogenic substrate (Coaset® FVII, Chromogenix, Mölndal, Sweden).
Bleeding time (Ivy method) was determined pre-dose and 4, 26, 72, and 432 h after idraparinux administration.

Statistical Analysis

Non-compartmental pharmacokinetics was performed using the software package WinNonlin V2.0 (Pharsight Corp., USA) by calculating AUC using the linear trapezoidal method, terminal half life, extrapolated AUC ($AUC_{0-\infty}$), Cl/F and Vd/F.
For the calculation of terminal half lives the values starting at 72 hours (3 days) and up to the final measurement 432h (18 days) were used. This decision was based on the values automatically selected by the program, followed by visual inspection. Non-zero pre-values were disregarded. Clearance/F was calculated as $Dose/(AUC_{0-\infty})$.
The influence of warfarin on idraparinux pharmacokinetics was estimated using the data from a previous study in which 6 healthy male subjects were dosed with 10 mg idraparinux sc [see 1]. As the effect of warfarin on idraparinux pharmacokinetics had to be apparent during the presence of warfarin (administered at 24 h, while vitamin K was administered at 96 h) AUCs from 24 h till 120 h were calculated and compared between groups using an unpaired t-test. An additional 'bioequivalence' analysis, in which the pharmacokinetic parameters (C_{max} and clearance) were compared between studies (also using the data of same comparator group), was performed to investigate the warfarin-effect on the pharmacokinetics of idraparinux. In this analysis, 90% bioequivalence

intervals on log-transformed parameters were calculated.
The PT and APTT values were corrected for the absolute pre-value (prior to t=0). Their response was characterised by calculating the Area Under the Effect Curve (AUEC) over the period from 24 to 96 hours (from the start of warfarin administration to the administration of vitamin K) dividing by the corresponding time span. All AUECs were calculated relative to baseline. Additionally, the maximal change from baseline was calculated for these parameters (Emax). Because the APTT response was already present immediately after idraparinux sodium administration, this parameter was furthermore quantified using the AUEC for 0–24h and the AUEC from 0-96h (i.e. the period before warfarin administration and the entire period until vitamin K administration).

RESULTS

The treatments were well tolerated and no clinically relevant adverse events were observed during the study. The most frequent reported events were development of haematoma(s) at puncture sites. None of the occurring adverse events was reason for premature discontinuation.

Pharmacokinetics

The pharmacokinetic estimates are summarised in Table 1. Shortly after idraparinux administration the maximal concentration was reached (T_{max} 3.6 hours; SD 1.3), after which plasma concentration declined with an elimination half-life of 129 hours (SD 6.5).
A summary of the results of the comparison with the comparator group is found in Table 2. The pharmacokinetic estimates were consistent with the data obtained in two previous studies [1, 2]. From these data it can be concluded that there is no influence of warfarin on the pharmacokinetics of idraparinux. This was confirmed using an unpaired t-test; the mean difference in $AUC_{24\text{-}120h}$ was 0.72 (95% CI: -9.47, 10.92; p = 0.88). The results of the 'bioequivalence' analysis are summarised in Table 2, and confirmed the absence of any warfarin-effect on the pharmacokinetics of idraparinux. Pharmacokinetic results are visualised in Figure 1.

Pharmacodynamics

Following both treatments an increase in PT values was found (Figure 2). The increase started shortly before 48 hours (i.e. just before the second warfarin administration) had a maximum at about 72 hours after which it decreased. Pre-dose values were reached between 5 to 7 days after idraparinux dosing. No differences between treatments could be found in the analyses of PT values (table 3).

Administration of idraparinux sodium was immediately followed by an increase in APTT, which persisted until 120 hours post-dosing (Figure 2). After 7 days APTT values were at the pre-dose level. In the group treated with warfarin alone, no changes in APTT were found until the administration of warfarin, after which a mean increase of approximately 10 seconds occurred. The treatment with the combination (idraparinux-sodium plus warfarin) resulted in statistically significantly higher AUECs for APTT compared to the warfarin-only treatment (for all AUECs $p<0.005$; table 3). The $AUEC_{0-24h}$ of the comparator group (mean $AUEC_{0-24h}$: 2.54 seconds (SD: 2.38)) was comparable to that of the combination. When the pre-warfarin (24h) APTT values were used as baseline, it was shown that the APTT prolongation in the idraparinux plus warfarin treatment group was 6.7% lower compared to the APTT prolongation in the warfarin alone treatment group. No differences between the two treatments were found in E_{max} values.

After administration of warfarin in the placebo group factor VII was, on average, statistically significantly decreased by approximately 64% for the AUC 24-96 h and 85% for the minimum observed mean ratio 24-96 h. After administration of warfarin to subjects in the idraparinux group factor VII was, on average, statistically significantly decreased by approximately 61% for the AUC 24-96 h and 79% for the minimum observed mean ratio 24-96 h. However, if the pre-warfarin (24 h) values were used as baseline no remarkably differences were revealed.

The post-dose bleeding time was more prolonged after idraparinux plus warfarin compared to the placebo plus warfarin treatment group, with a difference of up to 45% for the AUC 0-96 h and 47%

for the observed maximum mean ratio 0-96 h. Despite these substantial effect sizes, the differences did not reach statistical significance (due to the variability of the measurements). Because mean bleeding times were substantially lower in the idraparinux plus warfarin group at baseline, prolongation could be partly attributed to regression to the mean. A comparison of median post-baseline scores across treatment groups revealed that there was only a limited effect of idraparinux plus warfarin compared to placebo plus warfarin.

DISCUSSION

The objective of this study was to investigate possible pharmacokinetic and pharmacodynamic interactions between a single dose of idraparinux sodium and oral warfarin in healthy male volunteers. The design was chosen to partly mimic the current clinical anticoagulation practice for DVT. In this situation anticougalants with an immediate effect ((low molecular weight) heparin) are given and treatment with oral coumarins is started. After coumarin therapy has resulted in a sufficiently prolonged PT, the heparin-like anticoagulant is normally stopped. It is clear that in the situation when both drugs are given simultaneously, clinicians want to know that the other drug does not influence the measure they use to assess the coumarin therapy. This situation was mimicked in this study. This experimental design was chosen to avoid time-consuming attainment of steady-state oral anticoagulant levels, while providing an initial answer to the question of possible interactions. With this dose-regimen a full suppression of all vitamin K-dependent clotting factors will not be reached due to the long elimination half-life of factor X and II (30-36 and 50 h, respectively). Nevertheless, it provides relevant information for clinical studies or practice in which the switch from idraparinux sodium to oral anticoagulants is encountered [5].

The study was performed using a parallel group design. This is justified by the knowledge that idraparinux has shown to have very predictable pharmacokinetics with low inter-individual variability [1-2]. All studies conducted thus far confirm each other

with regard to safety, pharmacodynamics and pharmacokinetics. The co-administration of warfarin did not influence the pharmacokinetics of idraparinux.

Administration of warfarin-alone or the combination was followed by a small rise in the APTT. As the increase in APTT after both treatments was equal, an interaction seems absent. Although the data suggest that idraparinux increases APTT, this increase was minor; on average 8 sec over the first 24 hours. In addition, it appeared that the pre-dose values were higher in the group treated with warfarin alone. This would than be consistent with earlier observations [1,2] that idraparinux does not influence the APTT to a clinically significant extent. Furthermore, it appears that this parameter, which is commonly used for heparins, is not suitable for monitoring the effects of pentasaccharides.

The PT is the most commonly used test to monitor orally administered anticoagulants [6]. Treatment with warfarin-only or the combination of idraparinux sodium plus warfarin prolonged the PT. As it is known that the effects on factor VII mainly cause the increase in PT after initiation of coumarin therapy, this parameter was also evaluated. It was indeed shown that factor VII concentrations declined after warfarin. More importantly, with regard to the objectives of this study, it was demonstrated that idraparinux did not influence the warfarin induced decrease of factor VII. As the presence of the pentasaccharide did not influence the effect of warfarin on the PT, and the data of this study obtained in healthy volunteers can be extrapolated to patients, it can be concluded that PT is a parameter that can be used to monitor the effect of oral anticoagulants during a switch from anticoagulant treatment with idraparinux sodium to oral anticoagulant therapy.

Bleeding time was statistically significantly prolonged 26 h after administration of idraparinux and was not further increased by co-administration of warfarin. However, differences at baseline and variation within subjects prevented any firm conclusions with respect to bleeding time.

In conclusion, the pharmacokinetics of idraparinux appear to be unchanged after concomitant warfarin therapy. There were no

major pharamcodynamic interactions between idraparinux and oral warfarin, and the PT can be used to monitor the warfarin therapy.

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TABLE 1 **Pharmacokinetic estimates for idraparinux (10 mg; sc) in the presence of warfarin using non-compartmental analysis.**

	C_{MAX} (ng/mL)	$AUC_{0\text{-}LAST}$ (mg/L*h)	% AUC extrapolated	$AUC_{0\text{-}\infty}$ (mg/L*h)	$t_{1/2}$ (h)	Vz (L)	CL (mL/min)
Mean	2110	184	10.1	204	129	9.22	0.83
(SD)	(307)	(24.9)	(1.3)	(28.0)	(6.5)	(1.26)	(0.12)
range	1715–2670	148–215	8.4–12.0	165–238	122–140	7.75–11.11	0.70–1.01

Vz: apparent volume of distribution

TABLE 2 Effect of warfarin on idraparinux AUC (10mg sc)

TREATMENT	$AUC_{24\text{-}120h}$ (mg/L*h)	C_{MAX} (ng/mL)	CLEARANCE (mL/min)	N
idraparinux alone*	77.3 (6.60)	2182 (132)	0.82 (0.05)	6
idraparinux + warfarin	76.6 (9.88)	2110 (307)	0.83 (0.12)	8
90% bio-equivalence intervals**		92%–118%	90%–110%	

Results are reported as mean (SD)

* Results earlier study in healthy young male volunteers [1]

** Multiple comparison-testing idraparinux 10 mg sc versus idraparinux 10 mg sc + warfarin on log-transformed parameters

TABLE 3 Pharmacodynamic responses

A. PT parameters above pre-value

TREATMENT		$AUE_{24\text{-}96h}$ (s)	$E_{MAX\ 24\text{-}96h}$	
idraparinux + warfarin	Mean (SD)	6.19 (3.92)	11.02 (6.85)	
Warfarin	Mean (SD)	7.47 (5.01)	14.08 (9.16)	
idraparinux	Mean	-1.28	-3.06	
	(95%CI)	(-6.10 / 3.55)	(-11.74 / 5.61)	

B. APTT parameters above pre-value

TREATMENT		$AUE_{24\text{-}96h}$ (s)	$E_{MAX\ 24\text{-}96h}$	$AUE_{0\text{-}24h}$	$AUE_{0\text{-}96h}$
idraparinux + warfarin	mean (SD)	10.11 (2.38)	14.17 (2.84)	7.93 (1.84)	9.58 (2.09)
Warfarin	mean (SD)	4.63 (3.64)	11.89 (8.78)	-0.54 (1.69)	3.35 (3.00)
idraparinux	mean	5.49	2.28	8.48	6.23
	(95%CI)	(2.19 / 8.78)	(-4.72 / 9.28)	(6.58 / 10.37)	(3.45 / 9.00)

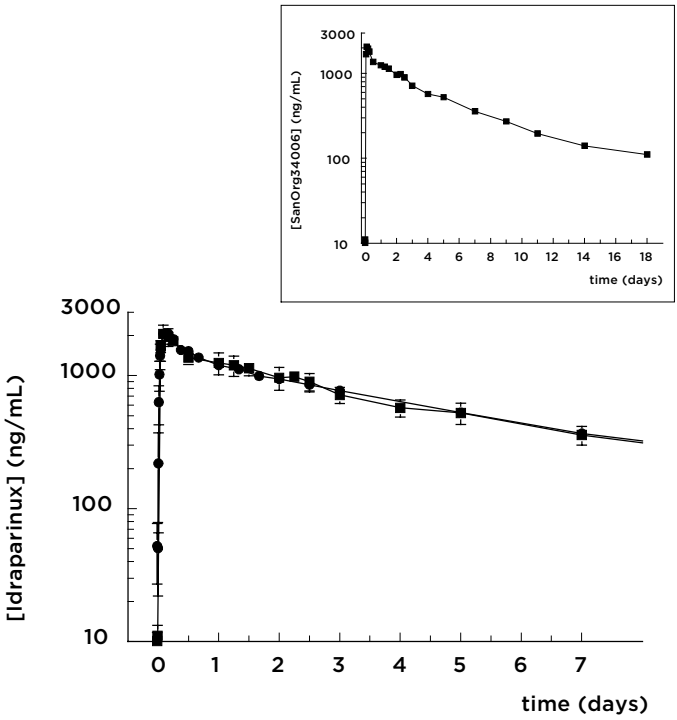

FIGURE 1 **Mean idraparinux concentration-time profile in two different studies**

●: Idraparinux alone (data from [1]), ■: Idraparinux + warfarin); inset: mean semi-logarithmic plasma profile of the combination over 18 days

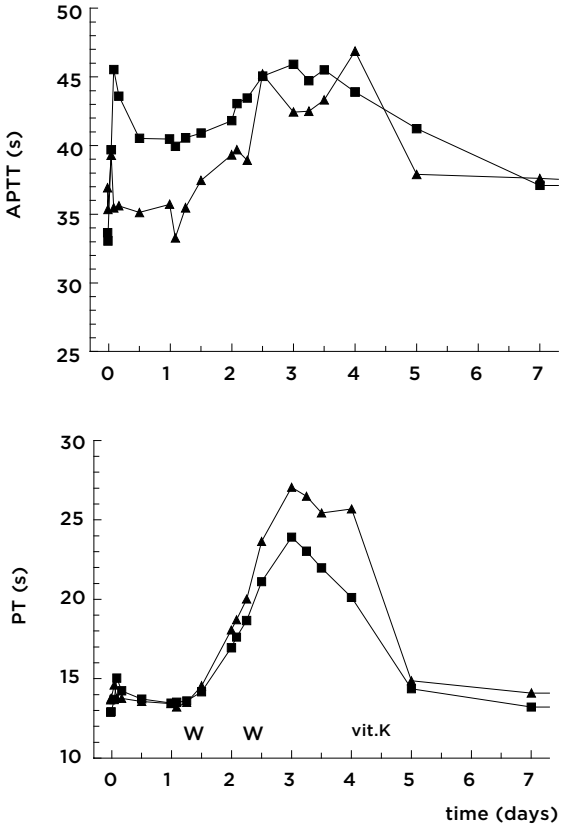

FIGURE 2 **Pharmacodynamic response (W and vit.K indicate warfarin and vitamin K administrations)**

■: Idraparinux + warfarin, ▲: warfarin alone)

CHAPTER 10

A PHASE I INTRAVENOUS RISING DOSE STUDY TO INVESTIGATE THE PHARMACOKINETICS/PHARMACODYNAMICS OF THE NOVEL ANTITHROMBOTIC GLYCOCONJUGATE ORG 36764 IN HEALTHY YOUNG MALE VOLUNTEERS

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ABSTRACT

Objectives

To investigate the tolerability, pharmacokinetics and pharmacodynamics of intravenous (iv) Org 36764, a novel anticoagulant compound obtained by full chemical synthesis.

Methods

Four (4) young healthy male volunteers received two single iv bolus injections (0.75 and 1.5 mg) according to an open study design with a washout period of at least one week between study occasions. After dosing, sampling for drug-activity assay (anti-Xa activity and anti-IIa activity), the Heptest®, AT and APTT/PT (INR), and measurement of safety and tolerability was performed regularly up to 120 hours. Assuming anti-Xa activity adequately reflects the pharmacokinetics of Org 36764, non-compartmental pharmacokinetic analysis was performed on the individual curves for this parameter.

Results

No serious adverse events were noted. Occurrence of haematomas at puncture sites (three times at each dose) and prolonged bleeding time (>240s; once at each dose) was observed. No obvious changes in the other pharmacodynamic parameters were detected. An unusual time course of drug levels after injection was observed for anti-Xa levels; these reached a plateau, which persisted for some time after the bolus injection. The maximum concentration increased non-linearly with the dose.

The clearance was independent of the dose as was the elimination half-life of about 45h. The apparent volume of distribution seemed slightly lower after the first dose. The anti-IIa activity showed low levels and no obvious differences between the two doses.

The results of the Heptest® were much more in line of expectation after iv bolus injection: the curves showed dose-dependent increase, with a time profile comparable to the anti-Xa activity but without a plateau phase.

Conclusions

The compound was safe over the limited dose range investigated. However, it appears that Org 36764 at the doses tested in this study showed signs of (excessive) pharmacological activity in healthy volunteers. The pharmacokinetics of Org 36764 are complex. The (indirect) pharmacokinetic assessments could be done with anti-Xa activity and results from the Heptest®, but the plasma-time profiles for anti-Xa activity and the Heptest® were discongruent. This precludes predictions on the pharmacokinetics.

INTRODUCTION

Glycocunjugates are carbohydrates covalently linked to a non-sugar moiety. The major representatives of this group of compounds are glycoproteins, glycopeptides peptidoglycans, glycolipids and the lipopolysaccharides. The novel compound Org 36764 (MW 3618) is a glycoconjugate consisting of two carbohydrate domains interconnected via an inert polyethylene glycol spacer (Figure 1). By means of its two domains the compound displays antithrombotic properties. One domain is a pentasaccharide with specific and high affinity for the plasma serine protease antithrombin (AT). The other domain, a persulphated cellobiose, binds thrombin based on electrostatic interactions [1].

Org 36764 mimics heparin in changing the conformation of endogenous AT to selectively accelerate the inhibition of Xa, as well as providing the template required for bringing AT and thrombin together, resulting in the interactions that lead to thrombin inhibition (based on its chain-length) [2].

The compound shows an anti-Xa activity of 800 U/mg and an anti-IIa activity of 10 U/mg [3]. Unlike heparin and heparin-like compounds, which are isolated from animal tissue and are chemically and biologically heterogeneous, this compound is obtained by full chemical synthesis [4]. This could make both dosage selection and interpretation of pharmacokinetics easier.

AT inactivates all serine protease coagulation factors (except factor VII), but its main activity is against factor Xa and IIa (thrombin). Factor Xa plays a central role in the coagulation

cascade being generated by multiple factors including factor VIIa/tissue factor (extrinsic route) and factor IXa (intrinsic route). Factor Xa boosters the factor VIIa generation and activates prothrombin to generate thrombin. Thrombin is a multi-active enzyme, both in blood coagulation and in cell-mediated processes [5,6]. Inhibition of factor Xa leads to a reduced thrombin generation and ultimately to reduced fibrin deposition and other thrombin dependent effects; additional inhibition of thrombin is anticipated to increase the antithrombotic effect.

Preclinical findings with Org 36764 have recently been published [3]. Importantly, these data showed that after iv administration, the compound displays the same plasma elimination curves for anti-Xa and anti-IIa activity, indicating its chemical stability *in vivo*. Toxicokinetic studies of Org 36764 were performed in rats and dogs (data on file). Other than changes induced by the pharmacological activity, no toxic effects were observed after administration to rats of 50 mg/kg, in dogs up to 5 mg/kg.

The compound appeared effective in several animal models of thrombosis, indicating Org 36764 could be a novel drug used in venous and arterial thrombosis. When the compound is intended to compete with heparin(-like) drugs (currently used for these indications, i.e. arterial thrombosis), a rapid onset of action is needed as the current medical practice for heparin therapy is to attain high levels rapidly and maintain these over various time spans. This means that either the subcutaneous absorption of Org 36764 should be very fast or that the first administration should be by intravenous (iv) injection. Possibly, subsequent doses could be given subcutaneously. Therefore, information on the pharmacokinetics of the compound after iv administration is important. This first-in-human single rising dose study was carried out to obtain a first indication of gross safety and tolerability, and to estimate the pharmacokinetic parameters and pharmacodynamic effects of iv Org 36764.

METHODS

Subjects and Design

The Ethics Committee of Leiden University Medical Center approved the investigational protocol. This open study was performed according to a single-center, single rising dose design. All subjects were dosed with 0.75 mg Org 36764 on the first study day and with 1.5 mg Org 36764 on the second day with a washout period of at least one (1) week between study occasions. The administrations took place as iv bolus injections over 20 seconds. Four healthy male subjects (aged: 21–29 yr., weight: 68.5–90.0 kg) with a normal coagulation screen participated in this study after written informed consent was obtained.

Study Procedures

The subjects were studied after an overnight fast. After study day preparations were completed the study drug was administered. During the next 24 hours sampling for drug/activity assay (anti-Xa activity and anti-IIa activity), Heptest® (a whole blood clotting assay relatively specific for anti-Xa activity), AT, APTT and PT (INR) was performed regularly, as well as measurement of vital signs, ECG and routine laboratory measures. The bleeding time (Ivy method) was determined pre-dose and at 15 minutes, 24 and 120 hours after administration of Org 36764. After discharge from the unit at approximately 24 hours after dosing the subjects returned to the unit for follow-up sampling up to 120 hrs after dosing.

Sampling

Blood samples were taken from an iv cannula contralateral to the infusion cannula (during the in-house period) or by venipuncture. Blood drawn from the iv cannula was taken after discarding the contents of the cannula, which was kept patent using intermittent flushing with saline solution (0.9%). No tourniquet was applied. Blood samples for the anti-Xa assay, anti-IIa assay, Heptest(APTT/PT assay, and AT assays were collected in tubes containing sodium citrate (0.129M).

Assays

Anti-factor Xa activity was assessed using a validated photometric assay, Coatest® LMW Heparin (Chromogenix, Mölndal, Sweden). The anti-IIa activity was determined with a photometric assay with thrombin substrate H-D-HHT-L-Ala-L-Arg-pNA.AcOH and was based on the inhibition of thrombin (=IIa) activity (Spectrolyse® Heparin anti-IIa, Biopool, Umea, Sweden). For the Heptest® a plasma sample was incubated with factor Xa at 37 °C and this mixture was recalcified by the addition of calcium and the clotting time was registered. This test was performed using the Accuclot™HEPTEST® (Sigma Diagnostics). For these three assays Org 36764 was used as calibrator.

Antithrombin (AT) was assessed using a photometric assay with S-2765 as a chromogenic substrate (Comatic® Antithrombin, Mölndal, Sweden). The APTT and PT assays were performed using standard procedures [7]. The reagents were STA APTT (Roche Diagnostics, Mannheim, Germany) and Thromborel S (sensitive human placental thromboplastin, ISI ≈ 1) (Behring Diagnostics, Marburg, Germany).

All assays were performed at the TNO-Gaubius Laboratories (Leiden, The Netherlands) on a STA® coagulation analyser (Roche Diagnostics, Mannheim, Germany).

Analysis

Non-compartmental pharmacokinetic analysis using actual sampling times was performed on the individual curves for the anti-Xa activities (expressed as ng/mL Org 36764) using standard procedures. These pharmacokinetic analyses were performed using WinNonlin V1.1 software (Scientific Consulting, Inc., Apex, NC). The drug-input regimen had to be selected as an extravascular bolus dose, because of the observed unusual time course of anti-Xa activity levels after injection.

This computational approach was chosen in order to avoid erroneous back-extrapolation to the Y-axis. The following non-compartmental pharmacokinetic parameters were derived from the profiles: the observed peak value (C_{max}) and the associated sampling time (T_{max}), the AUC from time zero up to the last

measurable concentration ($AUC_{0\text{-last}}$) using the linear trapezoidal method, the elimination rate constant associated with the terminal elimination phase (λ_z) and the terminal half life associated with λ_z ($t_{½}$), the AUC extrapolated to infinity ($AUC_{0\text{-}\infty}$), plasma clearance and the (apparent) volume of distribution (Vz).
The number of points used for λ_z calculation was automatically determined by the program and was visually checked for adequacy. As this was an exploratory study no formal statistical analyses have been performed.

RESULTS

General safety and tolerability

Org 36764 was safe over the limited dose range evaluated in this study, as there were no clinically significant changes in vital signs, ECG and routine laboratory measures. All adverse events were mild in severity and none required medical intervention. Apart from some non-specific events, the most striking event was the occurrence of haematomas at puncture sites (in three out of four subjects at each dose) and prolongation of bleeding time (above the commonly accepted upper range of 240 seconds; once at each dose).

Pharmacokinetic Parameters

The average curves for the anti-Xa activity are displayed in Figure 2. A summary of the pharmacokinetic parameters is given in table 1. For both doses, anti-Xa levels reached a plateau, which persisted for several hours after the bolus injection. Hence, higher T_{max} values than expected after an iv bolus injection were found. The C_{max} values increased non-linearly with the dose: the average value after the second dose was 3.1 times higher than the first dose. However, the clearance appears to be independent of the dose, as reflected by a dose-proportional increase in AUC values. It appears that for both doses the elimination occurs similarly; the elimination half-life is in the order of 43–49 hours.
The apparent volume of distribution estimated in this study was slightly higher after the first dose.
The anti-IIa activity showed such low levels that these could not

be measured against Org 36764 as standard. Therefore, the samples were assayed against the first international standard for LMWH (code 85/600; 665 IU anti-IIa activity/mL) obtained via the National Institute for Biological Standards and Calibration (UK). No obvious differences were found between the two doses (Figure 3).

The curves for the Heptest® showed a time profile comparable to the anti-Xa activity, however, no plateau phase was present (Figure 4a). In fact, these curves were much more in the line of the expectation one would have for drug effects after iv bolus injection. Maximal drug levels measured with the Heptest® were reached shortly after the administration and show dose-linearity (C_{max}: 142 ng/mL (SD: 24.3) and 281 ng/mL (SD: 19.9) for the 0.75 and 1.5 mg dose, respectively). The Heptest® AUC values also increased linearly with the dose. When the curves were plotted logarithmically, there are indications that in the Heptest® the drug showed a 2-compartmental behaviour (Figure 4b). Plotting of the concentration data for Org 36764 obtained with the anti-Xa assay and the Heptest® showed that at higher anti-Xa levels the results of the Heptest® levelled off (Figure 5).

Pharmacodynamics

No obvious changes in AT levels were detected after either dose; the levels remained in the same order of the pre-dose values of 100%, and although occasional deviations from this value occurred, these were not systematic. No significant abnormalities in APTT, PT/INR were noted after either dose. Although the average curve indicates a change in APTT after the second dose (1.5 mg), this is solely attributable to high values obtained in one subject at this dose (Figure 6).

DISCUSSION

In this study, the novel antithrombotic compound Org 36764 was administered for the first time in man. In pre-clinical experiments, a no-effect level for the compound could not be established. For instance, in dogs at all dose levels tested drug-induced haemorrhage occurred, most likely the result of the

pharmacological effect of this compound. Therefore, low doses of the compound were given to a limited number of subjects in order to get preliminary information on the drug. Over the limited dose range investigated in this study, iv administration of Org 36764 was safe as there were no clinically significant changes in vital signs, ECG, routine laboratory measures, and the time course of coagulation parameters (APTT and PT/INR). However, it appears that the compound at the doses tested (0.75 and 1.5 mg) showed signs of (excessive) pharmacological activity in healthy volunteers as a relatively high incidence of increased bleeding tendency was observed. At each dose level haematomas were noted at venipuncture sites and at the puncture sites used for the bleeding time assessments in 3 out of 4 subjects. This may indicate that secondary haemostasis was affected, which may be attributed to the (low) antithrombin activity of the compound.

The pharmacokinetic evaluation of Org 36764 is complicated. As a direct drug assay is not available, the assessment was based upon anti-Xa assay levels. In addition, anti-IIa levels and the Heptest® were used as indirect pharmacokinetic assays. Anti-IIa levels were virtually non-existing and hence non-informative. The results of the compound in the Heptest® were discongruent with the results obtained with anti-Xa assay. At lower anti-Xa levels the results from the Heptest® and anti-Xa activity are linearly related, but at higher anti-Xa levels the results from the Heptest® indicated consistently lower activity. Additionally, in case of low doses anticoagulant (as in this study), the Heptest® is known to sometimes overestimate the anticoagulant (i.e. cause shorter clotting-test results) due to additional activation of the intrinsic system. Therefore, the first part of the Heptest®-curve could be too steep (Abildgaard, personal communication), indicating this assay resulted in consistently lower anti-Xa levels over the whole range tested in this study.

It may also be that the discrepancy between the results is related to the sample handling procedure for the Heptest® assay. In this assay, linearity is present for the concentration to 300 ng/mL Org 36764, thus samples with higher concentration were diluted with pooled plasma. When high drug levels induce changes other

than changes in anti-Xa levels, this may have been diluted away. It can for instance be hypothesised that Org 36764 released TFPI. This may then be measured in the amidolytic anti-Xa assay, but not in the Heptest®. This hypothesis is supported by the expectation that heparin(-like) compounds induce TFPI release. Post-hoc analysis of the samples from the present study indicated that TFPI-levels were not different from baseline. The maximum levels were 16.1 ng/mL and 15.7 ng/mL at the low and high dose, respectively. Hence, it is unlikely that TFPI-release had occurred. Another hypothesis is that the thrombin-binding character of the molecule, affinity of the molecule for another plasma-component (or a combination of both) might have caused some non-treatment-related protein binding, comparable to the protein binding of medication, but saturated in the micromolecular range. However, this interference should have had a different influence in the different assays.

Though the Heptest® is relatively specific for anti-Xa activity, it does not exclusively reflect anti-Xa activity since agents containing exclusive anti-IIa activity influence it. In addition, the Heptest® is influenced by various other clotting parameters. The anti-Xa test might therefore be the more reliable test of the two tests, as used in this study. In addition, there is no other known cause to disturb the anti-Xa test than TFPI (Rosen, personal communication), which was excluded by the post-hoc analysis. If anti-Xa activity levels are considered the most reliable parameter for the pharmacokinetics of the drug then some findings are hard to understand. After iv bolus injection, drug levels attained a plateau for several hours. The reasons for this are unclear. It may be possible that an active metabolite is formed. However, animal data indicated that the compound is chemically stable [3]. Hence, breakdown of the compound is unlikely, but this can not be supported or refuted by this study, as the anti-IIa levels were much too low.

The elimination half-life of the compound (45 hours for both doses) could be adequately estimated and the percentage of extrapolation to measure the $AUC_{0-\infty}$ was 10-15%. The apparent volume of distribution was slightly higher after the first dose.

However, calculation of this value assumes a mono-exponential decay. As this is not the case for Org 36764, it is difficult to attribute a meaningful interpretation to this finding.
The increase in C_{max} between the two doses may be non-linear, although this may revert back to linearity when higher doses are studied.
To conclude, this study was carried out in 4 subjects only with two doses tested. Obviously, with the findings of this study (plateau of anti-Xa levels, nearly undetectable anti-IIa levels and the discrepancy between the (indirect pharmacokinetic) methodology used to profile the compound) it is impossible to predict the behaviour of the compound after higher doses or multiple doses in a reasonable manner.

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TABLE 1 Summary of pharmacokinetic parameters for two doses of Org 36764 after iv bolus administration (based on anti-Xa activity)

DOSE	C_{MAX} (ng/mL)	T_{MAX} (min)	$AUC_{0\text{-last}}$ (µg/ml*h)	$AUC_{0\text{-}\infty}$ (µg/ml*h)	% extrap.	$t_{1/2}$ (h)	CL (mL/min)	Vz (L)
0.75	180 (22)	87 (103)	8.7 (2.4)	9.8 (2.6)	12 (8)	49.4 (10.3)	1.33 (0.31)	5.50 (0.43)
range	151-199	15-240	7.1-12.2	7.5-13.6	6–24	41.8-63.8	0.92-1.66	5.07-6.00
1.50	553 (54)	80 (49)	16.3 (1.8)	19.0 (3.4)	14 (6)	43.2 (10.0)	1.35 (0.23)	4.90 (0.37)
range	498–621	30-125	14.3–18.3	15.6-23.3	8–22	32.7-56.7	1.07-1.61	4.55-5.26

Results are reported as mean (SD); ranges for each parameter are given at the 2nd line of each row. C_{max}: maximal concentration; T_{max}: time to maximal concentration; $AUC_{0\text{-last}}$: Area Under the Curve from zero time to last sample; $AUC_{0\text{-}\infty}$: Area Under the Curve from zero time to infinity; %extrap.: percentage of $AUC_{0\text{-}\infty}$ obtained by extrapolation; $t_{½}$ elimination half-life; CL: clearance; Vz: volume of distribution.

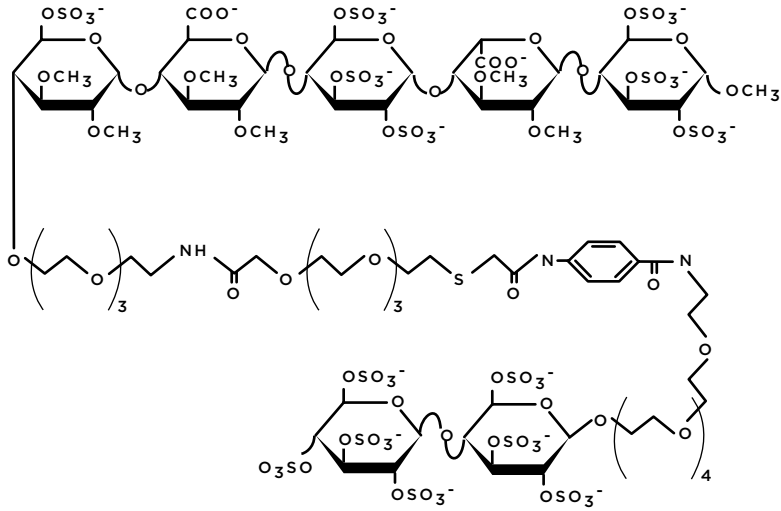

FIGURE 1 Molecular formula Org 36764

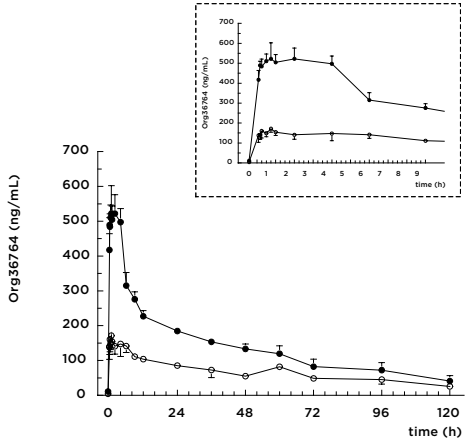

FIGURE 2 Average anti-Xa curves (+ SD) following both doses Org 36764 (anti-Xa activity expressed in ng/mL); ○: 0.75 mg, ●: 1.5 mg Org 36764

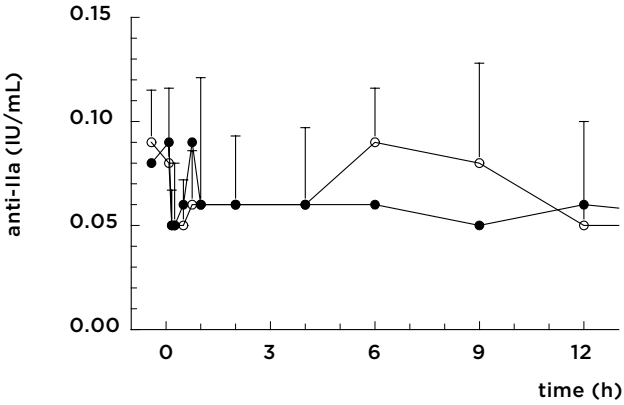

FIGURE 3 Average anti-II time curves over the first 12 hours post-administration; ○: 0.75 mg, ●: 1.5 mg Org 36764

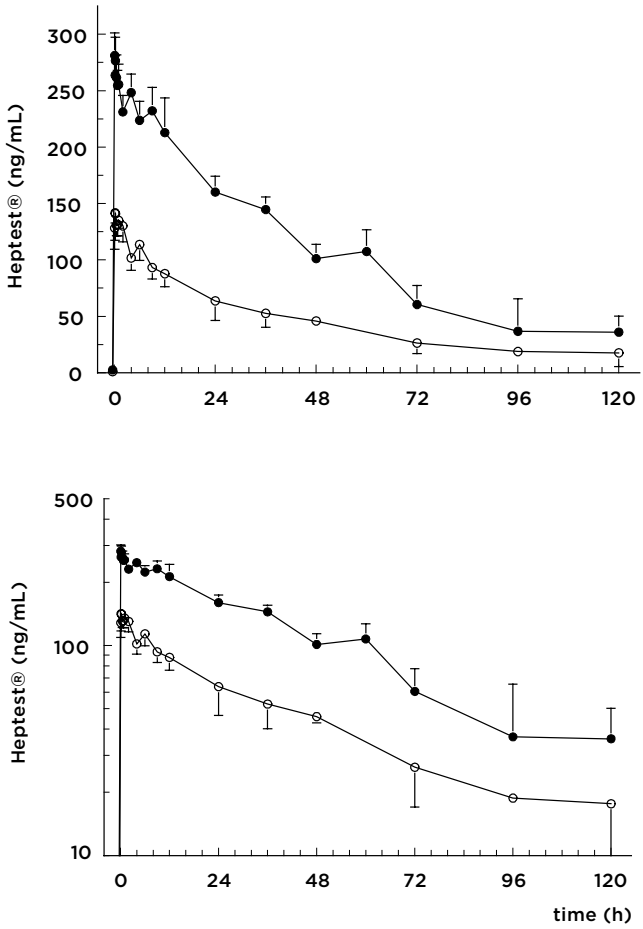

FIGURE 4 **Average Heptest® results**
(+ SD) (top graph: linear scale; bottom graph: logarithmic scale); ○: 0.75 mg, ●: 1.5 mg Org 36764

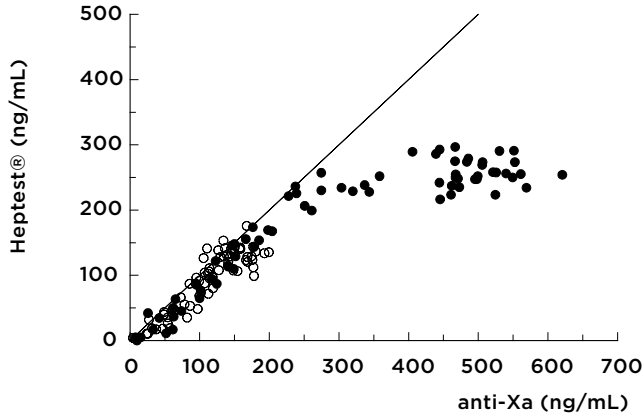

FIGURE 5 **Comparison drug level assessment using anti-Xa activity and the Heptest®**
(striped line: line of equality); ○: 0.75 mg, ●: 1.5 mg Org 36764

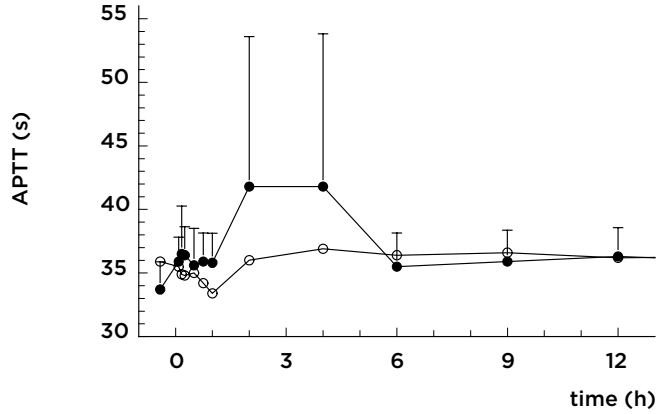

FIGURE 6 **Mean APTT time-curves over the first 12 hours post-dosing**
○: 0.75 mg, ●: 1.5 mg Org 36764

CHAPTER 11

THE EFFECT OF WARFARIN ON THE PHARMACOKINETICS AND PHARMACODYNAMICS OF NAPSAGATRAN IN HEALTHY MALE VOLUNTEERS

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ABSTRACT

Objective

To investigate the effect of oral warfarin on the pharmacokinetics and pharmacodynamics of the synthetic direct thrombin inhibitor napsagatran.

Methods

In an open, randomised, two-way crossover study, 12 healthy male volunteers were infused napsagatran (80 µg/min) for 48 hours. Each subject was administered 25 mg warfarin (Coumadin®) at the start of the infusion in either the first or second treatment period. Sampling was performed regularly over the treatment period and 24 hours thereafter for measurement of plasma levels of napsagatran, activated partial thromboplastin time (APTT) and prothrombin time (PT).

Results

The pharmacokinetic parameters of napsagatran were not significantly influenced by co-administration of warfarin. Napsagatran administration was followed by increases in APTT and PT. Co-administration of warfarin increased the AUEC calculated for the period 0–48 h (corrected for baseline) for APTT by 45% (95%CI: 28–65%) and for PT by 438% (95%CI: 272–678%) compared to the treatment with napsagatran alone.

Conclusion

Warfarin has no effect on the pharmacokinetics of napsagatran, but has a marked influence on the pharmacodynamic parameters (APTT, PT) of napsagatran. In clinical practice this interaction between the two compounds should be taken into account. The PT cannot be used to monitor the effect of oral anticoagulants during the switch from this group of direct thrombin inhibitors to full oral anticoagulant therapy.

INTRODUCTION

Currently, the treatment of acute deep venous thrombosis (DVT) consists of administration of unfractionated heparin (UFH) or low-molecular-weight heparins (LMWHs) overlapped and followed by oral anticoagulation with vitamin-K antagonists. Even though heparins have an established place in this treatment, this group of compounds has clear drawbacks [1,2]. These have fostered interest in the development of successor compounds. Thrombin is a multifunctional serine protease with a pivotal role in the coagulation cascade. It is the final common pathway for coagulation and as a consequence, inhibition of thrombin should be suitable for the treatment and the prophylaxis of thrombosis. As such, thrombin has become a major target for anticoagulation and cardiovascular disease therapy [3]. Theoretically, most of the drawbacks of the heparins can be overcome by the use of direct thrombin inhibitors. These compounds offer the advantage that they do not require antithrombin or heparin cofactor-II as a cofactor, and have the ability not only to inhibit 'free' thrombin, but also access clot- and tissue-bound thrombin [5,6]. Therefore, many new thrombin inhibitors have been developed during the recent years, and several of these agents are currently undergoing various phases of clinical trials [7]. Recent studies on treatment of DVT with direct acting thrombin inhibitors have shown promising results [8,9,10].

Napsagatran (formerly known as Ro 46-6240) is a synthetic, potent, competitive and reversible thrombin inhibitor of low molecular weight [11]. The development of the compound involves investigation of its efficacy in patients with diagnosed DVT (see the reports of the ADVENT trial [9,12]), and therefore the combined administration of napsagatran and oral anticoagulation is conceivable in practice. The current study was performed to assess the possible effects of oral warfarin on the pharmacodynamics and pharmacokinetics of intravenous (IV) napsagatran.

METHODS

Subjects and Design

The Ethics Committee of Leiden University Medical Center approved the study-protocol. Twelve healthy male volunteers (age: 22–27 yr., body-weight: 65–88 kg) participated in the study after written informed consent was obtained. The study was performed as an open, randomised, two-way crossover study with a two-week washout period. During each treatment period, the subjects received a 48-hour IV infusion napsagatran. The infusion rate was 80 µg/min, which resulted in a total dose of napsagatran of 230.4 mg (total volume of infusion: 384 mL). In either the first or second treatment period, just prior to the start of the infusion of napsagatran, 25 mg warfarin (Coumadin®, 5 mg tablets, Evans Medical Ltd.) was administered orally with 150 ml water.

Procedures

Subjects were studied after an overnight fast. They reported at the clinical research unit on the evening before drug administration. The next morning a cannula for administration of the medication was inserted in a suitable forearm vein. Another cannula was inserted in the contra-lateral arm for blood sampling. Samples for determination of plasma levels of napsagatran, activated partial thromboplastin time (APTT) and prothrombin time (PT) were taken pre-dose and frequently during the study-period. Because it was not the objective of the present study to investigate the possible influence of napsagatran on the pharmacokinetics of warfarin, no sampling was performed for warfarin-determination. The cannula for blood collection was kept patent by infusion of 0.9% saline. Blood was taken after discarding the contents of the cannula. Twenty-four (24) hours after the infusion was stopped (i.e. 72 hours after start of the treatment) the last blood samples were collected, a physical examination was performed and the volunteers returned home.

Laboratory Tests

The napsagatran assay was performed at the analytical laboratory of Roche Milano (Milano, Italy) under the responsibility of Dr A

Guenzi and consisted of the following steps: after addition of an internal standard (Ro 46-8970), proteins were precipitated by addition of acetonitrile. After concentration of the supernatant, the residue was chromatographed on a phenyl column (5 µm Spherisorb Phenyl) protected by a phenyl guard column (5 µm Brownlee Phenyl). The eluate fraction containing both napsagatran and the internal standard was switched to a reverse-phase column (5 µm Macherey-Nagel Nucleosil C_{18}), and quantified by fluorescence detection ($\lambda_{excitation}$=225 nm, $\lambda_{emission}$=350 nm). All columns were eluted with a mixture of 50 Mm KH_2PO_4 / acetonitrile 70:30 v/v, containing 0.025% triethylamine (flowrate of 1 mL/min). Under these conditions, napsagatran and Ro 46-8970 were eluted after approximately 11 and 14 minutes, respectively. The lower limit of quantification (LOQ) of the assay was 1 ng/mL using 1.0 ml of human plasma [13].
The coagulation assays were performed on a MLA Electra 1000c coagulation analyser (Pleasantville, New York, USA). APTT and PT were determined by standard procedures [14], using the Actin reagent (Dade Behring, Liederbach, Germany) and calcium chloride (0.02M) for the APTT and the Innovin reagent (Dade Behring, Liederbach, Germany) for the PT.
These assays were performed at the TNO-Gaubius Institute (Leiden, The Netherlands) under the responsibility of Dr C Kluft.

Data Analysis
Pharmacokinetic parameters for napsagatran were calculated using a standard two-compartment open pharmacokinetic model using the Siphar software package (Simed, Créteil, France).
The area under the concentration-time curve (determined by the linear trapezoidal rule) extrapolated to infinity ($AUC_{0-\infty}$), clearance (CL), half-life of the first exponential phase (t_{5l1}), half-life of the second exponential phase (t_{5l2}) and volume of distribution at steady state (V_{ss}) were computed using the fitted model. Additionally, the AUC until the last measured concentration ($AUC_{0\text{-last}}$), CL and the average steady state concentration (C_{ss} = rate of infusion/CL) were calculated non-compartmentally.

The pharmacodynamic effects were measured by calculating baseline corrected Area Under the Effect Curves (AUECs, determined using the linear trapezoidal method), the maximum effect (E_{max}), and time to reach maximum effect (T_{max}) for each parameter. AUECs were calculated from the timepoint of administration (t=0) until 48 hours post-administration. With the exception of T_{max}, all data were analysed after log-transformation to correct for the marked skewness of the data distributions. The different treatments were compared using paired t-tests and log-differences were backtransformed along with their 95% confidence intervals (CI) to yield measures which can be interpreted as percentage change induced by the combination treatment over the napsagatran alone administration. Calculations were carried out using SPSS (SPSS Inc., Chicago, U.S.A.) computer software.

RESULTS

All subjects completed the study and no serious adverse events were noted. None of the changes in the assessed safety parameters was of clinical relevance.

Pharmacokinetics

The mean semi-logarithmic concentration-time profiles for napsagatran after both treatments are displayed in Figure 1. The pharmacokinetic analyses are summarised in Table 1. No significant changes were observed in pharmacokinetic parameters of napsagatran after co-administration of warfarin.

Pharmacodynamics

The mean APTT-time profile is displayed in Figure 2 and PT-time profile in Figure 3. Results of the pharmacodynamic measures are summarised in Table 2. After napsagatran alone, increases were observed in these conventional coagulation parameters. Co-administration of warfarin resulted in a significant increase in $AUEC_{0\text{-}48}$ (corrected for baseline values) of 45% (95%CI: 28–65%) for the APTT. The maximum APTT value increased 20% (95%CI: 14–26%) after adding warfarin to the napsagatran infusion.

For the PT a significant increase was observed in $AUEC_{0\text{-}48}$ (above baseline) of 438% (95%CI: 272–678%). The addition of warfarin increased the maximum value for PT with 85% (95%CI: 55–119%).

DISCUSSION

The objective of the present study was to investigate the possible effect of oral warfarin on the pharmacodynamics and pharmacokinetics of the direct thrombin inhibitor napsagatran. Warfarin did not influence the pharmacokinetic properties of napsagatran. With the exception of the elimination half-life, all measured pharmacokinetic parameters for napsagatran were in accordance with those described earlier. Bounameaux et al. reported an elimination half-life of about 40 minutes [9], whereas Lavé at el. observed a mean value for this parameter of 1.7 hours [15]. The differences in elimination half-lives may be related to differences in the sampling schemes (e.g. sampling times, duration of the sampling-period).

Napsagatran has shown to be a potent inhibitor of thrombin thereby exerting a potent anticoagulant effect. Because napsagatran binds to the active site of thrombin, it influences both intrinsic and extrinsic coagulation pathways. This was reflected by increases in APTT and PT after the single drug administration. Co-administration of warfarin to the thrombin inhibitor treatment further decreases the overall activity of the intrinsic coagulation system. The APTT-curves differed between treatments from 6 hours after administration onwards.

The maximum difference in APTT between the two treatments occurred at 42 hours and remained similar until 48 hours. Therefore, the peak effect of the two compounds on the APTT has most probably been reached. After cessation of the napsagatran-infusion, a sharp decline in APTT was observed. After an additional 4 hours napsagatran was almost completely eliminated from the blood, and during the napsagatran-only treatment, the average APTT level at this timepoint was returned to baseline-values. Assuming that at this timepoint the influence of napsagatran on the APTT was negligible with the combined treatment, the remaining difference of about 4 seconds between

the two treatments at this time point is likely to be attributed to warfarin. This would imply that at 48 hours there is a supra-additive effect of about 4 seconds. In the remainder of the studied period the difference between the two treatments remained stable; at 72 hours the difference in APTT between napsagatran treatment and the combination treatment was still almost 4 seconds.

The activity of the extrinsic coagulation pathway is both influenced by napsagatran and warfarin. The maximum increase in PT after napsagatran alone was about 2 seconds, whereas the maximal increase in PT after the combination treatment was about 14 seconds at 36 hours and remained stable until 48 hours. After cessation of the napsagatran-infusion a decline of 2 seconds was expected for both treatments provided that the effect of warfarin on napsagatran is additive. However, in the first 2 hours after cessation of the infusion in the combination treatment a sharp drop of more than 6.5 seconds was observed, again suggesting a supra-additive effect. At this timepoint PT levels had returned to pre-medication levels for the napsagatran-only treatment, while there was a difference between treatments of almost 8 seconds. As at 48 hours the difference was 12.5 seconds, this would imply a supra-additive effect of about 5 seconds.

A further drop in PT of approximately 4.5 seconds is observed between 50 and 72 hours for the combination treatment.

The effects of napsagatran and warfarin could be additive, if this overall decline is a result of the normal decrease of warfarin due to a slow warfarin metabolism. Therefore, whether the decrease of the PT between 48 and 50 hours represents a supra-additive or an additive effect is uncertain. Nevertheless, the sharp drop of more than 2 sec in a relatively short time favours a supra-additive effect.

Since this study lacked a warfarin alone arm, historic controls were taken from the literature [16,17,18] in order to get comparative information about the time course of pharmaco-dynamic measures after administration of warfarin alone.

In these control studies healthy young male subjects were administered 25 mg oral warfarin as a single dose. The drawbacks

of using historic controls in this comparison are recognised (e.g. different analysing instruments and reagents). Nevertheless, it could provide an initial answer about the possible nature of the observed effects. The findings strengthen the idea about a supra-additive effect on PT when napsagatran and warfarin are co-administered. In order to investigate a possible interaction between these compounds, the maximum PT-values corrected for base line were compared. Even if the greatest increase in PT of the historic control-studies is taken, the summation of PT-effects of the single drug treatments is less than the increase induced by the combination of napsagatran and warfarin. The increase induced by napsagatran only is about 2 seconds, the increase induced by warfarin only about 9 seconds, whereas the increase induced by the combination of the two compounds is about 14 seconds.

In summary, it cannot be concluded whether the pharmaco-dynamic effects of oral warfarin and the synthetic thrombin inhibitor napsagatran are additive or supra-additive. This study shows that warfarin has no effect on the pharmacokinetic properties of napsagatran, but has a marked influence on the pharmacodynamics of the compound. In clinical practice one should be well aware of this interaction. Because the presence of napsagatran clearly influenced the effect of warfarin on the PT, it can be concluded that this parameter cannot be used to monitor the effect of oral anticoagulants during the switch from anticoagulant treatment with this kind of direct-acting thrombin inhibitors to full oral anticoagulant therapy.

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TABLE 1 **Pharmacokinetic parameters of napsagatran following both treatments and the effect of warfarin**

		treatment		
	PARAMETER	NAPSAGATRAN	NAPSAGATRAN + WARFARIN	DIFFERENCE*
Model-dependent	$AUC_{0-\infty}$ (mg/L*min)	559.5 ± 128.5	536.6 ± 122.6	-3.9% (-9.2% , 1.6%)
	Cl (mL/min)	431.0 ± 93.2	447.4 ± 87.4	4.1% (-1.5% , 10.1%)
	Vss (L)	25.8 ± 5.4	26.7 ± 7.3	2.4% (-10.7% , 17.5%)
	$t_{½\lambda 1}$ (min)	23.3 ± 10.5	22.1 ± 12.7	-12.2 (-47.2% , 45.9%)
	$t_{½\lambda 2}$ (min)	123.5 ± 36.5	105.8 ± 30.6	-14.7% (-36.6% ,14.9%)
Non-compartmental	$AUC_{0-\infty}$ (mg/L*min)	568.9 ± 144.4	533.8 ± 117.2	-5.4% (-10.7% , 0.2%)
	Cl (mL/min)	428.4 ± 105.3	448.9 ± 88.8	5.7% (-0.2% , 11.9%)
	C_{ss} (ng/mL)	197.5 ± 50.1	185.4 ± 40.7	-5.4% (-10.7% , 0.2%)

Results are reported as mean ± SD
* Percentage increase or decrease induced by co-administration of warfarin; reported with 95% CI

TABLE 2 Pharmacodynamic effects of both treatments and the effect of warfarin

		treatment		
	PARAMETER	NAPSAGATRAN	NAPSAGATRAN + WARFARIN	INCREASE*
APTT	E_{max} (s)	38.7 ± 5.7	46.3 ± 5.6	19.8% (13.9%/26.1%)
	AUC_{0-48} /48 (s)	10.9 ± 3.1 (8.8 / 13.0)	15.7 ± 3.9 (13.1 / 18.3)	45.3% (27.8%/65.2%)
	T_{max} (h)	24.7 ± 18.5	40.2 ± 10.1	15.5 (-0.3 /31.5)
PT	E_{max} (s)	14.2 ± 1.1	27.1 ± 7.8	84.5% (55.3%/119.2%)
	AUC_{0-48} /48 (s)	1.8 ± 0.8 (1.3 / 2.3)	9.8 ± 3.7 (7.3 / 12.3)	438% (272%/678%)
	T_{max} (h)	17.5 ± 20.1	35.0 ± 9.5	17.5 (4.7 / 30.5)

Results are reported as mean ± SD (95% confidence interval);
AUECs are divided by the corresponding time span and are baseline corrected; 95% confidence intervals may be used as a test for change from baseline.
*Percentage increase for E_{max} and AUEC, difference for T_{max}

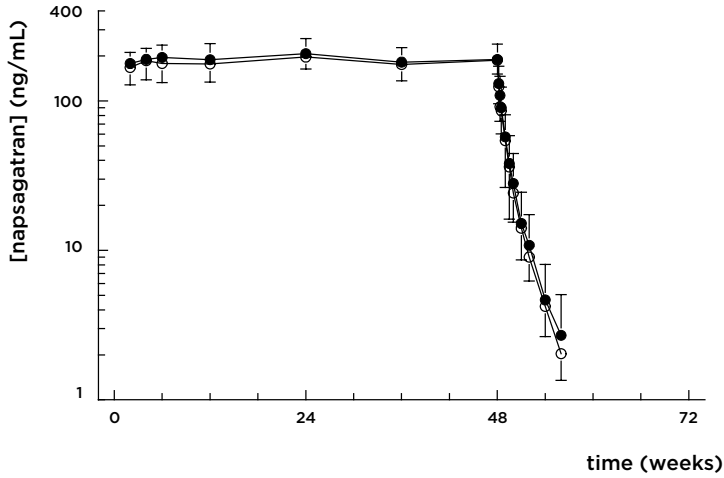

FIGURE 1 Average semi-logarithmic napsagatran concentration time profile

(± SD) with and without co-administration of 25 mg warfarin p.o. at t=0 (closed circles: napsagatran; open circles: napsagatran + warfarin). After the 58-hour timepoint results of this assay were below the LOQ (1ng/mL).

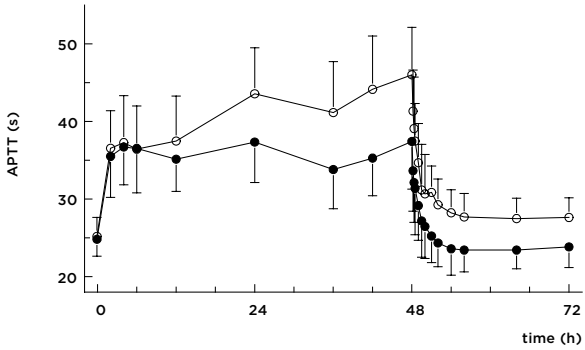

FIGURE 2 **Mean APTT time profile after napsagatran with or without warfarin co-administration**
(closed circles: napsagatran; open circles: napsagatran + warfarin; error bars represent the SD).

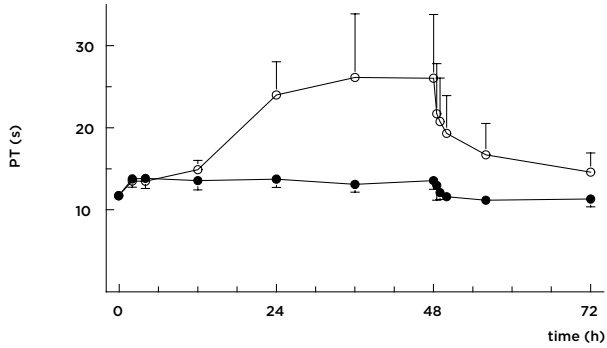

FIGURE 3 **Average PT time profile following co-administration of warfarin to IV napsagatran infusion**
(closed circles: napsagatran; open circles: napsagatran + warfarin; error bars represent the SD).

SECTION III

ALTERNATIVE ROUTES OF ADMINISTRATION

CHAPTER 12–14

CHAPTER 12

THE ORAL BIOAVAILABILITY OF PENTOSAN POLYSULPHATE SODIUM IN HEALTHY VOLUNTEERS

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ABSTRACT

Objective

Pentosan polysulphate sodium (PPS), a heparin-like drug, is supposed to be orally applicable. The objective of present study was to asses the oral bioavailability of PPS. However, since specific assays for PPS do not exist, this was done by using primary and secondary effect parameters.

Methods

The study was carried out using a three-way randomized crossover design with 18 healthy young male volunteers. The subjects received three treatments: PPS i.v. (50 mg), PPS orally (1500 mg) and placebo (orally). Blood sampling was done for activated partial thromboplastin time (APTT), anti-Xa activity, hepatic triglyceride lipase, lipoprotein lipase, tissue plasminogen activator (t-PA) activity, fibrin plate lysis, total triglyceride, total cholesterol, HDL and LDL.

Results

Intravenously administered PPS significantly increased APTT, anti-Xa activity, hepatic triglyceride lipase and lipoprotein lipase compared with placebo in a magnitude comparable to other i.v. heparin-like compounds. Orally administered PPS did not significantly influence any of the parameters when compared with placebo. Point estimates for the oral bioavailability of PPS were in the range of 0% with small confidence intervals (CIs).

Conclusion

The oral bioavailability of PPS is negligible in young healthy males.

INTRODUCTION

Pentosan polysulphate sodium (PPS), a highly sulphated semi-synthetic pentose polysaccharide, is manufactured from raw materials obtained from beechwood shavings [1]. It is developed from heparin-like drugs by chemical modification, and exhibits marked anticoagulant activity, fibrinolytic effects, antilipaemic

activity, mild antihypertensive effects, antiphlogistic effects and inhibits vascular smooth muscle cell proliferation [2–7], and thus may be useful in the prevention of deep vein thrombosis, thrombotic occlusions of cerebral and retinal vessels, coronary thrombosis and transient ischaemic attacks [8–10]. The compound is registered for these indications in several countries, e.g. Germany, France, Italy, Spain, Austria and South-Africa. Contrary to other heparin-like compounds, it has been suggested that PPS is effective after oral administration [10–14].

In addition, PPS has proved to be a highly potent and selective inhibitor of human immunodeficiency virus type I replication in vitro [15, 16]. Accordingly, it is currently being investigated in the treatment of HIV-infection. And PPS was recently approved by the US Food and Drug Administration (FDA) for its action as orally active urological compound [17].

In the search for perorally active agents for improving the endogenous fibrinolytic capacity the characteristics of PPS make the drug an interesting compound to be used in the prevention and treatment of illnesses with a multifactorial aetiology such as thrombosis and atherosclerosis. However, though it is claimed that PPS can be administered orally as well as parenterally there is no unanimous opinion in the literature regarding oral dosage [18, 19].

The available evidence suggest that PPS may be erratically and poorly absorbed from the gastrointestinal tract. It has been reported that 1–40% is absorbed following oral doses (10, 16, 20–22). So far, no studies have been performed to specifically assess the bioavailability of PPS. Therefore, the present study was designed to estimate the absolute bioavailability of PPS after oral administration.

Because no specific assays are available to measure PPS directly, indirect methods were used in this study. Since prolongation of activated partial thromboplastin time (APTT) and increase of anti-Xa activity are the most prominent effects observed after parenteral administration of PPS [5, 23, 24] these parameters, as well as hepatic triglyceride lipase [25], were chosen as primary criteria. Measures of endogenous fibrinolysis [tissue plasminogen

activator (t-PA) activity and fibrin plate lysis] and parameters reflecting fat metabolism (total triglyceride, total cholesterol, HDL, LDL and lipoprotein lipase) were chosen as secondary parameters.

For this study, an intravenous dose of 50 mg PPS was chosen because it was anticipated to be safe and to show significant effects on the coagulation system and the other parameters of interest. The oral dose employed in this study was selected on the basis of the following considerations:

1. It has been suggested that the oral dose should be at least 10 times the parenteral one in order to achieve the same effect [10, 26].
2. In studies oral therapy with PPS (300 to 400mg daily, for a period of 3 to 4 months) has not been associated with alteration of coagulation parameters [27, 28].
3. Daily oral doses of 900 mg for three months were found to be safe [29].

On account of the absence of a direct method and the variability in the chosen pharmacodynamic parameters to assess the bioavailability of PPS, a high oral dose of 1500 mg PPS was selected for this study. With this dose it could be anticipated that PPS, if orally available, should exhibit effects in the order of magnitude comparable with i. v. administration.

MATERIALS AND METHODS

Subjects and Design

Eighteen non-smoking normotensive healthy males with a normal coagulation screen (age: 19–32 years, body weight: 68–90 kg) participated in this study after written informed consent was obtained. The investigational protocol was approved by the ethics committee of Leiden University Hospital. All subjects received a full medical examination at screening and post-study. The study was carried out using a randomized, three-way crossover bioavailability design. The subjects were randomized to six

sequence groups, in such order that the study was balanced for all sequences. The subjects received an intravenous bolus injection of 50 mg PPS, an oral dose of 1500 mg PPS or an oral dose of placebo. The oral medication, which was administered double-blind, consisted of ten capsules of 150 mg and was administered with 400 ml of water. Wash-out between the study days was 2 weeks.

Procedures

The subjects reported at the Centre after an overnight fast from 24.00 hours. Alcoholic beverages were not allowed from 48 h before each study day to the end of the study days, and drinks containing caffeine and strenuous activity were not allowed 24 hours before the study days until the end of each occasion.
Upon arrival, a cannula was inserted into a forearm vein for blood sample collection. When indicated, another cannula for drug administration was inserted in the contra lateral arm.
The subjects were studied in a semi-recumbent position. Blood samples for baseline values were collected just prior to drug administration. After drug administration, blood sampling and measurements were done at regular time intervals, following intravenous administration at 5, 10, 15, 30, 45, 60, 120 min and 240 min after drug administration and after oral administration at 0.5, 1, 2, 3, 4, 6 and 12 h. Four hours after drug administration subjects were offered lunch, and on the study days on which oral medication was administered the subjects were also offered dinner. Subjects left the research unit by taxi after the last blood sample had been taken, the cannula removed and after a 'safety'-determination of APTT had been performed.

Medication

All medication was supplied by Bene-Arzneimittel GmbH Munich, Germany. The following preparations were used: ampoules Fibrezym® containing 50 mg Na-PPS, capsules containing 150 mg Na-PPS and placebo-capsules (150 mg).

METHODS

Vital Signs

Blood pressure and heart rate were measured with a non-invasive oscillometric system (MPV-7201, Nihon Kohden, Amsterdam, The Netherlands)

Sampling for APTT assay, anti-factor Xa assay and fibrin plate lysis assay

Blood samples (2.7 ml) were collected into tubes containing ice cold sodium citrate (0.3 ml of 0.129 mol.l^{-1}). Tubes were centrifuged at 5000 g for 6 min at 4°C. Plasma was divided into four aliquots and subsequently stored at -35°C.

Sampling for t-PA assay

Blood samples (4.5 ml) were collected into ice-cold Stabilyte tubes (Biopool, Umeå, Sweden) containing 0.5 ml acid anticoagulant in which the blood is immediately mixed and brought to pH 6.0 in order to stabilize t-PA activity [30]. The tubes were centrifuged at 5000 g for 6 min at 4 °C. Plasma was divided into two aliquots and subsequently stored at -70 °C.

Sampling for total triglyceride, total cholesterol, HDL and LDL assay, hepatic triglyceride lipase and lipoprotein lipase assay

After overnight fasting, blood samples (4.0 ml) were collected into glass tubes containing EDTA solution. Immediately following collection these tubes were slowly tilted backwards and forwards to bring the anticoagulant into solution and immediately cooled on ice. Within 20–30 min of collection, the blood samples were centrifuged at 5000 g for 6 min at 4 °C. Plasma was divided into five aliquots and subsequently stored at -35 °C.

Laboratory tests

APTT assay, t-PA assay and anti-factor Xa activity assay APTT was measured by standard procedures on an automatic haemostasis analyser STA (Boehringer/STAGO), using cephalin with microcrystalline silica-gel as activator [31]. t-PA activity was assessed using a solid phase enzyme bio-immunoassay [32]. Anti-Xa activity was assessed using a photometric assay (Coatest®),

commonly used for the determination of (low molecular weight) heparins in plasma [33-35].

Fibrin plate lysis assay
The fibrinolytic activity of the euglobulin fraction, prepared as previously described [36], on fibrin plates was assessed according to a validated method.

Total triglyceride, total cholesterol, HDL and LDL assay
Total cholesterol was measured with the enzymatic 'CHOD-PAP' method (Monotest, Boehringer) [37, 38]. HDL concentrations were determined in supernatant of serum which was obtained by precipitating the apo-B containing lipoproteins [39]. After this the 'CHOD-PAP' method was used. Triglyceride concentrations were measured using the enzymatic 'GPO-PAP' method (Peridochrom, Boehringer) [40, 41]. After this, the LDL concentrations were calculated according to the Friedewald formula [42].

Hepatic triglyceride lipase and lipoprotein lipase assay
Hepatic lipase and lipoprotein lipase were determined by an immuno-chemical method [43].

STATISTICAL ANALYSIS

Comparison between treatments was performed on the basis of AUECs (Areas under the effect curve) calculated with trapezoidal integration and using the protocol times. For the comparison of the three treatments AUECs from 0 to 240 min were calculated. For additional comparison of the two oral treatments AUECs from 0 to 720 min were calculated. For the calculation of the AUEC for anti-Xa activity, non-detectable levels were set to 0 and for hepatic triglyceride lipase and lipoprotein lipase, negative reported values were set to 0. The AUECs were log-transformed and analysed with ANOVA with factors subject, period and treatment. The study would be considered confirmatory in the case of significant overall effects in the ANOVA (Bonferroni adjusted alpha = 0.017). Then group comparisons were performed at the same level of alpha and data are reported as difference and 98.3%

confidence interval (98.3% CI). If the confirmatory goals were not reached, results were calculated with alpha = 0.05 and judged as exploratory. Data are then reported as difference and 95% confidence interval (95% CI). Oral administration PPS was considered efficacious if APTT, anti-factor Xa activity or hepatic triglyceride lipase AUECs differed significantly from placebo, after obtaining overall treatment significance in the ANOVA. Comparison of the AUCs calculated over 720 min after the oral treatments was performed using paired Student's t-tests.
The effects after oral administration were expressed in terms of bioavailability (F) using the formula:

$$F = [(AUEC_{oral} - AUEC_{plac}) / (AUEC_{iv} - AUEC_{plac})] * (D_{iv} / D_{oral}).$$

Using this method bioavailability was calculated for the following parameters: APTT, hepatic triglyceride lipase and lipoprotein lipase. Calculations were performed using SPSS/PC + V4.0.1 (SPSS, Chicago, Ill., USA) BMDP/Dynamic V7.0 (BMDP Statistical Software, Los Angeles, Calif., USA) and SAS for Windows V6.07 (SAS Institute, Cary, N.C., USA).

RESULTS

All subjects completed the study and no major adverse events were noted. No significant abnormalities were present at the post-study screening, either clinically or in the laboratory tests.

PRIMARY CRITERIA

APTT

Intravenously administered PPS increased APTT in all subjects (Fig. 1). The maximal effect occurred shortly after drug administration (T_{max} 5 min). The maximal APTT was 106 s (range 82–163 s). At 4 h after administration the effect had subsided and returned to pre-dose values. When expressed as the AUC over the first 4 h after administration, a highly significant difference of 44% (98.3% CI: 33%, 55%) between i.v. PPS and oral placebo was noted. The difference between i.v. and oral PPS was 47% (98.3% CI:

36%, 59%). After oral PPS the AUC for APTT over 4 h and 12 h was 2% lower compared with placebo (98.3% CI: -10%, +6%; for the $AUC_{0\text{-}240\ min}$). The point estimate for the oral bioavailability using the APTT as parameter was -0.1% (95% CI: -0.5%, +0.3%).

Anti-Xa activity
Following i.v. administration of PPS, anti-Xa activity increased rapidly (T_{max} 5 min) to a mean peak level of 0.17 $U.ml^{-1}$ (range: 0.11 - 0.26 $U.ml^{-1}$), and had a plateau around that level until t = 45 min, after which it declined (Fig. 2). Pre-dose values were reached at approximately 4 h after administration. Anti-Xa activity could not be detected following oral administration of PPS or placebo; therefore calculation of bioavailability was unsuccesful.

Hepatic triglyceride lipase
Peak levels of plasma hepatic triglyceride lipase (mean 376 $U.l^{-1}$; range: 60 - 550 $U.l^{-1}$) were reached after approximately 15 min following i.v. PPS (Fig. 3). This level returned to baseline approximately 4 h after administration. No changes in the plasma hepatic triglyceride lipase could be detected following oral administration of PPS or placebo. Intravenous administration of PPS resulted in 87-fold (98.3% CI: 52, 145-fold) higher AUC compared with placebo and a 69-fold (98.3% CI: 41, 114-fold) higher AUC compared with oral PPS. The calculated point estimate for the oral bioavailability of PPS using hepatic triglyceride lipase levels was 0% (95% CI: -0.01%, +0.03%).

SECONDARY CRITERIA

Lipoprotein lipase
Lipoprotein lipase levels increased after i.v. administration of PPS. Maximal levels were on average 105 $U.l^{-1}$ (range: 55–225 $U.l^{-1}$). Baseline values were reached at 4 h after injection. No changes in plasma lipoprotein lipase concentration could be measured after oral administration of PPS or placebo. Bioavailability was estimated to be 0% (95% CI: -0.1%,+0.1%).

t-PA activity and fibrin plate lysis

Compared with oral administration of PPS or placebo, AUC values for t-PA activity after intravenous PPS were 12% (95% CI: 0%, 26%) and 15% (95% CI: 3%, 29%) higher, respectively. Following oral PPS t-PA activity was 3% (95% CI: -8, +15%) higher compared with placebo. The results obtained with the fibrin plate lysis showed a similar trend; however, the differences between the treatments did not reach statistical significance.

Total triglyceride, cholesterol, HDL and LDL

Total triglyceride plasma level was slightly lower after all three treatments (Fig. 4); however, this was most marked after i.v. administration. The decrease in AUC between i.v. and oral administration reached statistical significance for the comparison between i.v. PPS and placebo only: mean AUC was 14% (95% CI: 2%, 24%) lower. The plasma concentration-time profiles for total triglycerides were identical after oral PPS and placebo (Fig. 4). None of the treatments significantly altered the plasma concentrations of total cholesterol, HDL or LDL. The outcome of the pharmacodynamic parameters is also summarized in Tables 1 and 2.

DISCUSSION AND CONCLUSIONS

Since heparins are active only after parenteral administration, an orally applicable anti-thrombotic drug which concomitantly increases endogenous fibrinolytic parameters and exerts favourable effects on fatty acid metabolism would be a great asset to the therapeutic armamentarium; e.g. it would have huge potential in the prophylactic treatment of patients at risk of atherosclerosis. PPS (formerly called SP54) was supposed to be such a drug. However, conflicting data are available regarding the oral bioavailability of the compound [12–14, 18–22, 44].

The objective of this study was to assess the absolute bioavailability of PPS after oral administration. Since specific assays for PPS do not exist, the bioavailability could only be assessed using pharmacodynamic measures. To ascertain a valid study design we also decided to compare the effects of i.v. PPS

and oral placebo. Secondly, a relatively high oral dose of PPS was selected to ensure that any effect occurring could be detected. PPS in doses up to 100 mg i.v. has been administered without any significant adverse effects [5, 23, 26, 45]; a dose of 50 mg i.v. was therefore chosen for this study, because it could be assumed to be safe and to show significant effects on the coagulation system and the other parameters of interest. The most prominent effect parameters known from previous experiments were chosen as primary criteria for the evaluation of bioavailability [5, 23–25]. Measures of endogenous fibrinolysis (t-PA activity and fibrin plate lysis) and parameters reflecting the fat metabolism (total triglyceride, total cholesterol, HDL, LDL and lipoprotein lipase) were chosen as secondary parameters.

This study showed that intravenously administered PPS changed APTT, anti-factor Xa activity, hepatic triglyceride lipase and the secondary criteria in an expected way comparable to other heparin-like compounds; it significantly increased APTT, anti-factor Xa activity, hepatic triglyceride lipase and lipoprotein lipase and showed marginal effects on t-PA activity, fibrin plate lysis, serum triglycerides and cholesterol. Oral administration of PPS resulted in very insignificant effects at most. These effects were not significantly different from placebo. When the effects after oral administration were expressed in conventional terms of bioavailability, it was shown that the oral bioavailability using the different parameters varied between -0.1% and 0.1%, even with the high oral dose of PPS. Probably even more significantly oral PPS did not differ from placebo in any respect. These findings can be explained in several ways. Firstly, estimation of 'bioavailability' using effect parameters can be less reliable because of the higher variability in comparison with ‘normal’ pharmacokinetic variability. Secondly, PPS absorption after oral administration may be so poor that no active drug reaches the systemic circulation. Using the data obtained in this study it can be calculated that the confidence intervals for the point estimations of the oral bioavailability were over a reasonably small range including zero. It is therefore highly unlikely that the variability in the parameters used to estimate the bioavailability

of oral PPS accounts for the lack of PPS effects in comparison with placebo. This strengthens the arguments in favour of the second explanation: PPS has a very low bioavailability either because of poor oral absorption or extensive pre-systemic breakdown. Thus the oral bioavailability of PPS, which was calculated reasonably precisely, was found to be negligible in young healthy males. This shows that PPS resembles other heparins, which all show significant effects when given parenterally but are virtually inactive after oral administration. These findings may have important implications for the rational prescription of PPS.

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TABLE 1 **Summary of pharmacodynamic parameters**

Mean area under the effect curve (with SD) for the first 4 h following drug administration] obtained after i.v. and oral administration of PPS in comparison with placebo.

	PPS (50 mg iv)	PPS (1500 mg po)	PLACEBO (po)
PRIMARY PARAMETERS			
APTT	13675 (2727)	9219 (1140)	9472 (1443)
Anti-Xa activity	19.5 (11.8)	NDL*	NDL*
HTL	50477 (18418)	779 (490)	608 (284)
SECONDARY PARAMETERS			
LPL	11704 (4759)	335 (326)	271 (209)
t-PA activity	108 (39)	96 (33)	93 (32)
Fibrin plate lysis	2847 (1688)	2408 (1642)	2102 (1318)
Triglyceride	156 (41)	171 (50)	182 (52)
Total cholesterol	901 (192)	932 (172)	910 (181)
HDL	237 (38)	243 (43)	238 (38)
LDL	592 (178)	617 (163)	590 (157)

NDL*: non-detectable levels

TABLE 2 **Summary of pharmacodynamic parameters**

Mean Area Under the Curve (with SD) for the first 12 hrs following drug administration] obtained after oral administration of PPS in comparison with placebo.

	PPS (150o mg po)	PLACEBO (po)
PRIMARY PARAMETERS		
APTT	27500 (3388)	27836 (3865)
Anti-Xa activity	NDL*	NDL*
HTL	2457 (1498)	1723 (983)
SECONDARY PARAMETERS		
LPL	916 (973)	766 (705)
t-PA activity	351 (100)	347 (101)
Fibrin plate lysis	11032 (5772)	10066 (5581)
Triglyceride	555 (161)	587 (174)
Total cholesterol	2811 (509)	2764 (542)
HDL	722 (134)	714 (120)
LDL	1844 (482)	1784 (465)

NDL*: non-detectable levels

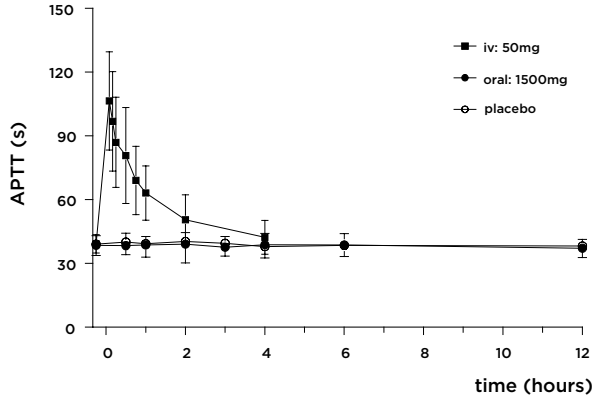

FIGURE 1 **APTT-values [mean with (SD); n=18] after i.v. PPS (pentosan polysulphate), oral PPS and oral placebo.**
No changes in APTT could be detected following oral PPS and placebo.

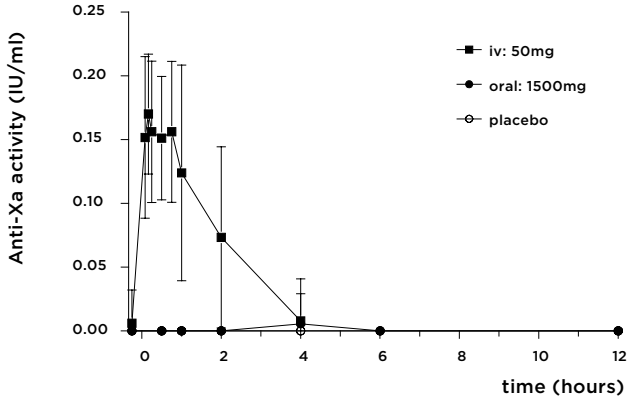

FIGURE 2 **Anti-Xa activity [mean with (SD); n=18] following i.v. and oral PPS (pentosan polysuphate) and oral placebo.**
Anti-Xa activity after both oral treatments barely exceeded base-line values.

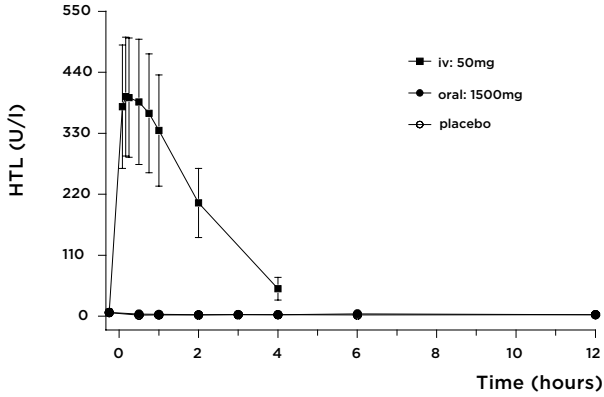

FIGURE 3 **Plasma hepatic triglyceride lipase (HTL) concentration [mean with (SD); n=18) following i.v. PPS (pentosan polysulphate), oral PPS and oral placebo.**

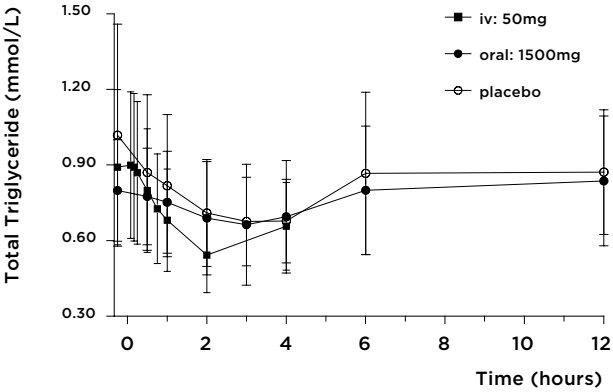

FIGURE 4 **Plasma concentration total triglyceride [mean with (SD); n=18] following i.v. PPS (pentosan polysulphate), oral PPS and oral placebo.**

The decrease between i.v. and oral administration reached statistical significance for the comparison between i.v. PPS and placebo.

CHAPTER 13

THE ABSOLUTE BIOAVAILABILITY AND TOLERABILITY OF SUBLINGUAL SYNTHETIC LONG ACTING PENTASACCHARIDE IDRAPARINUX SODIUM

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ABSTRACT

Objective

To investigate the tolerability, acceptance and the absolute bioavailability of a sublingual (SL) formulation of idraparinux sodium, a long-acting pentasaccharide anticoagulant.

Methods

Six (6) male subjects participated in this study. Each subject was given 9 mg idraparinux once, either SL or intravenous (IV). Blood samples for drug assay were taken up to 264h after dosing, sampling for APTT was done until 60 h. Local tolerability/ acceptance was investigated by regular inspection of the oral cavity and the subjects filled in visual analogue scales (VAS) over the first 24 h period after drug administration.

The pharmacokinetic parameters were estimated by non-compartmental and compartmental methods(using non-linear mixed effect modelling).

Results

Initially this study was planned as a cross-over design. However, following the first study occasion preliminary analyses revealed that the SL bioavailability of idraparinux was very low, and further investigation was not considered sensible. The analysis of this study comprises the pharmacokinetic analysis and the tolerability of the SL formulation of three subjects dosed SL and three subjects dosed IV. The results of the IV administration showed similar characteristics to previous studies. The compound showed a SL bioavailability of approximately 3.5%. Both curves (IV and SL) could be described using similar pharmacokinetic parameters. No obvious effects on oral tolerability were reported by the subjects or detected by the investigator.

Conclusions

Pharmacokinetic results found after IV administration of 9 mg idraparinux were comparable to previous studies. Idraparinux in the current formulation has a SL bioavailability of approximately 3.5%.

INTRODUCTION

Idraparinux is a fully chemically synthesised antithrombotic pentasaccharide. It works by activation of antithrombin (AT), a naturally occurring inhibitor of the blood clotting process. Unlike heparin, the compound selectively inhibits factor Xa because of its molecular size, too small to capture other coagulation enzymes like thrombin and accelerate inhibition by AT [1]. Apart from treatment of venous thromboembolism (VTE), patients need medication for secondary prophylaxis of recurrence of thromboembolic events. Such secondary prophylaxis ideally requires a drug with an extended elimination half-life, allowing a low dosing frequency. Idraparinux could be such a compound, as it is eliminated with a half-life of approximately 130 hours [2,3]. An alternative to injection of idraparinux could improve the tolerability and convenience of such a treatment. Pre-clinical experiments in dogs indicated that it was possible to deliver the drug to the systemic circulation via the sublingual (SL) route using formulations with sodium-taurocholate as absorption enhancer. The bioavailability was approximately 4% (data on file; NV Organon). The current study was performed to investigate the SL bioavailability of idraparinux in humans and to study the (local) tolerability and acceptability of the formulation.

METHODS

Subjects and Design

Six (6) healthy male subjects (age: 20-28 yr., weight: 61-77 kg) with a normal coagulation status participated in this single center study after written informed consent was obtained. The Ethics Committee of Leiden University Medical Center approved the investigational protocol. The study was initially planned according to a randomised, double blind cross-over design, in which each subject was dosed 9 mg idraparinux twice with a washout period of at least 8 weeks. However, analyses after the first study days showed that the SL bioavailability was so low that further investigation was not considered worthwhile. The study was discontinued and as a result, the data presented only comprises the pharmacokinetics and the tolerability of three subjects of 3 SL and 3 IV idraparinux administrations.

Medication

The medication for IV administration was provided as pre-filled syringes with either undiluted idraparinux (at a concentration of 3.80 mg/mL) or 0.9% saline. The volume of the syringes was just sufficient to enable the investigator to administer the required dose. The tablet for SL administration contained 9 mg idraparinux, 6.0 mg gelatin, 4.5 mg mannitol and 3 mg sodium-taurocholate, whereas the placebo formulation consisted of 13.5 mg gelatin and 10.1 mg mannitol and had an identical volume (150 µL). The SL placebo was therefore an identical formulation containing the inert substances but without active drug and without absorption enhancer.

Procedures

Subjects were studied after an overnight fast. Upon arrival at the research-unit a short medical history was taken and a physical examination was done to ensure compliance to the study protocol restrictions. Then the subjects emptied their bladder and an IV cannula for blood sampling was inserted in a forearm vein.
At zero time 9 mg idraparinux was administered either SL (together with a placebo IV injection) or IV (with a SL placebo tablet). The subjects remained in the unit for at least 60 hours post-dosing.
One investigator performed regular inspection of the oral cavity and the sublingual region using a standardised scoring sheet to document possible anatomical and/or pathological changes. Possible findings were also scored with regard to severity and location (on anatomical sketches). The subjects scored their experience with the SL tablets over the first 24 hours post-dosing using visual analogue scales (VAS). These were designed to evaluate changes within the oral cavity, taste of the formulation and their general experience.

Blood Sampling

No tourniquet was applied when blood was collected during the study period. At the research-unit an IV cannula used for blood sampling was kept patent by intermittent flushing with 0.9%

saline. Blood was taken after discarding the contents of the cannula.

Blood samples for drug-assay were drawn frequently until 264 h after administration of idraparinux. The Department of Drug Metabolism and Kinetics of Organon Development GmbH (Waltrop, Germany) performed the assessment of drug-concentrations using validated assays. The method is based on the inhibition of factor Xa by the AT/idraparinux-complex. A known, excessive amount of factor Xa is added to each undiluted plasma-sample followed by the addition of the chromogenic substrate S-2222. Unbound factor Xa induces hydrolysis of this substrate. The amount of hydrolysed substrate is measured by a spectrophotometer and is inversely proportional to the idraparinux-concentration. To screen the performance of the assay, each series of analyses was checked using quality control samples. The lower limit of quantification was 8 ng/mL.

Statistical analysis

Individual graphs were generated for the subject rated VAS results; these were visually inspected and no obvious abnormalities were found. No summary statistics or comparisons between groups were attempted because of the low number of measurements.

Non-compartmental pharmacokinetic analysis was performed using the software package WinNonlin V2.1 (Pharsight Corp., USA). Data points for calculation of terminal half-lives were determined by the program and accepted after visual inspection. Initial measurements for the intravenous data were back extrapolated to the y-axis (by the program). Sublingual data were analysed in two different ways: first by ignoring non-zero pre-values, while for the second analysis, average pre-values were calculated and subtracted from subsequent measurements. Non-compartmental pharmacokinetics was determined by calculating AUC using the linear trapezoidal method, terminal half-life, extrapolated AUC ($AUC_{0-\infty}$), Cl/F, Vz/F and V_{ss} (IV only). The absolute bioavailability after SL administration of idraparinux was calculated as the ratio of the average of the AUC values

obtained after SL administration over the average of the AUC values after IV administration.
Additionally, the pharmacokinetics of idraparinux after IV and SL administration was analysed using non-linear mixed effect modelling as implemented in NONMEM (Version V; NONMEM Project Group, UCSF, San Francisco, CA). First order conditional estimation was used with the 'interaction' option, assuming multiplicative inter- and intra-subject error distributions. A two-compartment model was used with first order absorption and a bioavailability fraction for the SL data. Presence of a constant (within an individual) endogenous level was assumed, due to low endogenous anti-Xa activity. This level was added to the pharmacokinetic equations and estimated [3].
Statistical analysis and calculations were performed using SPSS for Windows (SPSS, Inc., Chicago, IL, USA).

RESULTS

No clinically relevant adverse events were noted. The treatments were well tolerated and the tablets for sublingual dosing dissolved immediately after administration (within 0.5 minute). No changes within the oral cavity or the sublingual region were observed during the course of the study. The subjects reported minor differences between the active and the SL placebo treatment.

Pharmacokinetics

The mean plasma concentration profile of idraparinux is presented in Figure 1. Table 1 summarises the non-compartmental pharmacokinetic estimates. Absolute bioavailability of SL idraparinux calculated using $AUC_{0-\infty}$ ignoring (non-zero) pre-values is 7.2%. Calculation using $AUC_{0-\infty}$ after pre-value subtraction resulted in a bioavailability of 3.5%.
In the additional non-linear mixed effect modelling, initial analyses indicated that inter-individual variability could not be estimated for some pharmacokinetic parameters. This may be due to insufficient information or low inter-individual variability. In order to circumvent computational problems, these variability

estimates were fixed at zero. Subsequently, this pharmacokinetic analysis revealed that both the IV and SL curves could be described using similar values for the common pharmacokinetic parameters. Two different parameterisations were investigated; the results are summarised in Table 2. With this approach, bioavailability was estimated to be approximately 2% with high inter-individual variability (coefficient of variation of 37%).

DISCUSSION

The objective of this study was to investigate the tolerability, acceptance and absolute bioavailability of SL administered idraparinux in humans. In dogs, the bioavailability after SL administration was approximately 4%, which although low, warranted further investigation in humans. In the dog the tablets dissolved within 1-2 minutes, and it could not be excluded that the dogs had swallowed part of the tablets. Because SL tablets are easier to administer to humans, the expectation was that this could result in a higher SL bioavailability. However, the SL bioavailability of idraparinux was only 3.5%.

Initial non-compartmental pharmacokinetic analysis following SL administration resulted in a much longer elimination half-life than after IV administration (figure 1), as well as a T_{max} value of approximately 18 hours. This T_{max}-value might be due to a delayed delivery from a compoartment in which the drug is initially stored after absorption. Non-compartmental pharmacokinetic analysis following the SL administration was performed after subtraction of the pre-values as well. These pre-values were possibly due to low endogenous anti-Xa activity levels or difficulties with the assay (a 'matrix effect'), as these values are only slightly higher than the lower limit of quantification of the assay. This additional analysis resulted in a mean value for the elimination half-life similar to the IV results and compatible with earlier studies reported in this thesis.

Additional model-dependent pharmacokinetic analysis was performed in order to investigate whether the low-level error could account for the apparent difference in terminal half-life between SL and IV administration. By using nonlinear mixed

effect modelling more stable parameter estimates could be obtained than by using regular individual based non-linear regression [4]. It also allowed estimation of inter-individual variability in bioavailability. Analysis using a two-compartment model with first order absorption assuming a constant endogenous (or assay) level demonstrated that both the IV and SL curves could be described using similar values for the common pharmacokinetic parameters.

Both compartmental and non-compartmental (after subtraction of pre-values) pharmacokinetic analyses resulted in more or less similar conclusions, indicating that no serious model-misspecification existed in the model-dependent analysis. Because the SL and the IV curves could be described in a single analysis, these data are presented with regard to bioavailability. The compartmental analysis, which took into account the 'matrix-effect' of the samples with very low anti-Xa activity, also resulted in a terminal half-life comparable to the previous studies.

In order to obtain a first impression of the tolerability of the SL-formulation, the oral cavity and the sublingual region was inspected regularly. The subjects also rated the local tolerability/acceptance of the SL-tablet by VAS-scores. Although no apparent effects were seen during the course of the study, the subjects reported some minor abnormal feelings in the oral cavity. This might be due to the taurocholate in the active tablet. Based on these findings, in combination with the negative aspects in the safety profile of bile acids [5], the option of increasing the quantity of taurocholate in the active SL idraparinux sodium tablet in order to increase the SL bioavailability is excluded. Increasing the amount of idraparinux in the SL-formulation might lead to pharmaceutical difficulties (the tablet should contain a high dose and should also be administered SL).

The poor SL bioavailability with the high variability of the current formulation might preclude further investigations. Possible options to be followed may be the use of another absorption-enhancer as cyclodextrine or the development of a (sustained-release) buccal patch. Another alternative could be to adjust the dosing frequency. In order to compete with the

current oral anticoagulants, a compound should give a stable state of anticoagulation within 5 to 7 days. Using the pharmacokinetic model, and assuming constant pharmacokinetic parameters, the idraparinux time profiles for the three SL subjects were predicted after a once-daily dose-regimen (Figure 2). This figure indicates that a multiple dose-regimen might be an option although an actual multiple dose SL study must be performed to assess within and between subject variability in concentration profile. In conclusion, in its current form and dosing regimen, SL administration of idraparinux is not feasible but enhancements may be possible.

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TABLE 1 Mean (SD) pharmacokinetic parameters for idraparinux (non-compartmental PK-analysis)

	INTRAVENOUS		SUBLINGUAL; Ignoring pre-values		SUBLINGUAL; pre-values subtracted	
	Mean	SD	Mean	SD	Mean	SD
Lag time (min)			30	30	46	15
T_{max} (hr)	0.17	0.29	18.0	10.4	18.0	10.4
C_{max} (ng·mL^{-1})	2019	390	31.2	11.3	24.2	7.9
$AUC_{0\text{-last}}$ (mg·L^{-1}·hr)	116	14	5.5	1.6	3.5	0.6
Terminal half life (hr)	107	13	229	26	128	79
% extrapolated	13.9	2.9	42.4	4.5	22.3	17.7
$AUC_{0\text{-infinity}}$ (mg·L^{-1}·hr)	135	19	9.7	3.6	4.7	1.7
Cl/F (mL·min-1)	1.12	0.17	17.0	6.2	35.2	14.3
V_z/F (L)	10.4	0.9	327	87	327	103
V_{ss} (L)	9.5	0.8				

Bioavailability using $AUC_{0\text{-infinity}}$ ignoring (non-zero) pre-values: 7.2%
Bioavailability using $AUC_{0\text{-infinity}}$ after pre-value subtraction: 3.5%

TABLE 2 Model dependent pharmacokinetic analysis

	MEAN	SEM	CV (%)
CL (mL/min)	1.09	0.0762	14
Intercompartmental CL (mL/min)	4.80	0.488	0 (fixed)
Vc (L)	5.02	0.353	10
Vss (L)	9.68	0.278	2.3
Absorption half-life (hr)	7.87	2.35	35
Bioavailability (%)	2.16	0.451	36
Endogenous level (ng/mL)	8.81	1.30	17
Initial half-life (t_{5a}) (hr)*	5.44	0.488	0 (fixed)
Terminal half-life (t_{5b}) (hr)*	102	4.27	2.8
Residual variability (error)10%			

Mean: population average, SEM: approximate standard error of population average;
CV: coefficient of variation of inter-individual variability in population parameters;
V_c: central volume; V_{ss}: steady-state volume. *second parameterisation

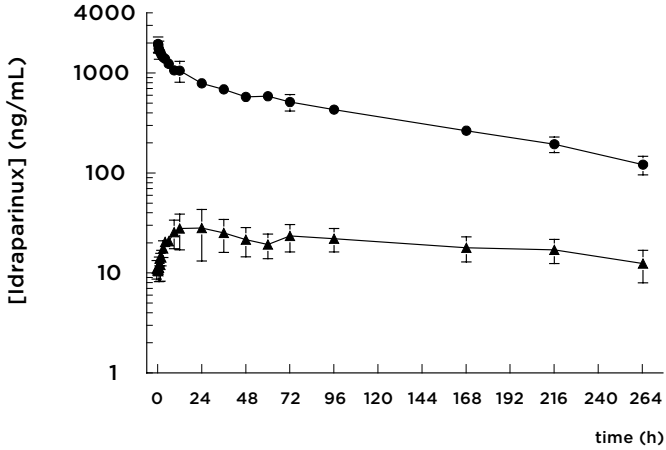

FIGURE 1 Mean (SD) concentration time profile of idraparinux after administration of 9 mg intravenously (●) or sublingually (▲).

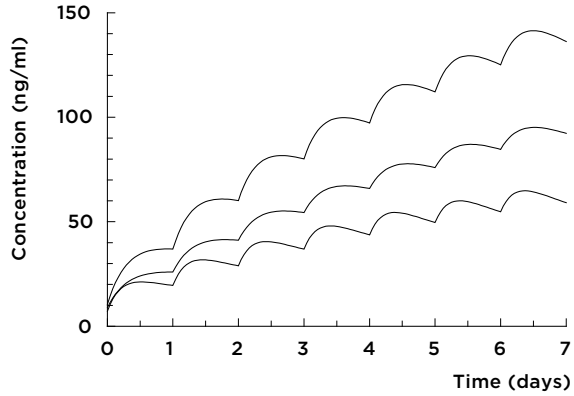

FIGURE 2 Predicted idraparinux time profiles using empirical Bayesian estimates for the three SL subjects in a once-daily dose-regimen.

CHAPTER 14

FIRST ADMINISTRATION OF AN ORALLY ACTIVE SYNTHETIC SPECIFIC FACTOR XA INHIBITOR YM 466IN HEALTHY MALE VOLUNTEERS

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ABSTRACT

Objective

YM 466 is a novel synthetic direct-acting selective coagulation factor Xa inhibitor, which is orally active in animals. This Phase I study was conducted to investigate the safety, tolerability, pharmacokinetics and pharmacodynamics of single doses of the compound.

Methods

The study was performed in healthy young male volunteers (aged: 19–44 yr., normal weight for height) without bleeding disorders. The study consisted of two parts. The first part was performed using a double blind, rising dose design in two panels of 9 subjects, which participated in alternating fashion. Per dose level the randomisation resulted in 6 subjects receiving active drug and 3 subjects placebo. Each subject received two escalating doses of oral YM 466 and a randomly allocated placebo dose. One panel was dosed with 10, 50 and 200mg YM 466, and the other panel received 20, 100 and 300mg of the compound. Washout between the study occasions was at least 14 days.

In a subsequent study, the absolute oral bioavailability of YM 466 and the effect of food on this bioavailability were investigated. This study was performed as an open randomised balanced single dose three-period crossover study with a Latin-square design in 12 subjects. The volunteers received a single 1-hour intravenous (iv) infusion of the compound at a dose of 5mg, while oral doses of 100mg were administered immediately after a standardised breakfast or under fasted conditions. Washout between the study days was at least 7 days.

Blood samples for drug-concentration, anti-Xa activity, APTT and PT was taken. Urine was collected in fractionated portions. Safety measurements (blood samples for routine determinations, urine samples, blood pressure, and 12-lead ECG recordings) were made regularly.

Results
YM 466 was well tolerated after both oral and iv administration. The most frequently reported adverse events included development of hematomas at puncture sites and flatulence. The compound was active following oral administration, but the bioavailability of YM 466 is low (about 2.5%). The elimination half-life of the compound was about 13 hours. The pharmacokinetic results showed a relative high inter-individual variability, and C_{max} as well as AUCs showed a disproportionate increase following dose-increases. The oral bioavailability was markedly reduced by concomitant food-intake to only about 26% of the fasted state. Dose-related post-dose increases of APTT and PT were observed. Maximum observed increases were 12 seconds for the APTT and 5 seconds for the PT, respectively, after the 300mg dose.

Conclusions
YM 466 may be a potential orally applicable antithrombotic drug. However, its oral bioavailability is low, further reduced by food intake, and shows substantial interindividual variability. Conventional coagulation tests (APTT, PT) should be monitored.

INTRODUCTION

Despite an enormously increased knowledge of the blood coagulation mechanism and many advances in therapy, treatment of venous thromboembolism (VTE) is still far from optimal and hampered by the disadvantages of the current antithrombotic therapies compromising safety and efficacy [1]. In the search for new compounds, as an approach to anticoagulation, thrombin has become a major target, because of its central role in thrombogenesis [2]. However, controlling thrombin generation by inhibition of the serine protease factor Xa represents an alternative to inhibition of thrombin. In theory, this should eliminate the continued production of thrombin by either extrinsic or intrinsic pathways without interfering with a basal level of thrombin activity necessary for 'normal' coagulation and would provide a slower more regulated control with minimal bleeding risk because some clot formation is still possible under

treatment. In addition, the thrombin catalysed activation of the protein C-pathway is still possible [3,4]. This has led to the development of many antithrombotic compounds which inactivate factor Xa, either indirectly via the potentiation of endogenous anticoagulant mechanisms [5] or directly [6].
YM 466 is a novel synthetic direct-acting selective inhibitor of factor Xa (Figure 1) supposed to be active after oral administration [7]. This report entails the first administration of YM 466 to humans and was aimed to investigate the safety, tolerability, pharmacokinetics and pharmacodynamics of single rising oral doses of YM 466 in healthy young male volunteers. In addition, the absolute oral bioavailability of the compound and the effect of food on this bioavailability were evaluated.

METHODS

Subjects

In total, 32 healthy male subjects (aged 19–44 yr., normal weight for height) participated in the study. This study was conducted according to the principles of the 'Declaration of Helsinki' and performed under GCP-guidelines. All subjects gave written informed consent before any screening procedures were performed. The protocol was approved by the Ethics Committee of Leiden University Medical Center.
Two (2) subjects participated in an open non-randomised pilot, which was performed to evaluate a single intravenous (iv) dose of the compound (5mg), administered as a 1-hour infusion.
The main study consisted of two parts. The first part was conducted according to a double blind, placebo-controlled, randomised study design in which two panels of subjects received single oral doses of YM 466. The randomisation resulted in 6 subjects receiving YM 466 and 3 placebo per dose level. Each subject received two rising doses of the compound and a randomly allocated placebo dose. Panels were dosed in an alternating fashion, in which one panel received 10, 50, and 200mg, while the other panel was administered 20, 100 and 300mg. YM 466 and placebo were supplied as matching capsules. Safety and tolerability were assessed during each dosing period and dose

escalation only proceeded after data combined with the pharmacodynamic results were regarded safe. Washout between study-occasions was at least 14 days. The second part of the study was performed in 12 subjects to explore the absolute bioavailability of YM 466 and the effect of food on the bioavailability. This part was conducted using an open randomised balanced three-period crossover with a Latin-square design. The compound was supplied as an iv infusion and capsules. Intravenous YM 466 was administered as a single 1-hour infusion at a dose of 5mg (in 250mL saline). The oral dose was 100mg immediately after a standardised breakfast or under fasted conditions, taken with 200 mL of water

Study Days

Subjects were studied after an overnight fast. In the morning a cannula for blood sampling was inserted in a suitable forearm vein. In case of the iv infusion, a second cannula was inserted in the contralateral arm. After pre-dose sampling and measurements the subjects received YM 466 or placebo under fasted conditions. In case of the administration after the meal, the subjects first had a high-fat breakfast (300mL of milk, 1 glass of orange juice, 2 slices of wheat bread, 15 g of margarine, 2 fried eggs, 1 slice of cheese and 1 slice of ham), and oral YM 466 was administered within 5 minutes of finishing breakfast. During the entire study period of 48 hours blood samples for YM 466 concentration and anti-Xa activity were collected frequently. Sampling for APTT and PT was done at lower frequency. Urine was collected in fractionated portions in pre-weighed plastic containers over the following time intervals: 0–4h, 4–8h, 8–12h, 12–24h and 24–48h. Blood samples for routine determinations (chemistry, haematology, coagulation status, urine samples (dipstick urinalysis), bleeding time measurements, blood pressure measurements and 12-lead ECG recordings were made at regular time-intervals. Subjects were allowed to leave the unit at 48 hours after drug administration. Washout periods of at least 14 days (first part) or 7 days (second part) separated the study-days. Subjects returned for a post-study visit within two weeks after the last study-period.

Sampling

No tourniquet was applied when blood was collected during the study days. The iv cannula used for blood sampling was kept patent by intermittent flushing with 0.9% saline. Blood was taken after discarding the contents of the cannula. Blood samples for base-line values were taken pre-dose. A pre-dose urine-sample was collected immediately before dosing.

To gain more insight in the mechanism of action of the compound, anti-IIa activity, tissue factor pathway inhibitor (TFPI), Fragment 1+2 (F1+2) and thrombin-antithrombin (TAT) complex levels were assessed as additional parameters in the first part of the study.

Pharmacokinetic Assessments

The analysis of YM 466 concentration in plasma and urine was performed at the Bioanalysis and Drug Metabolism Department of Yamanouchi Europe BV using a validated bioanalytical method. Briefly, the compound was extracted from the matrix by solid phase extraction on an Oasis column. After washing the column, YM 466 was diluted with methanol and the eluate was evaporated to dryness. The residue was reconstituted with 0.1% formic acid. An aliquot of the sample was injected into a HPLC-MS/MS system. Separation was achieved on a Symmetry C17 column and the drug was detected by a triple stage quadrupole mass spectrometer. For plasma the lower limit of quantification was 0.1 ng/mL, and for urine 1.0 ng/mL. Concentrations were expressed as the free base form of YM 466.

Pharmacodynamics Assessments

Anti-factor Xa activity was assessed using a validated photometric assay (Coatest LMW Heparin (Chromogenix, Mölndal, Sweden). As calibrator a sample of the corresponding dose of YM 466 was used. The activated partial thromplastin time (APTT) and prothrombin time (PT) assay were determined, in duplicate, in citrated plasma on a STA coagulation analyser (Boehringer Mannheim, Diagnostica Stago). The reagents were STA APTT (Boehringer Mannheim) and Thromborel S (sensitive human placental thromboplastin, ISI ≈1; Behring Diagnostics, Marburg,

Germany). Inter and intra assay precision (CV) for the PT and APPT was smaller than 5%.

Additional Measurements

Anti-factor IIa activity was assessed using a validated photometric assay with thrombin substrate H-D-HHT-L-Ala-L-Arg-pNa.AcOH and is based on the inhibition of thrombin activity (Spectrolyse® Heparin anti-IIa, Biopool, Umea, Sweden). A sample of the corresponding dose of YM 466 was used as calibrator. Tissue factor pathway inhibitor (TFPI) antigen levels were determined with an enzyme-linked sandwich immunoassay using the IMUBIND® Total TFPI Elisa kit (American Diagnostica Inc., Greenwich, USA). The quantative determination of human prothrombin Fragment 1+2 were performed using an enzyme immunoassay (Enzygnost® F1+2 micro (Behringwerke, Marburg, Germany)). The thrombin-antithrombin (TAT) complex levels were assessed using a validated assay (Enzygnost TAT (Dade Behring).
These assays were performed using a STA coagulation analyser (Boehringer Mannheim, Diagnostica Stago). All coagulation assays were performed at the TNO-Gaubius Laboratories, Leiden, The Netherlands.

Statistical Analysis

Non-compartmental pharmacokinetic analysis of the plasma concentration data was performed using WinNonlin version 1.5 (Scientific Consulting, Inc., Apex, NC, USA). The maximum concentration (C_{max}), the time to reach this maximum concentration (T_{max}), the area under the concentration curve from timepoint of administration up to the last quantifiable sample ($AUC_{0\text{-}last}$) using the linear-logarithmic trapezoidal rule, the area under the concentration curve extrapolated to infinity ($AUC_{0\text{-}\infty}$) and the terminal elimination half-life were calculated.
Urinary data were used to calculate the amount excreted unchanged in the urine from 0-48h ($Ae_{0\text{-}48h}$), the percentage of the dose excreted unchanged in the urine from 0-48h (% $Ae_{0\text{-}48h}$), and the renal clearance (CL_{renal}).
Using a fixed effects model that accounted for effects of different

subjects dose-proportionality was tested on AUC and C_{max}, as well as the effect of dose on the renal clearance of YM 466. In addition, the following parameters were calculated for the second part of the study: clearance (CL), volume of distribution during the terminal phase of plasma concentration vs. time profile (V_z) and at steady-state (V_{ss}) for the iv administration, and apparent clearance (CL/F) and apparent volume of distribution during the terminal phase of plasma concentration vs. time profile (V_z/F) for the oral administrations.
For the pharmacodynamic measures (anti-Xa activity, APTT and PT) the baseline-corrected Area under the Effect Curve (AUEC) was calculated from the zero timepoint (timepoint of administration) up to the last sampling point using the linear trapezoidal rule. The maximum values for these parameters were derived directly from the individual data.
Descriptive analyses were performed on the parameters to assess safety and tolerability data.

RESULTS

General

YM 466 was well tolerated after single oral administration up to 300mg, or 5mg iv to healthy young male volunteers. None of the abnormalities in the assessed safety indices was of clinical relevance. The most frequently reported adverse events included haematomas (mainly at puncture sites), flu and flatulence. The incidence of adverse events did not differ between the different treatments. No clinically significant effects were observed on the safety and laboratory measurements.

Pharmacokinetics

First part

The plasma concentration curves after the respective doses in this part of the study are displayed in Figure 2. A summary of the pharmacokinetic parameters is given in Table 1. Following the oral administrations YM 466 was rapidly absorbed with peak plasma concentrations between 1.2 and 2.8h after drug intake.
The maximum concentrations ranged from 2.8ng/mL (SD: 1.9) to

166.6ng/mL (SD: 66.0) over the dose range studied. The average observed terminal half-life was increased at the lower doses, to stabilise at a value of about 12.5 hours for the doses of 100mg and higher. Dose increases led to a greater than proportional increase in the expected exposure/peak: doubling the dose led to an approximate 2.25 times increase in $AUC_{0\text{-last}}$ (95%CI: 2.06-2.46) and approximately 2.17 increase in C_{max} (95%CI: 1.96-2.40). This was confirmed by the fixed effects model for dose-proportionality (p-values for the test were 0.0136, and 0.1016, respectively, for $AUC_{0\text{-last}}$, and C_{max}).
A summary of the result of the urinary parameters is shown in Table 1. This shows that renal clearance is dose-independent, which was confirmed by the result of the fixed effects model (point estimate of the slope of the curve of dose vs. renal clearance was -0.04 (95% CI: -0.15, 0.38; p=0.3890).

Second part

A summary of the pharmacokinetic results of this part of the study can be found in Table 2 and the plasma concentration curves are shown in Figure 3. Following the 5mg iv infusion the peak plasma concentrations averaged 118.9ng/mL (SD: 29.9).
The estimated mean terminal elimination half-life was 14.7h.
After oral administration with or without food, C_{max}-values were 6.4ng/mL (SD: 1.7) and 25.8ng/mL (SD: 8.5), respectively.
The fed-to-fasted C_{max}-ratio was 23.5% (90%CI: 16.6-33.6). The T_{max} was significantly prolonged after food intake (mean 3.5h) compared to fasted conditions (mean 2.4h; p=0.01). The mean apparent terminal half-lives were 12.9 and 14.2h, respectively, under fasted and fed conditions. Using $AUC_{0\text{-}\infty}$-values, the fasted mean absolute bioavailability of YM 466 was 2.59% (95%CI: 2.03-3.31). The $AUC_{0\text{-}\infty}$-ratio of fed over fasted oral treatment was 27.8% (95%CI: 22.9, 33.8). After iv administration the total plasma clearance (CL) was 7.33L/h (SD: 1.25), the volume of distribution associated with the terminal phase (V_z) was 156.9L (SD: 48.6) and at steady-state (V_{ss}) 81.0L (SD: 20.3). For the oral administrations the apparent clearance (CL/F) was 302.3L/h (SD: 105.8) and 1023L/h (SD: 204.6) under fasted and fed conditions, respectively.

Using the amounts of parent drug recovered in the urine after administration of YM 466 by the iv or oral routes, absolute and relative bioavailabilities were estimated (Table 2). The mean absolute bioavailability of YM 466 was 2.15% (95%CI: 1.40-3.29) and the $AUC_{0-\infty}$-ratio fed over fasted oral treatment was 23.6% (95%CI: 16.6-33.6).

Pharmacodynamics

Anti-factor Xa assay

Because this assay was performed using a sample of the corresponding dose of YM 466, the results were expressed in ng/mL. In all volunteers anti-Xa activity was measurable after YM 466-administration (Figure 4). Dose-related increases were observed for the oral administrations. After the highest dose in the first part (300mg orally) at the last timepoint, 48 hours, the mean anti-Xa activity still exceeded baseline values. The shape of the anti-factor Xa activity time curve mirrored the plasma concentration curve, however, overall the curve was substantially lower. Figure 5 displays the linear relationship between the plasma-concentration of YM 466 and the anti-Xa. Unexpectedly, this relationship differed between the routes of administration; the relationship for the iv-administration was more near the expected line of equality than the oral administrations.

Coagulation Assays

Post-dose increases above baseline were observed for APTT and PT. The prolongations of these parameters were most clearly present after the 5mg iv administration (Figure 6); the maximum increase at the end of the infusion was about 8 seconds in APTT (20%) and 3.5 seconds in PT (6%). Following oral doses, maximum increases in APTT and PT (E_{max}) were reached between 2 and 5 hours post-dosing. At twelve hours after dosing, APTT and PT returned to baseline values. The biggest observed prolongations in this study were almost 12 seconds for the APTT (32%) and 5 seconds for the PT (34%), respectively, in the 300mg group. As displayed in Figure 7, a clear linear relationship exists between the YM 466-dose and effect on APTT and PT.

Additional pharmacodynamic measurements (first part of the study only)

Anti-factor IIa Activity Assay

Anti-IIa activity remained virtually unchanged during the course of the study. Therefore, no relation could be discerned between the dose of YM 466 and the activity against factor IIa.

Thrombin fragment 1+2

In the placebo-group, the thrombin fragment 1+2 levels increased comparing pre-dose with 2h samples. This phenomenon was also observed after the lower doses of YM 466, however, following increasing doses the amount of increase of fragment 1+2 was reduced (Figure 8). No influence was seen on this parameter after 200 mg, and in the highest dose-group even a decrease was observed. Comparable to the plasma-concentrations of the compound the results for this parameter showed a high interindividual variability (see e.g. the 10mg dose-group; inset in Figure 8). Therefore, no conclusion could be drawn about the relationship between concentrations of YM 466 and this parameter.

TFPI Assay

This was measured at two timepoints only, pre-dose and 1 hours after dosing. In all treatment groups there was a trend towards a mean increase in the post-dose sample. However, the increase was only minor and was also observed in subjects receiving placebo, indicating that YM 466 did not influence TFPI levels over the dose-range tested.

TAT

The TAT-complex levels per dose-group increased slightly with dose. However, increasing TAT-levels were also seen in subjects receiving placebo. In addition, even pre-dose very high TAT-levels were found (e.g. approximately 230µg/L for the 50 mg dose-group; reference-values for this assay are 1.0–4.0µg/L).

DISCUSSION

Because the activated serine protease factor Xa is an essential enzyme at the converging point of the intrinsic and extrinsic

pathways of coagulation, intervention of the coagulation process at the level of factor Xa is an attractive option for the treatment of thromboembolic disorders. In contrast to thrombin, which is a multifunctional serine protease, to the current knowledge inhibition of factor Xa specifically affects the coagulation. Moreover, as this coagulation factor does not affect platelet function, intervention at this point in the coagulation cascade should notably decrease bleeding tendency. By acting primarily on and preventing the generation of thrombin, factor Xa inhibitors possess theoretical advantages over thrombin inhibitors, and a variety of specific factor Xa inhibitors are currently under development [8].

YM 466 was well tolerated in terms of the assessed safety indices after single administrations to healthy young male volunteers. No abnormalities of clinical relevance were noted. The most frequently reported adverse events included development of haematomas, mainly at puncture sites. The inhibitory effects on factor Xa activity of YM 466 observed in the present study demonstrate the compound is active after oral administration. This finding seems to be supported by the results of the fragment 1+2 assay, in which at the higher oral doses the formation of this prothrombin fragment was blocked or even showed a decrease, even in healthy volunteers without activated coagulation system. The anti-Xa activity curves mirrored the plasma concentration curves indicating the absence of significant amounts of active metabolites in human. Nevertheless, discordance for the linear relationship of this parameter and the plasma-concentration of the compound was found. Because a difference for this relationship was found between the iv and oral route of administration (with the iv curve more approaching the line of equality than the oral curve), a certain amount of active, unidentified, metabolites may have occured.

The terminal half-life of YM 466 was about 13-14 hours. This parameter appeared to increase with dose, but this was probably caused by the limited number of quantifiable samples at the terminal end-phase of the plasma profile for the lower doses. This finding was confirmed by the maximum extrapolated

fraction of the $AUC_{0-\infty}$, which was 11% for the 10mg oral dose in the first part of the study and about 5% for all other dose-levels, indicating the sampling scheme was adequate compared to the estimated value for $t_{½}$.

The apparent disproportionate increase in AUC and peak plasma concentrations might be indicative for dose-dependent pharmacokinetics. However, a wide scatter in these parameters was observed following the various doses resulting in considerable overlap in C_{max} and $AUC_{0-\infty}$ between dose-levels. Due to the limited number of data from the present study it is difficult to identify the pharmacokinetic mechanism responsible for the non-linearity. The clinical importance of this disproportionate increase remains to be established, especially since this was relatively minor.

The oral bioavailability in animals was between 3 and 9%.

In accordance with these preclinical studies [7], a low absolute bioavailability of the capsules was found. Co-administration of YM 466-capsules with food caused a decrease in oral bioavailability, whereas the time needed to reach peak plasma levels significantly prolonged. This latter effect is likely to be the result of delayed gastric emptying, a decreased dissolution rate in the presence of food or a combination of these effects. In contrast, the terminal elimination half-life after food was comparable to the other treatments, indicating only the (rate of) absorption of YM 466 is influenced by the concomitant intake of a meal. The route of administration did not markedly influence the relative high inter-subject variability in pharmacokinetics of the compound. It can be concluded that the low oral bioavailability is not the primary source of this variability. Other sources of the variability should be identified, but current information is insufficient. The low bioavailability may result from several different causes. For instance, there might be an interaction of the compound with contents of the gastrointestinal tract (e.g. bile salts), impairing its uptake by this tract. YM 466 may show a substantial first-pass effect, but also other causes (incomplete dissolution, poor intestinal permeability or competing reactions within the gastrointestinal tract) cannot be excluded at this stage.

The renal clearance of this direct-acting Xa inhibitor is a major route of elimination, because almost 60% of the administered dose was recovered in the urine following iv administration. As can be expected with the low oral bioavailability, after oral doses the urinary excretion of YM 466 was low. This ranged from 1.2 to 3.3% of the administered dose over the first 48 hours post-administration, which was comparable to about 60% of the bioavailability. As renal clearance was lower than the total plasma clearance, an extra-renal clearance pathway of YM 466 is likely. The renal clearance ranged from 3.5L/h (after a meal) to 6.47 L/h (100mg orally in Part I of the study). The protein binding of the compound is approximately 40% (in spiked plasma *in vitro*; data on file); correcting the renal clearance for this figure results in an unbound renal clearance exceeding the glomerular filtration rate (about 7.2 L/h in a 70-kg 20-year old male). Thus, YM 466 is likely to be both filtered and actively secreted by the kidneys. Any involvement of reabsorption cannot be discounted, but would this be the case, it must be less than secretion.
Using the urinary data the bioavailabilities were also estimated. The plasma pharmacokinetic estimates were confirmed and urinary data might, therefore, be used as replacement to estimate bioavailability. However, in other settings (i.e. not having the subjects lodged for the entire study period) ensuring complete urine collection might constitute a problem.

Compared to UFH, the group of LMWHs hardly influences the conventional coagulation assays (APTT/PT). Following this line, only minor increases in these assays were expected after YM 466-administration. Nevertheless, distinct post-dose increases were found. This finding might indicate that the highly selective AT-independent anti-Xa agent may have actions on other parts of the coagulation cascade. This is supported by the fact that the anti-Xa activity curves only mirrored the plasma-concentration curves. It was expected these curves would be identical, because anti-Xa activities as used in this study were determined with a sample of the compound as calibrator (and therefore reported in concentrations of the compound). Factor IIa was excluded as

being influenced by YM 466, as the anti-factor IIa levels were not influenced during the first part of the study. Given the structural homology between coagulation factors X, VII and IX [9,10], these latter factors seem candidates, which are influenced by YM 466. It may also be argued that the finding is due to the different mode of action compared to the pentasaccharides. For example, in addition to circulating 'free' coagulation factor Xa these direct-acting anti-Xa agents might also inhibit clot-bound Xa, comparable to the direct-acting thrombin inhibitors [11].
Finally, it has recently been shown in animals that inhibition of factor Xa may reduce tissue factor expression in the liver during endotoxemia [12]. Hence, it may be tentatively argued that factor Xa inhibition results also in a decrease of an important pro-coaugalnt factor (tissue factor) and that this is reflected in a prolonged PT. It is however unlikely that these phenomenona fully explain the findings in healthy young males. In addition, this study was not designed to answer the question and it is impossible to draw conclusions on this peculiar finding and further investigations to specifically adress this question should be planned.

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TABLE 1 Pharmacokinetic parameters of YM 466 for the first part of the study

A. Summary of pharmacokinetic parameters in plasma

DOSE (mg)	$AUC_{0\text{-last}}$ (ng*h/mL)	$AUC_{0\text{-}\infty}$ (ng*h/mL)	T_{MAX} (h)	C_{MAX} (ng/mL)	$t_{1/2}$ (h)
10 (n=6)	22.0 (13.6) [6.9–43.7]	24.2 (13.9) [8.78–46.8]	1.2 (0.7) [0.50–2.0]	3.5 (1.7) [1.5–6.1]	6.9 (2.2) [5.3–11.3]
20 (n=6)	54.8 (28.2) [23.2–91.4]	56.9 (28.5) [24.5–93.2]	1.3 (0.9) [0.5–3.0]	6.9 (3.8) [2.7–13.0]	9.2 (2.4) [6.6–13.1]
50 (n=6)	134 (33.7) [89.6–185]	138 (35.0) [92–192]	1.9 (1.0) [0.5–3.0]	15.4 (2.4) [11.9–18.3]	10.3 (1.6) [8.1–12.0]
100 (n=6)	372 (201) [159–705]	389 (209) [165–737]	2.8 (0.8) [2.0–4.0]	41.6 (23.3) [16.3–80.7]	12.5 (2.0) [10.9–16.6]
200 (n=6)	732 (198) [524–990]	761 (212) [535–1028]	1.9 (0.7) [1.0–3.0]	83.2 (27.3) [55.7–125]	12.3 (2.7) [9.3–15.9]
300 (n=6)	1315 (547) [704–2270]	1370 (564) [727–2358]	2.0 (1.0) [0.5–3.0]	157 (71.3) [91.7–292]	12.5 (2.8) [9.9–17.0]

B. Summary of urinary pharmacokinetic parameters

DOSE (mg)	$AE_{0\text{-}48h}$ (ng*h/mL)	% EXCRETED UNCHANGED	CL_{RENAL} (h)
10 (n=6)	0.1 (0.1) [0.1–0.2]	1.2 (0.7) [0.7–2.2]	5.2 (1.6) [3.4–8.2]
20 (n=5)	0.3 (0.1) [0.1–0.5]	1.8 (0.8) [0.6–2.8]	5.0 (0.5) [4.4–5.6]
50 (n=6)	0.6 (0.3) [0.3–0.9]	1.5 (0.6) [0.6–2.2]	4.5 (1.1) [2.9–6.0]
100 (n=5)	1.9 (0.8) [1.1–3.0]	2.3 (0.9) [1.3–3.6]	6.5 (1.3) [5.0–8.4]
200 (n=1)	5.5	3.3	5.6
300 (n=6)	5.8 (2.8) [2.4–10.7]	2.3 (1.1) [0.96–4.22]	4.3 (0.5) [3.5–4.9]

All results are reported as mean (SD) [range]

TABLE 2 Pharmacokinetic parameters of YM 466 for the second part of the study

A. Summary of pharmacokinetic parameters in plasma

DOSE AND ROUTE	$AUC_{0\text{-last}}$ (ng*h/mL)	$AUC_{0\text{-}\infty}$ (ng*h/mL)	T_{MAX} (h)	C_{MAX} (ng/mL)	$t_{1/2}$ (h)
5mg iv (n=10)	563.5 (118.3) [437–836]	589.5 (123.8) [458–873]	1.0 (0.1) [0.7–1.1]	122.9 (30.7) [88.5–178.4]	14.7 (3.2) [11.3–22.7]
100mg po fasted (n=12)	293.1 (82.1) [146–406]	307.4 (86.1) [154–419]	2.4 (0.9) [1.0–4.0]	27.6 (8.0) [17.0–38.3]	12.9 (1.8) [10.6–16.4]
100mg po fed (n=11)	80.3 (17.7) [53–121]	86.0 (18.1) [59–127]	3.5 (0.5) [3.0–4.0]	6.7 (3.2) [4.3–9.8]	14.2 (3.0) [10.8–19.0]

B. Summary of urinary pharmacokinetic parameters

DOSE (mg)	$AE_{0\text{-}48h}$ (ng*h/mL)	% EXCRETED UNCHANGED	CL_{RENAL} (h)
5mg iv (n=10)	2.4 (0.4) [1.6–2.8]	57.7 (11.0) [40.0–69.7]	4.4 (0.9) [3.0–5.6]
100mg po fasted (n=12)	1.2 (0.5) [0.6–2.3]	1.4 (0.6) [0.7–2.7]	4.1 (1.3) [2.0–6.1]
100mg po fed (n=11)	0.3 (0.1) [0.1–0.4]	0.3 (0.1) [0.1–0.4]	3.5 (1.5) [1.4–6.7]

All results are reported as mean (SD) [range]

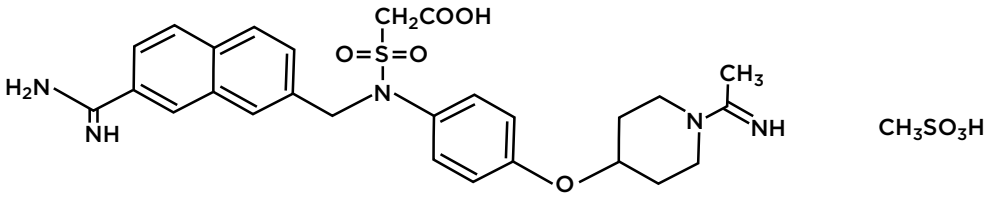


FIGURE 1 Molecular structure of YM 466.

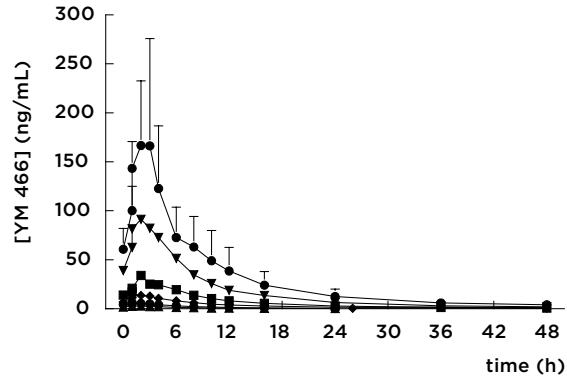


FIGURE 2 YM 466 plasma concentration time curves after the respective doses in the first part of the study (single rising oral doses 10 to 300 mg).

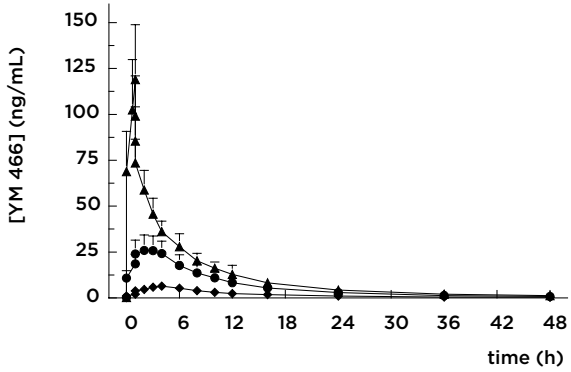


FIGURE 3 YM 466 plasma concentration time curves after the respective doses in the second part of the study (5mg iv, 100mg under fasted or fed conditions).

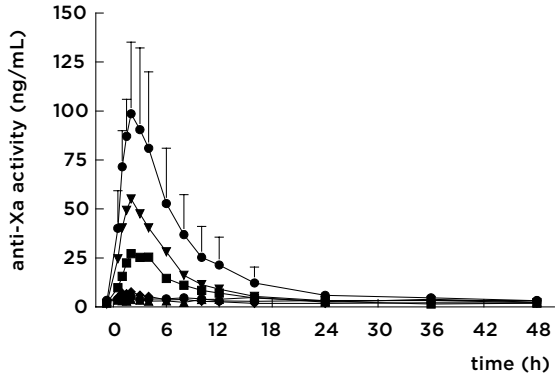


FIGURE 4 Anti-Xa activity time curves after the respective doses in the first part of the study.

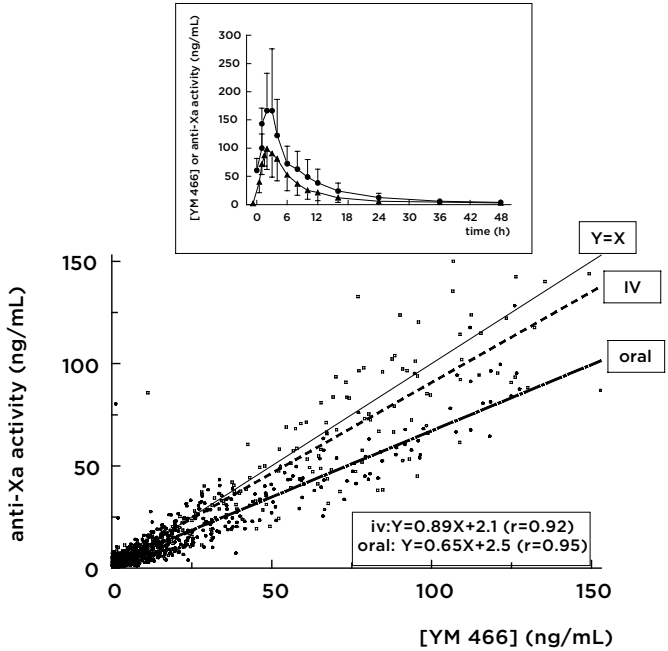


FIGURE 5 **Plot showing the linear YM 466 plasma concentration-anti-Xa activity relationship.**

The anti-Xa activity (determined with a sample of the compound as calibrator) was substantially lower. This was even more outspoken for the oral administrations. Inset: the YM 466 plasma concentration-anti-Xa activity time curves after the 300mg dose in the first part of the study; the Cmax for the plasma-concentration was 166ng/mL, whereas the maximum anti-Xa activity was 98ng/mL.

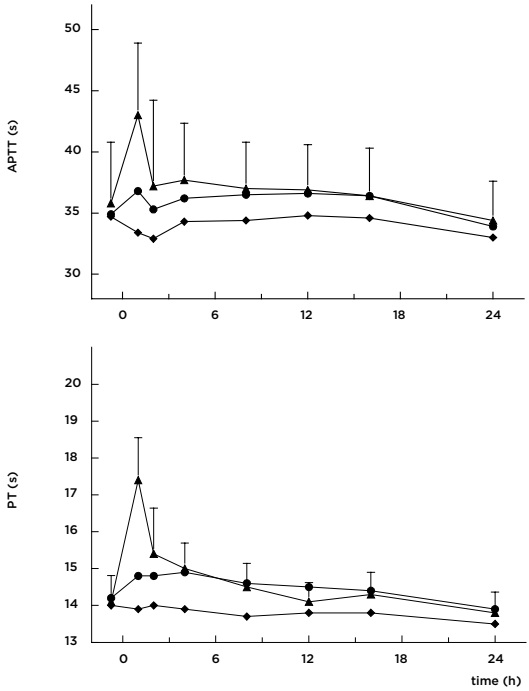


FIGURE 6 **APTT and PT time curves for the second part of the study.** After oral administration of 100mg YM 466 in the fasted state hardly any influence was seen; a mild prolongation in APTT of less than 2 seconds and less than 1 second in PT, whereas no influences on these parameters were observed when this dose was given after breakfast.

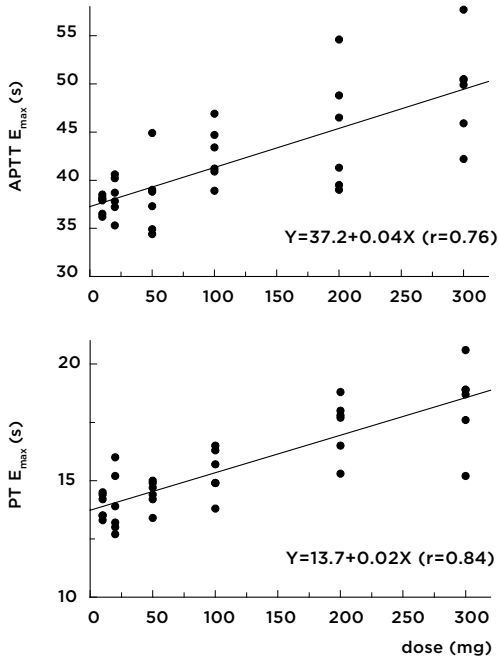
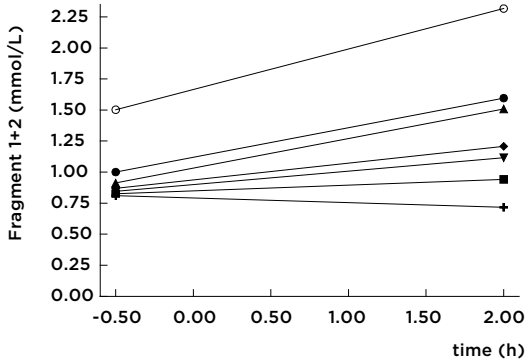


FIGURE 7 Maximum effect in coagulation-assay responses versus dose.



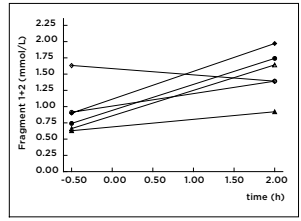


FIGURE 8 **F1+2 response to the rising doses in the first part of the study.**

The placebo response is the upper curve. Increasing the dose led to a steadily decreasing response, with the lower curve (300mg) which eventually indicated a decrease in response. The inset shows the individual data for the 10mg dose-group; the coefficient of variance for the pre-dose measurements was 41%, and for the 2-hour results 24%.

SECTION IV

METHOD EVALUATION

CHAPTER 15–17

CHAPTER 15

NEW ANTITHROMBOTICS IN PHASE I: A COMPARISON BASED ON ANTI-XA ACTIVITY

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ABSTRACT

Objective

Currently, the APTT and PT are the most widely used methods to compare novel antithrombotic drugs with existing compounds. However, these global assays may be inadequate for selectively acting agents and may result in unrealistic comparisons between older and new drugs.

Methods

The LMWHs dalteparin and nadroparin, the pentasaccharides fondaparinux and idraparinux, and the direct-acting anti-Xa inhibitor YM 466 were compared by investigation of the relationship between anti-Xa activity and APTT and PT. The anti-Xa activity was determined against the international standard for LMWH and APTT/PT were assessed using standard procedures. The best possible linear function describing the relationship between these parameters was determined.

Results

The baseline-ranges were comparable for the different compounds. The functions describing the linear fits between anti-Xa activity and APTT revealed higher slopes for the two LMWHs (slopes: 50 IU/s and 33 IU/s for dalteparin and nadroparin, respectively), lower slopes for the two pentasaccharides (slopes: 6 IU/s and 1 IU/s for fondaparinux and idraparinux, respectively) whereas the slope for YM 466 was in between the LMWHs (44 IU/s). Similar results were obtained for the PT-analysis.

Conclusions

Meaningful differences exist in the APTT and/or PT-response to partially or fully selective anti-Xa antithrombotics in man. This indicates that these conventional coagulation assays are biomarkers with compound-specific usefulness, which reflects overall effects in the coagulation system. This makes their use for monitoring the effects of new anticoagulants questionable.

INTRODUCTION

Anticoagulation is the cornerstone of therapy for venous thromboembolism (VTE), a condition that encompasses deep vein thrombosis (DVT) or pulmonary embolism (PE) [1]. Currently, LMWHs are emerging as the antithrombotic agents of choice for the prevention and treatment of these conditions [2], but subsequent to the search, which resulted in the development of the LMWHs, the increased knowledge of the pathophysiology of haemostasis and thrombosis and the specific actions of the traditional anticoagulant drugs has led to a spectacular growth and diversification of available antithrombotic compounds.

The assessment of the efficacy of these new anticoagulant treatments in patients with symptomatic thrombosis is hampered by the low incidence of the outcome measures of choice (symptomatic venous thromboembolic complications, or worse). The ultimate test for new antithrombotic agents is the clinical dose-finding study, which relates the antithrombotic effect to the plasma levels of the anticoagulants. Initially, the pharmacokinetic and pharmacodynamic effects of new compounds are characterised in the intact effector system (in healthy volunteers) [3]. At this stage, changes in the results of known tests are used as the primary endpoint. The changes on a biomarker, induced by a therapy, are expected to reflect changes in a meaningful clinical endpoint [4]. In order to prevent the large, time-consuming dose-finding trials, there is considerable interest in models that can serve as alternative efficacy outcome to relate the pharmacokinetics to the pharmacodynamics [5].

The group of newly developed antithrombotic drugs represents a wide group of agents that are targeted to modulate different biochemical pathways leading to thrombosis and include highly selective antiproteases, platelet inhibitors, and variants of traditional agents [6]. The marked structural and functional heterogeneity of these compounds has provided incentives to develop approaches for monitoring the alterations of haemostatic mechanisms. The choice of suitable biomarkers is important and not always easy. The most widely used models to investigate antithrombotic drugs are the *ex vivo* laboratory tests, the activated

partial thromboplastin time (APTT) and the prothrombin time (PT). Though the limitations of these tests are recognised, the broad clinical application of these tests provides extensive clinical experience. Nevertheless, the reliability of these assays should ideally be assessed for each new (class of) anti-coagulant. Unfortunately, a number of the new agents cannot be monitored using these traditional global coagulation assays as surrogate endpoint [3]. For instance, the LMWHs cause only mild prolongation of the APTT [2], but appeared at least as effective and safe as unfractionated heparin (UFH) in prevention and treatment of VTE.

Because factor Xa is important in the control of thrombogenesis, in the search for new agents there is much emphasis on anti-Xa selectivity. Therefore, coagulation factor X inhibition might be a useful biomarker in early phase thromboprophylactic studies. In addition, anti-Xa activity can be used as an appropriate and sensitive biologic marker of LMWH activity [7], correlates well with International Normalised Ratio (INR) values under warfarin therapy [8], and is not influenced by the presence of lupus anticoagulant [9].

Over the last years, phase I studies have been performed with novel drugs, which all had factor Xa inhibition as a common biological effect. In this paper, these compounds are compared by investigation of the agreement between the laboratory assay for anti-Xa activity and the commonly used overall coagulation assays APTT and PT. This might result possibly in a statement about the suitability of these conventional ex vivo tests as biomarker for monitoring of newer antithrombotic treatments.

METHODS

General

All antithrombotic compounds were administered in phase I studies in healthy young male volunteers. All subjects participated in these studies after written informed consent was obtained. The Ethics Committee of Leiden University Medical Center approved the investigational protocols. Because the studies were all phase I studies, in most cases first-entry-into-man

studies, many parameters were determined among which the traditional coagulation assays (APTT and PT).

Treatments
During the studies the following anticoagulant drugs were studied:

- Dalteparin (Fragmin®, Pharmacia&Upjohn), a low-molecular-weight heparin (LMWH) with a mean molecular weight (MW) of 5000D (range 2000 to 9000D) and an anti-Xa/anti-IIa ratio of 2.4 [10]. The administered dose was 2500 anti-Xa units, either subcutaneously (sc) or intravenously (iv).
- Nadroparin (Fraxiparine®, Sanofi-SynthéLabo), a LMWH with a mean MW of 4500D (range 2000 to 8000D) and an anti-Xa/anti-IIa ratio of 3.5 [11]. The administered dose was 7500 anti-Xa units, either sc or iv.
- The selective anti-Xa pentasaccharides fondaparinux and idraparinux (NV Organon, Oss, The Netherlands). fondaparinux was administered at a dose of 4mg (sc and iv), and a sc dose of 10mg.
  Idraparinux was dosed in a single rising dose fashion with a range of 0.25 to 14mg iv, and sc at a dose of 2 or 10mg.
- The synthetic direct-acting anti-Xa compound YM 466 (Yamanouchi Europe BV, Leiderdorp, The Netherlands). Dosing was in a range of 10 to 300mg orally and 5mg iv.

The commercially available LMWHs were obtained from one batch and supplied by the pharmacy of Leiden University Medical Center. The manufacturers supplied the other investigational compounds.

Sampling
Following drug administration, blood samples for APTT and anti-factor Xa-activity assay were drawn at regular time intervals. The PT was only determined in the studies with the three selective anti-Xa compounds. No tourniquet was applied when blood was collected during the studies. Blood samples were collected via a cannula, which was inserted into a forearm vein. The cannula was

kept patent by intermittent flushing with 0.9% saline. Blood samples were taken after discarding the contents of the cannula. Blood samples for base-line values were taken pre-dose.
In the studies with the LMWHs, venous blood was collected in 1/10 volume of 0.11M tri-sodium citrate. Plasma was obtained by centrifugation of the blood for 5 minutes at 5000*g and stored at or below –40°C until analysis.
For the studies with fondaparinux, idraparinux and YM 466, blood samples for the laboratory assays were drawn in (room temperature) tubes containing 0.129M sodium citrate (Vacutainer, Becton Dickinson & Co, Plymouth, UK). Tubes were centrifuged at 2000*g for 15 minutes at 4°C, within 30 minutes of collection. The separated plasma was subsequently snap frozen using a Carbon dioxide-Methanol mixture and stored at or below –20°C until determination.

LABORATORY PARAMETERS

General

All assays were performed at the TNO-Gaubius Laboratories (Leiden, The Netherlands) under the supervision of Prof. Dr. C. Kluft. Per study compound, all measurements were performed in one batch on a single instrument using plasma, which had been frozen and thawed only once.

Coagulation assays

The laboratory APTT and PT were determined, in duplicate, using standard procedures [12]. Inter and intra assay precision (CV) for the APTT and PT was smaller than 5%. For the LMWHs, the APTT assay was performed on an ACLTM Automated Coagulation Laboratory Analyser (Instrumentation Laboratory, Milan, Italy) with automated APTT reagent (General Diagnostics Inc., New Jersey, U.S.A.). For the pentasaccharides and YM 466, APTT and PT assays were determined on a STA® coagulation analyser (Roche Diagnostics, Mannheim, Germany). The reagents were STA APTT (Roche Diagnostics, Mannheim, Germany) and Thromborel S (sensitive human placental thromboplastin, ISI ≈ 1; Behring Diagnostics, Marburg, Germany), respectively.

Anti-Xa activity

Chromogenic amidiolytic assays were used to measure plasma anti-Xa activities. For the LMWHs, these assays were performed on an ACL™ Automated Coagulation Laboratory Analyser (Instrumentation Laboratory, Milan, Italy) using the Coatest Heparin kit (Kabi Vitrum, Stockholm, Sweden) according to Teien and Lie [13] with slight modifications. The anti-Xa activities after administration of dalteparin and nadroparin were calculated with the international standard for LMWHs. For fondaparinux, idraparinux and YM 466, anti-factor Xa activity was assessed on a STA® coagulation analyser (Roche Diagnostics, Mannheim, Germany) using a photometric assay (Coatest LMW Heparin, Chromogenix, Mölndal, Sweden).

Analysis

For each study, all timepoints at which samples were taken for anti-Xa activity assay and APTT (PT) determination were identified. In order to adequately compare the compounds, the anti-Xa activity was expressed in International Units anti-Xa activity determined against the first international standard for LMWH (WHO-code 85/600 [14]) obtained via the National Institute for Biological Standards and Control (UK). For each compound a calibration curve was constructed against the same standard. For each compound the baseline reference-range was calculated as the mean value 1.96 times the standard deviation (SD) using the pre-dose samples. Post-dose samples below the lower limit of quantification (LOQ) for anti-Xa activity were not included in the analysis. The anti-IIa activity of the compounds was plotted on X-axis of graphs with the corresponding APTT-values on the Y-axis. The relationship between the anti-Xa activity and APTT or PT, as it could be described by the best possible linear function, was determined by regression-analysis. The slope and the intercept of these functions are reported with 95% confidence intervals (95%CI).

RESULTS

APTT

The APTT-anti-Xa plots for all individual compounds are displayed in Figure 1. For the more direct comparison the compounds are

grouped in Figure 2. Figure 2 also describes the relationship between the influence of the different compounds on anti-Xa activity and APTT, by means of the best possible linear fit.
The functions describing the linear fits are summarised in Table 1. From these graphs it is clear the baseline-ranges were comparable for the different compounds. The intercepts of the respective functions were also comparable between compounds. The mean intercept for all compounds was 33.8 seconds (SD: 2.7) for the APTT. The different LMWHs influence the APTT already at very low anti-Xa activities. This effect is somewhat more outspoken for dalteparin (slope 50.4 s/anti-Xa unit; 95%CI 45.5, 55.4) than nadroparin (slope 33.3 s/anti-Xa unit; 95%CI: 30.2, 36.4). The two different pentasaccharides hardly induce changes in this parameter. The slopes are 6.1 s/anti-Xa unit (95%CI: 4.6, 7.6) and 0.9 s/anti-Xa unit (95%CI: 0.8, 1.1) for fondaparinux and idraparinux, respectively. The direct-acting YM 466 already induces an increase in APTT (slope 43.5 s/anti-Xa unit; 95%CI: 36.3, 50.7) at very low doses. The relationship between anti-Xa activity and APTT for YM 466 was almost identical to this relationship for the LMWHs (Table 1).

PT

The PT-anti-Xa graphs for the pentasaccharides fondaparinux and idraparinux, and the direct-acting YM 466 are displayed in Figure 3. The overall PT-comparison of these latter compounds is grouped in Figure 4. This latter figure also describes the relationship between the influence of the different compounds on anti-Xa activity and PT. The functions describing the linear fits are summarised in Table 2.
Comparable to the APTT-analysis, the baseline-ranges were in agreement for the different compounds and the intercepts of the respective functions were also comparable between compounds. The mean PT-intercept for all compounds was 13.9 seconds (SD: 0.2). The results of the PT-analysis for the pentasaccharides and YM 466 confirmed the APTT-results, as it revealed almost identical results. The two different pentasaccharides hardly induce PT-changes, whereas YM 466 already induces an increase at very low doses.

DISCUSSION

In order to justify an alteration in practice patterns any change must show to be 'better' than the current standard (more effective, fewer/less severe side-effects, more convenient or less expensive). Therefore, in the first stages of evaluation, the biological activity of a new compound is usually compared with that of known compounds. Practical obstacles thwart a direct pharmacodynamic comparison between existing and new antithrombotic compounds. UFH is still the most widely used anticoagulant compound, but controversies remain about the optimal UFH-levels (as 'the golden standard') in monitoring [15]. In clinical practice, the need of an adequate assay to monitor anticoagulant/antithrombotic therapy is obvious.

Until recently, the APTT and PT have been the mainstay in the laboratory assessment of coagulation and monitoring anticoagulants, however, the development of new antithrombotic agents has shown the limitations of these assays and the fact they do not provide complete information of the mechanisms of coagulation. Though this study was formally not designed to compare different compounds, it was tried to avoid random variation by extraneous factors and to maximise the abilities to demonstrate associations. This was done by ensuring that all timepoints at which both a sample was taken for APTT/PT as well as anti-Xa activity assay were included in the analysis. In addition, all studies were performed at one center, and all assays were performed by one laboratory.

The results of the present study data indicate that meaningful differences exist in the APTT and PT response to partially or fully selective anti-Xa antithrombotics (with different modes of action) in man. The small differences observed in APTT-response per anti-Xa unit between the LMWHs in this paper can clearly be related to the differences in thrombin inhibition between dalteparin and nadroparin as these compounds have different anti-Xa/anti-IIa ratios. The pentasaccharides (fondaparinux and idraparinux) hardly influenced the conventional coagulation assays.

No influence whatsoever was observed on APTT for the long-acting pentasaccharide idraparinux, not even at very high anti-Xa levels.

In contrast, the direct-acting YM 466 already caused an increase in APTT at low anti-Xa levels. For this compound, the relationship between anti-Xa activity and APTT was almost identical to the relationship observed for the LMWHs. Comparable results were found for the PT-analysis confirming the APTT-findings.
These data suggest that, at least for the antithrombin-dependent heparinomimetic compounds, it turns out that the greater the specificity of a compound for anti-Xa, the lower the influence on the conventional coagulation assays. This is indeed reflected in the findings of the pentasaccharides, which lack anti-IIa activity, and did not influence the APTT/PT at all.

In the search for the 'ideal' antithrombotic compound [16], the group of newly developed drugs represents a heterogeneous group. The valid assessment of qualities pivotal to a successful clinical development of innovative antithrombotics remains a complex and exciting challenge. Because the use of real clinical endpoints is often not feasible with regard to thromboprophylaxis-research (as well as in general in clinical trials), surrogate endpoints are used. It appears to be impossible to integrate all crucial determinants for thrombosis as defined by Virchow in a single model or parameter for the valid assessment of novel antithrombotic therapies.
Despite the clinical importance of the APTT and PT, the pharmacodynamic effects observed with novel anticoagulants in these assays cannot be simply extrapolated to clinically relevant effects. The results of these tests are known to be affected by numerous pre-analytic and analytic variables [17], and in light of the current opinion on blood clotting in vivo, it is clear that both APTT and PT are highly artificial in vitro systems with major limitations.
In this study antithrombotic levels were determined using antifactor Xa assays as biomarker. It remains to be understood how anti-Xa levels can be related to haemostasis in vivo effects, although there might be a relationship between anti-Xa levels and the risk of bleeding [18,19]. Currently, this parameter should only be considered as a biologic marker for the effects of the compared compounds on coagulation. The results of the present study

indicate that an APTT-prolongation induced by a certain compound is not the same than the APTT-increase induced by another compound, as there are considerable differences in anti-Xa activity between compounds.

If an APTT-prolongation induced by a certain compound is not the same as the APTT-increase induced by another compound, a question raised by the results of the current study are if a certain level of anti-Xa activity induced by one compound is the same as the exact level induced by another compound? The biologic and pharmacokinetic differences among LMWHs have been recognised, but up till now attempts to standardise the antithrombotic potency of LMWHs based on anti-Xa activity have failed [20]. In addition, this might also depend on the mode of action of the compound. Another question is how the anti-Xa levels can be translated to clinical significant parameters (adverse effects such as bleeding complications)? These questions cannot be answered using Phase I clinical trial data. Nevertheless, a comparison between compounds or with positive controls (LMWHs in this study) is possible using the methodology as in this study. The anti-Xa activity is a more physiologic biomarker than the more indirect artificial biomarkers (APTT/PT), and as such a better measure in comparing new anticoagulant compounds acting against activated factor X. Unfortunately, current data are insufficient to determine if each agent should have an individual target anti-Xa range [21]. The goal for future biomarkers is to develop in vitro and ex vivo models that can accurately relate the pharmacokinetics to the anticoagulant and antihaemostatic effects in human [5].

To conclude, this study indicates that meaningful differences exist in the pharmacodynamics responses to different anti-Xa antithrombotics (with different modes of action) in man. For early phase studies with novel anticoagulants, strategies different from those currently used are needed. The APTT and PT are simple, reproducible, relatively inexpensive and are fully automated. Despite their widespread use they are not the most

suitable tools for investigation and monitoring of new anticoagulant agents. But assuming the results of the present study hold true, and the specific anti-Xa activity inhibitors do not influence these assays, they might still be useful to categorise new compounds based on the specificity for activated coagulation factor X.

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TABLE 1 **Functions describing the linear fits for the relationship between the anti-Xa activity of the respective compounds and their influence on APTT.**

COMPOUND	INTERCEPT	SLOPE	r
dalteparin	31.0 (30.3, 31.7)	50.4 (45.5, 55.4)	0.74
nadroparin	31.4 (30.7, 32.0)	33.3 (30.2, 36.4)	0.76
fondaparinux	37.3 (36.5, 38.2)	6.08 (4.57, 7.59)	0.42
idraparinux	33.8 (33.3, 34.4)	0.93 (0.79, 1.01)	0.38
YM 466	35.5 (35.1, 36.0)	43.5 (36.3, 50.7)	0.44

Results are reported as mean (95%CI). r = correlation coefficient

TABLE 2 **Functions describing the linear fits for the relationship between the anti-Xa activity of the selective anti-Xa inhibitors and their influence on PT.**

COMPOUND	INTERCEPT	SLOPE	r
fondaparinux	4.0 (13.5, 14.6)	2.15 (-1.33, 5.63)	0.11
idraparinux	13.7 (13.5, 13.9)	0.31 (0.24, 0.37)	0.32
YM 466	13.9 (13.8, 13.9)	12.9 (11.7, 14.1)	0.66

Results are reported as mean (95%CI). r = correlation coefficient

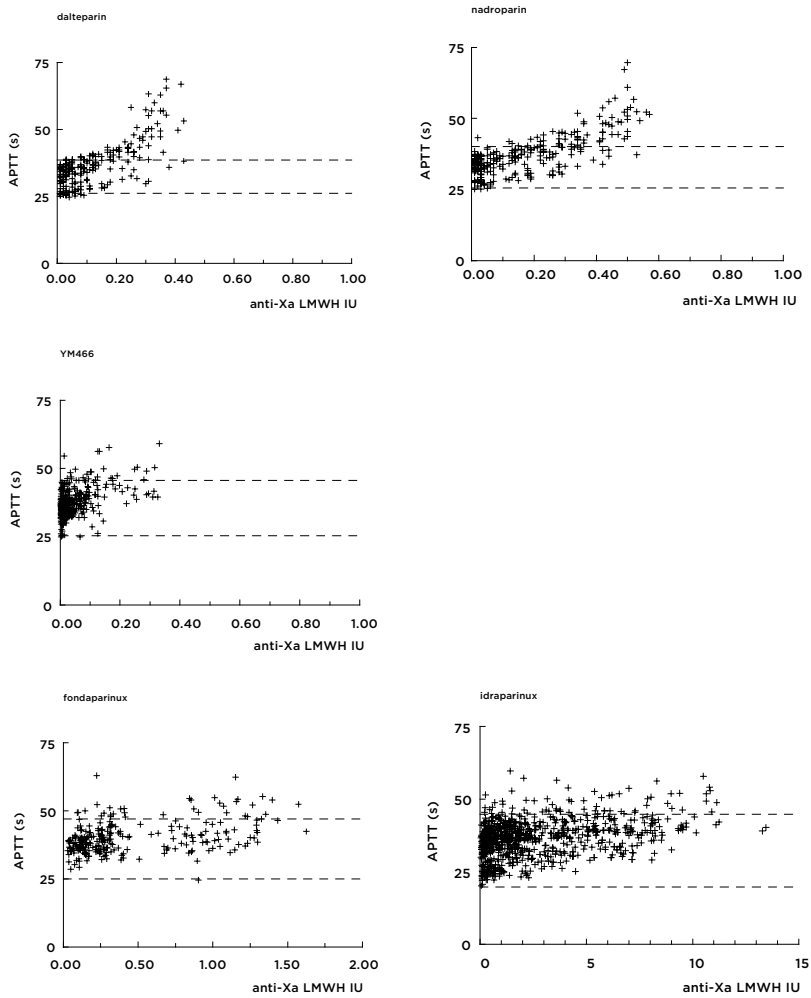

FIGURE 1 **Anti-Xa activity versus APTT for the different compounds.** The striped lines indicate the reference ranges per compound. Note that the X-axis for the pentasaccharides differs from the other curves.

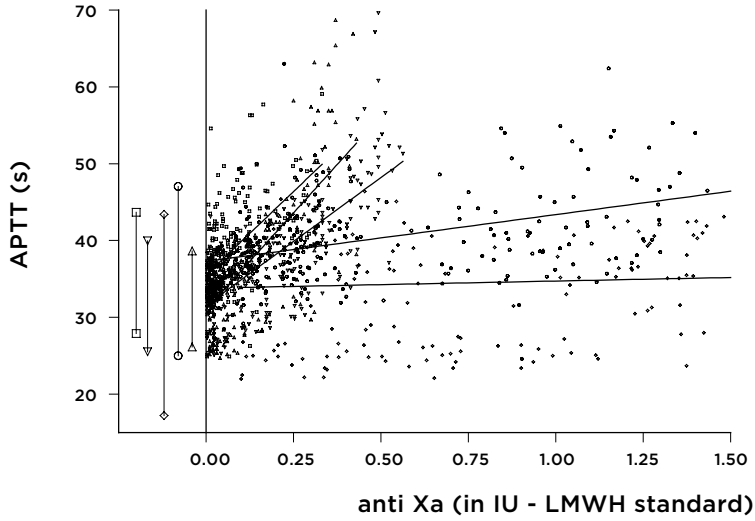

FIGURE 2 **Overall APTT group comparison, including the linear fit for the relationship between anti-Xa activity and APTT.**
Vertical lines (left) indicate the reference-ranges per compound.

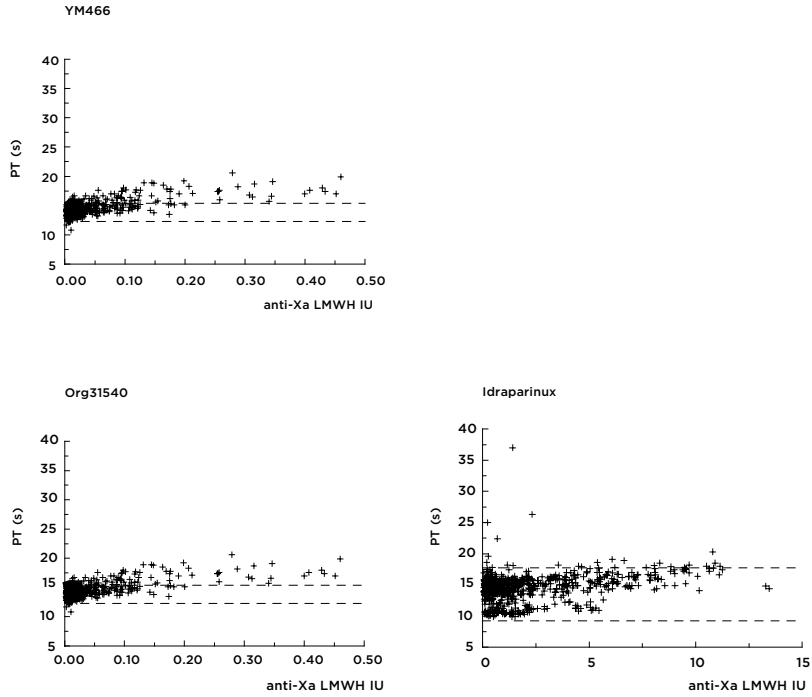

FIGURE 3 **Anti-Xa activity versus PT for the selective anti-Xa inhibitors.**

The striped lines indicate the reference ranges per compound. Note that the X-axis of the curve for idraparinux differs from the other curves.

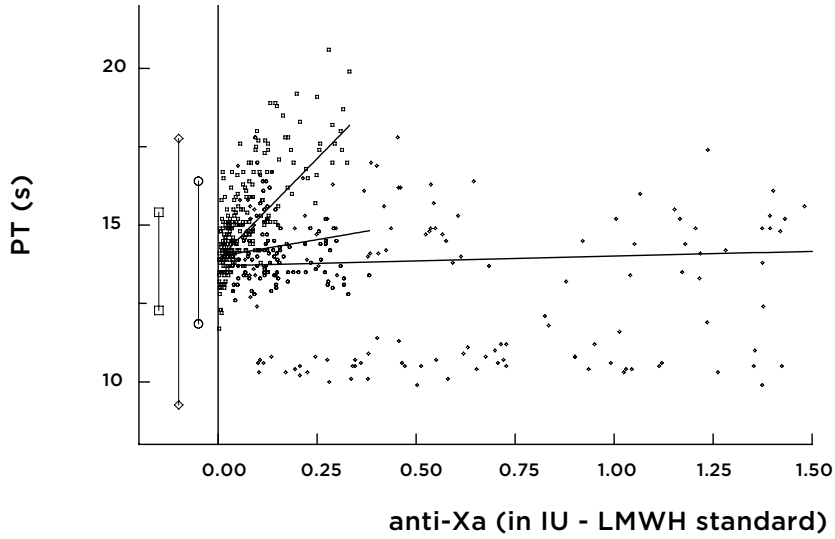

FIGURE 4 **Group PT-comparison for the anti-Xa inhibitors, including the linear fit for the relationship between anti-Xa activity and PT.**

Vertical lines (left) indicate the reference-ranges per compound.

CHAPTER 16

APTT: AN OUTDATED BIOMARKER TO COMPARE ANTITHROMBOTICS

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ABSTRACT

Background

The APTT is the most widely used method to compare antithrombotic drugs. However, this generalized assay may be inadequate for selectively active agents and may result in unrealistic comparisons between older and new drugs.

Methods

The LMWHs dalteparin and nadroparin, and the direct-acting thrombin inhibitor efegatran were compared by investigating the relationship between anti-IIa activity and APTT. The anti-IIa activity was determined using the international standard for LMWH and APTT was assessed using standard procedures. The best possible linear function describing the relationship between the parameters was determined.

Results

The relationship between APTT vs. anti-IIa activity was almost identical for the two LMWHs (slope: 116 s/IU for dalteparin and 110 s/IU for nadroparin), whereas the slope for efegatran was lower (25 s/IU).

Conclusions

The APTT-response to partially or fully selective anti-IIa antithrombotics differs in a compound-specific manner suggesting that its usefulness to monitor new anticoagulants is questionable, and may result in biased comparisons in clinical studies.

INTRODUCTION

The increased knowledge of the pathophysiology of haemostasis and thrombosis and the specific actions of the traditional anticoagulants has led to a spectacular growth and diversification of available antithrombotic compounds. The optimal efficacy assessment of new anticoagulant treatments is the incidence of symptomatic thromboembolic complications. However, these events have a relatively low incidence [1]. In order to prevent large, time-consuming trials, alternative outcome measures (biomarkers) are used, which are most commonly developed in early phase I studies [2]. During this first evaluation, the activity of the new agents is usually also compared with that of known compounds in order to obtain an indication of the dose range needed for antithrombotic efficacy.

In the search for the ideal antithrombotic [3], there is emphasis on selectivity against thrombin, because this is the final enzyme in the coagulation cascade [4] and plays a key role in acute coronary ischaemic syndromes. Many thrombin inhibitors have been developed recently with several of these agents currently undergoing various clinical trials [5]. Studies on the treatment of DVT with direct acting thrombin inhibitors have shown promising results [6,7,8], but uncertainty remains with regard to the benefits of these compounds on major cardiovascular outcomes [9].

One of the biomarkers, used in both the early phase trials and the pivotal clinical trials with selective thrombin inhibitors is the activated partial thromboplastin time (APTT). This test has become established to monitor unfractionated heparin (UFH). In addition, the APTT has been applied to compare newer antithrombotic agents with UFH. However, this strategy is not without risk. It may well be that drugs with a different mechanism of action also influence the APTT differently. This may be the case for direct thrombin inhibitors such as hirudin. Theoretically, hirudin could be a more reliable, safer, and more efficacious compound than heparin which was seemingly confirmed by encouraging early studies. However, the GUSTO-II [10], the TIMI-9 [11] and the HIT-III [12] trial were stopped

prematurely, because of a higher incidence of bleeding. The GUSTO-II and the TIMI-9 studies were later restarted at lower doses and failed to show benefit over heparin. An explanation for these findings may be the inappropriate use of a target APTT as pivotal biomarker in these studies. Therefore, it is important investigate to what extent the conventional coagulation assay APTT correlates with a common feature of the drugs such as their anti-IIa activity and whether this would provide a better measure to compare compounds. This may possibly lead to better understanding of the discrepancies found in earlier and latter trials. The objective of this paper is to compare some anticoagulant compounds using the relationship between the laboratory assay for anti-IIa activity in a uniform manner and their effect on the coagulation cascade assessed with the APTT.

METHODS

General

Over the course of several phase I studies, antithrombotic compounds were administered to healthy volunteers. All subjects participated in these studies after written informed consent was obtained. The Ethics Committee of Leiden University Medical Center approved the investigational protocols. In these studies many parameters were determined among which included the anti-IIa activity of the compounds and the APTT.

Treatments

During the studies the following anticoagulant drugs were investigated:

- Dalteparin (Fragmin®, Pharmacia &Upjohn), a low-molecular-weight heparin (LMWH) with a mean molecular weight (MW) of 5000D (range 2000 to 9000D) and an anti-Xa/anti-IIa ratio of 2.4 [13]. The administered dose was 2500 anti-Xa units, either subcutaneously (sc) or intravenously (iv).
- Nadroparin (Fraxiparine®, Sanofi-SynthéLabo), a LMWH with a mean MW of 4500D (range 2000 to 8000D) and an anti-Xa/anti-IIa ratio of 3.5 [14]. The administered dose was 7500 anti-Xa units, either sc or iv.

- The synthetic direct-acting anti-IIa compound efegatran (Lilly Research Centre Ltd., Windlesham, Surrey, UK) which was dosed in a range of 0.05 to 0.4mg/kg iv.

The commercially available LMWHs were obtained from one batch and supplied by the Leiden University Medical Center pharmacy and efegatran was supplied by the manufacturer.

Sampling

Blood samples were taken pre-dose (as a baseline) and then regularly after drug administration. Free flowing venous blood was obtained from an indwelling intravenous cannula after discarding the contents of the cannula. The samples were collected in 0.5 mL of citrate containing plastic tubes (Sarstedt, 3.8% sodium citrate). Immediately after collection, plasma was obtained by centrifugation (5000*g for 6 minutes) and stored at –70°C until analysis.

Assays

Per study compound, all measurements were performed in one batch on a single instrument using plasma, which had been frozen and thawed only once. The APTT was determined using standard procedures [15] on an ACL™ Automated Coagulation Laboratory Analyser (Instrumentation Laboratory, Milan, Italy). Plasma anti-IIa activity was assessed using a chromogenic amidiolytic assay [16]. This was also done using an ACL™ Analyser with the 'IL Test' heparin kit (Instrumentation Laboratory, Milan, Italy). For each compound a calibration curve was constructed against the first international standard for LMWH (WHO-code 85/600 [17]) obtained via the National Institute for Biological Standards and Control (UK) and the anti-IIa activity was expressed in International Units (IU).

Analysis

The time points at which samples were taken concomitantly for anti-IIa activity and APTT were identified. The baseline APTT reference-range for each compound was calculated as the mean value ± 1.96 times the standard deviation (SD) from the pre-dose

samples. For the post-dose samples in which both parameters were measured, the anti-IIa activity was plotted on X-axis of graphs with the corresponding APTT-values on the Y-axis. The relationship between these parameters was determined by regression-analysis. The slope and the intercept of these functions are reported with 95% confidence intervals (95%CI).

RESULTS

The plots showing the APTT vs. anti-IIa relationship are shown for each compound in Figure 1, and for a more direct comparison in one graph in Figure 2. In the latter figure, the best possible linear fit for the relationship between anti-IIa activity and APTT is also indicated. The results of the linear fits are summarised in the table. The data indicate that the baseline-range (intercepts) for the compounds was comparable. The slopes of the plots for the two LMWHs were almost identical. For efegatran, however, the slope of the curve is shallower compared to the slope of the LMWHs. This indicates that at a target APTT of 60 seconds, efegatran's anti-IIa activity will be approximately 4.5-fold higher than that of the 0.25 IU for the LMWH's.

DISCUSSION

The results of this study data indicate that meaningful differences exist in the antithrombotic activity after partially or fully selective anti-IIa antithrombotics (with different modes of action) assessed by the APTT in man. This is an important observation as a target APTT is commonly used as a biomarker to compare antithrombotics, which assumes that identical APTTs reflect identical antithrombotic states. The data from this study suggest that the APTT is an inappropriate biomarker and hence, may result in erroneous comparisons.

The selection of a suitable biomarker is important, and yet not always easy. During the development of new antithrombotic agents the limitations of the traditional coagulation assays as biomarkers was previously shown [2]. However, it is frequently the case that biomarkers are used because they are available rather than because they are responsive to needs. A rational

choice may be to use a selective biomarker that the drugs that are compared have in common.

For thrombin inhibitors, the biological activity against factor IIa, as used in this study, might be a more appropriate biomarker. Anti-IIa activity directly reflects the action of the drugs and should bear a relationship with the clinical events. It is realised that it remains to be determined how precisely anti-IIa levels associate with clinical endpoints (e.g. bleeding, recurrent thrombosis), because in most studies the number of events is too low to allow statistically reliable evaluation, but that is true for the APTT as well [18]. Our data indicate that it is apparent that APTT is unsuitable to be used in comparative studies. It adequately indicates therapeutic and toxic doses of UFH but does not necessarily reflect the *in vivo* antithrombotic potential of newer compounds, such as the thrombin inhibitors. Moreover, an equivalent effect on this generalized assay of coagulation induced by different compounds does not correspond to equivalent antithrombotic effects in clinical practice. Indeed, the present study clearly indicates that in order to obtain similar APTT levels, much higher anti-IIa activities will be present with a specific thrombin inhibitor compared to LMWHs. This is corroborated by the results of Carteaux et al. in an animal model [19].

When the present results are extrapolated to UFH, this will result in a steeper slope for the relationship of anti-IIa vs. APTT compared to the LMWHs. Assuming that hirudin has a comparable anti-IIa vs. APTT relationship as efegatran, then heparin and hirudin will differ like LMWHs and efegatran. This implies that at therapeutic APTT, much higher anti-IIa-levels will be present after hirudin compared to heparin. Even if hirudin would have a steeper slope compared to efegatran, the huge difference between the LMWHs and efegatran in this study makes it plausible that this will hold true for the comparison between UFH and heparin. Our findings might then well explain the safety observations of the pilot phase of the HIT-III study [12], as well as the interruption of the TIMI 9a [11] and the GUSTO-IIa [10] trials. It also provides a rationale to explain that direct thrombin-inhibitors, which were initially thought to be safe and effective, did not live up to their

promise in large clinical trials as GUSTO-II and TIMI-9 trial [20,21]. Others have tried to explain these findings alternatively by showing that hirudin blocks thrombin generation, using prothrombin fragment F_{1+2}, more effective than heparin [22]. However, if hirudin and heparin are compared at a certain APTT, this is synonymous with higher plasma anti-IIa levels after hirudin. Thus the equilibrium reaction of prothrombin to thrombin will be shifted to the left and thrombin generation, reflected by the split-product of this reaction F_{1+2}, is inhibited more. Thus it is unnecessary to assume different thrombin-generation capabilities of the compounds. The use of a common feature of heparin and hirudin as anti-IIa (assessed using a single standard as in this study) might have prevented this bias. Because the results of clinical trials with direct thrombin inhibitors in VTE were encouraging [6,7,8], further studies are required to define the full potential of these agents in arterial indications, particularly at lower doses.

In conclusion, this study indicates that the APTT is a biomarker with compound-specific characteristics. This makes its value to monitor the effects of new (more specifically acting drugs) questionable and may easily result in a biased comparison if a target-APTT is used in clinical trials. It is likely that a direct reference as the anti-IIa activity may be more appropriate.

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TABLE 1 **Mean (95% confidence interval) for the slope and intercept of the linear relationship between APTT and the anti-IIa activity of dalteparin, nadroparin and efegatran.**

COMPOUND	SLOPE (sec/UI anti-IIa)	INTERCEPT (sec)	r
Dalteparin	116.3 (102.7–130.0)	31.6 (30.9–32.3)	0.68
Nadroparin	110.3 (98.2–122.3)	31.4 (30.7–32.2)	0.70
Efegatran	25.3 (23.4–27.3)	31.8 (31.2–32.4)	0.79

r = correlation coefficient

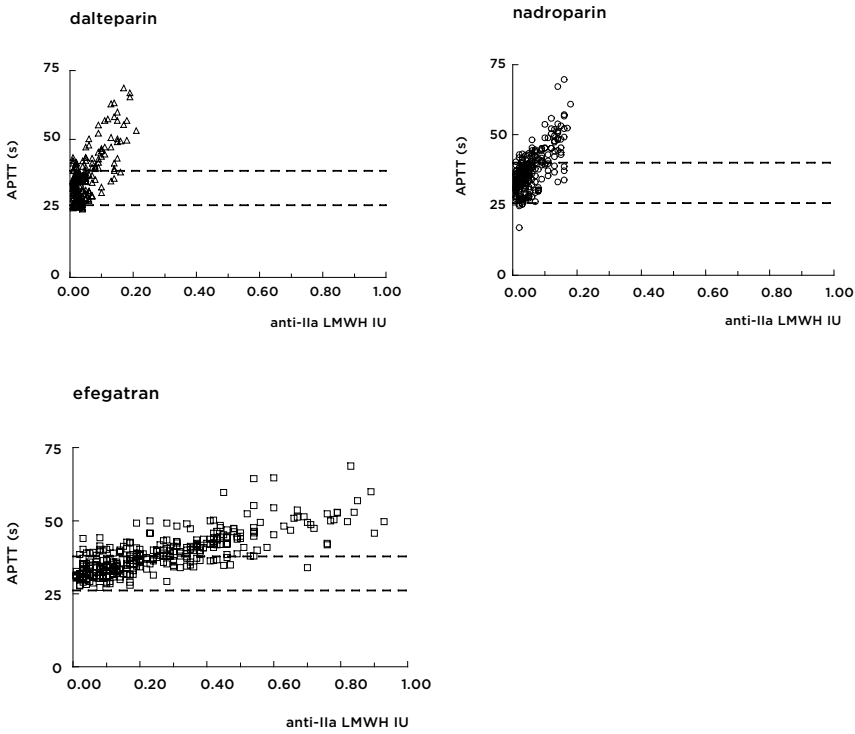

FIGURE 1 **APTT vs. anti-IIa activity for the LMWHs dalteparin and nadroparin and the thrombin inhibitor efegatran.**
The dotted lines indicate the reference range per compound.

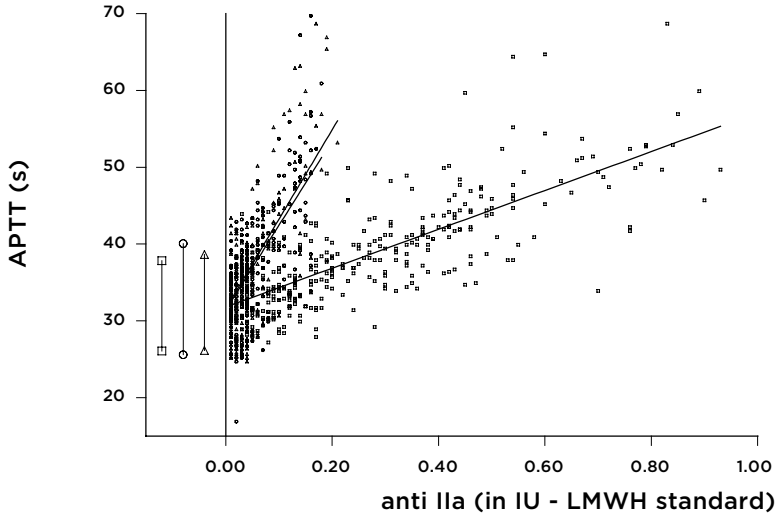

FIGURE 2 **APTT (in seconds) vs. anti-IIa activity (in international LMWH units), including the linear fit for the relationship between anti-IIa activity and APTT for dalteparin (△), nadroparin (○) and efegatran (□).**
The vertical lines at the left indicate the baseline range for each compound.

CHAPTER 17

DISAGREEMENT BETWEEN BEDSIDE AND LABORATORY ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT) AND INTERNATIONAL NORMALISED RATIO (INR) FOR VARIOUS NOVEL ANTICOAGULANTS

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ABSTRACT

During studies on warfarin, heparin and various anticoagulants with novel mechanisms of action, the activated partial thromboplastin time (APTT) and the (apparent) international normalised ratio (INR) from a bedside monitor (Coagucheck Plus®) were compared with laboratory assay results. Data were compared using the Bland and Altman method of comparison where systematic differences result in significant slopes of the regression line. During heparin treatment, the bedside monitor largely underestimated the APTT (slope=-0.80). During treatment with the direct thrombin inhibitor Napsagatran (slope=0.99), the pentasaccharides fondaparinux (slope=0.77) and idraparinux (slope=0.35), and Warfarin (slope=0.60), at lower APTT levels the bedside monitor underestimated the APTT while at higher APTT levels the bedside monitor overestimated the laboratory values. The bedside monitor slightly overestimated the INR during treatment with Warfarin (slope=0.33). Apparent INR was largely overestimated during treatment with fondaparinux (slope=1.38), idraparinux (slope=0.97), Napsagatran (slope=1.23), and recombinant tissue factor pathway inhibitor (slope=1.48, and $p<0.001$ for all regression lines).
These results indicate that a substantial disagreement in APTT or (apparent) INR exists between the bedside monitor and laboratory assay during treatment with the studied 'classic' and novel anticoagulants. The amount of disagreement depended on the anticoagulant given.

INTRODUCTION

In clinical practice, the effects of anticoagulant drugs on the intrinsic and extrinsic pathway of blood coagulation are measured using the activated partial thromboplastin time (APTT), and prothrombin time (PT) or its international normalised ratio (INR). Commonly, these parameters are assessed in citrated plasma using laboratory assays. The laboratory assays are accurate and reproducible. A disadvantage is the relatively time consuming procedure, compared to the directly available results from a bedside monitor. Bedside monitors are simple to handle, require

little training and produce APTT or PT/INR values within 3 minutes. However, for interpretation of the bedside APTT and INR results, knowledge of the agreement between both methods and repeatability of the measurement is necessary.

Numerous studies have assessed the agreement of the bedside monitor and the laboratory assay for the APTT values. Reported agreements are, however, ambiguous; excellent correlations [1-4] as well as large differences in bedside and laboratory APTT values [5,6] have been reported. All reports on APTT assessment have studied the agreement of the bedside monitor and the laboratory assay during heparin treatment. The reliability of the bedside monitor to assess non-heparin-induced changes in APTT is unknown.

Agreement for the INR values has been assessed in many warfarin studies in clinical and outpatients settings. Again, results are ambiguous; satisfactory agreement between the bedside and laboratory assays [7-11] and systematic difference or disagreement with other assays [12-15] have been reported. Mostly, an agreement was found for an INR value of approximately 3 [12,16].

As agreement has only been assessed during treatment with warfarin, the reliability of the bedside monitor to evaluate changes in the extrinsic pathway by drugs other than vitamin K antagonists is unknown.

As new anticoagulant drugs with different mechanisms of action emerge on the market, the reliability of the bedside monitor needs to be assessed for each new (class of) anticoagulant. At the Centre for Human Drug Research, bedside monitors have been used to obtain initial safety INR and APTT values during several studies with anticoagulant drugs. Blood sampling for bedside and laboratory APTT and INR were performed concomitantly, enabling comparison between the laboratory assay and the bedside monitoring. Here we report on the comparison between bedside and laboratory assays during treatment with the 'standard' anticoagulant drugs heparin and warfarin as well as with new anticoagulant drugs with various mechanisms of action on the coagulation cascade.

METHODS

Treatments

The data from a number of studies with different anti-coagulant drugs are reported here. The following drugs were studied; heparin (Spruyt Hillen, Utrecht, The Netherlands), warfarin (Coumadin®), the direct thrombin inhibitor napsagatran [17] (F. Hofmann-La Roche Ltd., Switzerland), the selective anti-Xa pentasaccharides fondaparinux [18] and idraparinux [19] (NV Organon, Oss, the Netherlands), and recombinant tissue factor pathway inhibitor (rTFPI) (G.D. Searle Corporation, Skokie, IL, USA). In addition, various combinations were studied (Table 1).

Subjects

All studies were conducted according to the principles of the 'Declaration of Helsinki' and in accordance with the Guidelines for Good Clinical Practice. The study protocols were approved by the Committee on Medical Ethics of the Leiden University Medical Center. All subjects were included after giving written informed consent. The subjects were healthy as assessed by medical history, physical examination, 12-lead ECG, routine clinical laboratory screening (blood chemistry, hematology and urinalysis) and faecal occult blood testing.

Blood sampling and assays

APTT and PT/INR were assessed prior to dosing the study drug, and frequently at subsequent time points after dosing. Blood for laboratory assay was drawn concomitantly with the bedside measurement of APTT and PT/INR. The sampling schedule depended on the studied drug. Blood for the laboratory assays was drawn in (room temperature) tubes (Vacutainer, Becton Dickinson & Co, Plymouth, UK) containing 0.129 M sodium citrate. Within 30 min blood was centrifuged at 2000 g for 15 min at 4 °C. Plasma was frozen in plastic tubes at –40 °C until assay. Following these blood handling procedures no effect of freezing on PT and APTT was observed (data on file). During the rTFPI study blood was drawn in pre-chilled tubes and immediately kept on ice water until centrifugation. Currently, the 0.129 mol/L sodium citrate

is no longer recommended by the WHO for INR determinations. The laboratory APTTs and PTs were determined, in duplicate, in citrated plasma on a STA® coagulation analyser (Roche Diagnostics, Mannheim, Germany). The reagents were STA APTT (Roche Diagnostics, Mannheim, Germany) and Thromborel S (sensitive human placental thromboplastin, ISI≈1) (Behring Diagnostics, Marburg, Germany). During napsagatran treatment the PT was assessed with Innovin reagent (Dade Behring, Liederbach, Germany) on a MLA 1000c coagulation analyser. Laboratory INR was calculated using the PT normal, which was determined for each batch of thromboplastin, and the ISI, as provided by the manufacturer. See table 1 for the ISI values of the thromboplastin lots used during the various studies. Inter- and intra-assay precision (CV) for the APPT and PT was smaller than 5%. The bedside measurements were made using three Coagucheck Plus® (formerly called Biotrack 512®) coagulation monitors (Roche Diagnostics, Mannheim, Germany) following manufacturer instructions. The monitor is a laser photometer with ready-to-use APTT- and PT-cartridges (Roche Diagnostics). For the measurement, one drop of fresh whole blood was applied on the (pre-inserted and pre-warmed) cartridges. Blood was drawn into the monitor by capillary action. PT and APTT are defined as the time from application until clot detection. The PT cartridge contains a low-sensitive rabbit brain thromboplastin (ISI ≈ 2). The APTT cartridge contains soybean phosphatides and bovine brain sulfatide reagent. The APTT from the bedside monitor is calibrated against Organon Teknika Auto reagent (Organon Teknika, Boxtel, The Netherlands) (data from Roche Diagnostics, Almere, The Netherlands). The APTT normal range is 21 to 41 seconds and has a measurement range from 18 to 150 seconds. The within-day and between-day precision (CV) for assessment of APTT is reported to range from 3 to 7% and from 4 to 8% respectively (2,4]. The PT normal range is 10 to 13.8 seconds; normal is defined as 12 seconds; measurement range is 8 to 50 seconds (INR; 0.5-18). The within-day and between-day precision (CV) for PT assessment is reported to range from 3 to 5% and from 2 to 7 % respectively [2,10]. The inter-instrument variability was

1.8% for the APTT and 2.9% for the PT measurements. Before each study the apparatuses were validated on the entire range using PT and APTT Coagucheck Plus quality controls. Quality controls consisted of fixed erythrocytes and human plasma that was diluted and subsequently applied on the cartridges to assess system performance on two different levels, following manufacturer instructions. On each study-day the microprocessor and optical laser system were checked using electronic quality control cartridges. Throughout the studies different lots of cartridges have been used (see table 1 for ISI values). The monitor electronically corrects lot-to-lot variability in reagents using information coded on the test cartridge.

Data analysis

Bedside and laboratory agreement in APTT and INR were calculated according to the method of comparison described by Bland and Altman [20]. The differences were plotted against the average value of both methods. In the plot, three observations can be made; firstly, the average difference (bias) between the two methods, secondly, the variability of the differences, as shown by the scatter, and thirdly, a possible trend in the difference. The latter observation indicates whether a tendency of the mean difference to increase or decrease with increasing magnitude exists [21]. It should be noted that, if a trend is observed, the magnitude of the average difference is dependent on the data range. For the observed trends Pearson correlation coefficients were calculated. As correlation coefficients are influenced by the data range, in addition, slopes of the regression lines were calculated. A genuine change in the difference with increasing magnitude will be reflected by a regression line with a non-zero correlation and slope.

Correction of PT values with the International Sensitivity Index (ISI) ($INR = (PT_{patient} / PT_{mean\ normal})^{ISI}$) should lead to zero INR differences and slopes. The precision of the agreement was calculated as mean bias ± 1.96 standard deviations (95% limits of agreement). Precision (repeatability) of the individual methods was assessed by determining the coefficient of variation (CV)

expressed in percentage (as the SDx100 divided by the mean) for all pooled duplicate baseline samples.
Data handling and testing were performed using SPSS statistical software (V9.0.1, SPSS Inc. Chicago, IL, USA). P-values smaller than 0.05 where regarded significant.

RESULTS

APTT agreement (Fig. 1 and Table 2)
Pre-dose the bedside monitor underestimated the laboratory APTT.

Heparin

During heparin treatment an increasing underestimation of the APTT by the bedside monitor was observed with increasing APTT. This is reflected in the large mean difference (-30.7 seconds) and the negative slope for the change in difference.

Other anticoagulants

During treatment with the direct thrombin inhibitor napsagatran, the pentasaccharides fondaparinux and idraparinux, and warfarin, at lower levels the bedside monitor underestimated the APTT while at higher APTT levels the bedside monitor overestimated the laboratory values. The shift in the difference, as expressed by the slope of the regression line, depended on the anticoagulant drug given ($p<0.001$ for all regression lines).

PT agreement (Table 3)
During treatment with warfarin and idraparinux the bedside monitor underestimated laboratory PT (a negative mean difference was present) and negative correlation for the differences in PT values was observed. During treatment with fondaparinux, napsagatran and rTFPI, a positive correlation was observed for the trend in the difference between bedside and laboratory PT.

INR agreement (Fig. 2, Table 3)
Translation of the PT values to INR led to a general overestimation by the bedside monitor of laboratory INRs.

Warfarin

INR values from the laboratory were overestimated by the bedside monitor (bias 0.36) and an increase in the difference was observed with increasing magnitude (slope = 0.33, $P<0.001$).

Other anticoagulants

During treatment with the other anticoagulants an increase in the difference in apparent INR was observed with increasing magnitude (all $p<0.001$). The rate of overestimation, as described by the mean bias and the slope of the regression line, depended on the anticoagulant drug given.

Repeatability
Laboratory APTT variability (CV) of the duplicate baseline measurements was 10.2%. Bedside APTT variability was 20.6%. Laboratory PT variability was 3.0%, and bedside PT variability was 6.7% seconds.

DISCUSSION

In this study, the agreement of the bedside monitor with the laboratory assay was assessed according to the method of comparison as described by Bland and Altman [20]. For none of the anti-coagulant treatments studied a good agreement in APTT values was observed between the bedside monitor and laboratory assay. For all treatments, a genuine shift in the difference was observed with increasing APTT. Calculation of INR values to correct for the thromboplastin used in the two methods did not produce a sufficient agreement between the bedside monitor and laboratory assay. During all treatments the bedside monitor overestimated laboratory INR values and this overestimation became more prominent at higher INR values. The rate of under- or overestimation for APTT and INR assessment depended on the drug given.

The 95% limits of agreement of the difference between two methods are determined by a trend in the difference and the scatter of the individual differences. The latter is dependent on the repeatability of the individual methods. The repeatability, therefore, limits the amount of agreement between two methods. The agreement of the APTT and PT between bedside and laboratory was, in part, limited by the relatively poor repeatability of the bedside monitor. Precision may be improved by performing bedside measurements in duplicate. However, the poor agreement can be mainly attributed to the trends in the difference with increasing magnitude.

The difference between bedside and laboratory APTT values during heparin treatment may be caused by a variety of factors that affect the APTT measurement system. As the APTT response is affected by the type of clot detection system used, the contact activator, and the phospholipid composition of the reagent [22], factors that differ largely between the two systems, not surprisingly a disagreement between the results of the bedside monitor and laboratory assay was observed. Different commercial APTT reagents are known to vary in their responsiveness to heparin [22,23]. This may account for the differences observed between the bedside monitor APTT which is calibrated against Organon Technika auto reagent and the laboratory APTT which has been determined with the STA APTT reagent. In addition to the difference in measurement system, the assays differ in the medium in which the APTT is measured. Differences in handling of blood samples are known to have considerable effect on the APTT [24]. Sample processing to acquire citrated plasma may result in removal of endogenous compounds that are present in whole blood. In addition, neutralisation of circulating heparin by platelet factor 4 released from platelets during collection of blood from anticoagulated patients could result in an underestimation of the actual *in vivo* heparin effect [25].

During warfarin treatment the bedside monitor overestimated laboratory INR levels with an increasing difference at higher INRs. In our studies we used blood sampling tubes containing 0.129 mol/L sodium citrate. This may affect the ISI and hence

the INR results [26]. However, due to the low ISI of the Thromborel S, the effects of sodium citrate on INR will be small [27]. In addition, if having an effect on the INR, the 0.129 mol/L sodium citrate will increase INR and hence tend to decrease the differences we observed between bedside and laboratory INR. The disagreement in INR levels is more likely to be caused by the relatively short warfarin treatment (3 days) in our kinetic studies. For assessment of ISI values, prothrombin times are calibrated using plasma from patients stabilised on oral anticoagulant treatment [28]. The ISI may therefore not be valid in all other situations. The decrease in the levels of the coagulation factors during warfarin treatment depends on their respective half-lives [29]. During the first 2-5 days of treatment, the prolongation of the PT is mainly the result of a reduction of functional factor VII and partially because of reduction of factor X. PT tests have a variable response to depletion of factors VII and X due to a difference in thromboplastins. The disagreement in INR levels may be attributed to a difference in sensitivity of the thromboplastins towards changing levels of the coagulation factors.

The thromboplastin from the bedside monitor is less sensitive than the thromboplastin used in the laboratory resulting in higher PT levels during warfarin treatment. This is corrected with the ISI resulting in almost equivalent INR values. As the ISI corrects the PT ratio, in part, for the thromboplastin used it was expected to normalise likewise the differences between PT values from both methods during treatment with non-vitamin-K antagonist anticoagulants. However, during treatment with all non-vitamin-K antagonist anticoagulants on higher PT the bedside monitor overestimated the laboratory PT (i.e. the relationship of the difference in PT between both methods had a positive slope) and the INR was largely overestimated by the bedside monitor. This indicates that the correction of PT ratios with ISI values may only be used during treatment with K-antagonists and not with the studied pentasaccharides, thrombin inhibitor and recombinant TFPI. As the INR has been defined exclusively for patients stabilized on oral anticoagulants, this

finding is not expected, but to our knowledge has not yet been reported for the studied anticoagulants.

Based on the laboratory INR assay in citrated plasma, the optimal intensity of anticoagulant therapy during vitamin-K antagonist treatment has been investigated extensively [30]. A clear relationship between INR values and the most important events observed during anti-coagulant treatment, *i.e.* thrombo-embolism or bleeding, has been assessed leading to an optimal INR range. For the APTT, so far, no clear relationship between the APTT increase induced by heparin and its clinical efficacy or safety has been demonstrated [31]. For clinical practice, it would be interesting to investigate the relationship between APTT and PT/INR values during treatment with the various anticoagulants and incidence rates of thrombo-embolic or bleeding events, in order to establish an optimal bedside monitor APTT or PT/INR during treatments with these drugs. Possibly, results from the bedside monitor may have a more predictable value. The bedside monitor system has some advantages; results are easy and rapidly obtained which may, in part, improve outcome. In addition, due to less processing of the sample, results from the bedside monitor may be more reliable and give a more 'true' estimate of blood coagulation status.

The degree of agreement between bedside and laboratory assay is dependent on the anti-coagulant treatment given. As results may differ largely, the results from the bedside monitor may not be representative for the laboratory assay results. For some anticoagulants differences are marginally and the bedside assessment may serve as a rapid method to determine the anti-coagulant status of a patient. In this respect it should also be noted that there is a difference between statistical significance and clinical relevance [8,32]. The interpretation of the bedside monitor should depend on the anticoagulant treatment and clinical setting in which the apparatus is used. Development of normograms to correct for the differences may serve to help to use the bedside monitor and the laboratory assay interchangeably.

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TABLE 1 Overview of anticoagulant drugs studies and ISI values of laboratory and bedside PT assay

TREATMENT	NUMBER OF SUBJECTS	AVERAGE AGE (RANGE) IN YEARS
Heparin	9	21 (19–23)
Napsagatran	12	24 (22–27)
Fondaparinux	12	22 (19–27)
rTFPI	8	23 (20–28)
Idraparinux	12	23 (20–30)
Warfarin	12	22 (19–27)
Warfarin + napsagatran	12	24 (22–27)
Warfarin + fondaparinux	12	22 (19–27)
Warfarin + idraparinux	16	24 (18–35)

iv = intravenous, sc = subcutaneous, ISI = International Sensitivity Index, ND = not done, bedside PT normal was 12.0 seconds for all studies

	DOSE AND MODE OF ADMINISTRATION	ISI	
		Laboratory	Bedside
	2000 IU *iv* for 40 min	ND	ND
	80 µg/min *iv* for 48 hours	0.92	2.03
	4 mg *sc* OD for 5 days	1.08	2.05
	0.2 ng/kg/min *iv* for 4 hours	1.09	2.04
	10, 12 or 14 mg *iv* or 10 mg *sc*	1.08	2.04
	10 or 15 mg *oral* OD for two days	1.08	2.05
	single dose *oral* warfarin 25 mg with *iv* napsagatran 80 µg/min for 48 hours	0.92	2.03
	5 x sc fondaparinux (4mg) at 24 h intervals + *oral* warfarin at the 4th (15mg) and 5th (10mg) injection	1.08	2.05
	single *sc* 10 mg dose idraparinux followed by *oral* warfarin at 24h (15 mg) and 48 h (10 mg)	1.10	2.04

TABLE 2 Mean differences between bedside and laboratory APTT

With 95 % limits of agreement, correlation coefficients and slopes of the regression for the observed trends in the difference

TREATMENT	N	MEAN DIFFERENCE (SECONDS)	95% LIMITS OF AGREEMENT	CORRELATION COEFFICIENT	SLOPE OF REGRESSION
Heparin	27	-30.7	-68.9/7.5	-0.89‡	-0.80‡
Napsagatran	108	15.4	-12/42.8	0.94‡	0.99‡
Idraparinux	164	-9.2	-22.9/4.5	0.29‡	0.35‡
Fondaparinux	155	-5.2	-18/7.8	0.66‡	0.77‡
Warfarin	152	-6.6	-24.0/10.7	0.50‡	0.60‡
Warfarin+fondaparinux	151	-4.6	-19.5/10.3	0.38‡	0.42‡
Warfarin+idraparinux	78	-13.1	-25.8/-0.3	0.62‡	0.81‡
Warfarin+Napsagatran	108	18.1	-15.5/51.7	0.82‡	0.88‡

N= number of measurements, ‡ p<0.001, * p<0.05

TABLE 3 Mean differences between bedside and laboratory PT and INR

With 95 % limits of agreement, Pearson correlation coefficient and slopes for the observed trends in the difference

		PT			
TREATMENT	N	MEAN DIFFERENCE (SECONDS)	95% LIMITS OF AGREEMENT	CORRELATION COEFFICIENT	SLOPE OF REGRESSION
Warfarin	105	-3.1	-9.5/3.3	-0.83‡	-0.81‡
Fondaparinux	107	-0.3	-2.7/1.9	0.38‡	0.45‡
Idraparinux	85	-2.0	-4.1/0.1	-0.24*	-0.25*
Napsagatran	108	2.5	0.3/4.7	0.62‡	0.41‡
rTFPI	179	1.0	-3.3/5.3	0.81‡	0.57‡
Warfarin+fondaparinux	106	-3.2	-10.4/3.9	-0.85‡	-0.77‡
Warfarin+idraparinux	64	-2.1	-7.2/3.3	-0.85‡	-0.60v
Warfarin+Napsagatran	107	-0.7	-9.0/7.6	-0.85‡	-0.74‡

N= number of measurements, ‡ p<0.001, * p<0.05

	INR			
	MEAN DIFFERENCE	95% LIMITS OF AGREEMENT	CORRELATION COEFFICIENT	SLOPE OF REGRESSION
	0.36	-0.25/0.96	0.47‡	0.33‡
	0.27	-0.20/0.74	0.89‡	1.38‡
	0.18	-0.15/0.51	0.77‡	0.97‡
	0.52	-0.19/1.22	0.96‡	1.23‡
	1.00	-0.96/2.96	0.99‡	1.48‡
	0.42	-0.51/1.35	0.46‡	0.40‡
	0.27	-0.41/0.95	0.71‡	0.42‡
	0.56	-0.33/1.45	0.71‡	0.47‡

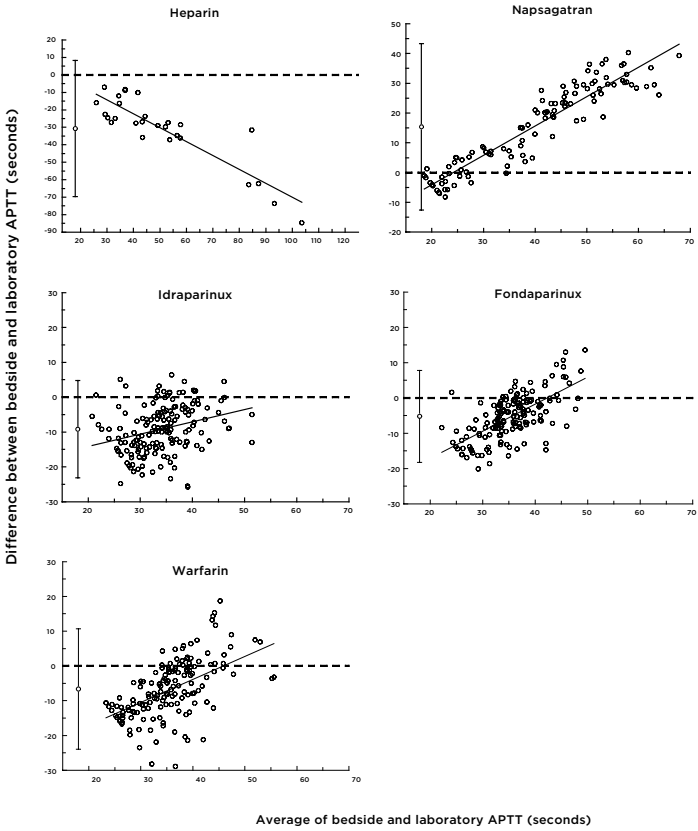

FIGURE 1 **Scatter (○) of the difference in APTT between the bedside monitor and laboratory assay against the average values of both methods during treatment with heparin, napsagatran (direct thrombin inhibitor), idraparinux and fondaparinux (pentasaccharides), or warfarin.**

The scatter above the (dashed) zero line indicates overestimation of the APTT by the bedside monitor while the scatter under the zero line indicates underestimation by the bedside monitor. The solid lines represent the regression lines for the observed trends in the difference (all significant; $p<0.001$). The markers with error bars indicate the mean difference and the 95% limits of agreement.

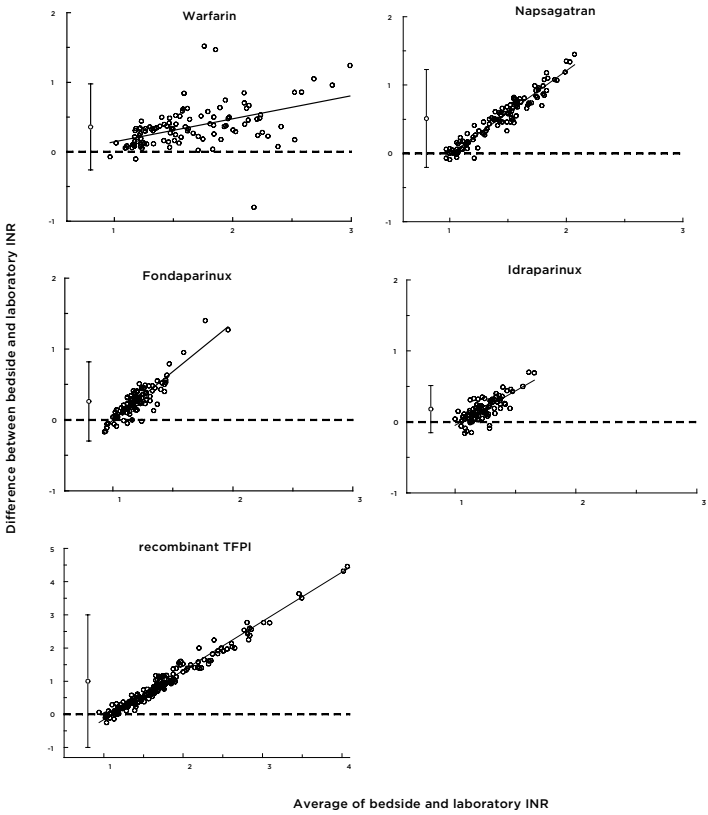

FIGURE 2 **The difference in INR between the bedside monitor and laboratory assay against the average value of both methods during treatment with warfarin, idraparinux and fondaparinux (pentasaccharides), napsagatran (thrombin inhibitor), and recombinant TFPI.**

The scatter (D) above the (dashed) zero line indicates overestimation of the INR by the bedside monitor while the scatter under the zero line indicates underestimation by the bedside monitor. The continuous lines represent the regressions line for the observed trends in the difference (all significant; $p<0.001$). The markers with error bars indicate the mean difference and the 95% limits of agreement.

SECTION IV

CONCLUSIONS

CHAPTER 18–19
+
CURRICULUM VITAE

CHAPTER 18

SUMMARY AND CONSIDERATIONS

Haemostasis and thrombosis are extraordinarily complex phenomena, which, despite a tremendous research effort, are still incompletely understood. The importance of understanding the events leading to thrombosis and its prevention are obvious. The explosion of research in this area and the expanding armamentarium of antithrombotic drugs have furthered our knowledge of thrombogenesis. The development of successors of the conventional compounds is also fostered by the drawbacks of the presently available conventional anticoagulant therapies. Although the prevention and management has greatly improved over recent years, existing therapies fail to prevent VTE in a proportion of cases in high-risk procedures, and are associated with important adverse effects. The need for more effective and safer agents has led to the development of novel indirect and direct coagulation inhibitors [1].

An ideal antithrombotic agent should reliably prevent thromboembolic events without impairing haemostasis. It is increasingly recognised that people at risk of thrombotic disorders need to be treated over an extensive period of time (months, years, or even life-long). Prolonged antithrombotic treatment causes significant inconvenience. Based on these considerations, the development of antithrombotic agents with more defined and specific action has emerged during the last decades [2]. At present, further studies are required in all indications, including cost-effectiveness studies of thromboprophylaxis with these new compounds. [1].

The low molecular weight heparin products have already improved our ability to control anticoagulant therapy because drug concentrations are more predictable in patients receiving these products. In addition, low molecular weight heparins produce a more consistent, predictable anticoagulant response, giving clinicians a new pharmacological tool which may readily lend itself to patient-controlled, home-based anticoagulant pharmacotherapy [3].

Current interests in thromboprophylactic research are the development of inhibitors of TF, factor VIIa, factor VIIa-TF, factor IXa, factor XIIa, and factor XIIIa. Although inhibition of specific enzymes appears a logical approach to control of thrombogenesis,

each of these enzymes or enzyme/activator complexes represents a specific site with limited activation potential. Nevertheless, all of these proteases and activators, except for factor XIIIa, also augment the generation of Factor Xa. Therefore, inhibition of Factor Xa represents a more comprehensive approach to control thrombogenesis [4].

CHARACTERISATION OF NOVEL ANTITHROMBOTICS

Previous experience with pentasaccharides suggested that with this group of compounds a highly selective antithrombotic effect can be elicited. The first of this new class of synthetic inhibitors of factor Xa tested thus far (fondaparinux) has been shown to be safe in pre-clinical and human studies, both in healthy volunteers and patients [5,6,7,8,9,10]. The drug appears to be eliminated mainly by renal excretion and has been reported to have an elimination half-life of its biological effect of approximately 15–20 h.

In this thesis some important issues regarding the use of pentasaccharides were addressed. In **CHAPTER 3** the result are presented regarding the influence of renal function on the pharmacokinetics of fondaparinux. It was demonstrated that the clearance of this drug decreased proportionally with renal function impairment. With an estimated $r^2 = 0.90$ for the linear relationship between the creatinine-clearance (used as a measure for renal function), only about 10% of the variability in clearance is due to other causes. As renal function was assessed with a simple method (creatinine clearance calculation using a single plasma creatinine concentration), this provides an easy tool for dose adaptation of this drug if deemed necessary in clinical practice. Indeed, the methodology can be used to preliminary predict the influence of renal function. If all administrations from studies described in **CHAPTERS 3, 4** and **5** are recalculated to the currently preferred prophylactic dose of 2.5mg once-daily subcutaneously (Pentathlon-study) the concentration time profiles can be calculated (Figure 1). The figure also shows the peak and trough concentrations.

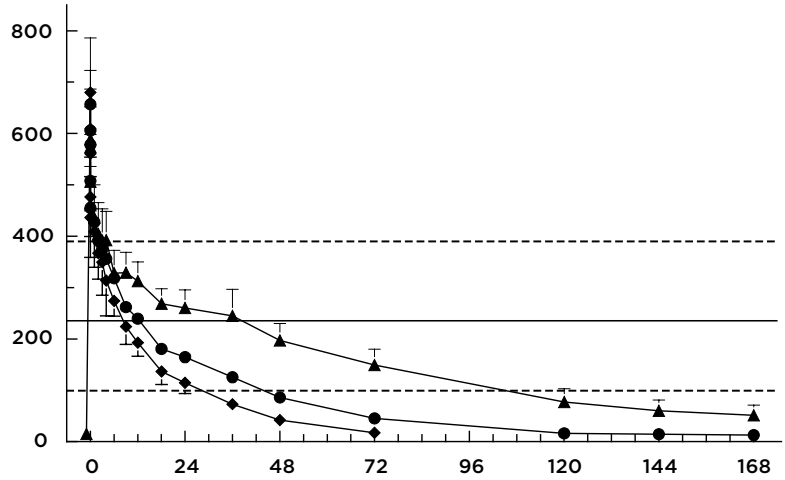

FIGURE 1 **Fondaparinux plasma-concentration time profiles recalculated to a once daily sc dose of 2.5mg.**
The three curves display the average curves from the fifteen subjects with an impaired renal function. The horizontal dashed lines indicate clinically important concentrations; upper line : peak concentration; lower: trough concentration. The solid line indicates the trough-level if the drug were to be given in a sc dose of 6mg once daily

The figure indicates that dose-adaptation may be only necessary in the patients with moderate (CL_{CR} >30-60 mL/min) or severe renal impairment (CL_{CR} 10-30 mL/min). The simulation with the 6-mg dose (which was discontinued in the phase II dosing study) clearly shows that this dose will result in plasma concentrations that are higher than the 2.5-mg peak level for the greater part of the day. Hence, it could have been expected that this dose would result in an unfavourable benefit-risk ratio.
It is increasingly recognised that people at risk of thrombotic disorders need to be treated over an extensive period. As this causes significant inconvenience, a longer acting safe anti-thrombotic compound would be preferable. The longer-acting pentasaccharide idraparinux shows a specific activity of about 1400 anti-Xa units/mg. The higher activity of idraparinux

compared to its shorter acting predecessor was reflected in a higher antithrombotic efficacy in different animal models of thrombosis. It was shown that idraparinux required 2 times less amount of drug to display the same effect [11].
The first-entry-into-man study with this long-acting pentasaccharide (**CHAPTER 6**) demonstrated its long elimination half-life, its linear pharmacokinetics and its 100% subcutaneous bioavailability in young volunteers. These findings were reconfirmed for elderly volunteers (**CHAPTER 7**) and the results of this study suggested that age-related effects on the pharmacokinetics and pharmacodynamics of the drug are probably minor.
In order to mimic the current clinical practice, a multiple dose study with the compound idraparinux was conducted in people recently treated for a VTE (DVT or PE). They received idraparinux subcutaneously in a once-weekly schedule for 4 weeks subsequent to their initial treatment period of at least 3 months (**CHAPTER 8**). For the peak levels, steady-state was already reached after the second dose, and for the trough levels this was reached after the third administration. With the results of this study (and the previous studies), the profile of idraparinux, as if it were administered for a longer period of time, could easily be calculated.

In **CHAPTER 4, 5** and **9** interaction studies with the pentasaccharides are described. It was shown that there was no pharmacokinetic interaction for this group of compounds with warfarin. These results can most likely be extrapolated to the entire groups of the coumarin-derivatives. Another important finding is the fact that the biomarker Prothrombin Time (PT, currently expressed in INRs) can still be used to monitor the effect of the coumarins during the initial concurrent administration as occurs in clinical practice. For the short-acting pentasaccharide no pharmacokinetic interaction could be found with the non-steroidal anti-inflammatory drugs (NSAIDs).
Using the appropriate pharmacodynamic measures as biomarker, the different effects of the compounds in this interaction study could easily be separated. This suggests that no pharmacodynamic

interaction between the pentasaccharide and NSAIDs will occur. Although the differences between the shorter and the longer-acting pentasaccharide are obvious, it is unlikely that they will behave differently with regard to the interactions with the NSAIDs. It thus seems that pentasaccharides provide an important addition to the therapeutic armamentarium, although some caution must be expressed with regard to the influence of renal function.

On theoretical grounds, most of the drawbacks of the currently used antithrombotic drugs might be overcome by the use of direct thrombin inhibitors. Indeed, preclinical studies with these drugs generated considerable excitement and anxious anticipation regarding their prospects as antithrombotic agents [12]. Therefore, many thrombin inhibitors have been developed, and several of these agents are currently undergoing various phases of clinical trials during the recent years [13]. The combined administration of direct-acting thrombin inhibitors and oral anticoagulant therapy with coumarin derivatives is conceivable in clinical practice. In CHAPTER 11 summarises the results of a study in which the effect of warfarin on napsagatran in healthy male volunteers was investigated. Warfarin was shown to have no effect on the pharmacokinetics of napsagatran, but to markedly influence the pharmacodynamics of napsagatran. Although large differences exist between compounds of the heterogeneous group direct-acting thrombin inhibitors, it can clearly be concluded from this study, that the PT should not be used as biomarker during the switch from any direct-acting thrombin inhibitor to oral anticoagulant therapy.

Another class of compounds that may be promising antithrombotics are drugs of a single chemical entity obtained by synthesis possessing both anti-IIa and anti-Xa activity as for instance Org 36764. The dual mechanism of action is achieved by linking a pentasaccharide structure (anti-Xa activity) while at the same time providing (by glycoconjugation) the chain-length required for the coupling of AT and thrombin (anti-IIa activity). The first administration of this compound to man showed that if anti-Xa

activity levels are used to describe the pharmacokinetics of the drug an unusual time course of drug concentrations was observed. Plasma concentrations reached a plateau, which persisted for some time after the bolus injection, and the maximum concentration increased non-linearly with the dose. Using the anti-IIa activity only low levels were observed with no obvious differences between the doses. At present no satisfactory explanation for these findings can be provided. However, it is clear that this compound, although conceptually attractive, is yet unsuitable to be further developed.

ALTERNATIVE ROUTES OF ADMINISTRATION

Although factor Xa inhibitors are a promising alternative to thrombin inhibitors a major drawback is that they can only be used parenterally. The present efforts aimed at developing high affinity synthetic factor Xa inhibitors will be especially successful when they yield an orally applicable compound [4]. Section III of this thesis contains the description of some experiments that focused on routes other than the conventional parenteral administrations.

In CHAPTER 12 it is demonstrated, that it is to no avail to administer LMWH-compounds to humans orally. Although plasma-concentrations of pentosan polysulphate sodium could not be measured directly, the indirect pharmacodynamic methods clearly showed that the oral bioavailability of this semi-synthetic compound was negligible in healthy young, male volunteers. It should thus be concluded that the development of oral compounds from this class is futile.

CHAPTER 13 describes an experiment in which a sublingual formulation of the pentasaccharide idraparinux was tested. The data indicated that this formulation has a poor sublingual bioavailability with high variability. Nevertheless, if this would be interpreted as preclusion of further investigations this towards such a formulation, because enhancements could certainly be possible. Indeed, simulations with the data obtained suggest that frequent administration of the formulation used may result in therapeutic plasma concentrations. It is also

conceivable that the use of other absorption enhancers as for instance cyclodextrines may improve the delivery characteristics of such a sublingual or buccal formulation as shown for testosterone [14].

In CHAPTER 14 the first study in humans with the orally active and supposedly selective direct-acting Xa inhibitor YM 466 is described. It was shown that YM 466 is a potential clinically applicable oral antithrombotic, but the oral bioavailability is low, highly variable, and further reduced by food intake. This may be disadvantageous in clinical practice particular for antithrombotic therapy where always a fine balance has to be found between effective anticoagulation but low bleeding tendency. For compounds with variable pharmacokinetics it will be difficult to design an 'one suits all' regimen, which makes its clinical application unattractive. In addition, and in contrast to the pentasaccharides, this compound was not a selective anti-Xa inhibitor. as demonstrated by its influence on the conventional coagulation tests (APTT, PT). The increases in APTT and PT after administration of YM 466, have also reported for other direct-acting anti-Xa compounds [15,16,17]. This thus seems to be a class-effect of these compounds. It may be argued that this is due to the different mode of action compared to the pentasaccharides. For example, in addition to circulating 'free' coagulation factor Xa these direct-acting anti-Xa agents might also inhibit clot-bound Xa, comparable to the direct-acting thrombin inhibitors [18]. Recently, it has been shown in animals that inhibition of factor Xa may reduce tissue factor expression in the liver during endotoxemia [19]. Hence, it may be tentatively argued that factor Xa inhibition results also in a decrease of an important procoaugulant factor (tissue factor) and that this is reflected in a prolonged PT. It is however unlikely that these phenomena fully explain the findings in healthy young males. Whatever the reason for the observations, it seems inevitable that the use of these drugs require monitoring, which is disadvantageous compared to the pentasaccharides. A way to avoid expensive and laborious laboratory monitoring may the use of point-of-care (POC) devices. Indeed, YM 466 activity could be very adequately

be monitored using a POC device (Figure 2). The device appears to be sensitive enough to detect PT-changes over the entire anti-Xa concentration range achieved in this study. A high correlation was established for the dose-response relationship of YM 466 and the (apparent) POC international normalised ratio (INR).

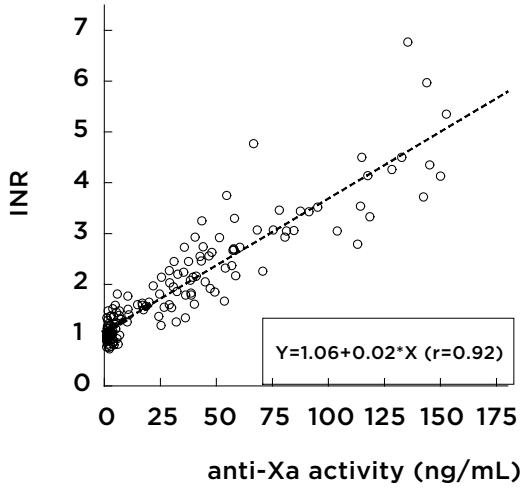

FIGURE 2 **The YM 466 anti-Xa-POC INR curve yielded high correlation.**
This may represent an effective bedside tool for monitoring the response to this novel, direct, selective factor Xa inhibitor. Nevertheless, further evaluation is warranted.

MONITORING OF ANTITHROMBOTICS

Another important point in antithrombotic therapy is the establishment of reliable and effective monitoring methods. This is addressed in section IV. The choice of suitable biomarkers is important and not always easy.
Additionally, the development of new antithrombotic agents has shown the limitations of the traditional global coagulation assays as biomarkers [20]. **CHAPTER 15** describes a comparison of antithrombotics agents in various phase I studies based on their

respective anti-Xa activities. Meaningful differences exist in the APTT and/or PT-response to partially or fully selective anti-Xa antithrombotics in man. It seems, at least for the AT-dependent heparinomimetic compounds, that the more specific a compound inhibits factor Xa, the lower the influence on the conventional coagulation assays APTT and PT. This is indeed reflected in the findings of the pentasaccharides, which lack anti-IIa activity, and did not influence the APTT/PT at all. Moreover, the slope of the anti-Xa/APTT-correlation curve for idraparinux differs by a factor 6 compared to fondaparinux, possibly reflecting the higher affinity of the longer acting pentasaccharide for AT (and thus factor Xa). These results indicate that the APTT and PT are biomarkers with compound-specific usefulness. This could make their use for monitoring the effects of new anticoagulants questionable. Nevertheless, these assays might be useful to categorise novel compounds based on the specificity for activated factor X.

In CHAPTER 16 a comparable study is described with regard to a comparison of compounds, which all showed activity against thrombin (factor IIa), and were compared using the relationship between their anti-IIa activity and the APTT. The results of this study data demonstrate that inappropriate use of a certain biomarker can unequivocally result in erroneous comparisons. By now, it has become clear that an equivalent effect on the APTT induced by different compounds does not necessarily correspond to equivalent antithrombotic effects in clinical practice. In a comparison of these kinds of agents it may therefore be preferable to use more selective biomarkers, such as biologic activities of compounds.

The current broad clinical application of the APTT and PT provides extensive clinical experience. That's why it can be assumed these assays will keep on being used, at least in the near future. Moreover, recently different apparatuses have been introduced, which can be applied for 'bedside' assessment of these conventional coagulation assays. In CHAPTER 17 one of these POC-monitors (the Coagucheck Plus®) is evaluated while determining the APTT and/or PT during treatment with various anti-

coagulants in several Phase I studies. This study demonstrates there is a substantial disagreement in APTT or (apparent) INR between
the bedside monitor and laboratory assay during treatment with the studied 'classic' and novel anticoagulants. None of the anti-coagulants showed a good agreement in APTT and INR values. With increasing results for APTT or INR, a genuine shift in the difference between the two assessment methods was observed. The amount of disagreement (under- or overestimation) for APTT and INR assessment depended on the anticoagulant given. The (clinical) implications of these findings are at present unclear as it remains to be determined if for each agent individual target ranges should be defined.

PERSPECTIVES

This thesis highlighted several important developments to improve the current antithrombotic strategies. It is apparent that the pentasaccharides provide a major step forward. But there still questions to be answered. The influence of impaired renal function on the benefit-risk ratio with widespread use of these compounds is still unknown. This issue deserves attention as it can be anticipated that a large part of the target population will consists of elderly people who may have an impaired clearance of the drug. Also, the pentasaccharides can still only used parenterally, and in this respect they do not offer advantages over the LMWHs currently in use. The results described on alternative routes of administration show that is possible to develop orally active anti-Xa agents, which is encouraging. However, it remains to be established how these agents will perform clinically, because it seems that anti-Xa selectivity is not guaranteed. Also, the preliminary data on sublingual administration of pentasaccharide suggest that this approach can be pursued further.
This thesis also emphasis the need to develop proper tools to measure the effects of novel drugs. This is already an issue in early drug development. The era that novel antithrombotics can be characterised with routine methodology as for instance

the APTT, PT or variants thereof (e.g. markers reflecting the effects on the 'overall' coagulation status) is definitely over. There should be a search for the appropriate biomarker to characterise the drug and this should include the effects of the currently available drugs on this biomarker. More importantly, validation of biomarkers beyond the stage of a bioassay should be undertaken. It is therefore that efforts should be made to investigate and establish the relationship between biomarkers and clinical endpoints. At present, this seems poorly developed, as the used markers show a robust relationship with clinically relevant endpoints [21]. This is remarkable since most of the biomarkers used (both previously and currently) meet the criteria defined for proper biomarkers in the field of antipsychotics [22]. There is no doubt that antithrombotics show a clear, consistent response across studies. Also, a clear response of the biomarker to a therapeutic dose, and a dose (concentration)-response relationship has been established. In addition, there is a relationship between the biomarker, the pharmacology of the drug and the pathogenesis of the disease. Apparently, this is not sufficient to capture the relationship between drug action and clinical outcome. It may be speculated that the complexity of the coagulation process, the underlying pathology, unknown factors during the intervention with drugs in interplay with each other form such a complex situation that is too hard to capture with this biomarker concept. It may also reflect our insufficient understanding of the biological processes during thrombotic events while we think we understand them. Although it is recognised that at present the biomarker approach is the best available approach for drug development, it should not be forgotten that it may still be insufficient. Suggestions to improve the biomarker paradigm may be simultaneous use of multiple biomarkers, to provide a more comprehensive understanding of the pathology of thrombosis, and a strict application of the biomarker approach in pre-clinical development, providing a more detailed insight into the pharmacology of the drug. This will obviously be a major task, but provides a challenging opportunity for (clinical) pharmacologists.

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CHAPTER 19

SAMENVATTING

SECTIE I / INLEIDING

Het verlies van bloed door beschadigingen als gevolg van een letsel aan de bloedvaten wordt voorkomen door bloedstolling of hemostase. Hemostase is het chemische proces waarbij vloeibaar bloed wordt omgezet in een vaste vorm (stolsel). Dit proefschrift start met een algemene inleiding, welke dit proces beschrijft volgens de huidige inzichten.

Wanneer door een verwonding een bloedvat is beschadigd, wordt het stollingsproces in gang gezet door het vrijkomen van verschillende stollingsfactoren en doordat bloed in contact komt met lucht. De verschillende stollingsfactoren reageren in min of meer vaste volgorde met elkaar (de 'stollingscascade') en het resultaat is dat fibrine wordt gevormd. Een bloedstolsel bestaat uit een mengsel van fibrine, bloedplaatjes en rode bloedcellen. Fibrinedraden vormen een kleverig netwerk over de opening in het bloedvat, waarna zich bloedplaatjes aan dit netwerk vasthechten en de opening afsluiten. Normaal voltrekt de bloedstolling zich in 3–6 minuten. Dit fysiologische systeem wordt nauwlettend gereguleerd door verschillende processen: het feit dat bloed stroomt, dat geactiveerde bloedplaatjes slechts beperkt beschikbaar zijn, en op verschillende plaatsen en stadia door circulerende remmers. Het gevolg is dat in een gezond persoon het stolsel beperkt blijft tot de plaats van de wond.

Onder normale omstandigheden stolt het bloed niet in de bloedvaten, maar soms is het mechanisme dat de bloedstolling reguleert, van slag en ontstaat een stolsel in een bloedvat (trombose). Hoewel trombose in principe in elk bloedvat en op iedere plaats in het lichaam kan ontstaan, treedt het in verreweg de meeste gevallen op in de beenaderen (diep veneuze trombose, DVT). Een bloedstolsel kan zich vormen wanneer de aderwand ergens is beschadigd of wanneer de stroomsnelheid van het bloed lager is dan normaal (bijvoorbeeld tijdens langdurige bedrust). Dan krijgen bloedplaatjes de kans om zich aan de vaatwand te hechten en zo een stolsel te vormen. Bijvoorbeeld dikke mensen, bejaarden, zwangere vrouwen, en vrouwen met de combinatie roken en pilgebruik lopen een verhoogd risico om trombose te krijgen. Een trombus is een solide massa, die bestaat uit bloed-

cellen ingebed in een netwerk van fibrinevezels. Het gevolg is dat weefsels verstoken blijven van voedingsstoffen, en deze weefsels kunnen ten gronde gaan als gevolg van het gebrek aan zuurstof. Derhalve is een dergelijke afsluiting van een bloedvat door een trombus verantwoordelijk voor twee van de meest dodelijke aandoeningen van de geïndustrialiseerde wereld, het hartinfarct (myocardinfarct: MI) en het herseninfarct ('beroerte', cerebrovasculair accident: CVA). Naast overlijden, kan het MI verantwoordelijk zijn voor chronisch hartfalen, en het CVA voor verlammingen. In de (onderste) ledematen kan een bloedvatafsluiting in de arteriën leiden tot functionele stoornissen (bijvoorbeeld etalagebenen, claudicatio intermittence) of weefselverval. Tevens kunnen fragmenten van een verse trombus losschieten, meegevoerd worden met de bloedstroom en vastlopen in de bloedvaten van de longen (longembolie, 'pulmonary embolism': PE). Dit is een belangrijke doodsoorzaak van postoperatieve sterfte.
De behandeling van trombose bestaat uit toediening van medicijnen die de bloedstolling remmen. Hierdoor wordt de verdere groei van het stolsel en het ontstaan van nieuwe stolsels voorkomen; het lichaam zorgt dan zelf voor de afbraak van het reeds gevormde stolsel. Deze geneesmiddelen ('bloedverdunners', ofwel antitrombotica) zijn geneesmiddelen die de neiging van het bloed om te stollen verminderen.
De meest gebruikte soorten zijn: 1) stollingsremmers, die de aanmaak van bepaalde stollingsfactoren remmen doordat ze de werking van vitamine K tegengaan en 2) de meer direct werkende stollingsremmers (bijv. heparine), die rechtstreeks aangrijpen op het bloedstolsel. Echter voor beide groepen geldt dat het gebruik van stollingsremmers voortdurende controle vergt, omdat overdosering tot bloedingen kan leiden.

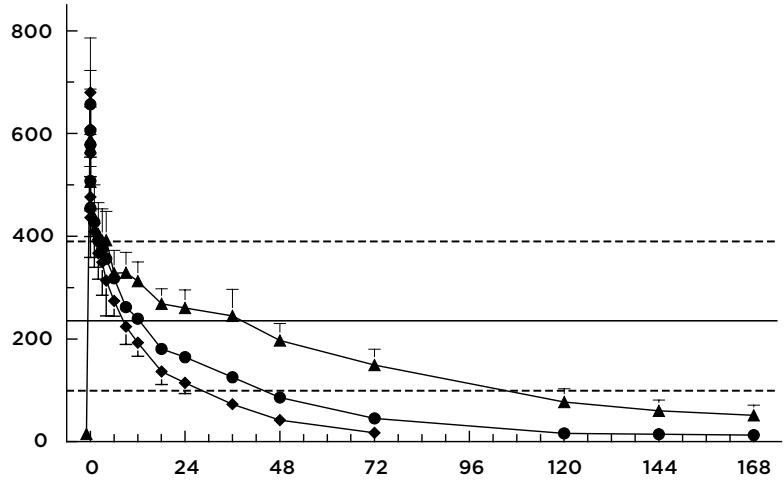
Achtergrond van dit proefschrift
Stolling en trombose zijn zeer complexe processen, die, ondanks veel onderzoek, nog steeds niet geheel zijn ontrafeld. Het belang om het proces leidend tot trombose te doorgronden en de preventie zijn duidelijk. De explosieve groei van onderzoek in dit gebied

en het groeiende arsenaal aan mogelijke antitrombotica hebben bijgedragen aan ons begrip van de trombogenese.
En ondanks dat trombose preventie en behandeling de laatste jaren sterk zijn verbeterd, wordt de ontwikkeling van nieuwe geneesmiddelen in dit gebied m.n. gestimuleerd door de tekortkomingen van de op dit moment beschikbare geneesmiddelen. Bestaande therapieën schieten nog steeds bij een belangrijk deel van de patiënten tekort, en gaan gepaard met bijwerkingen.
De vraag naar meer effectieve en veiliger middelen heeft geleid tot de ontwikkeling van nieuwe stollingsremmers [1].
Het ideale antitromboticum zou aan de ene kant tromboemboliëen moeten voorkomen echter zonder aan de andere kant de bloedstolling te beïnvloeden. Daarnaast wordt het steeds meer onderkent, dat mensen met een tromboserisico voor langere tijd behandeld moeten worden. Zo'n lange behandeling zorgt voor een duidelijk ongemak. Dus is er de laatste tientallen jaren een zoektocht gaande naar antistollingsmiddelen met een meer omschreven werking dan de huidige middelen [2].
De laagmoleculair gewicht heparines (LMWHs) hebben ons vermogen antistollingsbehandeling te controleren al verbeterd, omdat plasmaconcentratie en het effect op het stollingssysteem, beter voorspelbaar zijn. Tevens hebben clinici hiermee geneesmiddelen gekregen, die zich lenen voor patiëntgecontroleerde thuisbehandeling [3].
In het verlengde hiervan is er onderzoek gaande om remmers van TF, factor VIIa, factor VIIa-TF, factor IXa, factor XIIa, en factor XIIIa te ontwikkelen als antitrombotica. Alhoewel het specifieke remmen van genoemde enzymen een rationele benadering is om trombogenese te controleren, lijkt het dat elk van deze enzymen of enzym/activator-complexen een specifieke plaats is met een mogelijk beperkte werking. Ook beïnvloeden al deze enzymen, met uitzondering van factor XIIIa, uiteindelijk de aanmaak van geactiveerde factor X (factor Xa). Derhalve lijkt remming van factor Xa de meest logische keuze om het stollingsproces te beïnvloeden [4]. Dit proefschrift beschrijft een deel van de zoektocht naar stoffen, die mogelijk een verbetering zouden kunnen zijn t.o.v. de huidige behandelingsmogelijkheden

voor trombo-embolische aandoeningen. Met voorafgaande in het achterhoofd was het focus vooral gericht op remmers van factor Xa.

SECTIE II / NIEUWE ANTITROMBOTICA

De meest 'natuurlijke' groep opvolgers van de heparines is de groep van de pentasaccharides en derhalve begint dit deel van dit proefschrift met een aantal studies, die betrekking hebben op deze stoffen. Uit eerdere ervaring met de pentasaccharides was duidelijk geworden, dat met deze stoffen een zeer selectief antistollingseffect wordt bewerkstelligd. De eerste uit deze groep van volledig synthetische remmers van stollingsfactor Xa (Org31540 / SR90107A), nu geregistreerd als Arixtra® (fondaparinux), is veilig, zoals gebleken was uit preklinische studies en eerdere studies in mensen, zowel in gezonde vrijwilligers als patiënten [5–10]. Deze stof wordt voornamelijk renaal geklaard en heeft een halfwaarde tijd van het biologische effect van 15 tot 20 uur. In dit proefschrift zijn belangrijke uitgangspunten voor het gebruik van pentasaccharides aangestipt.
In Hoofdstuk 3 wordt aangetoond dat voor fondaparinux de klaring rechtevenredig afneemt met de nierfunctie. Met een berekende $r^2 = 0.90$ voor de lineaire relatie tussen de creatinine-klaring (als maat voor de nierfunctie) en plasmaklaring van de stof, wordt slechts zo'n 10% van de variantie in de klaring van fondaparinux door andere oorzaken verklaard. In de dagelijkse klinische praktijk kan de dosering van de pentasaccharide makkelijk worden aangepast, omdat de creatinine-klaring in deze studie werd berekend via een éénmalige plasma ceatinine bepaling gebruik makend van de formule van Cockroft & Gault [11]. Als alle toedieningen uit de studies beschreven in Hoofdstukken 3, 4 en 5 worden herberekend naar dosis van 2.5mg eenmaal daags onderhuids (=subcutaan; uit de Pentathlon-studie was gebleken, dat dit de dosis van keuze is als profylaxe in orthopedische chirurgie), kunnen de gemiddelde plasma-concentratie tijd curven worden voorspelt (Figuur 1). Deze figuur laat ook de piek- en dalconcentraties zien (onderbroken lijnen).



FIGUUR 1 **Fondaparinux plasmaconcentratie tijdprofielen herberekend volgens het doseringsschema van 2.5mg eenmaal daags subcutaan.**

De drie curven geven de gemiddelden aan van de 15 proefpersonen met een verminderde nierfunctie. De horizontaal gestreepte lijnen zijn klinisch belangrijke concentraties; de bovenste lijn de gemiddelde piekconcentratie, de onderste lijn de gemiddelde dalconcentratie, respectievelijk. De ondoorbroken lijn geeft de gemiddelde dalspiegel weer, wanneer fondaparinux zou worden gegeven volgens een schema van 6mg eenmaal daags.

De figuur maakt duidelijk dat aanpassing van de dosering van fondaparinux in de klinische praktijk waarschijnlijk alleen nodig is bij patiënten met een matige of een ernstige achteruitgang in nierfunctie (creatinine-klaring tussen 30 tot 60 mL/min of kleiner dan 30 mL/min, respectievelijk).
De simulatie met de 6-mg dosering (deze dosering en 8mg werden gestopt in de Fase II-studie a.g.v. de bijwerkingen) laat zien dat, gedurende het grootste deel van de dag, de plasmaconcentratie hoger ligt dan het piekniveau van het 2.5mg schema. Het kon dus verwacht worden dat hieruit een slechter risicoprofiel naar voren zou komen.
Het blijkt dat mensen, behorend tot de risicogroepen voor het ontwikkelen van een trombo-embolische ziekte, voor een lange

periode behandeld moeten (maanden, jaren, 'levenslang'). Omdat dit een achteruitgang is in de kwaliteit van leven, zou een langwerkend veilig antitromboticum een uitkomst kunnen zijn. De langwerkende pentasaccharide idraparinux heeft een specifieke activiteit van 1400 anti-Xa eenheden/mg. Deze hogere activiteit in vergelijking tot de korter werkende voorganger (fondaparinux) kwam tot uiting in een hogere antitrombotisch werking in verschillende diermodellen voor trombose. Van idraparinux was ongeveer de helft van de stof nodig om hetzelfde effect te bewerkstelligen [12]. De eerste toediening aan de mens van deze stof bevestigde de lange halfwaarde tijd, de lineaire farmacokinetiek (de lotgevallen van de stof in het lichaam, d.w.z. wat doet het lichaam met idraparinux) en de 100% biologische beschikbaarheid na subcutane toediening aan gezonde jonge mannen (Hoofdstuk 6). Omdat in de klinische praktijk anti-stollingsmiddelen vooral bij oudere mensen worden gebruikt, werd een studie uitgevoerd in oudere vrijwilligers (60 jaar en ouder). De resultaten van deze studie kwamen overeen met de eerdere bevindingen en suggereren dat het leeftijdseffect op de farmacokinetiek en farmacodynamiek (de effecten van de stof, d.w.z. wat doet idraparinux in het lichaam) minimaal is (Hoofdstuk 7).
Vervolgens werd, om de dagelijkse praktijk na te bootsten, een studie uitgevoerd waarbij meerdere doses idraparinux worden toegediend. Dit werd gedaan bij mensen, recent behandeld voor een trombo-embolische aandoening (DVT of LE). Zij kregen idraparinux subcutaan eenmaal per week gedurende 4 weken, aansluitend aan hun initiële behandeling van tenminste 3 maanden (Hoofdstuk 8). Voor de piekconcentraties werden stabiele plasmaniveaus ('steady-state') reeds bereikt na de tweede toediening, voor de dalplasmaspiegels was dit het geval na de derde toediening. Met de resultaten van deze studie (en de voorafgaanden) kon de plasmaconcentratie tijdcurve, alsof idraparinux voor langere tijd werd gegeven, worden voorspelt.

In Hoofdstukken 4 en 9 worden interactiestudies van penta-saccharides met warfarine beschreven. In de klinische praktijk

vindt instelling op de cumarines plaats gedurende een overlapfase met het antistollingsmiddel, dat gebruikt is voor de acute behandeling; deze situatie werd in de studies nagebootst. Er is voor deze groep van stoffen geen farmacokinetische interactie met warfarine, wereldwijd gezien het meest gebruikte cumarinederivaat. Deze resultaten kunnen waarschijnlijk worden geëxtrapoleerd naar de gehele groep van vitamine K-antagonisten. Een tweede belangrijke bevinding was het feit, dat de Protrombine Tijd (PT, tegenwoordig uitgedrukt als International Normalised Ratio; INR) nog steeds gebruikt kan worden om het effect van de cumarines te vervolgen tijdens een overlapfase met pentasaccharides.
In Hoofdstuk 5 worden de resultaten van een interactiestudie van fondaparinux met piroxicam weergegeven. Voor de kortwerkende pentasaccharide bestaat geen farmacokinetische interactie met het non-steroidal anti-inflammatory drug (NSAID). Ook de farmacodynamische effecten konden duidelijk worden gescheiden, hetgeen suggereert dat er ook geen farmacodynamische interactie bestaat. Hoewel er verschillen bestaan tussen de kort- en de langwerkende pentasaccharide, is het onwaarschijnlijk dat zij zich verschillend gedragen ten opzichte van de interacties met de NSAIDs. De pentasaccharides kunnen dus een belangrijke toevoeging zijn op de huidig beschikbare antitrombotica, alhoewel enige voorzichtigheid betracht moet worden met betrekking tot de invloed van de nierfunctie.

Op theoretische gronden zouden de meeste tekortkomingen van de huidige antitrombotica kortgesloten kunnen worden door het gebruik van direct werkende trombine remmers. En inderdaad werden de eerste preklinische studies met veel enthousiasme ontvangen met betrekking tot de mogelijke toepassing als antitrombotica [13]. Vervolgens werden meerdere trombine remmers ontwikkeld, en verscheidene van deze stoffen bevinden zich in verschillende fasen van het klinische onderzoek in de laatste jaren [14]. Het is denkbaar dat in de klinische praktijk direct-werkende trombine remmers gelijktijdig worden toegediend met orale antistolling met een cumarinederivaat.

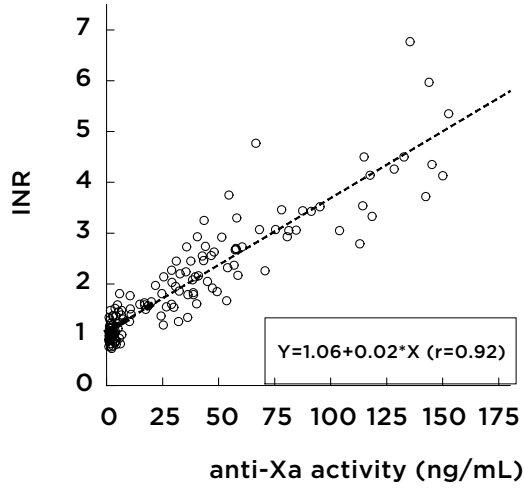
Hoofdstuk 11 vat de resultaten van een studie, waarin het effect van warfarine op napsagatran werd onderzocht, samen. Warfarine had geen effect op de farmacokinetiek, maar wel duidelijk de farmacodynamiek van deze stof. Ook al bestaan er grote verschillen tussen de verschillende stoffen van de heterogene groep direct werkende trombine remmers, kan het worden geconcludeerd, dat de PT niet gebruikt moet worden als parameter tijdens de overgang van een direct werkende trombine remmer naar orale antistollingstherapie.
Een andere groep van veelbelovende antitrombotica zijn de volledig chemisch gesynthetiseerde stoffen, die (net als de heparines) een 'dubbel' werkingsmechanisme vertonen met zowel anti-IIa als anti-Xa activiteit. Eén van die stoffen is Org 36764. Deze heeft een pentasaccharide structuur (anti-Xa activiteit), terwijl via een inerte 'spacer' met een disaccharide structuur voldoende ketenlengte wordt bereikt om zowel Antitrombine (AT) en trombine te binden (anti-IIa activiteit). De eerste toediening van deze stof aan de mens (Hoofdstuk 10) laat een ongebruikelijk plasmaconcentratie tijdprofiel zien, als de anti-Xa activiteit van de stof gebruikt worden om de farmacokinetiek te beschrijven. Concentraties bereiken een plateau, welke enige tijd na het toedienen van een intraveneuze bolus injectie aanhoudt, én maximum concentraties nemen niet evenredig met de dosering toe. Gebruikt men de anti-IIa activiteit van Org 36764, dan worden er zeer lage concentraties waargenomen zonder duidelijke verschillen tussen de verschillende doseringen. Er bestaat voor deze resultaten geen voldoende verklaring. Het is echter duidelijk, dat deze theoretisch interessante stof (nog) niet geschikt is verder ontwikkeld te worden.

SECTIE III / ALTERNATIEVE MANIEREN VAN TOEDIENEN

Stollingsfactor Xa remmers kunnen een veelbelovend alternatief worden voor de huidige antitrombotica. Een nadeel is echter dat ze nog steeds parenteraal toegediend moeten worden. Derhalve zal de ontwikkeling van deze groep stoffen nog succesvoller zijn, als hier een makkelijker toepasbare stof (oraal ?) uit naar voren komt [4]. Dit deel van het proefschrift beschrijft studies, waarbij alternatieve routes van toedienen werden onderzocht.

In Hoofdstuk 12 wordt duidelijk gemaakt, dat het geen nut heeft LMWHs in tabletvorm aan mensen te geven. Al konden plasmaconcentraties van pentosan polysulfaat niet direct gemeten worden, de gebruikte indirecte parameters demonstreren dat de biologische beschikbaarheid van deze toedieningsvorm verwaarloosbaar is in gezonde jonge mannen.
Het kan geconcludeerd worden, dat ontwikkeling van een oraal toepasbare LMWH zinloos is.
In Hoofdstuk 13 wordt een studie beschreven met een sublinguale formulering (onder de tong) van de pentasaccharide idraparinux. De resultaten laten zien, dat de huidige tablet een lage biologische beschikbaarheid met een hoge variabiliteit. Dit sluit echter verder onderzoek naar een dergelijke formulering niet uit, want er zijn zeker mogelijkheden voor verbetering. Simulatie experimenten, met de in deze studie verkregen data, laten zien dat frequente toediening (eenmaal daags) tot therapeutische plasmaconcentraties zou kunnen leiden. Het is ook denkbaar, dat andere absorptieverbeteraars de opname van idraparinux via een sublinguale of buccale (in de wang) formulering zouden kunnen verbeteren, zoals bijvoorbeeld ook het geval voor testosteron (met cyclodextrines als absorptieverbeteraar) [15].
De eerste toediening aan de mens van de direct-werkende Xa remmer YM 466 wordt weergegeven in Hoofdstuk 14. Deze stof is potentieel toepasbaar, maar de orale biologische beschikbaar is laag, zeer variabel en neemt nog verder af door inname tegelijk met voeding. Speciaal voor antitrombotica kan dit een nadeel zijn, omdat in de klinische praktijk een balans gevonden moet worden tussen effectieve antistollingstherapie enerzijds en een laag bloedingrisico anderzijds. Met een dergelijke farmacokinetisch variabele stof zal het moeilijk worden een doseringschema te vinden, dat voor alle patiënten zal kunnen gelden, hetgeen gebruik onaantrekkelijk kan maken. Tevens, en in tegenstelling tot de pentasaccharides, lijkt YM 466 geen selectieve factor Xa-remmer te zijn, gezien het effect dat deze stof heeft op de conventionele stollingstesten APTT (Activated Partial Tromboplastin Time) en PT. Dergelijke stijgingen in deze testen werden ook gerapporteerd voor andere direct-werkende anti-Xa

stoffen [16–18]. Het zou dus als een 'klasse' effect van deze groep van stoffen beschouwd kunnen worden. Het kan beargumenteerd worden, dat dit voorkomt uit de andere werking in vergelijking met de pentasaccharides. Deze direct-werkende stoffen zouden bijvoorbeeld niet alleen het 'vrij' circulerende factor Xa kunnen remmen, maar ook de reeds in een bloedstolsel gebonden factor Xa, net als direct-werkende trombine remmers [19]. Dit kan echter niet de verklaring zijn in studies met gezonde jonge mannelijke vrijwilligers. Ongeacht de verklaring lijkt het dat deze stoffen toch weer strikte controle vereisen, hetgeen een nadeel is ten opzichte van de pentasaccharides. Een mogelijke manier om dure (zowel in tijd als geld) laboratoriumcontroles te vermijden zou het gebruik van een 'bedside'-apparaat kunnen zijn. Recent zijn nl. apparaten geïntroduceerd, waarmee aan de operatietafel (de APTT) of thuis door de patiënten zelf (de PT, in geval van orale antistolling [20]), deze conventionele antistollings-bepalingen uitgevoerd kunnen worden.



FIGUUR 2 De YM 466 anti-Xa 'bedside'-INR curve, welke een hoge correlatie oplevert.

Gebruik makend van een dergelijk apparaat (de Coagucheck Plus®), kon de YM 466 activiteit adequaat vervolgd worden. Dit apparaat bleek gevoelig genoeg om PT-verandering over de gehele anti-Xa concentratie breedte te beschrijven. Voor de dosis-effect relatie tussen YM 466 en de (ogenschijnlijke) INR werd een hoge correlatie gevonden. Dit zou een effectieve sneltest kunnen zijn om het gedrag van deze nieuwe direct-werkende factor Xa remmer te controleren. Echter, verder onderzoek is noodzakelijk.

SECTIE IV / CONTROLE VAN ANTISTOLLINGSMIDDELEN

Zoals juist hierboven al aangetoond, is een belangrijk punt in de antistollingstherapie het gebruik van de juiste, betrouwbare en effectieve methoden om de antistollingsmiddelen te controleren. Dit wordt in dit deel van het proefschrift aan de orde gesteld. De keuze van de meest geschikte biologische maat (biomarker) is belangrijk en niet gemakkelijk. Bovendien heeft de ontwikkeling van nieuwe antitrombotica de beperkingen van de traditionele stollingstesten als biomarkers aan het licht gebracht [21]. Gebruikmakend van resultaten uit verschillende Fase I-studies wordt in Hoofdstuk 15 een vergelijking gemaakt tussen diverse antitrombotica, met als gemeenschappelijk kenmerk hun activiteit tegen factor Xa. Voor de gedeeltelijk of volledig specifieke anti-Xa remmers bestaan belangrijke verschillen in de reactie op de APTT en PT. Voor de AT-afhankelijke stoffen lijkt het hoe specifieker de stof factor Xa remt, des te geringer is de invloed op deze conventionele stollingstesten.
De pentasaccharides, die in het geheel geen anti-IIa activiteit hebben, beïnvloeden de APTT/PT totaal niet. In vergelijking met de curve voor fondaparinux verschilt de richtingscoëfficiënt van de anti-Xa/APTT-correlatie grafiek voor idraparinux zelfs nog een factor 6, waarmee waarschijnlijk het verschil in affiniteit voor AT (en dus factor Xa) wordt. De APTT en PT zijn dus biomarkers met stof-afhankelijke effecten, waarmee er vraagtekens gezet kunnen worden bij het gebruik van deze testen voor het in de gaten houden van nieuwe antitrombotica. Deze bepalingen zouden echter wel gebruikt kunnen worden om de specificiteit van een bepaalde stof voor geactiveerde factor X te testen.

In Hoofdstuk 16 wordt een studie beschreven gelijk aan die in Hoofdstuk 15, echter nu worden stoffen bekeken, die allemaal activiteit tegen trombine (factor IIa) vertonen. De vergelijking in deze studie was gebaseerd op de relatie tussen de anti-IIa-activiteit en de APTT. Tegenwoordig is het bekend, dat eenzelfde effect op de APTT, veroorzaakt door verschillende stoffen, niet noodzakelijkerwijs overeenkomt met eenzelfde antitrombotische werking. Ook uit deze studie komt duidelijk naar voren, dat oneigenlijk gebruik van een biomarker leidt tot verkeerde conclusies. In vergelijkende studies met antitrombotica zou het de voorkeur hebben meer selectieve markers te gebruiken (bijvoorbeeld de biologische activiteit). Er is zeer veel klinische ervaring met de APTT en de PT door het reeds jarenlang wijdverbreide gebruik van deze bepalingen. In de nabije toekomst zullen dit de meest gebruikte testen blijven. In Hoofdstuk 17 werd één van de 'bedside'-apparaten (de Coagucheck Plus®) geëvalueerd voor het bepalen van de APTT of PT, na toedienen van verscheidene antitrombotica in verschillende Fase I studies. Deze studie demonstreert dat er substantiële verschillen zijn tussen de uitkomst van een APTT- of PT-bepaling met een dergelijk apparaat en de 'gouden' standaard (de laboratoriumbepaling) tijdens behandelingen met klassieke of nieuwe antitrombotica. Voor geen van de stoffen werd een goede overeenkomst in APTT- of PT uitkomsten gevonden. Het verschil tussen de verkregen resultaten werd zelfs steeds groter bij hogere APTT of PT uitslagen. De mate van het verschil (over- of onderschatting) was afhankelijk van het toegediende antistollingsmiddel. De (klinische) implicaties voor deze resultaten kunnen nu nog niet overzien worden, omdat voor iedere stof de therapeutische breedte nog vastgesteld moet worden.

CONCLUSIES

Dit proefschrift beschrijft enige belangrijke ontwikkelingen, waarmee de huidige antitrombotische strategieën verbeterd zouden kunnen worden. De pentasaccharides zijn een stap in de goede richting. Er zijn echter nog wel vraagtekens. De mogelijke invloed van een verminderde nierfunctie op de risico-baten verhouding bij uitgebreid gebruik van deze stoffen is nog

onbekend. Aan dit punt zal zeker aandacht besteed dienen te worden, omdat een groot deel van de toekomstige gebruikers bestaat uit oudere mensen, die fysiologisch een lagere klaring van de stof zullen hebben. Ook moeten de pentasaccharides nog steeds parenteraal gegeven moeten worden, wat geen voordeel oplevert boven de tegenwoordige LMWHs. Het is mogelijk om oraal toepasbare anti-Xa middelen te ontwikkelen, maar er blijven nog enige kanttekeningen. Het moet afgewacht worden hoe deze stoffen zich klinisch gaan gedragen, omdat de selectiviteit bij deze orale Xa-remmers niet zo groot is als men tevoren aannam. De voorlopige data met de sublinguale tablet van de langwerkende pentasaccharide suggereren dat dit een benadering is, die zeker aandacht verdient.

In dit proefschrift wordt ook de noodzaak benadrukt de juiste biomarkers te gebruiken om de effecten van nieuwe geneesmiddelen te kunnen bepalen. Dit is reeds in de vroege fase van de ontwikkeling van een geneesmiddel een belangrijk item.
Het tijdperk, waarin nieuwe antitrombotica gekarakteriseerd konden worden middels routine methoden (APTT, PT of varianten hierop, d.w.z. testen, die het effect van een middel op het gehele stollingsysteem weergeven) is voorbij. Om een bepaald geneesmiddel te kunnen volgen zal de geëigende biomarker gevonden moeten worden. Hierbij dienen ook de effecten van de op dit moment beschikbare middelen op deze biomarker onderzocht te worden. Het zal nog belangrijk blijken, de valideren van dergelijke methoden voorbij het stadium van een bio-assay te tillen. Er zal dus veel moeite gestoken moeten worden in het onderzoeken van de relatie(s) tussen biomarkers en klinische eindpunten. Op dit moment vertonen de gebruikte biomarkers vaak slechts een matige relatie met klinisch relevante uitkomsten [22]. Dit is opmerkelijk, omdat de meeste biomarkers (zowel de vroeger als de tegenwoordig gebruikte) wel aan de criteria voor een goede biomarker voldoen [23].
Antitrombotica vertonen een gelijkblijvende respons over verschillende onderzoeken. Er zijn dosis (concentratie)-effect relaties vastgesteld, en er bestaat ook een duidelijke reactie in

de biomarkers op therapeutische doseringen. Tevens bestaat er een (cor)relatie tussen de farmacologie van een antitromboticum, de biomarkers en de pathogenese van de ziekte. Dit blijkt echter onvoldoende om de relatie tussen geneesmiddelactie en klinische uitkomst vast te leggen. Dit zou kunnen komen door de complexiteit van het stollingsproces, de onderliggende pathologie, onbekende factoren tijdens het ingrijpen met geneesmiddelen hierin, òf combinaties hiervan, met een dusdanig moeilijke situatie tot gevolg die zich niet middels het biomarker concept laat beschrijven. Het kan natuurlijk ook zo zijn, dat ons begrip van het ontstaan van trombotische complicaties in het stollingssysteem tekort schiet, terwijl wij denken dat wij dit biologische proces begrijpen.

De benadering van de ontwikkeling van geneesmiddelen via het concept van de biomarkers lijkt op dit moment de best mogelijke optie. Zoals duidelijk gemaakt, kan dit model toch tekort schieten. Ter verbetering hiervan, zou men meerdere biomarkers tegelijkertijd kunnen gebruiken, gevoegd bij een verbeterd begrip van de pathologie van trombose. Een strakke toepassing van deze benadering in de preklinische ontwikkeling van een geneesmiddelen zal ons dan meer inzicht opleveren in de farmacologie van antitrombotische stoffen. Dit is een moeilijke taak, maar in ieder geval een uitdaging voor (klinisch) farmacologen.

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CURRICULUM VITAE

Richard Faaij werd op 26 februari 1969 te Gouda geboren. Na het behalen van het Atheneum diploma (Rijksscholengemeenschap te Gouda) in 1987, werd in hetzelfde jaar een aanvang gemaakt met de studie Geneeskunde aan de Erasmus Universiteit te Rotterdam. Het doctoraal examen werdbehaald in 1993. Het artsexamen werd afgelegd in september 1995.

Vanaf augustus 1995 was hij als arts-onderzoeker werkzaam bij het Centre for Human Drug Research te Leiden (directeur: Prof. dr A.F. Cohen), waar onder andere de onderzoekingen zoals beschreven in dit proefschrift werden verricht.

In maart 2001 is hij in dienst getreden van het Academisch Ziekenhuis Rotterdam, eerst een periode als AGNIO Klinische Geriatrie (gedetacheerd in het Havenziekenhuis, Rotterdam), vervolgens sinds januari 2002 als AGIO voor dat specialisme (Opleider: dr T.J.M. van der Cammen).

Stellingen behorend bij het proefschrift 'Clinical Pharmacology of Novel Antithrombotic Compounds'

1 Suikergoed geeft bloedverdunning. – *Dit proefschrift*

2 Bij het voorschrijven van pentasaccharides dient er rekening gehouden te worden met de nierfunctie van de patiënt. – *Dit proefschrift*

3 Pentasaccharides vertonen geen geneesmiddelinteracties met de veel gebruikte vitamine K-antagonisten en de niet-steroïde anti-onstekingsmiddelen (NSAIDs). – *Dit proefschrift*

4 Het feit, dat ouderen ondervertegenwoordigd zijn in geneesmiddelonderzoek [1], gaat niet op voor de pentasaccharide idraparinux. – *Dit proefschrift*

[1] Schmucker DL, Vesell ES. Are the elderly underrepresented in clinical drug trials? *J Clin Pharmacol* 1999; 39: 1103-1108

5 Bij het eventueel willen toepassen van pentosan polysulfaat als middel tegen HIV [2] of Creutzfeld-Jacob [3] gaat men voorbij aan de werking van deze stof op de stolling *in vivo*. – *Dit proefschrift*

[2] Baba M, Nakajima M, Schols D, Pauwels R, Balzarini J, De Clercq E. Pentosan polysulfate, a sulfated oligosaccharide, is a potent and selective anti-HIV agent in vitro. Antiviral Research 1988; 9: 335-343. [3] Farquhar C, Dickinson A, Bruce M. Prophylactic potential of pentosan polysulphate in transmissible spomgoiform encephalopathies. Lancet 1999; 353: 117

6 Het gedrag van het glycoconjugaat Org 36764 laat zich niet voorspellen. – *Dit proefschrift*

7 De direct-werkende anti-Xa remmer YM 466 kan met een 'bedside' antistollingsmeting adequaat vervolgd worden. – *Dit proefschrift*

8 De toepassing van nieuwe, specifiek-werkende, antithrombotische geneesmiddelen zal leiden tot het verdwijnen van de gebruikelijke evaluatiemethoden van het stollingssysteem in de monitoring. – *Dit proefschrift (J Burggraaf, 26 maart 1998)*

9 Wetenschap wordt gevoed door twijfel. Derhalve zullen, nu de wetenschap er zich mee bemoeit [4,5], de controversen over voetbalspelregel 11 alleen nog maar gaan toenemen.

[4] Sanabria J, Cenjor C, Márquez F, Gutierrez R, Martinez D, Prados-Garcia JL. Oculomotor movements and football's Law 11. Lancet 1998; 351: 268 [5] Oudejans RRD, Verheijen R, Bakker FC, Gerrits JC, Steinbrückner M, Beek PJ. Errors in judging 'offside' in football. Nature 2000; 404: 33

10 Dementie gaat gepaard met psychologische en lichamelijke veranderingen. De directe omgeving heeft het met deze dubbele achteruitgang bijzonder moeilijk; huisartsen zouden meer oog moeten hebben voor deze problematiek van mantelzorgers.

11 Gezien het feit, dat bij de behandeling van een intracapsulaire collumfractuurbehandeling van een oudere patiënt een zorgvuldige evaluatie van de mentale status essentieel geacht wordt [6], dient dit laatste òf onderdeel te worden van de specialistische opleiding heelkunde òf dient er een intensieve samenwerking opgezet te worden tussen de chirurgische staf en de geriaters.

[6] L. van Dortmont. The intracapsular femoral neck fracture in relation to mental state. PhD-thesis Rotterdam 2001

12 Elk bereikt doel is weer het begin van een nieuwe tocht, en zo tot in het oneindige. *(Arthur Schopenhauer)*

Richard A. Faaij , 2 oktober 2002, Leiden

UITNODIGING VOOR DE PROMOTIE VAN RICHARD FAAIJ

De promotie zal plaatsvinden op 2 oktober 2002 om 15.15 uur in de Senaatskamer van het Academiegebouw, Rapenburg 73, Leiden.

Tot de promotie wordt met het oog op de beperkte ruimte in de Senaatskamer uitsluitend toegang verleend op vertoon van een bewijs van toegang, verkrijgbaar bij de promovendus of het CHDR (tel: 071 – 5246400).

Met tijdrovende parkeermoeilijkheden bij het Academiegebouw moet rekening worden gehouden.

De receptie vindt plaats in het Academiegebouw na afloop van de promotie van 16.15 – 17.00 uur.

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