A QUESTION BASED APPROACH TO DRUG DEVELOPMENT

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VOOR MIJN OUDERS

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A question based approach to drug development

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CHAPTER 1

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Drug development project management by a new question based approach and decision analysis support

Introduction

The value of knowledge

Peter is a clinical research manager at a pharmaceutical company and his current job is to set up a clinical development program for a new promising antipsychotic drug. His plan is to perform phase I studies in healthy volunteers (single ascending dose/multiple ascending dose/drug metabolism studies/food interaction), followed by phase II trials in well defined small groups of patients to select the optimal dose which he intends to use in the large phase III trials.

John just started working at the same company after having worked as an academic neurologist and he is unsure if the drug will penetrate the brain. Therefore, he has suggested adding a brain imaging study with positron emission tomography (PET) study immediately after the first study in man. This will require the development of a special radioactive labelled molecule and may delay the project by more than a year. John and Peter have discussed this with their manager. They have a short meeting about this during which the research director shows a spreadsheet (table 1).

TABLE 1 **Project valuation by Net Present Value (NPV)**

Peter's plan (xM$)										
Out	1	2	4	20	30	40	0	0	0	
In	0	0	0	0	0	0	100	100	300	
Balance	-1	-2	-4	-20	-30	-40	100	100	300	
John's plan (xM$)										
Out	1	2	2	4	20	30	40	0	0	0
In	0	0	0	0	0	0	0	100	100	300
Balance	-1	-2	-2	-4	-20	-30	-40	100	100	300
Net Present Value (NPV)										
Peter's plan (xM$)	241									
John's plan (xM$)	226									
Difference (xM$)	15									

"Sorry John but as you can see there is no chance we will do this. Delaying the project will not only cost us two million dollars extra, but worse, you reduce the value of our project by 15 million. I know that the board is unlikely to allocate enough priority to this project so we probably have to cancel

it altogether. Anyway, we will have to assess the efficacy of the new drug in the phase II studies in the patients. Also, John, please remember that we are a commercial company. Our job is to make money -not write interesting papers. With a planned yearly turnover of about 500 million dollars this is what I see as the cost of a year's delay!"
John feels there is something wrong with the logic of this reasoning and ponders two possible scenarios. Of course the drug may be developed according to Peter's plan, but what if the drug does not penetrate the blood brain barrier? In that case the first indication of this will only come in expensive phase II or III trials.

TABLE 2 **Recalculation of the plans for the scenario that the drug does not penetrate the brain.**

Peter's plan (xM$)										
Out	1	2	4	20	30	40	0	0	0	
In	0	0	0	0	0	0	0	0	0	
Balance	-1	-2	-4	-20	-30	-40	0	0	0	
John's plan (xM$)										
Out	1	2	2	4	0	0	0	0	0	0
In	0	0	0	0	0	0	0	0	0	0
Balance	-1	-2	-2	-4	0	0	0	0	0	0
Net Present Value (NPV)										
Peter's plan (xM$)	(74)									
John's plan (xM$)	(8)									
Difference (xM$)	(66)									

John wants to convince his boss that in the latter case, his development plan would have saved considerably more resources than Peter's. While the initial value of his plan was lower according to his boss's estimation, he feels the project valuation did not adequately value the contents of his program or the value of the early discontinuation of the development. He is not quite sure why the calculation of the Net Present Value of his plan does not seem to reflect exactly what he sees as value. He manages to get his manager's spreadsheet and recalculates it for the situation that the project is discontinued after Phase III for Peter's plan because the drug is not effective in schizophrenia. In John's plan the PET study may have given unequivocal evidence that the drug does not get into the brain and the project is stopped immediately after the PET study (Table 2). To his surprise this shows a very different picture. Now the value of both projects is negative because there is no income anymore but Peter's plan produces much more negative value

than his does! John goes back to the research director and presents this again. “John, my dear fellow, you seem to be making an academic exercise out of everything you do! I do not rate the probability that this happens very high. You would be best advised to just do what you are paid for- show that our new drugs work in patients.”

John is disappointed. His boss obviously judges the probability of certain scenarios differently, but why? It surely can’t be as black-and-white as this? How can he find a way to communicate with his colleagues about these matters? John leaves the office and wonders how he can express all these different facts so that his point does become clear -he has never been the type that gives up easily.

Problem with modern drug development

Every year 200.000 compounds are examined on potential medicinal properties worldwide. About twenty new drugs are introduced every year. This implies that one in ten thousand compounds make it through the drug development program. The discovery and development of new medicines is an expensive and time-consuming process. It takes an average of 12-15 years to discover and develop a new medicine. Most of that time is spent testing the drug to make sure it is safe. The average cost of bringing one new medicine to market in 1990 was estimated at $500 million. The Tufts University Center for the Study of Drug Development found that the time from synthesis of a new drug to marketing approval has increased over time. While in the 1960s the approximate time from synthesis to approval was about 8 years, this has increased to 14.2 years in the 1990s. Recent figures show that although pharmaceutical companies spend more on research and development of new drugs, the number of new compounds launched decreases (Figure 1). There is no denying that many of the diseases that fuelled the enormous explosion in profit and turnover of the pharmaceutical industry in the 1980’s and 90’s (like asthma and gastric ulcers) are now well controlled and this reduces the potential added value of any new treatment. Furthermore, the many diseases that remain inadequately treated are chronic with complex pathophysiology and difficult outcome measurements. Good examples are neuro-psychiatric diseases or cancer. Therefore, pharmaceutical companies need to rely on a few highly successful products to fund the high costs of innovative research and development (R&D). The data show

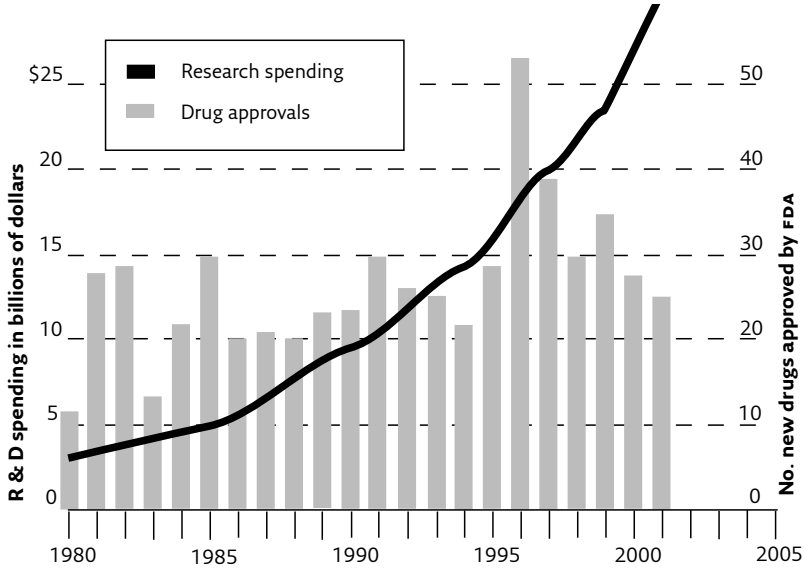
ref. 1

ref. 2

ref. 3

that it is increasingly difficult to develop new drugs for the treatment of the complex diseases that remain inadequately treated. To limit the costs of drug development it pays to discontinue failures as early as possible.

FIGURE 1 **Growing drug development costs and declining number of registrations of new drugs**



In order to cope with this changing perspective, several attempts are made to optimise the development of new drugs:

Target optimisation

Several individual approaches are introduced to optimise the process of identifying new lead compounds both in quantity and selectivity:

- **Computer aided drug design**

The use of computational techniques to design and optimise molecular targets has increased the number of new chemical entities (NCE's). Furthermore, the selectivity of the NCE's is enhanced by evaluating and optimising the binding affinity to selected targets *in situ*.

- **Combinatorial chemistry**

Combinatorial chemistry techniques (often automated using synthesis

robots) have facilitated and increased the number of synthesised NCE's with potential biological activity.

- **High throughput screening**

The increased number of synthesised compounds is easily screened using molecular biological techniques usually referred to as high throughput screening. This usually implies that several hundreds of related compounds can be simultaneously screened for activity at receptor level using fluorescent activation markers. Therefore, the most potent compounds at receptor level can be selected from the wide range of available compounds.

- **Genomics**

The availability of the human genome has generated a wealth of new possibilities for new drug targets. New insights to the origin of complex diseases are under investigation. Furthermore, gene chips are available to evaluate the effects of new drugs at DNA/RNA/protein production level providing more detailed knowledge on the mechanism of action of new and existing drugs.

There is no doubt that these approaches are producing many molecules that bind to biological targets. However, for these to be successful as medicines much more is needed. New drug targets do not necessarily mean that the relation of these to disease is well understood and realising this understanding may be very time consuming. An immediate payback of these techniques is therefore not expected. ref. 4

Process optimisation

Optimisation of the discovery of new drugs is complemented by optimisation of the development process.

- **Optimisation of resources**

Pharmaceutical companies have merged in an effort to increase the company's pipeline of new investigational drugs and to combine expertise on different indication areas as well as reduce overhead costs. This has largely failed from this point of view. The percentage of turnover spent on research and development has remained constant for companies before and after mergers. This gives no indication of any economy of scale. Clearly there may have been other advantages of the increased market share that are beyond the scope of this paper.

- **Rigorous selection of investigational new drugs**

Identifying and stopping development of drugs that will fail to reach registration as soon as possible once it has entered the clinical development phase is highly rewarding. For this reason, more and more effort is put in early selection of the compounds.

- **Inclusion of biomarkers for effects in an early phase**

Part of the early selection and cost reduction is the inclusion of biomarkers at an early stage and introduction of early proof of principle or proof of concept studies.

- **Project value estimation and portfolio analysis**

In order to select the most profitable project within a company's pipeline, each project is valuated in advance, usually using Net Present Value (NPV; see box 1) calculations. The highest NPV is achieved by projects with the highest estimated market value combined with the lowest development costs and shortest time to registration. Throughout the development process the milestones to monitor the project progress are usually defined by the classical development phases 1 (small healthy volunteer studies on safety, kinetics and tolerability), 2 (small patient population studies on mechanism of action and therapeutic window) and 3 (large multi centre trials to confirm efficacy and safety). A description of these clinical phases by the US Food and Drug Administration (FDA) is given in box 2.

Because Peter's program optimised many procedural aspects of the development including time and costs, his project had a high NPV as estimated by his boss. John assumed that the bottleneck for the potential antipsychotic could be the penetration in the brain and he proposed to spend additional time and money to get an early confirmation of the critical question. Subsequently, the NPV of his project was lower than Peter's but it was unclear to them how to value the early increase of critical knowledge. For some reason they communicated about procedural aspects but seemed to lack a device to communicate about the content of their project. Whilst the procedural aspects were covered by numbers any discussion about the probabilities of certain events occurring was done intuitively.

Improving the discovery or the process has not resolved the main problem of the apparent slack in drug innovation. One of the matters that has not been dealt with is the integration of both procedural and knowledge aspects of drug development. We therefore postulate a question-based approach to drug development that integrates the two into a comprehensive concept.

Question-based development (QBD)

During the classical phases 1 to 3 (and 4), a number of generic questions need to be answered (Figure 2). The detailed questions have to be determined on a case-by-case basis but the questions groups may give some structure to the list.

1 Does the biologically active compound/active metabolites get to the site of action?

This main generic question contains several issues that need to be determined such as absorption, distribution, metabolism and excretion of the drug. Not only the parent compound, but also any possible active metabolites should be included in answering this question. Additional items can be relevant for certain drugs such as ability to penetrate the blood-brain-barrier for CNS active drugs. Unexpected biologically active metabolites can be formed *in vivo*, or unexpected sites of action can be discovered, which should be incorporated in this main question as soon as observed.

2 Does the compound cause its intended pharmacological / functional effect(s)?

Answering this question includes the demonstration of the mechanism of action of the investigational drug. For example a new drug for hyperlipidemia will at least have to reduce the plasma cholesterol in a dose or plasma concentration dependent manner.

3 Does the compound have beneficial effects on the disease or its pathophysiology?

This question reflects the question traditionally answered in the classical phase 3 studies to establish the effects on the disease but also the alteration of other physiological systems resulting in clinical side-effects.

4 What is the therapeutic window of the new drug?

The therapeutic window of each investigational drug needs to be established in order to select the optimal dose that is clinically efficacious at tolerated levels. This question includes important sub-questions: Which dose regimen will keep the drug's concentration within the therapeutic window? What is the optimal dosing interval relative to the intended indication (chronicity of intended drug exposure)? Can controlled drug delivery improve the product's action? What is the forgiveness of the product (i.e. the difference between the product's post-dose duration of effective therapeutic action and the recommended interval between doses)?

ref. 5

ref. 6

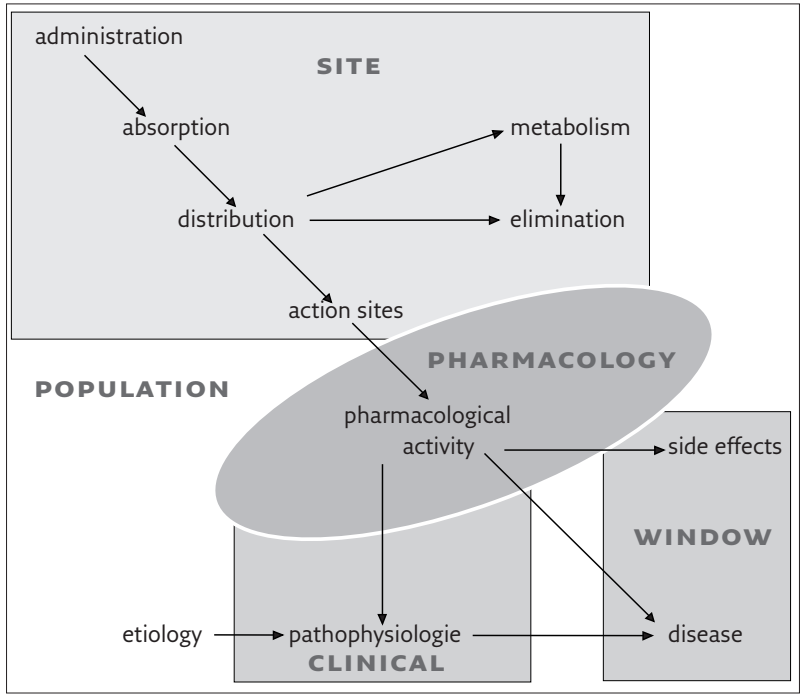
5 How do the sources of variability in drug response in the target population affect the development of the product?

The sources of variability in drug response have been defined as: Dose (formulations and compliance), Pharmacokinetics (absorption, distribution, metabolism and elimination), Pharmacodynamics (sensitivity, maximum response) and other (disease, other drugs, circadian rhythms). The main question should include: Are there any specific factors in the target population that may affect dosage? A general sub-question can be: is there any food-interaction with this compound? But also more drug/population specific questions can arise. The regular use of co medication within the

ref. 7-9

target population may require extra drug interaction studies. Ethnopharmacological issues and pharmacogenomics can play a key role in some development programs (*e.g.* for introduction of a 'western' drug in Japan).

FIGURE 2 **Schematic representation of the course of action of drugs (from administration to effects) and the questions from the question-based development plan**



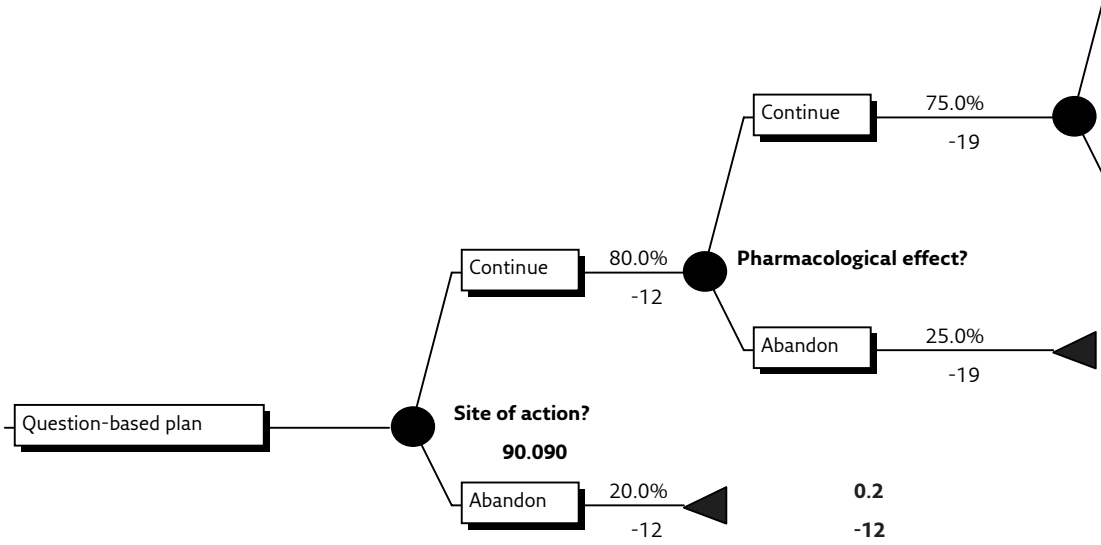
One question can be concealed in several studies and one study can provide partial answers to multiple questions. However, when a project is monitored by its progression through the traditional phases, little is learned about what questions are actually being answered. Managers will have to assume this is being done adequately. The first time the answers are sometimes critically examined is by the regulatory authority that has to give approval for marketing. Extreme disappointments and losses can occur in such cases. In 2002 the company Bristol Myers Squibb paid several billion dollars for a small biotech company Imclone with an interesting anticancer agent (Erbitux). A fee of 200 million dollars was paid when the dossier was sent

to the FDA who subsequently judged the data to be insufficient. The details will probably never be known but the questions lingers how experienced drug developers at BMS were able to miss something that was found after review of the dossier by the FDA.

The question-based approach is designed to make the central issue in drug development projects explicit rather than implicit. This central issue can be described concisely as "Are all the relevant questions asked AND answered adequately?" If so, a regulatory authority can confidently give market authorisation. During the development period, managers can monitor the progress of the study by the questions that are answered and the length of the remaining list. Obviously, it remains important to answer these questions as rapidly and as cheaply as possible, but the tools for doing so should be in place in any sensible company.

QBD assumes that the costs of an answer and the probability an answer can be given adequately can be estimated. These probabilities and costs can be estimated using either expert opinions or historical data. Subsequent sensitivity analysis can reveal the relative impact of the estimations on the project value. Each set of probabilities and costs (combined with market value) will have its own optimal priority sequence. Therefore, early evaluation

FIGURE 3 **Question-based drug development plan**

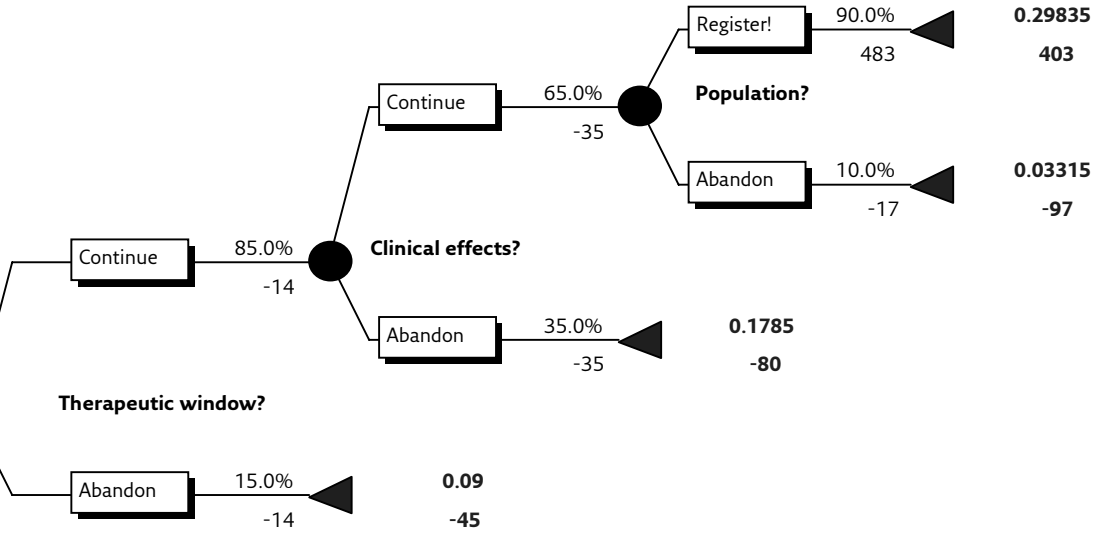


studies on the most critical questions are highly rewarding, since these prevent expenditure on projects that are unlikely to produce a positive cash flow. NPV analysis just indicates that such evaluation studies only cost time and money. NPV calculation inadequately values the increases in knowledge.

ref. 10

The option-based theory takes such probabilities of success into account. However, this method is rarely used and if so, it uses the classical phases of drug development as decision points (or knots in the project's decision tree). These phase definitions are not relevant as drug development targets but are merely classifications based on the type and number of patients involved in such studies.

We therefore propose a question-based approach that uses decision points that are relevant to the development of knowledge in the drug development



process (the five standard questions). The question-based approach now moves one closer to an adequate reflection of true risks and values of the uncertainties that are faced during the development of a new drug.

Additionally, the question-based approach can demonstrate how the project value varies with increased knowledge generated by early evaluation studies. The unique combination of probabilities on successfully answering the question in combination with the costs (and market value) determines

the optimal development strategy that varies for each drug. Furthermore, it displays the bottlenecks within the development and can contribute substantially to the early discontinuation of failures. The estimation of the market value of the new drug can be less accurate in this question-based approach as long as the market value far outweighs the costs. As the probability of successfully answering the questions is determined both by the compound's potential and the availability of methods to demonstrate effects, the question-based approach incorporates the value of both knowledge about methodology and additional early evaluation studies contrary to conventional NPV calculations.

If John and Peter's boss had used the question-based approach to develop the antipsychotic drug, he would have estimated the probabilities and costs of answering the questions. He would have wanted the input from his fellow project team member's (including pre-clinical scientists as well as John and Peter) opinion on the compounds potential and the availability of adequate methods to successfully answer the questions. Together, they might have reached consensus that the 'site of action' question for the potential anti-psychotic had the lowest probability of success and therefore, this question would have required the highest priority in the program. The addition of John's suggested PET study combined with the 'traditional' phase I studies would have adequately answered the 'site of action' question at the earliest possible stage. The question-based approach to drug development is especially valuable for stopping development of drugs that have a high probability of failing by identifying the critical issues that will lead to the discontinuation and dealing with these first. Early discussions about probabilities are also an excellent device to promote communication about critical issues within the project team and to higher management.

The decision analysis tree shown in figure 3 now shows Peter's plan with the probabilities as estimated by the team. The input parameters for the development plan are shown in table 3. The team has decided that the overall probability the compound will make it to registration is comparable to the historical probability of a drugs making it through the clinical phases; *i.e.*, about 30%. They used the cost and payoff estimates from the NPV calculations: total development costs will be M$ 97 and the payoff will be M$ 500.

Decision analysis revealed that the estimated project value taken these success probabilities into account would be M$ 90.1. According to the original NPV analysis, addition of the PET study according to John's

suggestion would increase the overall costs with M$ 2. A lively discussion begins in the team how the input parameters will change if the PET study is performed. One team member argues that the probability that too low a dose will be selected for further development is substantially decreased if the PET study is performed. The team agrees with the assumption that with this study a dose can be found that shows minimum receptor occupancy.

TABLE 3 **Input parameters for Peter's development program**

Parameter	Value
Success action site	80%
Success pharmacological effect	75%
Success clinical efficacy	65%
Success therapeutic window	85%
Success population	90%
Costs action site	12 M$
Costs Pharmacological effect	19 M$
Costs Clinical effect	35 M$
Costs Clinical window	14 M$
Costs Population	17 M$
Estimated marketvalue	500 M$

According to the literature, lower than 90% occupancy can not have any clinical effect. This consideration would increase the probability that clinical effects will be observed with the selected dose and the successful defining of the therapeutic window will also be enhanced because the lower limit is determined. The team does not want to be too optimistic towards management so they decide to increase the estimated probabilities of success on both the 'clinical' and the 'window' question both with only 1%. The additional costs of the PET study are divided amongst the 'clinical' (M$ 36) and 'window' (M$ 15) questions. The project value is recalculated and instead of the loss of M$ 15 according to the NPV analysis, the estimated value is increased with M$ 2.6 to M$ 92.7!!

John decides to investigate how much more costs can be introduced in Peter's plan if the success probabilities are affected more than the estimated 1%. He calculates the project value for a series of different success probability combinations. He varies both the 'clinical' and the 'window' question up to ± 10% of the base values. This two-way sensitivity analysis is displayed in table 4. John realises that his gut feeling that Peter runs a higher risk to lose relatively large amounts compared to his plan is confirmed by this analysis.

John and Peter needed some time to explain their question based plan and the decision analysis that demonstrated that an early PET study actually had a high probability to be very cost effective. However, management agreed in the end. It was agreed to add a question-based Gantt chart to the traditional project and regularly review the progress in answering questions as well as the conventional progress in the studies.

TABLE 4 **Break-even table for success probabilities 'clinical' and 'window'; the bold project value is the expected value for 1% increase in both probabilities**

	Success probability 'window'										
Success probability 'clinical'	0.77	0.79	0.81	0.83	0.84	0.86	0.88	0.89	0.91	0.93	0.95
0.59	67	69	71	73	76	79	81	84	87	89	92
0.61	70	72	74	76	79	81	84	87	90	92	95
0.62	72	74	77	79	81	84	87	90	93	95	98
0.63	75	77	79	82	84	87	90	93	96	98	101
0.65	78	80	82	85	87	90	93	96	99	102	105
0.66	80	83	85	88	90	**93**	96	99	102	105	108
0.67	83	85	88	90	93	96	99	102	105	108	111
0.69	86	88	90	93	96	99	101	105	108	111	114
0.70	88	91	93	96	99	102	104	107	111	114	117
0.71	91	93	96	99	102	104	107	110	114	117	120
0.73	94	96	99	102	105	107	110	113	117	120	123

BOX 1 NET PRESENT VALUE CALCULATION

The Net Present Value (NPV) of a project is calculated by adding the present value of all future cash flows and subtracting the initial investments. The calculation of the present value is performed by discounting the future cash flows with a percentage, which reflects the required return of investment of the project according to the following formula:

$$NPV = \sum_{t=o}^{n} \frac{CF(t)}{(1+i)^t}$$

Where
NPV= Net Present Value; CF(t) = Cash flow in period t (including investments), incoming +, outgoing −; t = Period; n = Number of periods; i = Required return of investment (or discount factor)

The NPV assumes an incremental cash flow. Therefore, the NPV calculation compares the situation where the project is performed and the situation that results if the project is cancelled. If the NPV of a project is positive, the project adds value to the company and is therefore worthwhile.

NPV calculation requires the *a priori* determination of the required return of investment. This factor correlates with the risk associated with the investment in the project. The higher the risk of the investment, the higher the return of investment should be. Usually, the discount factor is determined using the Weighted Average Cost of Capital:

$$WACC = K_d(1-T_c)\left[\frac{MVD}{TDE}\right] + K_e \frac{MVE}{TDE}$$

Where
WACC = Weighted Average Cost of Capital; Kd = Interest rate; Ke = Treasury rate; Tc = Tax return; MVD = Market Value of Debt; MVE = Market Value of Equity; TDE = Total Debt and Equity = MVD + MVE

BOX 2 PHASES IN CLINICAL DRUG DEVELOPMENT

PHASE I

Research using small groups of healthy volunteers. Traditionally, this phase mainly focuses on if the human body tolerates the new drug and on finding a dose where the level of tolerance is acceptable. In general, this phase takes about 1 to 2 years. The centre for drug evaluation and research of the United States Food and Drug Administration (FDA) states:

"*Phase 1 includes the initial introduction of an investigational new drug into humans. These studies are closely monitored and may be conducted in patients, but are usually conducted in healthy volunteer subjects. These studies are designed to determine the metabolic and pharmacologic actions of the drug in humans, the side effects associated with increasing doses, and, if possible, to gain early evidence on effectiveness. During Phase 1, sufficient information about the drug's pharmacokinetics and pharmacological effects should be obtained to permit the design of well-controlled, scientifically valid, Phase 2 studies. Phase 1 studies also evaluate drug metabolism, structure-activity relationships, and the mechanism of action in humans. These studies also determine which investigational drugs are used as research tools to explore biological phenomena or disease processes. The total number of subjects included in Phase 1 studies varies with the drug, but is generally in the range of twenty to eighty.*"

PHASE II

Research on a group of patients where the first proof for efficacy is established. More characteristics of the NCE are determined and a safe and well-tolerated dose is determined where the drug is efficacious. According to the FDA:
"*Phase 2 includes the early controlled clinical studies conducted to obtain some preliminary data on the effectiveness of the drug for a particular indication or indications in patients with the disease or condition. This phase of testing also helps determine the common short-term side effects and risks associated with the drug. Phase 2 studies are typically well-controlled, closely monitored, and conducted in a relatively small number of patients, usually involving several hundred people.*"

PHASE III

The potential new drug is tested on thousands of patients in multi-centre research projects to investigate the side effects of the drug at a set dose in more detail. Furthermore, the efficacy of the drug at the determined dose is compared to existing medication. Further research is conducted to investigate possible side effects after long-term treatment and development of the drug for different indications is investigated. The FDA describes:
"*Phase 3 studies are expanded controlled and uncontrolled trials. They are performed after preliminary evidence suggesting effectiveness of the drug has been obtained in Phase 2, and are intended to gather the additional information about effectiveness and safety that is needed to evaluate the overall benefit-risk relationship of the drug. Phase 3 studies also provide an adequate basis for extrapolating the results to the general population and transmitting that information in the physician labelling. Phase 3 studies usually include several hundred to several thousand people.*"

PHASE IV

The registered drug is monitored closely to examine the occurrence of unexpected side-effects and interactions with other drugs.

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Outline of this thesis

As shown in the previous case study, changing the development plan from phase/time oriented to question based can improve the insights on the information that needs to be obtained and will help display the priorities within the program. In conventional phase-based drug development, timing is not the most important issue, as long as studies are performed rapidly. In this thesis, it is shown that the order in which studies are performed has a significant impact on the efficiency and quality of the drug development process. The impact of this novel approach can best be demonstrated by calculation of the financial consequences of resolving the right questions at the right time, during the development of new compounds. This calculation is based on the real-option theory, applied to drug development questions. Simple decision analyses suffice to determine the best sequence of research projects, and detailed pharmaco-economic models are unnecessary for this purpose. The thesis also provides some examples of research projects that were performed at different stages of drug development, with widely different consequences for the values of the projects concerned.

This thesis consists of four main sections.

SECTION 1 *Literature evaluation* describes some examples of evaluating existing biomarkers for clinical effects in healthy volunteers as helpful tools for early phase drug development. A structural procedure was adopted to evaluate the methods used in healthy volunteer trials using antipsychotics (Chapter 2) and benzodiazepines (Chapter 3). The use of REM sleep reduction as a frequently used method to evaluate the effects of antidepressants is reviewed in Chapter 4.

SECTION 2 *Developing a new formulation* describes four clinical studies with rilmenidine -a centrally acting antihypertensive agent- that investigate and define the optimal characteristics of sustained release rilmenidine formulations. Chapter 5 investigates the *in vivo* properties of a sustained release formulation in healthy volunteers. Furthermore, these *in vivo* pharmacokinetic characteristics are related to the *in vitro* sustained release properties. Chapter 6 defines the pharmacokinetic/pharmacodynamic relationship between rilmenidine concentrations and the development of side effects in healthy volunteers. In Chapter 7, the pharmacokinetic / pharmacodynamic relationship between rilmenidine concentrations and the reduction of blood pressure is investigated in mild to moderate hypertensive patients. Finally, in Chapter 8 the effects of multiple doses of sustained release formulations are investigated in mild to moderate hypertensive patients.

SECTION 3 *Bridging the gap to Japan* exemplifies two ways of comparing Japanese and Caucasian subjects with the aim of reliably extrapolating clinical data from Caucasian subjects to Japanese subjects. Chapter 9 describes an interethnic comparative study between Japanese and Caucasian volunteers. A Japanese study on the pharmacokinetic/pharmacodynamic relationship of nitrazepam is repeated in Caucasian subjects matched for gender, age and body size and the results are subsequently compared. Chapter 10 describes a simultaneously performed bridging study on a new oral contraceptive agent where the single dose and steady-state pharmacokinetics are compared between Caucasian female subjects and Japanese female subjects.

SECTION 4 *Market advantage* shows that early in the drug development program a small study can be performed to investigate potential advantages of newly developed agents over existing drugs. Chapter 11 describes a study in healthy volunteers to compare two doses of a potential anxiolytic drug with lorazepam and placebo to investigate the central nervous system effects of the new agent.

Each section is concluded with a value estimation, which discusses the impact of the presented studies on the drug development program using the question-based approach.

SECTION 1

SECTION 2

SECTION 3

SECTION 4

Literature evaluation

Developing a new formulation

Bridging the gap to Japan

Market advantage

CHAPTER 2

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Biomarkers for the effects of antipsychotic drugs in healthy volunteers

Abstract

Studies of novel antipsychotics in healthy volunteers are traditionally concerned with kinetics and tolerability, but useful information may also be obtained from biomarkers of clinical endpoints. A useful biomarker should meet the following requirements: a consistent response across studies and antipsychotics; a clear response of the biomarker to a therapeutic dose; a dose-response relationship; a plausible relationship between biomarker, pharmacology and pathogenesis. In the current review, all individual tests found in studies of neuroleptics in healthy volunteers since 1966 were progressively evaluated for compliance with these requirements. A MedLine search yielded 65 different studies, investigating the effects of 23 different neuroleptics on 101 different (variants of) neuropsychological tests, which could be clustered into seven neuropsychological domains. Subjective and objective measures of alertness, and of visual-visuomotor-auditory and motor skills were most sensitive to antipsychotics, although over half of all the studies failed to show statistically significant differences from placebo. The most consistent effects were observed using prolactin response and saccadic eye movements, where 96% and 83% of all studies resp. showed statistically significant effects. The prolactin inducing dose equivalencies relative to haloperidol of nineteen different antipsychotic agents correlated with the lowest recommended daily maintenance dose ($R^2 = 0.52$). This relationship could reflect the clinical practice of aiming for maximum tolerated levels, or it could represent a common basis behind prolactin release and antipsychotic activity (probably D_2-antagonism). The number of tests used in human psychopharmacology appears to be excessive. Future studies should look for the most specific and sensitive test within each of the domains that are most susceptible to neuroleptics.

Introduction

There is a growing pressure on the drug development process to enhance the relevance of studies at all stages. Traditionally, phase 1 studies were mainly concerned with kinetics and tolerability of a new compound in healthy volunteers, but increasing efforts are now made to include potential biomarkers of clinical endpoints. This approach is particularly useful in areas where phase 2 studies are cumbersome, for practical or ethical reasons. This is the case for many neuropsychiatric indications, including psychosis and schizophrenia. Patient studies can be complicated by factors associated with the disease, such as concomitant or previous treatments, adaptation

of dose and duration of treatment to clinical responses, different types and severity of psychopathology and overlap between symptoms and side effects of treatment. Also, a heterogenic patient population may augment individual variability for example due to differences in intelligence and motivational aspects.

Studies in healthy volunteers lack most of these methodological and logistic problems, but are faced with others. Healthy volunteers are usually studied using single (ascending) doses, as opposed to chronic treatment in patients. They obviously also lack the disease characteristics that serve to measure the treatment effects, although some studies use healthy subjects with schizotypy-like personalities to approach clinical relevance. The information derived from studies in healthy volunteers could also be enhanced with appropriate biomarkers, which can be defined as indicators of biologic, pathogenic or pharmacologic processes or responses to therapeutic interventions.

ref. 1-4

Currently, no validated biomarkers for psychosis or antipsychotics are available, but a useful marker should meet the following requirements:

1 a clear, consistent response across studies (from different groups) and antipsychotics
2 a clear response of the biomarker to a therapeutic dose of the antipsychotic
3 a dose (concentration)-response relationship
4 a plausible relationship between the biomarker, the pharmacology of the antipsychotic and the pathogenesis of the disease.

In the current review, these requirements were used to evaluate all potential biomarkers that have been used in healthy volunteer studies of antipsychotic agents over the past 30 years.

Methods

Structured literature evaluation

An extensive MedLine search (keywords: (antipsychotic or neuroleptic) and healthy) revealed a large number of individual tests, which differed widely in their sensitivity and specificity for detection of central nervous system (CNS) drug effects, with a lack of standardisation between the studies even for the

same tests. In addition, many studies used different antipsychotic dosages, usually at single doses. A structured procedure was adopted in order to obtain an overview. First, the results for all individual tests, drugs and dosages were put into a database. Most studies used different tests, which were all treated as independent measures of drug effect. The tests could then be roughly divided into neuropsychological/ motor skills, subjective assessments, and neurophysiological and neuroendocrine measurements. This approach allowed the preservation of individual study data in early stages, followed by a progressive condensation of results in logical clusters.

The test results could not be recorded quantitatively, considering the large diversity of methods, parameters and treatments. Instead, the ability of a test to show a statistically significant difference from placebo was scored as + (improvement/increase), = (no significant effect) or - (impairment / decrease). Although statistical significance is not only determined by the test variance but also by other factors like group size, this approach at least allowed an evaluation of the applicability of a test as a biomarker. No efforts were made to further quantify the level of statistical significance. A more quantitative approach was possible only for prolactin, where the peak concentration relative to baseline could be determined from most studies.

The chance that a test will detect a difference from placebo is expected to grow with increasing dose. To investigate this possibility, for each individual neuroleptic and test it was determined whether the number of statistically significant results increased with the dose. In this way, the most frequently used tests and drug dosages could be compared for dose-dependency. In many cases however, the number of tests or doses was too small to determine a relationship. To obtain an overview of dose-effects across neuroleptics, drug dosages were pooled into 'lower', 'medium' and 'higher' dosages. The 'medium' dose was determined as the lowest recommended therapeutic starting dose, as shown in Table 1. If the starting dose could not be retrieved, half the lowest recommended maintenance dose was used. The 'lower' and 'higher' doses were all dosages below or above this level.

ref. 5-6

This approach allowed the identification of tests showing a consistent response across studies and antipsychotics, and those with a clear response to a therapeutic dose of the antipsychotic (requirements 1 and 2 from the introduction). All measurements fulfilling these criteria were further tested for compliance with requirements 3 and 4: the existence of dose-response relationship and the plausibility of a mechanistic relationship, by reference to the original publications and the neuropharmacological literature.

TABLE 1 **PRL-inducing dose equivalencies for prolactin release, therapeutic dose and receptor affinities for antipsychotic drugs. (See text for explanation of PRL-inducing dose equivalence)**

Drug	Maintenance dose (mg/day)	Reported study range (mg)	PRL-inducing dose equivalencies	D2 receptor affinity Ki (nM)*	5-HT2/D2 ratio *
Amisulpride	300-1200	20-400	56.6		
DU -29895	?	3-10	2		
Remoxipride	60-300	0.5-150	9.29	272	>40
Sulpiride	100-200	100-400	97.8	31	40
Zetidoline	10-30		10-40		0.81
Raclopride	4-8	0.1-16	1.72	7.0	1429
Mazapertine	?	5-50	-		
Zotepine	50-300	25-100	17.2	13	0.07
Pimozide	2-6	2-6	7.89	1.2	5
Setoperone	15-120	5-40	14		
Olanzapine	5-20	3-5	-		
Clozapine	150-300	12.5-50	309	152	0.02
Risperidone	4-8	1-2	0.1	3.1	0.05
Chlorpromazine	75-300	25-100	39.6	19	0.14
Prochlorperazine	25-50	2.5-5	8.3	3.1	2.4
Trifluoperazine	20-30	4	5.1	4.3	2
Perphenazine	16-64	1	2	6.5	0.66
Haloperidol	4-10	0.25-10	1.11	1.2	2.3
Fluphenazine	2.5-5	0.35	1.89	1.9	1.8
Thiotixene	20-30	0.25-0.5	0.56	2.5	39
Thiethylperazine	10	10	4.37	4.5	11
Molindone	30-100	5	1.13	25	94
Thioridazine	200-800	10-75	-	16	0.26

ref. 49 *Leysen et al. *Psychopharmacology (Berl)* 1993; 112:40-54

Neuropsychological/motor skill

In the first phase of the literature review, tests from different studies were only grouped if they were equal as judged from name and description or literature reference (e.g. all Digit Symbol Substitution Tests (DSST)), but all variants or related forms of the tests (DCCT, SDST etc.) were treated separately.

TABLE 2 **Progressive condensation of all reported tests; from test to cluster to domain (after Spreen et al, 1998, ref. 7)**

Test	Cluster	Domain
WAIS vocabulair		
WAIS similarity		**Achievement**
WAIS block design		
WAIS picture composition	Intelligence	
Blue-Brown visual inhibition		
H-mask visual inhibition		
Auditory Latent inhibition		
Visual Latent inhibition		
Stroop colour word		
Simple reaction (conflict task)		
Cognitive Set switching	Inhibition task	
Logical reasoning		**Executive**
Decision making time		
Rapid info processing		
Perceptual maze		
Simulated driving		
Visual search	Complex info process	
Time estimation		
Time perception	Time estimation	
Visual search		
Attentional search		
Symbol copying		
Letter cancellation		
Alphabetic cross-out		
D2 cancellation		
Brickenkamp D2	Search	
DCCT		
SDST		
DSST	DSST like	
Digit Vigilance		
Vigilance		**Attention**
Auditory vigilance test		
Wesnes/Warburton Vigilance task		
Rapid info processing		
Continuous attention	Other vigilance	
CRT + Tracking		
Divided attention		
Selective attention		
Focussed attention Task		
Emotional attention Task	Divided attention	
Auditory Flutter fusion		
Flash fusion		
CFF	Flicker discrimination	
Paired associate learning		
Word list learning		
15 word test		
Introductory conditioning	Learning	**Memory**
Delayed word recall		
Delayed word recognition		
Delayed picture recognition	Delayed recall	

Test	Cluster	Domain
Word presentation		
Word recognition		
Numeric working memory		
Numerical memory		
Memory scanning		
Auditory Brown/Peterson		
Visual Brown/Peterson		**Memory (continued)**
Visual spatial memory		
Fragmented picture test	Immediate recall	
Pauli test		
Block Span		
Digit span		
Digit Span (forward)		
Digit Span (backward)	Span tests	
WAIS vocabulair		
WAIS similarity		**Language**
Word fluency		
Verbal fluency	Language	
Performance time (Delayed word recogn.)		
Performance time (Nummeric working memory)		
Performance time (Digit vigilance)		
Performance time (Rapid info processing)		
Performance time (Delayed picture recognition)		
Performance time (Visual information processing)	Performance time	
Simple Reaction Time		
CRT		
Complex RT visual		
Visual 2choice RT		
VRT		
Visual response speed		
SDSTART		**Visual, visuomotor and auditory**
Acoustic RT	Reaction time	
Wire Maze Tracing		
Archimedian spiral		
Critical tracking task		
Trail making		
Tracking		
Complex Tracking		
Wiener Geraet	Eye-hand coördination	
Flexibility of closure		
WAIS block design		
WAIS picture comp.		
Digit copying	Other	
Manipulative motor		
Feinmotorik		
Graphological analysis		
Tapping	Manipulation	**Motor**
Hand arm lateral reach coordination		
Visual arm random reach		
Motor contr.&coord.		
Motor behaviour	Motorcontrol	

Next, all tests that could be regarded as variants from a basic form were clustered as indicated in Table 2. Thus, all tests determining the ability to discriminate flash- or flicker frequencies were grouped as 'flicker discrimination'. These data were used to determine the consistency of results within test clusters and to identify potential dose-effects.

Although many different methods are used to evaluate the functional effects of neuroleptics, most actually measure a limited number of core features. Neuropsychological/ motor skills-tests can be categorised according to a catalogue of neurocognitive tests (attention, executive etc.), as presented in Table 2. This catalogue divides tests according to different neuropsychological domains, assuming that the results of each test are mainly determined by one of these domains. To determine the domains that are most affected by neuroleptics in healthy subjects, all tests within a neurocognitive domain were bundled. The number of statistically significant differences from placebo was scored and compared to the total number of studies within the domain.

ref. 7

Subjective assessments

For the subjective assessments, most individual scales corresponded to individual lines for the subscales 'alertness', 'mood' and 'calmness', proposed by Norris and applied to CNS-drug evaluation by Bond and Lader. Other scales could be grouped under 'anxiety', 'subjective (psychotropic) drug effects' and '(extrapyramidal) side effects'.

ref. 8-9

Neurophysiological assessments

ELECTROENCEPHALOGRAPHY (EEG) EEG is sensitive to a wide range of centrally active substances, although the exact mechanism is hardly ever known. EEG-studies differ in numbers of leads or technical settings, but they usually report effects per EEG-frequency band, which are divided into delta (0.5-3.5 Hz), theta (3.5-7.5 Hz), alpha (7.5-11.5 Hz) and beta (above 11.5 Hz; sometimes subdivided into beta 1 (11.5-30Hz) and beta 2 (above 30 Hz)). In the current review, statistically significant differences from placebo were scored for the four major frequency bands.

ref. 10-14

EYE MOVEMENTS Smooth pursuit and saccadic eye movements have been extensively validated to assess CNS-drug (side)-effects. Saccadic eye

ref. 15-20

movements provide information on the sedative properties of antipsychotic drugs. These effects are not specific for a class of drugs, but rather quantify sleep/wake transition. Although there are different techniques to measure eye movements, most studies report peak velocity for visually guided saccades or sometimes anti-saccades (where subjects are instructed to look away from the target). Smooth pursuit eye movements are reported as deviations from the time that the eyes closely followed the target. Statistically significant differences from placebo were reported, and dose-response relationships were investigated for consistent responses.

ref. 21-22
ref. 23-24

EVOKED POTENTIALS Schizophrenic patients exhibit abnormalities in event related potentials (ERP) that are postulated to reflect characteristic changes in stimulus discriminability and decision making. Typically, these consist of a reduction in the amplitude and a prolongation of the latency of the P300 component.

ref. 3-4, 25-35

There were not enough healthy volunteer studies to warrant (semi-) quantitative evaluation of these tests, but the results are described because of the apparent relevance of this method in schizophrenia research.

Neuroendocrine assessments

PROLACTIN (PRL) Neuroendocrine tests and particularly the PRL response to antipsychotic agents have been reviewed in several publications. PRL response to antipsychotics is clinically related to hyperprolactinemia and is therefore thought undesirable during drug development. However, the prolactin response to antipsychotics is a direct consequence of dopamine antagonism, since pituitary PRL secretion is inhibited by dopamine. Dopamine antagonism is one of the core characteristics of antipsychotic agents, and abnormal dopamine activity is a widely accepted central pathophysiological abnormality in psychosis. The PRL-response to neuroleptics is frequently studied in healthy volunteers, and usually the maximum PRL-response is reported. This response is determined by the dose of a neuroleptic, and by its PRL-inducing potency. The value of prolactin as a biomarker would be particularly large, if for a range of neuroleptics the PRL-inducing potencies were closely related to the therapeutic doses. Such a comparison can only be made directly on the basis of well-defined PRL-inducing potencies determined from complete dose-response relationships for each neuroleptic. The literature did not provide this information for most neuroleptics; only haloperidol yielded enough data to plot a curve over a wide

ref. 36-41
ref. 42
ref. 43
ref. 44-45
ref. 44-46

dose range, as described in the results-section. Therefore, an alternative approach was chosen where the PRL-inducing potency of each neuroleptic was expressed relative to this haloperidol dose-response curve. Neuroleptic doses that caused a larger PRL-response than observed with haloperidol were not plotted on this reference curve; *i.e.* data were not extrapolated beyond the extent of the curve. In this way, for each neuroleptic dose an equipotent haloperidol dose could be determined, that would theoretically cause the same peak PRL-response. Next, each dose was normalised to haloperidol 1 mg, and the mean of these values was calculated per neuroleptic. This constituted a PRL-inducing dose equivalence (relative to haloperidol) for each neuroleptic.

ref. 47-48

To examine the value of prolactin release as a biomarker for therapeutic efficacy, these mean PRL-inducing dose equivalencies were compared to the lowest recommended daily therapeutic maintenance doses (see Table 1). The relationships of individual PRL-inducing dose equivalencies with some key pharmacological features for the antipsychotics (D_2 affinity (K_i) and 5-HT/D_2 antagonism ratio) were examined. The K_i values (Table 1) were assessed using the same methods, allowing inter-drug comparison.

ref. 5-6

ref. 49

CORTISOL AND GROWTH HORMONE (GH) 5-HT agonists and antagonists have been found to have an effect on plasma cortisol and growth hormone levels, but the data are inconclusive. These hormones have been used to evaluate antipsychotic drug action on serotonergic function, particularly 5-HT_2 which may play a role in the mechanism of action of atypical neuroleptics. The number of studies was too low to allow any quantitative analysis. The statistically significant differences from placebo were reported.

ref. 50-54

ref. 49

Statistical evaluation

To allow the calculation of average responses with confidence intervals for binomial proportions, responses were coded as follows. Impairment / decrease was coded as 0, no change was coded as 0.5 and improvement / increase was coded as 1. A cumulated response code was calculated by multiplying the number of occurrences for each response by the coding, and adding this over the 3 responses. A proportion was calculated by dividing the cumulated response code by the total number of responses. This yields an average response between 0 (impairment/decrease) and 1 (improvement / increase). For these proportions, exact confidence intervals for binomial

proportions were calculated using the cumulated response code and the total number of responses. Exact confidence intervals were calculated using SAS for Windows V6.12 with the ExactPCI V1.2 procedure provided by SAS Inc, (SAS Institute Inc, Cary, NC).

Results

The literature search yielded 65 different studies, published since 1966. These studies investigated 23 different neuroleptic agents, with 2.2 doses per study on average. Olanzapine was only given at slightly subtherapeutic dosages and mazapertine was not registered, but 76% of the doses of all other agents were at 'medium' or 'higher' levels. Thus, most studies were able to comply with the requirement that a useful biomarker should respond to therapeutic doses. Eighteen studies were solely devoted to haloperidol, and 12 studies used haloperidol as a reference for other neuroleptics. On average, there were 17 healthy participants (range 5-110) per study.

ref. 2, 19, 47-48, 52-53, 55-107

Neuropsychological/motor skill

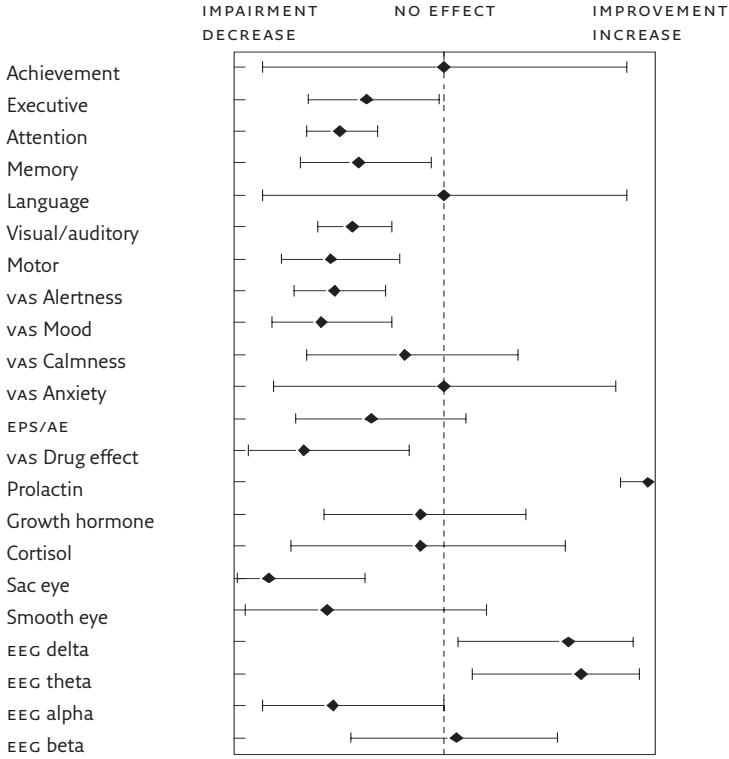
There were 101 different test-(variants), as shown in Table 2; 51 of these were used only once. Six tests were used more than ten times: critical flicker fusion (32 times), choice reaction times (32x), finger tapping (18x), time estimation (15x), simple reaction times (15x) and DSST (14x). At least 33% of all tests that were used twice or more (by different groups) showed statistically non-significant or conflicting differences between neuroleptics and placebo. For the five most frequently used test, these percentages were 53%, 47%, 39%, 53%, 40% and 43%, respectively.

Fifteen individual tests showed statistically significant impairment in all cases (100%), but nine of these were only used once (one dose of one antipsychotic), and the six other tests were only used by a single research group: alphabetic cross-out (8x), wire maze tracing (3x), Pauli test (3x), delayed picture recognition (2x), delayed word recognition (2x), and performance time for digit vigilance (2x).

Subsequently, comparable tests or variants were clustered as shown in Table 2. Reaction times showed significant prolongation in 46% of the 52 times this method was used. Complex information processing tasks were used 39 times, showing significant impairment in 46%. Flicker fusion was

employed 38 times, demonstrating significant impairment in 45%. The 21 DSST-like tests showed statistically significant impairment in 48%, no change in 48% and an improvement in 4%. Significant impairment on search tasks was found in 70% of 20 cases. Medium or higher doses were used in all cases except two. Manipulative motor tasks were performed 31 times, and showed significant impairment in 48%. A significant impairment was found in 41% of the 34 times that eye hand co-ordination was studied. Clustering of comparable tests thus did not increase the number of significant results.

FIGURE 1 **The averaged significant effects of antipsychotics on neuropsychological domains, subjective assessment, neuroendocrine and neurophysiological parameters**



However, the larger number of studies within clusters allowed a better estimation of dose-dependency. In most cases, consideration of only

'medium' or 'higher' doses did not appreciably increase the percentages of significant results. Only flicker fusion and complex information processing showed modest increases in the percentages of tests demonstrating impairment, when the lower dosages were omitted (from 45% to 57%, and from 46% to 51%, respectively).

No individual neuropsychological/motor skill-test or cluster of related test variants showed a consistent response to antipsychotics, and this did not improve to any extent when a dose-effect relationship was taken into account. To evaluate which neuropsychological domains are most clearly affected by neuroleptics in healthy volunteers, tests were categorised as indicated in Table 2. The percentages of statistically significant test results are presented in Figure 1. These results show that the most sensitive neuropsychological domains are attention, visual/auditory/visuomotor skills, and motor function.

It was subsequently determined for these most sensitive areas, whether there were systematic differences between effects of 'classic' (haloperidol, thioridazine and chlorpromazine) and 'atypical' neuroleptics (all others -see Table 1). In addition, an overview was obtained for differences between individual agents, although this effort was restricted by the limited number of assessments per drug. Such differences did not appear to exist. In each of the most sensitive areas, at least 48% of the 'atypical' antipsychotics caused impairment. Similar or even lower percentages were found for the 'classical' neuroleptics.

Subjective assessments

Thirty-one different subjective assessment scales were employed; five of which only once. The scales used most often (by more than one research group) were: simple visual analogue scales for alertness (17 times), mood (13x) and attention (10x), and the combined scales from Bond & Lader (11x) and the Von Zerssen Befindlichkeitsskala (10x). The latter test was most consistent (significant results in 8 cases), but these were all from the same group; the only other group using this method obtained non-significant results. The other frequently used tests showed impairment in 38-59% of cases. Thus, none of the individual neuropsychological/motor skill tests or subjective assessments exhibited a consistent response across studies and antipsychotics. None of the subjective assessments showed an improvement, except one positive mood change with 2 mg haloperidol.

ref. 70, 74, 107
ref. 82

Assessments were clustered into scales for 'alertness' (57 measurements; significant deterioration in 53%), 'mood' (28x; 50%), 'calmness' (16x; 19%), 'anxiety' (5x; 0%), 'subjective (psychotropic) drug effects' (14x; 57%) and 'extrapyramidal side effects' (21x; 29%). Most subjective assessments showed indications for dose-dependency. After deletion of 'lower' doses, scales for 'alertness' became significant in 64%, 'mood' in 70%, 'calmness' in 33%,'subjective (psychotropic) drug effects' in 80% and 'extrapyramidal side effects' in 43%.

Neurophysiological parameters

ELECTROENCEPHALOGRAM (EEG) EEG was measured 17 times employing six different antipsychotics. The observed trend is an increase in delta (59%) and theta (65%) and a decrease in alpha (59%) and beta (29%) frequencies, as shown in Figure 1. These effects can be observed with a number of other psycho-active drugs and generally indicate sedation. Consideration of only 'medium' and 'higher' doses did not appreciably change these results.

EYE MOVEMENTS Saccadic eye movements were used more frequently than smooth pursuit eye movements (18x vs 9x) (Figure 1). No more than three different antipsychotics were evaluated by saccadic eye movement. Saccadic peak velocity showed significant impairment compared to placebo in 83%. Only 56% of the smooth pursuit eye movement recordings showed impairment (increased saccadic intrusions). These percentages increased slightly to 85% and 57% after discarding the 'lower' doses. However the effects of the neuroleptics on eye movements were found to be indistinguishable from the effects of benzodiazepines. Saccadic eye movements appear to remain a sensitive nonspecific marker for the sedative properties of a drug.

ref. 19

EVOKED POTENTIALS The effects of oral sulpiride 150 and 300 mg on ERP's have been studied recently in healthy volunteers. Sulpiride induced an increase in P200 and P300 latencies. The amplitude response to sulpiride of ERP parameters was bidirectional; the amplitude of subjects with a high initial value decreased while those with low initial values increased. It is remarkable that comparable results were obtained with the dopamine agonist bromocriptine. However, a recent study showed that the dopamine agonist apomorphine (0.75 mg s.c.) had no effect on the P300. Assessing the potential of ERP as a biomarker is difficult. First of all, no clear quantitative relationship between abnormalities in ERP components and schizophrenic

ref. 89

ref. 108

ref. 12

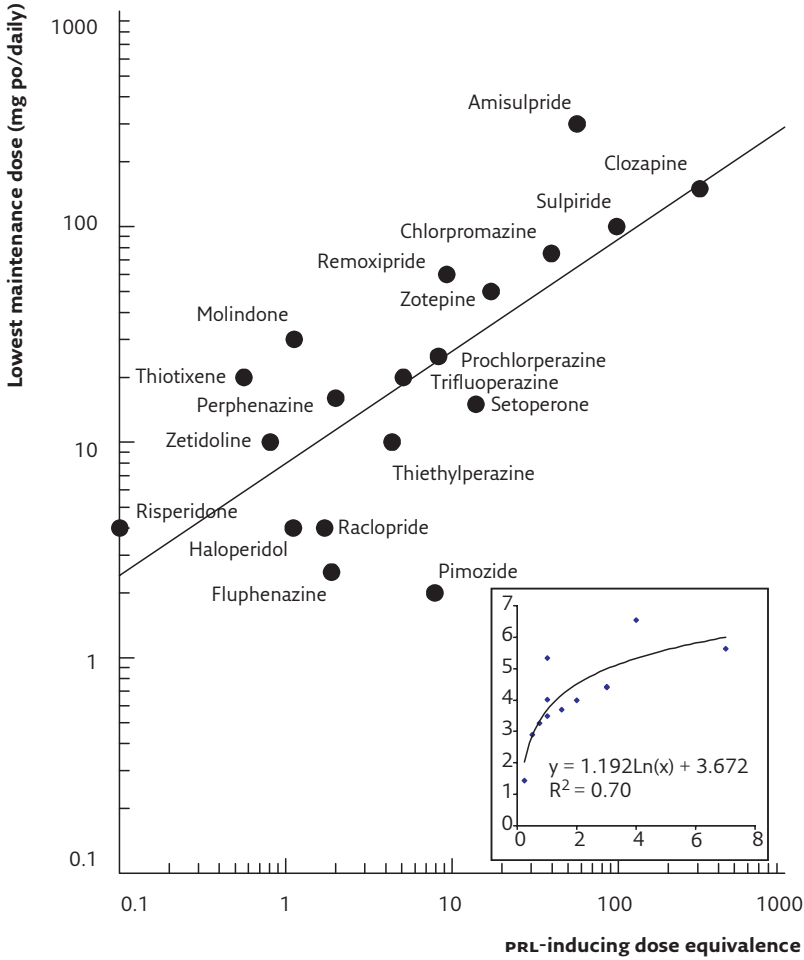
symptomatology exists. Secondly, the relationship between the latency / amplitude and stimulus perception/processing is speculative. Also, the P300 is markedly influenced by the subjective expectancy of a stimulus by an individual subject. Given that the effect of antipsychotic drugs on ERP in healthy volunteers has been assessed in very few studies, ERP is as yet unsuitable as a biomarker in the development of antipsychotic drugs.

Neuroendocrine parameters

PROLACTIN (PRL) Plasma prolactin response to antipsychotic agents was assessed 79 times using 21 different antipsychotics. Three statistically non-significant responses were measured, for the lowest dose (0.1 mg po) of raclopride and for clozapine 12.5 and 50 mg. 'Lower' doses showed 96% statistically significant PRL responses. Consideration of only the 'medium' and 'higher' dosages increased these percentages to 97%. These uniform PRL responses allowed an examination of the relationship between PRL response and therapeutic effect of antipsychotics, as described in the methods-section. The normalisation of different doses was accomplished by reference to a logarithmic dose-response curve for haloperidol, constructed using eleven haloperidol dosages reported in the literature (range 0.25-7 mg; relative PRL increase = 1.192Ln(dose) + 3.672; R^2 = 0.70; see insert in Figure 2). This range of haloperidol doses caused peak PRL-increases of 1.4-6.6 times baseline. All doses of the other neuroleptics that did not exceed the maximum PRL-increase observed with haloperidol were plotted on this curve. For each neuroleptic, the geometric mean of the equivalent haloperidol-doses was calculated as a measure of PRL-inducing dose equivalence. Nineteen neuroleptic doses caused PRL responses beyond the range of the haloperidol reference line (range 7.6-10.6 times PRL elevation). This completely excluded mazapertine from the analysis, as well as several doses of amisulpride, raclopride, remoxipride, risperidone, sulpiride and zetidoline.

The PRL-inducing dose equivalencies and their concomitant lowest therapeutic maintenance doses are shown in Table 1 and Figure 2. The neuroleptics showed a good correlation between these two characteristics (R^2 = 0.52, $p<0.001$). Compounds to the right of haloperidol in the graph are less potent PRL-releasers than haloperidol, although higher doses may still cause more PRL-release. Clinically potent drugs with a minimal PRL-releasing propensity are expected in the lower right hand corner of the graph, which only includes pimozide. There is no clear distinction between 'classic' and 'atypical' neuroleptics (*e.g.* clozapine).

FIGURE 2 **PRL-inducing dose equivalencies compared to lowest daily therapeutic maintenance dose for various antipsychotics (see text for explanation). Insert: reference curve for haloperidol dose (x-axis) and PRL-increase relative to baseline (y-axis)**



ref. 48 Prolactin release is generally attributed to inhibition of the D_2-receptor, whereas the antipsychotic effect may be more related to the ratio of

ref. 109, 110 5-HT_2/D_2-antagonism. This was further investigated by correlating these parameters (shown in Table 1) with the therapeutic and PRL-inducing dose equivalencies. There were no significant relationships between the

5-HT$_2$/D$_2$-ratio and the PRL-inducing dose equivalence (R^2 = 0.05) or the therapeutic dose (R^2 = 0.03). Weak correlations were found between D$_2$-K$_i$-values and PRL-release (R^2 = 0.34, $p<0.05$) or the recommended maintenance dose (R^2 = 0.63, $p<0.001$).

CORTISOL AND GROWTH HORMONE (GH) It seems that 5-HT function is reflected by Cortisol and GH release; agonists elevate hormone levels and antagonists reduce the hormone response. Decreased levels of both hormones are expected after neuroleptics, since most antipsychotic agents have some 5-HT antagonistic properties (particularly 5-HT$_2$ and 5-HT$_{1A}$). Cortisol response to antipsychotic drugs was measured eleven times; GH was evaluated in 18 instances. Significant changes from baseline were rare. Cortisol levels were changed in only two studies with antipsychotics in healthy volunteers. Only 11% of the GH responses to neuroleptics showed significant decreases (2 out of 18). This percentage decreased if only 'medium' and 'higher' doses are taken into consideration. Decreased levels from baseline of both hormones are difficult to measure due to detection limits. Baseline levels can be increased using heat stress (cortisol) or exercise (GH). Both methods were used once with neuroleptics and both yielded significant decreases. For now, cortisol and GH responses are unreliable biomarkers, but may become measures of 5-HT antagonism in studies using baseline-induction techniques.

Discussion

The aim of this review was to evaluate the usefulness of methods used to assess neuroleptic effects in healthy subjects. A striking number of different neurocognitive tests was identified, and only very few methods were used frequently enough to allow individual evaluation. Consequently, tests had to be grouped, to observe trends for relationships with neuroleptic effects. Several different meaningful ways to group tests were used in this review, although each method inevitably led to a loss of information. Even grouping tests with the same name and/or description bypasses differences among research groups or test variants. Some methods used by individual research groups may have all the characteristics of ideal biomarkers, but this may have been missed in this review. Some of these tests consistently showed effects of different neuroleptics (*e.g.* alphabetic cross-out, wire maze tracing, Pauli test), but it is difficult to evaluate their usefulness in drug development if they are not generally applied, and more studies are needed to allow a judgement on these tests.

Even after clustering of comparable tests (DSST-like, flicker discrimination-like etc, as shown in Table 2), most methods were still applied relatively infrequently. Six of twenty test clusters were performed more than twenty times, and the effects of neuroleptics were inconsistent in over half of these cases. Thus, no single widely applied test or test-cluster appeared to stand out.

Despite the large number of test forms, most primarily address a single neuropsychological function, or a limited number of functional domains (Table 2). Therefore, tests were further grouped according to their primary neuropsychological domain. This showed that certain functional and subjective drug effects were more consistently affected by neuroleptics than others (Figure 1), notably attention (DSST like, flicker discrimination and search clusters), visual/auditory visuomotor responses (reaction time cluster), motor skills (manipulative motor skill cluster), and subjective effects (mood, alertness, and 'drug effect'). Tests aiming for achievement, executive function, memory, language and (extrapyramidal) side effects showed little or no change, although they were used quite regularly. This information is useful for planning future studies with neuroleptics in healthy subjects, because it allows the targeted selection of a few specific tests within each sensitive domain. Attention for instance is one of the most sensitive domains to single dose neuroleptics, and some 24 different test clusters (or more than fifty different individual tests) were used within this domain. Not all of these tests have been systematically compared, but whenever comparisons were made, saccadic eye movement (peak velocity) was the most sensitive measure of alertness/attention caused by a wide range of drugs or circumstances. The sensitivity of saccadic eye movements to neuroleptics was confirmed in the current review, as shown in Figure 1. More comparative studies are necessary to determine the most useful tests for the other neuropsychological domains.

ref. 21, 23

Electroencephalography (EEG) has also been claimed to be sensitive to antipsychotic medication. On average, the EEG showed a decrease in alpha, and an increase in delta and theta frequencies, but the sensitivity was not as large as for saccadic eye movements. The evaluation of evoked potentials as potentially useful biomarkers was severely impaired by the small number of studies using this technique.

Baseline levels of growth hormone and cortisol are relatively low, and decreases therefore rarely reach significance in small groups. Significant changes were only detected after pre-drug growth hormone and cortisol

levels were elevated by exercise or heat. Prolactin response showed a pronounced consistent effect across studies, antipsychotics and dosages. The relative dose equivalence to induce a PRL-response was clearly related to the affinity for D_2-receptors and to the recommended therapeutic starting dose. Theoretically, this information could be used to predict a likely therapeutic (starting) dose for a new neuroleptic, by plotting its PRL-inducing dose equivalence on the curve of Figure 2. In practice, this application could be limited by the logarithmic scaling, and by the lack of reference data for (potential) neuroleptics that cause more PRL-release than haloperidol. Also, the data were derived from a large variation in studies and methods, and the applicability could benefit from a systematic characterisation of dose-PRL-response curves for a range of antipsychotics.

Despite these practical limitations, the findings clearly show that PRL response is the best validated biomarker for 'clinical' effects of antipsychotic drugs, although it is unclear what these effects are. At first glance, Figure 2 suggests that PRL-release directly reflects the antipsychotic potency, because both may be related to D_2-antagonism. Our review indicates that D_2-affinity is significantly (albeit weakly) related to clinical potency. Neither relationship showed a difference between older/'classic' and newer/'atypical' neuroleptics. This is in agreement with a postulated common action of antipsychotics on cortical D_2-receptors, irrespective of class. There may also be another explanation for the close relationship between the PRL-inducing and therapeutic potencies. Both in drug development and medical treatment it is common practice to look for a maximum tolerated dose, to increase the chance of a therapeutic success. Consequently, many recommended antipsychotic doses are too high. By increasing the dose to maximum tolerated levels, any therapeutic selectivity that may exist between 'classic' and 'atypical' neuroleptics (or amongst novel antipsychotics) could disappear. In this case, the PRL-inducing dose equivalence is as much a measure of tolerability as of clinical efficacy. This would explain why no differences were found between the two classes in any of the more sensitive neuropsychological or subjective domains, including motor skills reflecting extrapyramidal side effects. Ideally, this suggestion can be examined by comparing the effects on a biomarker for efficacy to the prolactin dose equivalence and/or therapeutic dose. However, no validated biomarker for efficacy is currently available.

ref. 47-48

ref. 110

ref. 111

ref. 112

Obviously, the clear relationship between PRL-inducing and therapeutic potencies does not imply that all mentioned antipsychotics will necessarily cause clinical cases of hyperprolactinæmia. Clinical hyperprolactinæmia

typically develops during prolonged treatment, and is usually characterised by higher levels of prolactin than measured in the single-dose experiments reported here. Thus, chronic and acute prolactin elevation may differ, and we cannot exclude that 'classic' and 'atypical' neuroleptics have different long-term effects on PRL-release. The maximum extent to which neuroleptics can cause prolactin release (E_{max}) cannot be determined from the PRL-inducing dose equivalence relative to haloperidol, shown in Table 1 and Figure 2. This can only be derived from fully characterised individual dose-response relationships.

ref. 42, 43

In conclusion, the number of different neuropsychological, subjective, neurophysiological and neuroendocrine tests that are used to measure effects of antipsychotic agents in healthy volunteers, far outweigh the number of studies. This greatly impairs the usefulness of these tests in drug development. Only a few neuropsychological domains appear to be sensitive to neuroleptics in clinically relevant single doses, notably subjective and objective measures of decreased alertness, and of reduced visual-visuomotor-auditory and motor skills. Most studies used several methods, which in part overlapped in these domains, and in part were aimed at insensitive areas. Useful biomarkers should be particularly sought in the most specific and sensitive tests within each of these susceptible domains. All neuroleptics caused an increase in prolactin, which was closely related to the therapeutic dose. This relationship could reflect the clinical practice of aiming for maximum tolerated levels, or it could represent proximity of pathways involved in prolactin release and antipsychotic activity. The number of tests used in human psychopharmacology appears to be excessive and reduction of the number of tests as well as further evaluation and validation is long overdue.

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CHAPTER 3

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Biomarkers for the effects of benzodiazepines in healthy volunteers

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Abstract

Studies of novel centrally acting drugs in healthy volunteers are traditionally concerned with kinetics and tolerability, but useful information may also be obtained from biomarkers of clinical endpoints. A useful biomarker should meet the following requirements: a consistent response across studies and drugs; a clear response of the biomarker to a therapeutic dose; a dose-response relationship; a plausible relationship between biomarker, pharmacology and pathogenesis. In the current review, all individual tests found in studies of benzodiazepine agonists registered for anxiety in healthy volunteers since 1966 were progressively evaluated for compliance with these requirements. A MedLine search yielded 56 different studies, investigating the effects of 16 different benzodiazepines on 73 different (variants of) neuropsychological tests, which could be clustered into seven neuropsychological domains. Subjective and objective measures of alertness were most sensitive to benzodiazepines. The most consistent effects were observed on saccadic peak velocity (SPV) and visual analogue scores (VAS) of alertness, where 100% and 79% of all studies resp. showed statistically significant effects. A dose-response relationships could be constructed for temazepam and SPV, which was used to determine dose equivalencies relative to temazepam, for seven different benzodiazepines. These dose equivalencies correlated with the lowest recommended daily maintenance dose ($R^2 = 0.737$, $p<0.05$). This relationship between SPV-reduction and clinical efficacy could reflect the clinical practice of aiming for maximum tolerated levels, or it could represent a common basis behind SPV reduction and anxiolytic activity for benzodiazepines (probably sedation). The number of tests used in human psychopharmacology appears to be excessive and their sensitivity and reproducibility low.

Introduction

Traditionally, phase 1 studies are mainly concerned with the pharmacokinetics and tolerability of a new drug in healthy volunteers. However, increasing efforts are made to include measures for efficacy as early as in

phase 1 studies. This is especially the case for neuropsychiatric disorders where phase 2 studies in patients can be difficult to realise due to practical or ethical issues such as concomitant or previous treatment, adaptation of dose, and the wide variety of types and severity of psychopathology.

Studies in healthy volunteers evade most of the methodological and logistic problems of patient studies, but other complications arise. Most early phase 1 studies are highly dependent on the used biomarker. However, useful information on the potential therapeutic effects of the investigational drug at an early stage could enhance the drug development program of the new compound.

Although no validated biomarker for anxiolysis exists, in general a useful biomarker for activity of a drug class should meet the following criteria:

1. a clear, consistent response across studies (from different research groups) and drugs from the same class
2. a clear response of the biomarker to therapeutic doses
3. a dose (concentration)-response relationship
4. a plausible relationship between the biomarker, the pharmacology of the drug class and the pathogenesis of the therapeutic area.

ref. 1

Previously, these criteria were used to evaluate the usefulness of biomarkers for the effects of antipsychotic drugs in healthy volunteers. In the current review, the effects of benzodiazepines in healthy volunteers were evaluated using the same methodology. Benzodiazepines are registered for different indications (like anxiety disorders, epilepsy treatment, insomnia and pre-medication in anesthesiology), often with various doses and formulations for each indication. To facilitate the review, it was limited to (doses of) benzodiazepines that are registered or investigated for the treatment of anxiety disorders.

Methods

Structured literature evaluation

A broad MedLine search, (keywords: (anxiety or anxiolytic) *and* (model or parameter or effect or *dynamic) *and* healthy *and* (subjects or volunteers)) revealed a large number of individual tests, with an apparent lack of standardisation between the studies even for the same tests. First, all studies where an anxiolytic benzodiazepine was administered were filtered out.

The results of these studies for each individual test, drug and dosage were put into a database (Microsoft® Access 97 SR-2, Microsoft Corporation, Redmond, WA, USA). Most studies used different tests on different doses of a benzodiazepine, which were all regarded as independent measures of drug effect. The tests could then be roughly divided into neuropsychological/motor skills, subjective assessments, and neurophysiological measurements. This approach allowed the preservation of individual study data in early stages, followed by a progressive condensation of results in logical clusters.

Grouping of individual test results

ref. 1 A structured procedure described previously was adopted in order to obtain an overview. This method includes progressive evaluation of all the reported tests on the basis of the mentioned criteria. The purpose of this review was to identify generally applicable biomarkers of benzodiazepine action. Results from tests that were used only once or by one research group could not be generalised, and were therefore not individually analysed. Such tests were grouped with other comparable tests. The first step in this process included grouping of tests that could be regarded as variants from a basic form (*e.g.* all tests determining the ability to discriminate flash- or flicker frequencies grouped as the test cluster 'flicker discrimination'). Subsequently, a catalo-
ref. 2 gue of psychological tests was used to group these test clusters further to the neuropsychological domain it actually measures. The results of the effects on these domains were also reviewed.

In most cases, individual test results could not be recorded quantitatively, considering the large diversity of methods, parameters and treatments. Instead, the ability of a test to show a statistically significant difference from placebo or baseline was scored as + (improvement/increase), = (no significant effect) or - (impairment/decrease). Although statistical significance is not only determined by the test variance but also by other factors like group size, this approach at least allowed an evaluation of the applicability of a test as a biomarker in typical early drug development studies. No efforts were made to further quantify the level of statistical significance at this stage.

Dose normalisation

The chance that a test will detect a difference from placebo is expected to increase with dose. To investigate this possibility, it was determined for each

individual benzodiazepine and test whether the number of statistically significant results increased with the dose. In this way, the most frequently used tests and drug dosages could be compared for dose-dependency. In many cases however, the number of tests or doses was too small to determine a relationship. To obtain an overview of dose-effects across benzodiazepines, drug dosages were pooled into 'lower', 'medium' and 'higher' dosages. The 'medium' dose was determined as the lowest recommended therapeutic dose. The 'lower' and 'higher' doses were all dosages below or above this level. Benzodiazepines often have different doses for different indications. In such cases, the recommended anxiolytic starting dose was chosen.

This approach allowed the identification of tests showing a consistent response across studies and benzodiazepines and those with a clear response to a therapeutic dose of the anxiolytic (requirements 1 and 2 from the introduction). All measurements fulfilling these criteria were further tested for compliance with requirements 3 and 4: the existence of dose-response relationship and the plausibility of a mechanistic relationship, by reference to the original publications and the neuropharmacological literature. In this case, the original test-results were used if possible, rather than statistical significance and effect direction.

Neuropsychological/motor skill tests

In the first phase of the literature review, tests from different studies were only grouped if they were equal as judged from name and description or literature reference (*e.g.* all Digit Symbol Substitution Tests (DSST)), but all variants or related forms of the tests (DCCT, SDST *etc.*) were treated separately.

Next, all tests that could be regarded as variants from a basic form were clustered as indicated in Table 1. Thus, all tests determining the ability to discriminate flash- or flicker frequencies were grouped as 'flicker discrimination'. These data were used to determine the consistency of results within test clusters and to identify potential dose-effects.

Although many different methods are used to evaluate the functional effects of benzodiazepines, most actually measure a limited number of core features. Neuropsychological/ motor skills-tests can be categorised according to a catalogue of neurocognitive tests (attention, executive *etc.*),

ref. 2

TABLE 1 **Neuropsychological tests reported and clustered with similar tests and the affected domain**

Test	Cluster	Domain
arithmetic addition test	Intelligence	**Achievement**
differential reinforcement of low response rate		
divided visual attention	Divided Attention	
continuous attention		
DSST		
SDST	DSST-like	
critical flicker fusion		
tone discrimination		
two flash fusion	Flicker Discrimination	**Attention**
addition		
auditory discrimination task		
Binaural stimulation test		
number of minisleeps		
number vigilance		
vigilance		
visual vigilance	Other Vigilance	
card rotations		
card sorting		
logical reasoning		
mean RT signal identification		
repeated acquisition		
repeated acquisition (2nd order)		**Executive**
sequence completion		
signal identification		
subjektieve Leistungseinschätzung	Complex Information Processing	
Prepulse inhibition		
Stroop colour word test	Inhibition Task	
15 words test (delayed)		
auditory recall (delayed)		
cued recall test		
long term visual memory		
picture recall (delayed)		**Memory**
word recall (delayed)		
word stem completion	Delayed Recall	
15 words test (immediate)		
auditory recall (immediate)		
immediate visual memory		

Test	Cluster	Domain
number recognition		
picture recall		
picture recognition		
Randt memory test		
running word recognition		**Memory (continued)**
verbal memory		
Williams' word memory test		
word recall (immediate)		
word recognition	Immediate Recall	
memory scanning	Learning	
finger tapping	Manipulation	
anterior tibialis activation latency		**Motor**
body sway		
functional reach	Motor Control	
pursuit aiming		
pursuit rotor		
subcritical wheel tracking		
trace sine-wave		
tracking		
visual motion integration		
visual tracking task		
Wiener Gerät	Hand-Eye coordination	
AERP reaction time		
auditory reaction time		
choice reaction time		
complex choice reaction time		**Visual, visuomotor and auditory**
reaction time		
simple choice reaction time		
simple reaction time		
Sternberg memory test		
visual reaction time	Reaction time	
Bourdon cancellation		
letter cancellation		
rotated designs matching to sample		
symbol cancellation		
visual attention		
visual search	Search	

as presented in Table 1. This catalogue divides tests according to different neuropsychological domains, assuming that the results of each test are mainly (although not exclusively) determined by one of these domains.

Subjective assessments

For the subjective assessments, most individual scales corresponded to 'alertness', 'mood' and 'calmness'. These are similar to the scales proposed
ref. 68-69 by Norris and applied to CNS-drug evaluation by Bond and Lader. Other subjective scales could be grouped under 'craving', 'dizziness', 'drug effect', 'psychomimetic', 'sleep' and 'symptoms'.

Neurophysiological assessments

ELECTROENCEPHALOGRAPHY (EEG) EEG is sensitive to a wide range of centrally active substances, although the exact mechanism is hardly
ref. 10-14 ever known. EEG-studies differ in numbers of leads, technical settings and EEG-quantification methods, but they usually report effects per EEG-frequency band, which are divided into delta (0.5-3.5 Hz), theta (3.5-7.5 Hz), alpha (7.5-11.5 Hz) and beta (above 11.5 Hz; subdivided into beta 1 (11.5-30Hz) and beta 2 (above 30 Hz) if possible). Results describing the total EEG-spectrum were scored under the cluster EEG.

EYE MOVEMENTS Smooth pursuit and saccadic eye movements have
ref. 3-5 been frequently used to assess CNS-drug (side)-effects. Saccadic eye movements provide information on the sedative properties of benzodiazepines. Although there are different techniques to measure eye movements, most studies report peak velocity for visually guided saccades or sometimes anti-saccades (where subjects are instructed to look away from the target). No- and antisaccadic movements involve more complex cognitive processing than stimulus-evoked saccades and are considered as a separate cluster. Smooth pursuit eye movements are also treated separately. They are often reported as deviations from the time that the eyes closely followed the target. Eye blink is the cluster containing tests concerning spontaneous eye blinking. Dopaminergic pathways are thought to be involved in spontaneous
ref. 6-8 eye blinking. Startle eye blinks can be elicited by sudden noise bursts. They are part of the polysynaptic startle reflex and occur involuntarily as fast as 20 – 150 ms after stimulus onset. The tests clustered under 'startle reflex' were 'startle blink' and 'acoustic startle'.

Analysis of relationship with therapeutic efficacy and in vitro pharmacology

Biomarkers that complied with the first three mentioned criteria were subsequently evaluated for potential relationships between the biomarker and the therapeutic effects of the drugs. Establishing such relationships would require clear dose-response relationships for each drug, to determine potency measures for the biomarker and therapeutic effects. For the validation of the biomarker finding a close relationship between the potency of the drug to show an effect on this biomarker and the therapeutic doses would be extremely valuable. Establishing this relationship is only possible with well-defined potencies to affect the biomarker determined from dose-response relationships for each found benzodiazepine. For most benzodiazepines this relationship was not provided by the literature. As an alternative approach, a reference curve was constructed for each of the biomarkers, using quantitative results from the most frequently used benzodiazepine. Next, the potencies of other benzodiazepines were expressed relative to this reference agent, by plotting the observed effect of the benzodiazepine on the curve and determining the corresponding dose of the reference drug. Benzodiazepine dosages that caused a larger response than observed with the reference drug were not plotted on the reference curve; *i.e.* data were not extrapolated beyond the extent of the curve. In this way, for each benzodiazepine dose an equipotent reference drug dose was determined, that would theoretically cause a similar response. Subsequently, the mean of these values was calculated per benzodiazepine. Comparing these mean biomarker-affecting potencies to the lowest recommended daily therapeutic maintenance dose was the next step in examining the value of a biomarker for predicting the eventual therapeutic efficacy. Finally, the mean biomarker-affecting potencies were plotted against *in vitro* K_d affinities for the benzodiazepine binding site to evaluate the relationship between the biomarker and the *in vitro* pharmacology of the drugs. This investigation of a plausible relationship between the biomarker, the pharmacology of the drug class and the pathogenisis of the therapeutic area (the last defined requirement of a useful biomarker) was performed using data from studies that include effects of drugs from the benzodiazepine class irrespective of their registered indication.

ref. 9-10

Dose-response reference curve could only be constructed, if for a particular test (cluster) enough quantifiable data were available for a single benzodiazepine. Often, the number of studies with the potential reference drug was too low, or the presentation of results too variable. In these cases, doses

of different benzodiazepines were represented ('normalised') as fractions of the medium therapeutic dose. Similarly quantified test results were plotted against these 'normalised' doses, to identify relationships between the biomarker and the therapeutic (anxiolytic) benzodiazepine doses.

Results

ref. 14-67

The literature search yielded 56 different studies using 16 different benzodiazepines, published since 1966. There were 173 different tests used, on average 3.1 tests per study. On average 20 subjects participated in each study (range 4 to 145 subjects). On average 1.2 doses were given per study. All reported psychological tests and the relevant clusters and psychological domains are represented in Table 1. The benzodiazepines reported in the reviewed articles are listed in Table 2 with the therapeutic dose ranges for the various routes of administration. Fifty-eight tests that never showed any significant effect are listed in Table 3.

Neuropsychological/motor skill tests

There were 73 different test (-variants) as shown in Table 1. Seventeen of these were used only once and 55 tests were used less than five times in combination with a benzodiazepine dose. Sixteen tests never showed any significant effects at all. Tests that showed a consistent response across different benzodiazepines include the digit symbol substitution task (DSST), which was measured 33 times and showed significant impairment in 21 of these cases. Tracking showed impairment in 8 out of 9 cases and visual reaction times showed impairment in 3 out of 5 cases. Similarly, the choice reaction time showed impairment in 53% of the 15 observations. The critical flicker fusion was used 16 times and showed impairment in 6 cases but all these cases include high benzodiazepine dose. Both DSST and tracking showed significant responses at therapeutic doses. The only observation of effects on visual reaction time at a therapeutic dose was not significant. Choice reaction times results were similar at low, medium or high dose; impairment was observed in half the cases. The responsiveness of both DSST and tracking improved after discarding the low dose results.

Subsequently, comparable tests were clustered. The clusters 'complex information processing' (9 out of 21), 'DSST-like' (25 out of 38), 'flicker discrimination' (6 out of 20), 'hand-eye coordination' (17 out of 34),

'manipulation' (4 out of 11), 'other vigilance' (8 out of 17), and 'reaction time' (19 out of 34) showed consistent responses across studies. However, at therapeutic dose, only 'DSST-like' and 'hand-eye coordination' showed responses in half the cases or more. 'DSST-like' tests showed the clearest dose response-relationship (25% significant results for low dose, 67% for medium and 94% for high dose).

TABLE 2 **Benzodiazepines reported in the reviewed articles, therapeutic dose ranges, dissociation constants at benzodiazepine binding site and SPV dose equivalences (see text for explanation). i.m, intramuscular; i.v, intravenous; p.o., per os**

DrugName	Route	Lowest therapeutic dose (mg)	Highest therapeutic dose (mg)	Kd at benzodiazepine site (nM)	SPV dose equivalences (10 mg Temazepam)
Aldipem	PO	50	50		
Diazepam	PO	6	10	9.8 (11.2)	4.3
	IV	7.5	15		
	IM	7.5	15		
Camazepam	PO	10	10		
Adinazolam	PO	20	20		
Chlorazepate	PO	15	15		
Clobazam	PO	20	20		
Flutoprazepam	PO	2	2	(12.0)	
Lorazepam	PO	1	1	3.8 (2.6)	1.7
	IV	2	2		
Medazepam	PO	15	15	(2322)	
Oxazepam	PO	30	30	39 (37.4)	
Premazepam	PO	25	25		
Abecarnil	PO	10	10		
Alprazolam	PO	0.75	0.75	10.6 (13.8)	0.6
	IV	1	1		
Midazolam	PO	10	15	4.86	2.5
	IV	7.5	15		
Quazepam	PO	15	15	66	10.2
Temazepam	PO	10	20	58	10.8
	IV	10	20		
Bretazanil	PO	0.5	0.5		
Bromazepam	PO	4.5	4.5	(39.8)	5.5
	IV	4.5	6		
Flunitrazepam	IV	0.5	1	6.2	

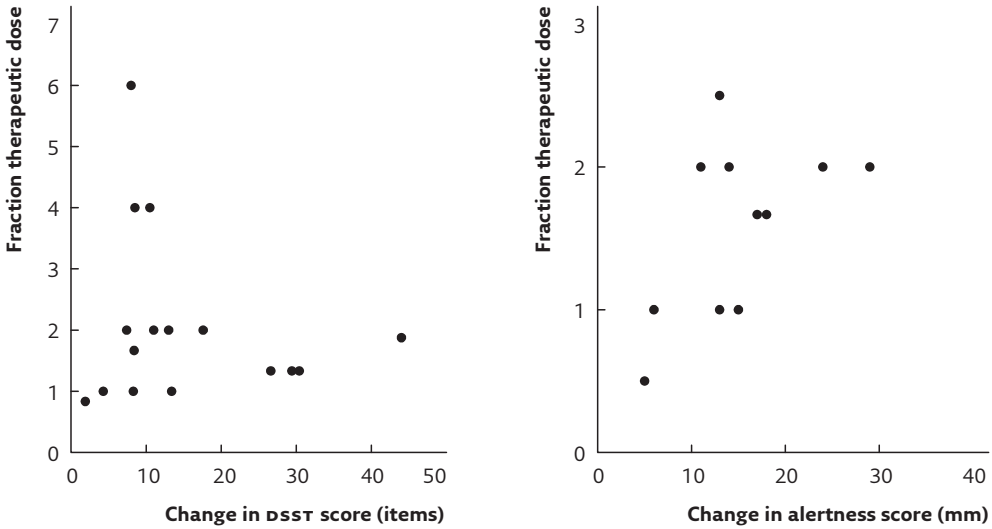
TABLE 3 **Tests or parameters that never showed any significant effect after administration of a benzodiazepine registered or investigated for anxiety**

Test	ref ID	Test	ref ID
Antisaccadic peak velocity	46	Mentally slow-quickwitted	30
Antisaccadic velocity	63	Most-least nauseated	29
Anxiety	25, 26, 27, 56	Normal-easily telded	60
Attentive-dreamy	30	Peaceful-tense	60
Basle mood scale	44	Performance	44
Blood pressure	25	Prepulse inhibition	27
Bodily symptom scale	33	Prolactine	32
Calm-anxious	60	Puff duration	26
Clearheaded-muzzy	30	Pulse rate	25
Clyde mood scale	24	Pursuit aiming	21
Compensatory effect	21	Repeated acquisition (2nd order)	26
Contendedness	27, 33, 34, 60, 64	Restless-calm	29
Cortical excitability	38	Self-rated concentration ability	65
Differential reinforcement of low response rate	26	Sequence completion	30
Divided visual attention	16	Serum gastrin levels	37
Drug liking	26	Sternberg memory test	42
Fatigue	21	Stroop colour word test	21
Functional reach	11	Subjective drug potency scale	26
Gastric acid secretion	37	Subjektieve Leistungseinschätzung	58
Happy-sad	29	Subjektieve Stimmung	58
High	26	Tension	39
Hopkins symptom checklist	41	Tone discrimination	16
HAVA	40	Two flash fusion	55
Incompetent-capable	30	VAS Mood Scale (no Bond & Lader)	41
Inter-puff-interval	26	Visual attention	16
Logical reasoning	56	Visual search	56
Long term visual memory	16	Well coordinated-clumsy	30
Maddox wing	67	Wiener Gerät	53, 44
Max force	25	Worst-best ever	29

Attempts were made to construct a reference dose-response curve for the 'DSST-like' cluster. There were too many different parameters to allow clear dose-response-relationships for any of the 11 benzodiazepines that were studied with 'DSST-like' tests. Some studies measured "number of correct substituted symbols over 90 seconds". Others measured "time needed to substitute 90 symbols" or "power of DSST (correct number

divided by time needed for correct substitutions)". The most commonly reported parameter ("score/90 seconds") was plotted against the fraction of therapeutic dose for all benzodiazepines in Figure 1. No clear relationship was observed between this 'normalised' therapeutic dose used and the result on the DSST.

FIGURE 1 **The effects on DSST (R^2=0.03) and subjective alertness (R^2=0.29) of benzodiazepine doses normalised to fraction of therapeutic dose**



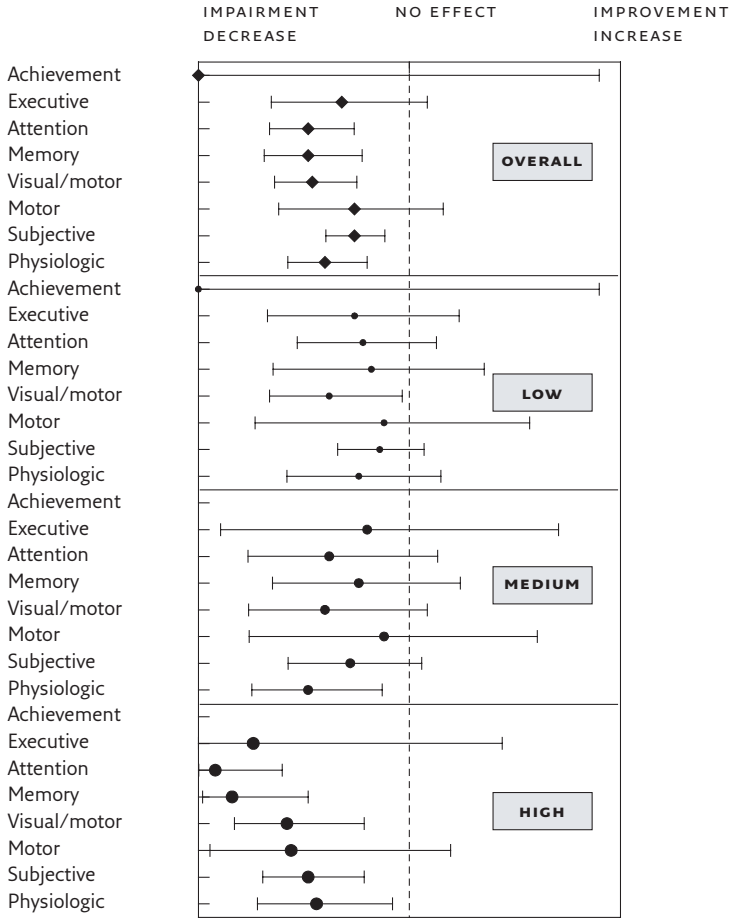
In order to investigate the overall effects of benzodiazepines on the major neurocognitive domains, the clusters were further condensed to domains. The results are displayed in Figure 2. The results for low, medium and high dose are represented in the same Figure. This differentiation showed that most domains are affected by high dose benzodiazepines.

Subjective assessments

Fifty-eight different subject assessments were used. Thirty tests never showed any significant effects. Most tests were used fewer than five times (48 assessments) and 15 tests were only used once. The most consistently responsive scale was 'alertness', which was significantly impaired in 11 out of 14 cases. Other responsive scales included 'sedation' both scored by the

subjects and by the investigator (11 out of 14 and 3 out of 5 times significant results respectively). However, the scale 'sedation' showed improvement in 3 cases and impairment in 8 cases. Similarly, the investigator-rated 'sedation' scale showed improvement once and impairment thrice.

FIGURE 2 **The averaged significant effects of benzodiazepines on neuropsychological domains, subjective assessment and neurophysiological parameters (see text for explanation). Averaged overall scores (◆) and effects after low dose (•), therapeutic (medium) dose (●) and above therapeutic (high) benzodiazepine dose (●)**



Clustering the results for subjective assessments showed that the scales 'alertness', 'mood' and 'calmness' as described by Norris and adapted by Bond and Lader were frequently employed. The most consistently responding scale was 'alertness', which showed 35 reductions and 4 improvements out of the 94 times it was used.

ref. 68
ref. 69

Alertness also complied with the second and third requirement. All observations at medium doses were significant reductions. A quantitative analysis was performed to assess the fourth requirement as shown in Figure 1. This analysis showed no relationship between a decrease in alertness and the different dosages ('normalised' for therapeutic dose) of benzodiazepines assessed by alertness. There were too few results to perform a more quantitative analysis of the test alertness.

Neurophysiological assessments

Sixty-two different neurophysiological parameters were identified. Twelve parameters never showed any significant effect and 22 parameters were used only once. Thirty-seven parameters were used less than five times.

ELECTROENCEPHALOGRAM (EEG) Inconsistent responses were observed for EEG Theta: 2 increases, 3 decreases and 1 non-significant result. EEG Delta was increased in 2 cases whereas remained unaffected in 3 cases. EEG alpha showed significant reductions in 5 out of 8 cases and EEG Beta was increased in all 5 instances.

EYE MOVEMENTS Eye movement tests were the most consistently responsive tests. Smooth pursuit eye movement recordings (measured 12 times) showed impairment in 50%. No-/antisaccadic eye movements were used 13 times and showed impairment in 54%. Saccadic latency showed impairment in 4 out of 9 observations. Saccadic eye movements showed impairment in 80% of all cases and were measured most frequently (31 times). The most frequently used parameter was saccadic peak velocity (11 times) and it showed significant impairment compared to placebo in all cases.

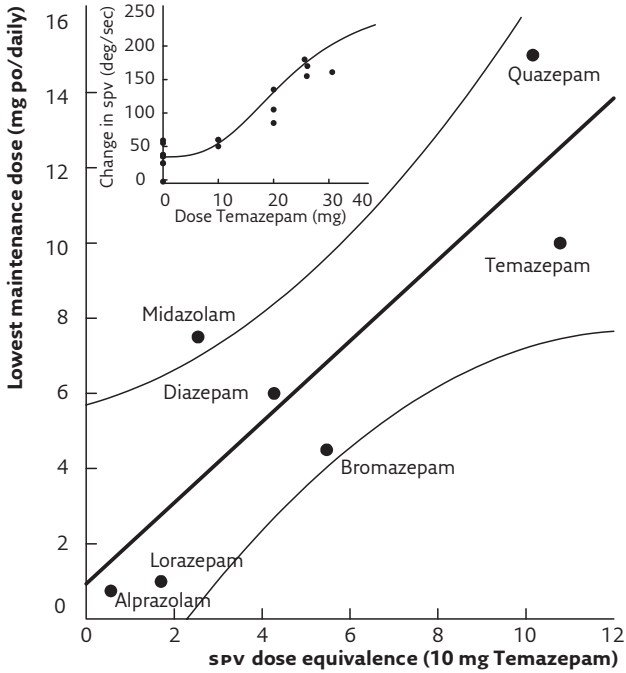
Saccadic peak velocity (SPV) also showed consistent effects at therapeutic doses. A reference dose response curve could be constructed for temazepam, since saccadic peak velocity was reported in all studies where saccadic eye movements were used with this drug. An E_{max} model with E_0 (placebo

response) was used to construct a reference curve using 9 placebo responses and 10 temazepam responses at various doses according to the following equation:

$$\Delta\text{SPV} = 34.3 + \frac{(243.7 * \text{Dose}^{2.9})}{\text{Dose}^{2.9} + 23.1^{2.9}}$$

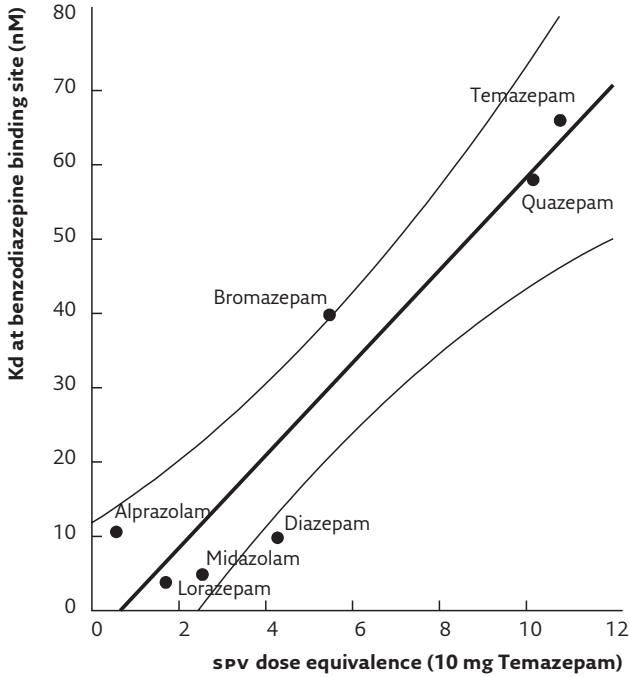
Subsequently, ΔSPV responses of all benzodiazepines were used to calculate the corresponding temazepam dose. These values were averaged for each benzodiazepine and plotted against the lowest recommended therapeutic maintenance dose as shown in Figure 3. A significant correlation was observed for seven benzodiazepines according to the equation ($R^2 = 0.737$, $p<0.05$): *Lowest maintenance dose = 0.94 + 1.08 * SPV dose equivalence*.

FIGURE 3 **SPV-decreasing dose equivalencies compared to lowest daily therapeutic maintenance dose for various benzodiazepines (see text for explanation). The 95% confidence interval (95% CI) of the linear regression is shown in thin lines. Insert: reference curve for temazepam dose (x-axis) and SPV-decrease relative to baseline (y-axis)**



Furthermore, the SPV dose equivalences of these seven benzodiazepines strongly correlated to the K_d at benzodiazepine binding sites (R^2 = 0.894, p<0.01) as shown in Figure 4: *K_d at benzodiazepine binding sites = -4.06 + 6.24 * SPV dose equivalence*.

FIGURE 4 **SPV-decreasing dose equivalencies compared to dissociation constants at benzodiazepine binding site for various benzodiazepines (see text for explanation). The 95% confidence interval (95% CI) of the linear regression is shown in thin lines**



EVOKED POTENTIALS Evoked potential tests were used 3 times and showed impairment in all. Evoked potentials were measured in two studies using two benzodiazepines. Auditory evoked response potentials (AERP) P300 was used once, as was AERP slow wave positivity. These results came from the same study. The auditory 40 Hz response amplitude was used also once in another study.

STARTLE REFLEX Startle reflex tests were used 4 times and in each case showed benzodiazepine-induced reductions. 'Startle blink' was used three times in one study with one benzodiazepine. 'Acoustic startle' was used once.

Discussion

The aim of this review was to evaluate the usefulness of methods used in healthy volunteer studies, to assess effects of anxiolytic benzodiazepines. A strikingly large number of different neurocognitive tests were identified (173). About a third of all tests used in combination with benzodiazepines (58) never showed any significant response to a benzodiazepine dose. Only very few methods were used often enough to allow individual evaluation. Consequently, tests had to be grouped, to observe trends for relationships between comparable tests and benzodiazepine effects. Several different meaningful ways to group tests were used in this review, although each method inevitably led to a loss of information. Even grouping tests with the same name and/or description could bypass differences among research groups or test variants. Some methods only used once or twice or by a single research groups may have had all the characteristics of ideal biomarkers, but this would have been missed in this review, simply because part of the definition of 'ideal' was general widespread use of the biomarker. Evoked potentials and startle responses for instance showed consistent results, but only in less than a handful of studies from even fewer research groups. At this stage, it is difficult to evaluate the usefulness of these techniques in drug development, and more studies are needed to allow definite judgements. Also, useful methods were defined in this review as tests that produced a statistically significant result in typical healthy volunteer studies, *i.e.* with small subject numbers. Some tests may be very useful biomarkers in larger studies, but these would not be identified in this review.

As expected, increasing doses caused more significant results for many tests. The sedative properties of benzodiazepines at high doses caused some impairment in most of the neurocognitive domains, probably secondary to reduced alertness. However, a useful biomarker should show responses at therapeutic levels (preferably also at low dose to allow dose-response relationships). This precludes 'critical flicker' discrimination tests, which despite widespread use only seems to respond to high dose levels of benzodiazepines. The more useful biomarkers identified in this review (saccadic eye movements, 'DSST-like' tests and subjective scores of alertness) seem to all be related to the sedative properties of benzodiazepines which apparently correlate with the therapeutic effects of the selected benzodiazepines. Effects of other sedating compounds have been demonstrated with saccadic eye movements, suggesting that saccadic eye movements quantitatively reflect alertness.

ref. 70

All benzodiazepines caused an impairment of saccadic peak velocity, which was closely related to the therapeutic dose. There are several possible explanations for this close relationship. Firstly, it could reflect the clinical practice of aiming for maximum tolerated levels. Secondly, the anxiolytic effects of benzodiazepines could be linked to sedation 'in parallel', if both are regulated by closely related neurobiological systems (*e.g.* different GABA-receptor subtypes or different components of the ascending reticular activating system; the latter probably connects saccadic eye movements to alertness/sedation). Thirdly and perhaps less plausibly, the link could be 'in series', if reduced alertness would be the basis for reduced anxiety (*e.g.* by reduced susceptibility to (disturbing) exogenous and endogenous stimuli). Research on partial agonist benzodiazepines that potentially discriminate between sedation and anxiolysis should include saccadic peak velocity as the most sensitive measure of sedation. Similarly, the effects of non-benzodiazepine anxiolytic agents could show a different effect profile. A review of the effects of such variable compounds (similar to the current benzodiazepine review) would be difficult, because the diverse effect profiles would hamper any relationship between biomarkers and pharmacology of the drugs. Also, most of these drugs are registered for multiple indications (*e.g.* depression).

CNS drug development is likely to increase as the attention of the pharmaceutical industry shifts further in the direction of this area with the largest unmet therapeutic need. Additionally the improvements in biological knowledge through genomics will undoubtedly produce new targets that require further validation. Early evaluation of these new drugs must be done with the best possible methodology and it is highly surprising that the field apparently uncritically uses untested and often insensitive methodology. Healthy volunteers should not be exposed to procedures that can a priori be assumed not to produce any useful data. In addition, the cost of these studies is high especially when no or possibly confusing data arise from this. A large number of the methods included in this review are actually used for studies that eventually appear in dossiers for registration and the uncritical approach to this methodology seems to extend to the registration authorities in many countries.

ref. 71

Similar to the conclusion of a review on the effects of antipsychotic drugs in healthy volunteers, this review confirms that the number of tests used in human psychopharmacology appears to be excessive and reduction of the number of tests as well as further evaluation and validation is long overdue.

ref. 1

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CHAPTER 4

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REM sleep effects as biomarker for the effects of antidepressants in healthy volunteers

Abstract

This review investigated the potential use of Rapid Eye Movement (REM) sleep effects as a biomarker for the therapeutic effects of antidepressants in healthy volunteers. A literature search was performed to select studies investigating the effects of antidepressants on REM sleep. To assess the specificity of REM sleep effects as a biomarker, the effects of other CNS drugs on REM sleep were also investigated. A significant REM sleep reduction was shown for 16/21 investigated antidepressants after single dose (mean reduction 34.1%) and for 11/13 drugs after multiple dose administration (mean reduction 29.2%). The median increase in REM latency was about 60% after single- or multiple-dose administration. REM sleep effects were linearly normalised to therapeutic doses, by dividing the REM sleep effect by the investigated dose and multiplying by the therapeutic dose. Normalised REM sleep effects were highly variable (range −27.0 to 81.8% for REM sleep and range −17.0 to 266.3% for REM latency) and showed no relationship with relevant pharmacological properties of the investigated drugs. No quantifiable dose-response relationship could be constructed after single and multiple dose administration. REM sleep effects were not specific for antidepressants. Benzodiazepines for instance caused an average dose normalised REM sleep reduction of 8.7% and a median 8.6% increase of REM latency. This review demonstrates that although REM sleep effects occur with most of the antidepressants, it is by itself of limited value as a biomarker for antidepressant action. Its specificity for antidepressants is limited, and it does not show a quantitative dose-response relationship to antidepressant agents. This is at least partly due to the complex relationships between drug pharmacokinetics and the variable time course of REM and other sleep stages throughout the night. Models that take these complex relationships into account may provide more comprehensive and quantifiable results.

Introduction

Nearly 10% of the population suffers from a depressive disorder making it one of the most common diseases of the world. Pharmacological treatments of depressive disorders include three major classes of antidepressants: selective serotonin reuptake inhibitors, tricyclic antidepressants and monoamine oxidase inhibitors. The exact therapeutic mechanism of action of antidepressants is still not known, although the binding to specific receptors has been elucidated and most antidepressants enhance monoaminergic neurotransmission. Although antidepressant activity can be fairly reliably

ref. 1-2

predicted from these specific pharmacological characteristics and from preclinical animal models, it remains difficult to establish efficacy in clinical practice, mainly because of the heterogeneity of the patient population, fluctuations in disease severity, and a delayed onset of antidepressant action. Consequently, it is difficult to establish therapeutic doses, and compounds can still fail in later phases of development. Therefore, efforts have been made to identify biomarkers for the therapeutic effects of new antidepressants as early as in phase I.

Some investigations have suggested that the therapeutic effects of antidepressants are caused by suppression of Rapid Eye Movement (REM) sleep. In 1975, it was suggested that antidepressants do not have a consistent effect on sleep in healthy volunteers. Since then, many more studies have been published on the possible effects of new antidepressants on sleep in healthy volunteers. The aim of this review is to investigate the relationship between different antidepressants and the effect on REM sleep in healthy volunteers and to examine the potential use of REM sleep effects as a biomarker for depression in healthy volunteers. To identify the specificity of REM sleep effects as typical biomarker for the effects of antidepressants, the effect of other Central Nervous System (CNS) drugs on REM sleep was also investigated.

ref. 3

Methods

A literature review was performed by a PubMed search (keywords: antidepressive agents, REM and healthy human) for all antidepressants. From this search, studies determining the effects on REM sleep of one or more antidepressant dose (single or multiple dose) in healthy subjects were selected. The results of all selected studies were stored in a database. To allow a quantitative comparison of study results, the reported average REM sleep effects were expressed as a percentage of the difference between REM sleep effects during treatment and REM sleep effects at baseline. Assuming linear dose-effect relationships, REM sleep effects were normalised to therapeutic dose by dividing the REM sleep effects of each antidepressant by the used dose, and multiplying the result with the therapeutic daily starting dose (for single dose results) and lowest therapeutic maintenance dose (for the multiple dose results). To determine whether relevant drug concentrations were reached during the night, the time of drug administration was compared with the expected T_{max}, for all single-dose studies where this information was available. The effects after single and multiple dose administration were compared to investigate potential differences in REM sleep effects during prolonged treatment.

Single dose

For each individual antidepressant, the REM sleep effects after a single dose normalised to hypothetical responses at therapeutic doses were averaged (assuming linear dose-effect relationship), to investigate the average response at therapeutic doses. Next, the relationships between REM sleep effects and specific receptor affinities were investigated. Affinity was expressed as the antidepressants' equilibrium dissociation constant (K_d), and was obtained from the same source for all antidepressants for serotonin, noradrenaline, dopamine and muscarinic receptors. The normalised REM effects were plotted against the affinity constants of the various receptors and the specificity ratio of serotonin over noradrenaline. Finally, changes in REM sleep effects of antidepressants that are typically referred to as selective were compared to non-selective ('classic') agents.

ref. 1-2

Multiple dose

REM sleep effects and normalised REM sleep effects were plotted for each antidepressant individually against the number of days of administration. Normalised REM sleep effects of studies with constant repeated doses and the same number of administration days were reviewed to predict response to antidepressant therapy. Normalised REM sleep effects of different antidepressants with the same amount of days of administration were averaged and compared with each other. Finally, the same receptor affinity relationships that were investigated for single dose administration were examined.

Other CNS drugs

To assess the specificity of REM sleep effects as a potential biomarker for particularly the effects of antidepressants, a PubMed search was performed (keywords: CNS drug, REM, healthy human) to determine the effects of other CNS drugs on REM sleep.

Results

The literature search yielded 41 articles published since 1977. Twenty-four articles could be used, because some collected articles were reviews, investigated REM sleep effects in only depressive patients or effects of other drugs.

ref. 4-27
ref. 3, 28-43

Sleep ElectroEncephaloGraphy (EEG) recordings to determine REM sleep were sometimes performed ambulantly but most frequently in a sleep laboratory. Standard sleep polygraphy was typically performed for 8 hours and daytime napping was prohibited. Most of the studies had on average two adaptation nights before the EEG recording night(s). The sleep EEG recordings were typically visually scored according to the criteria of Rechtschaffen and Kales. On average, antidepressants caused 34.1% REM sleep reduction after single dose administration and 29.2% after multiple doses in healthy volunteers. Other CNS drugs caused on average 12.2% REM sleep reduction.

ref. 9, 11, 18, 20, 22-23, 26, 30

ref. 44

Single dose

Twenty articles, investigating 21 different antidepressants and 45 different doses of an antidepressant, were reviewed for the effects on REM sleep after single dose administration. In the cases where this information was available (17 of 32), times of drug administration seemed to be based on the predicted T_{max}-values. Paroxetine was always administered in the morning before the sleep recording, which is in agreement with the long duration of action of this compound. Only fluoxetine was given relatively late ('at lights out') considering the T_{max} of 4-8 hr and the accumulation of an active metabolite. In one imipramine study, a low dose was given at 21:00 hr, leading to an early peak concentration before midnight. In all other cases, the expected T_{max}-ranges lay between 23:00 and 03:00 hr, covering most of the normal REM sleep period.

ref. 5, 19

ref. 27

REM sleep effects of all reported antidepressant doses are presented in Table 1. Sixteen antidepressants showed significant, consistent responses on REM sleep reduction. Inconsistent or non-significant results were reported for five antidepressant agents. Nefazodone never showed a significant reduction but rather an increase in one case and no effect in the other, consistent with previous findings in patients. REM sleep reductions that were statistically significant varied widely, from 1.8% for 100 mg trimipramine to 81.8% for 75 mg oxaprotiline. The antidepressant doses used ranged from 50% to 600% of the therapeutic dose. Consistent significant increases in REM latency were exhibited by fifteen of twenty antidepressants, although the magnitudes of the responses varied widely. The median increase of dose-normalised REM-latency for all agents was 60.1% (range –15.1% to 266%).

ref. 42-43, 45

Twelve antidepressant therapeutic doses were administered. Eight dosages showed reduction, three showed no significant change and one dose

resulted in a significant increase of the REM sleep. This includes the mentioned two doses nefazodone (increase in one case and no effect in the other). Trimipramine 25 mg and fluoxetine 20 mg did not produce significant effects on REM sleep at therapeutic doses either. After normalising to hypothetical effects at therapeutic dose the normalised effects varied between −27.0% for trimipramine and 81.8% for oxaprotiline.

TABLE 1 **Single dose antidepressants and observed REM sleep effects in healthy volunteers**

Antidepressant (dose)	Reference	Δ REM sleep (min)[a]	Δ REM sleep (%)[b]	Δ REM sleep normalised (%)[c]	Δ REM latency normalised (%)[c]
Amitriptyline (25mg)	(Nakazawa et al., 1977)	37	32.3	64.6	-
Amitriptyline (75mg)	(Riemann et al., 1990)	60.9	67.2	44.8	122.7
Clomipramine (50mg)	(Maeda et al., 1990)	70.3	80.7	80.7	266.3
Dexnafenodone (20mg)	(Jobert et al., 1999)	55.5	64.3	-	-
Dothiepin (100mg)	(Wilson et al., 2000)	16.0 n.s.	19.3 n.s.	- n.s.	-
Fluoxetine (20mg)	(Nicholson et al., 1988)	9.3 n.s.	9.0 n.s.	9 n.s.	-17.0 n.s.
Fluoxetine (40mg)	(Nicholson et al., 1988)	3.2 n.s.	3.1 n.s.	1.5 n.s.	-1.8 n.s.
Fluoxetine (60mg)	(Nicholson et al., 1988)	45.9	44.4	14.8	8.4 n.s.
Fluoxetine (60mg)	(Nicholson et al., 1989)	36.4	31.4	10.5	-0.4 n.s
Fluvoxamine (100mg)	(Wilson et al., 2000)	36	43.4	43.4	53.8
Imipramine (40mg)	(Yamadera et al., 1998)	49.1	43.8	27.4	53.1
Imipramine (75mg)	(Jobert et al., 1999)	54	62.6	20.9	115.3
Indalpine (25mg)	(Nicholson et al., 1986a)	55.5	48	-	-
Indalpine (50mg)	(Nicholson et al., 1986a)	85.3	73.7	-	-
Indeloxazine (40mg)	(Kajimura et al., 1991)	43.2	35.5	-	-
Lofepramine (140mg)	(Hopes, 1989)	50.6	56.1	40.1	85.3
Lofepramine (140mg)	(Herdman et al., 1993)	43.3	49.5	35.4	48.5
Maprotiline (75mg)	(Nicholson et al., 1986a)	38.1	32	10.7	-0.7 n.s.
Maprotiline (150mg)	(Nicholson et al., 1986a)	54.6	45.9	7.7	4.3 n.s.
Mianserin (20mg)	(Maeda et al., 1990)	17.6	16.7	25	40.1
Mianserin (20mg)	(Nicholson et al., 1986b)	33.4	29.9	44.9	54.1 n.s
Mianserin (40mg)	(Nicholson et al., 1986b)	45.8	41	30.8	105.1
Mirtazapine (30mg)	(Ruigt et al., 1990)	-21 n.s.	-13.7 n.s.	-6.9 n.s.	18.2
Nefazodone (200mg)	(Sharpley et al., 1996)	14.0 n.s.	13.5 n.s.	13.5 n.s.	-13.3 n.s.
Nefazodone (200mg)	(Ware et al., 1994)	-24.4	-27	-27	-16.9 n.s.
Nomifensine (100mg)	(Nicholson et al., 1986a)	55	49.3	24.7	34.2 n.s.
Nomifensine (100mg)	(Nicholson et al., 1986a)	48.1	40.5	20.2	47.2
Nomifensine (100mg)	(Nicholson et al., 1986a)	44.9	38.8	19.4	-11.3 n.s.

Nomifensine (100mg)	(Nicholson et al., 1988)	38.9	37.7	18.8	1.7 n.s.
Nomifensine (50mg)	(Nicholson et al., 1986b)	25.3	22.6	22.6	34.8 n.s.
Nomifensine (100mg)	(Nicholson et al., 1986b)	31.9	28.6	14.3	18.7 n.s.
Oxaprotiline (75mg)	(Gnirss, 1986)	76.5	81.8	81.8	125.8
Paroxetine (20mg)	(Sharpley et al., 1996)	39.0	37.5	37.5	140.0
Paroxetine (20mg)	(Saletu et al., 1991)	26.6	34.1	34.1	102.8
Paroxetine (30mg)	(Saletu et al., 1991)	34.6	44.3	29.5	105.5
Paroxetine (40mg)	(Saletu et al., 1991)	42.9	54.9	27.5	93.2
Trazodone (100mg)	(Ware et al., 1994)	12.33	13.6	20.5	86.7
Trazodone (100mg)	(Yamadera et al., 1998)	-10	-8.9	-13.4	14.6
Trimipramine (25mg)	(Nicholson et al., 1989)	13.6 n.s.	11.7 n.s.	11.7 n.s.	-0.4 n.s.
Trimipramine (50mg)	(Nicholson et al., 1989)	28	24.1	12.1	13.9 n.s.
Trimipramine (75mg)	(Nicholson et al., 1989)	25.1	21.6	7.2	11.3 n.s.
Trimipramine (100mg)	(Feuillade et al., 1992)	1.6	1.8	0.5	15.1
Venlafaxine (75mg)	(Salin-Pascual et al., 1997)	99.9	79.7	79.7	-
Zimelidine (100mg)	(Nicholson et al., 1986a)	43.2	38.7	77.5	106.7 n.s.
Zimelidine (200mg)	(Nicholson et al., 1986a)	83.2	74.6	74.6	118.5

a REM sleep (min) after drug dose - REM sleep (min) placebo or baseline
b (ΔREM sleep (min) / REM sleep placebo or baseline (min)) x 100 %
c % ΔREM effects normalised to the therapeutic starting daily dose if possible
- unknown therapeutic starting daily dose
n.s. not significant

ref. 4-6, 8, 11, 13-14, 18-21, 27

For ten antidepressants, two or more dose levels were investigated. As doses increased, nine antidepressants showed greater reductions in REM sleep, with the exception of trimipramine. Fluoxetine did not produce significant results at doses of 20 mg (the therapeutic dose) and 40 mg but showed significant reductions at 60 mg (300% of therapeutic dose). It was impossible to quantitatively determine dose-response relationships because of the lack of sufficient data. No clear linear dose-response relationship was observed for most antidepressants.

Table 2 shows the relationships with receptor affinities for each antidepressant. The antidepressants are presented in order of decreasing acute effect on REM sleep. The REM sleep effects plotted against the serotonin, noradrenaline and dopamine transporter affinities and muscarinic receptor affinities did not show any consistent relationship with the REM sleep effects at therapeutic dose. 5-HT/NA selectivity ratios were used to examine relationships with serotonergic selectivity, rather than absolute affinity. Zimelidine and clomipramine have higher 5-HT/NA selectivity values than many other drugs and cause the most significant REM sleep reductions.

TABLE 2 **Antidepressants (in descending order of normalised REM sleep reduction), equilibrium dissociation constants (Kd's) for human serotonin (5-HT), noradrenaline (NA) and dopamine transporters and the 5-HT/NA selectivity value (from Kd values)***

Antidepressants	Serotonin Kd (nM)	Noradrenaline Kd (nM)	Dopamine Kd (nM)	Muscarinic Kd (nM)	5-HT/NA
Oxaprotiline	3900	4.9	4340	2900	0.0012
Clomipramine	0.28	38	2190	37	130
Venlafaxine	8.9	1060	9300	-	120
Zimelidine	152	9400	11700	13000	62
Amitriptyline	4.3	35	3250	18	8.1
Fluvoxamine	2.2	1300	9200	24000	580
Lofepramine	70	5.4	18000	-	0.077
Mianserin	4000	71	9400	820	0.018
Paroxetine	0.13	40	490	-	300
Imipramine	1.4	37	8500	90	27
Nomifensine	1010	15.6	56	250000	0.015
Maprotiline	5800	11.1	1000	570	0.0019
Fluoxetine	0.81	240	3600	2000	300
Trimipramine	149	2450	3780	58	16
Trazodone	160	8500	7400	324000	53
Mirtazapine	>100000	4600	>100000	-	-
Nefazodone	200	360	360	-	1.8

- unknown value

ref. 1-2 * Tatsumi et al., 1997 and Richelson et al., 1984

However, fluoxetine, paroxetine and fluvoxamine also have high 5-HT/NA selectivity values, but did not cause as large REM sleep reductions as zimelidine and clomipramine. The 'selective' antidepressants caused REM sleep reductions ranging from 12-34%. The non-selective antidepressants amitriptyline and dothiepin showed high affinities for all investigated receptors, but they did not cause consistent REM sleep reductions, ranging from no significant effects to nearly maximum REM sleep reduction. Thus, no associations could be found between the REM sleep effects caused by antidepressants and any pattern of receptor binding affinity.

Multiple dose

Antidepressant effects typically develop over time, so studies using prolonged treatment were evaluated separately. Twelve articles were

ref. 5-7, 9-10, 13-16, 23-25

included, investigating the REM sleep effects of 13 different antidepressants and 28 evaluations after multiple dose administration. Sometimes, doses were increased gradually. The final doses ranged from 50% to 210% of the therapeutic dose, and duration of treatment ranged from 2 to 25 days. A consistent significant REM sleep reduction was observed for most drugs (11 of the 13 investigated antidepressants showed only significant results). Nomifensin 3 de die 25 mg (3dd25 mg, 150% of the therapeutic dose) for

ref. 15

5 days and nefazodone 2dd100 mg (67% of the therapeutic dose) for 4 days, 2dd200 mg (134% of the therapeutic dose) for 16 days and 1dd200mg for

ref. 5, 7, 23

eight days and 1dd400 mg for 16 days demonstrated non-significant REM sleep effects. A significant REM sleep induction was observed after nefazodone 1dd400 mg (134% of the therapeutic dose) on day two of

ref. 23

treatment. The significant REM sleep reductions per drug varied from respectively 17.2% for indeloxazine 1dd40 mg (unknown therapeutic dose)

ref. 16

for 3 days and 20.9% for paroxetine 1dd30 mg (150% of the therapeutic dose)

ref. 10

for 25 days, to 100% for venlafaxine 1dd150 mg (200% of the therapeutic

ref. 24

dose) for 4 days. Similar results were found for REM-latency, although this was not reported in all articles. After multiple dose treatment, consistent significant increases in REM-latency were exhibited by eight of eleven antidepressants. The median increase of dose-normalised REM-latency for all agents was 62.1% (range –10.1 to 236%).

Normalisation to the hypothetical effect at therapeutic dose did not reduce the variability in significant response. The significant REM sleep reductions normalised to therapeutic dose varied from 13.9% for paroxetine 1dd30 mg

ref. 10

(150% of the therapeutic dose) for 25 days to 91.1% for venlafaxine 1dd75 mg

ref. 24

(at the therapeutic dose) for 2 days after multiple dose administration. Four antidepressant doses were at therapeutic dose and showed responses varying from 39.6% for fluoxetine (1dd20 mg for 6 days) to 91.1 for venlafaxine (1dd75 mg for 2 days).

For the relationship between antidepressants and REM sleep effects, each study was investigated individually, due to the wide variability in the design of the different studies. Most drugs (amitriptyline, clomipramine, imipramine,

ref. 6, 14, 13

indeloxazine, lofepramine, paroxetine and dexnafenodone) caused a REM

ref.16, 9, 5, 13

sleep reduction which diminished over time. REM-reductions did not

ref.14, 23, 24

diminish with mianserin, trazodone or venlafaxine. For most antidepressants,

ref.23

REM-latency-effects also diminished over time, except for trazodone and

ref.13

dexnafenadone. The averaged REM sleep and the averaged normalised REM sleep effects of the antidepressants with the same number of treatment days demonstrated no consistent relationship with the number of treatment days.

A dose-response relationship with a REM sleep response could not be evaluated without the confounding factor of time.

Relationships with transporter affinities of each antidepressant were only investigated for antidepressants with the same number of administration days and a constant repeated dose. Only four studies produced comparable results, which showed no relationship with any of the affinities.

Other CNS drugs

To obtain an impression of the specificity of REM sleep effects for (monoaminergic) antidepressant, a literature search was also performed for other CNS-active drugs. Most of these articles were on benzodiazepines (15 studies investigating 13 different benzodiazepines). The number of CNS-active drugs from other classes was too limited to get a comprehensive overview of class-effects on REM sleep. The results for 2 nonbenzodiazepine hypnotics, 2 antipsychotics and 1 antihistamine are presented for reference. The 19 articles found, together with results of a review that investigated the effects of drugs on sleep in healthy volunteers and patients published in 1996 provided the following results:

ref. 46-60

ref. 54-55, 61-64

ref. 65

BENZODIAZEPINES With the exception of 3 benzodiazepines (fosazepam, nitrazepam and doxefazepam) sleep effects of all drugs were determined after single dose administration. Fosazepam and nitrazapam were administered on two consecutive days and doxefazepam was administered for 30 days. Six (brotizolam, flurazepam, fosazepam, lorazepam, midazolam and nitrazepam) of the 13 investigated benzodiazepines caused consistent REM sleep reductions. Brotizolam and midazolam were administered at therapeutic dose and showed 24% and 26% REM sleep reduction, respectively. Flunitrazepam (at 200, 400 and 800% of the therapeutic dose), oxazepam (50 and 83% of the therapeutic dose) and triazolam (200 and 400% of the therapeutic dose) showed both non-significant and significant REM sleep reductions in different studies. Clorazepate (at a therapeutic dose), doxefazepam (unknown therapeutic dose), nordiazepam (33 and 67% of the therapeutic dose) and temazepam (150% of the therapeutic dose), showed no significant REM sleep effects. Together with the findings of the review published earlier, it seems that about half the studies with benzodiazepines caused a REM sleep reduction (normalised to the therapeutic dose) of 8.7%, on average (range −4.5-35.0%). The median increase of dose-normalised REM-latency for all benzodiazepines was 8.6% (range −1.6% to 39.1%).

ref. 48, 48, 47

ref. 55, 51, 58, 60, 48, 50, 57, 48-56

ref. 46, 51-52, 58

ref. 49, 57

ref. 58-59

ref. 60

ref. 47

ref. 53-54

ref. 65

OTHER CNS DRUGS The antihistaminic agent promethazine at doses of 50mg, 100 mg and 200 mg (above the therapeutic dose of 25 mg) showed REM sleep reductions of 21%, 35% and 45%, respectively. The non-benzodiazepine hypnotic zopiclone (at therapeutic dose) also showed a REM sleep reduction of 31%, but results for its congener zolpidem were less clear: at therapeutic doses, REM sleep reductions were significant (10%) in one study, but non-significant effects in another. The antipsychotic agent olanzapine showed non-significant REM sleep effects below therapeutic dose and a 39% REM sleep reduction at therapeutic dose. The antipsychotic pimozide (at 400% of the therapeutic dose) demonstrated no significant effects on REM sleep.

ref. 61
ref. 55
ref. 64, 54
ref. 63
ref. 62

Discussion

This review aimed to systematically evaluate the use of REM sleep effects as a potential biomarker for therapeutic effects of antidepressant agents in healthy volunteers. A systematic stepwise approach to literature evaluation was adopted. Firstly, the usefulness of REM sleep effects as a biomarker was assessed by investigating the consistency of responses across various antidepressants. Secondly, the responses at therapeutic levels were determined. The effects on REM sleep of other different CNS drugs also were investigated, to get an impression of the specificity of REM sleep effects for antidepressants. Next, possible dose-response relationships were evaluated. Finally, attempts were made to relate the responses to the pharmacology of the drugs. This approach showed several links between the depression, REM sleep and monoaminergic antidepressants.

Significant reductions of REM sleep were observed with most of the investigated antidepressants, both after single (on average 34.1% normalised REM sleep reduction) and multiple (on average 29.2% REM sleep reduction) dose administration. The median increase in REM latency was about 60% after single- or multiple-dose administration. Statistically significant effects of single therapeutic doses were found in about three-quarters of all antidepressants for REM sleep and in two-thirds for REM latency. Responses generally increased with rising doses, but REM effects were too variable to identify a meaningful dose-response relationship. Statistically significant REM sleep reductions were also found for other CNS-active agents (at similar rates for benzodiazepines), but the effects were generally less consistent and smaller (on average 8.7% reduction of normalised REM sleep and median 8.6% increase of REM latency for benzodiazepines).

There are several clues for relationships between REM sleep and the pathophysiology of depression. Sleep disturbances of various types are among the most frequent symptoms of major depressive disorders. Aside from sleep continuity disturbances, sleep in depressed patients is characterised by a reduction of slow wave sleep (SWS), a reduction of REM latency, an increased amount of REM sleep, a prolongation of the first REM period and an increased number of eye movements during REM periods. Sleep deprivation causes short-term improvement of depressive symptoms. REM sleep is regulated by a complex mechanism in the brain, which is still not completely understood but seems to involve monoaminergic activity. It is suggested that laterodorsal tegmental (LDT) and pedunculopontine (PPT) neuron activity is high and both serotonergic and noradrenergic cells have their lowest discharge rates during REM sleep. Serotonergic/noradrenergic activity is believed to suppress LDT and PPT activity and thereby reduce REM sleep. REM sleep is probably also generated, in part, by stimulation of the muscarinic cholinergic receptors in the medial pontine reticular formation. Serotonin and noradrenaline have been shown to inhibit brainstem cholinergic neurons.

ref. 66
ref. 36
ref. 67
ref. 68

Despite these biochemical links between depression, REM sleep and monoaminergic antidepressants, none of the investigated drugs evidenced relationships between their pharmacological characteristics and their REM sleep effects. Also, REM sleep effects showed a considerable overlap among different CNS-active drug classes, although the effects were generally more consistent and larger with monoaminergic antidepressants. Finally, in spite of a general increase of REM sleep reduction with rising doses, REM effects were too variable to show a meaningful quantitative dose-response relationship. These limitations thwart the practical applicability of REM sleep reduction as a biomarker during development of monoaminergic antidepressants. Nonetheless, the associations with REM sleep reduction seem stronger for antidepressants than for other CNS-active drugs. There may be many reasons why even a strong relationship would not become apparent in a literature review. One important factor is common to all the explored levels of potential relationships: the method used to quantify REM sleep reduction. Most of the reviewed articles determined REM sleep as absolute periods of time, or the percentage REM sleep within the total sleep EEG. The individual results were recalculated as percentage reduction, to allow quantitative comparisons in the current review. The hypnographic sleep recordings are typically scored by determining for each consecutive 30 second epoch, whether the subjects are awake, in REM sleep, or in non-REM sleep stages 1, 2, 3, or 4. In healthy young adults, these stages show a regular

pattern. After 80-90 minutes of non-REM sleep, the subject goes into REM sleep, which is followed by alternating cycles of non-REM and REM sleep with a period of about 100 minutes. In most hypnographic analyses, sleep stage scores are aggregated over the night, resulting in the various parameters including REM sleep duration. This makes it impossible to examine the time course of the effects on sleep and the relationships with the pharmacokinetics of the investigational drug. Most studies based their time of administration on the expected T_{max} of the antidepressant agent, apparently aiming for pharmacologically active concentrations when REM sleep is most abundant. However, the individual variabilities in T_{max}-values and REM sleep patterns, and the constant transitions of the various sleep stages require more complex concentration-effect analyses, than are possible with aggregated REM sleep effects. For temazepam, the overall sleep effects did not show any significant pharmacokinetic/pharmacodynamic (PK/PD) relationships. Useful concentration-effect relationships were only found, after analyses with a first-order Markov mixed effect model, using the individual time-arrays of individual hypnographic 30-second epochs and each subject's pharmacokinetic estimates. This analysis yielded descriptions of the probability of changes in sleep stages as a function of time, and quantified the influence of drug concentrations on these probabilities. Similar methods are probably needed, to show dose- (concentration)-REM sleep effect relationship, and potential correlations with the pharmacological characteristics of antidepressants. Without such complicated analyses, the practical usefulness of REM sleep reduction as a predictive biomarker for antidepressant action is limited.

ref. 69

ref. 70

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The value of research on biomarkers

Estimating the hypothetical additional value of reviews on biomarkers assumes that useful knowledge about biomarkers for the effects of CNS drugs in healthy volunteers allows better selection of the appropriate marker for answering a relevant question. In the traditional drug development plan, this would lead to a higher probability of success to enter phase II (or a lower probability the drug will be abandoned after phase I). To investigate the impact of the phase I to phase II transition probability, a general drug development plan is constructed using historical data. This development plan has a limited amount of options during the execution of the plan: drugs can 'fail' or 'succeed' in phase I, phase II and phase III. Success in phase III means payoff of the estimated market value.

The marketvalue is arbitrarily chosen as M€ 400 making the overall project value positive (otherwise, the drug will not be developed at all). Failure in any of the phases obviously implies no payoff and a negative outcome (equal to the cumulative costs made in the phases until failure). An historical probability is assigned to each of the phase transitions. The defined costs throughout the three clinical phases are also based on historical data [1-2]. The input parameters for this general drug development program are listed in table 1.

TABLE 1 **Hypothetical input parameters of the classic development plan**

Parameter	Value
Success probability phase I	70%
Success probability phase II	50%
Success probability phase III	85%
Costs phase I	M€ 15
Costs phase II	M€ 60
Costs phase III	M€ 100
Market value	M€ 400

The project value at the start of the development of this hypothetical plan would have to take all the probabilities into consideration. The drug can fail in phase I, II or III or it can successfully be introduced on the market. Each of these options has

a value defined by the probability it will happen multiplied by the net cash flow at that point. The estimated project value is the sum of these probability-corrected cash flows of all possible events. In the presented example, the estimated value of the program equals M€ 27. This is represented in table 2.

TABLE 2 **Value estimation of all possible outcomes of the classic development plan**

Possible development outcomes	Probability	Costs (M€)	Payoff (M€)	Profit (M€)	Profit* Probability (M€)
Successful development	0.2975 (70%*50%*85%)	175	400	225	66.9375
Abandoned after phase III	0.0525 (70%*50%*15%)	175	0	-175	-9.1875
Abandoned after phase II	0.35 (70%*50%)	75	0	-75	-26.25
Abandoned after phase I	0.3 (30%)	15	0	-15	-4.5
Total:	1 (100%)	440	400	-40	27

Another way to represent this is by using a decision tree (generated by decision tools®) showing that the positive project value makes the decision to go ahead with the development of the drug is 'TRUE' (Figure 1).

The fact that drug development is a risky process [4-6] can be reflected in the risk profile of a decision tree [7]. The risk profile of this example is presented in Figure 2. The graph is a graphical display of table : all possible 'profits' are displayed on the x-axis and the probability this will occur is represented on the y-axis. From this graph it can be concluded that it is most likely the development of the drug will be abandoned after phase II losing M€ 75 (35%).

Assuming that knowledge on biomarkers for the effects of CNS active drugs eventually has impact on the probability of success from phase I to phase II, sensitivity analysis on this input value quantitatively estimates the effect of each change of 1% success probability. Varying the success probability phase I 10% around the initial 70% and recalculating the project value yields a linear relationship between the two parameters (Figure 3).

The corresponding linear trendline equation suggests that for every 1% improvement of the probability that the compound will successfully enter phase II, there is an estimated *a priori* project value increase of about M€ 0.18).

FIGURE 1 **Decision tree for a classical drug development program**

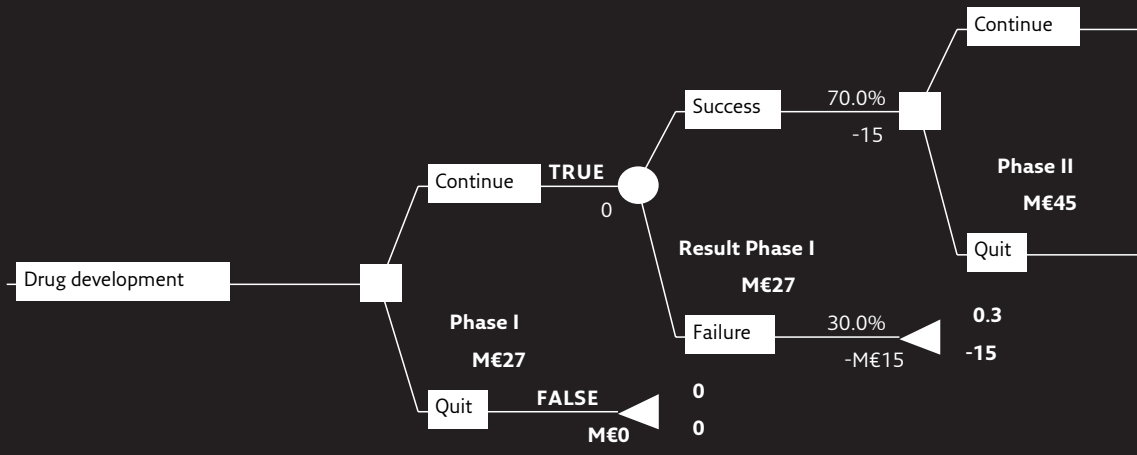
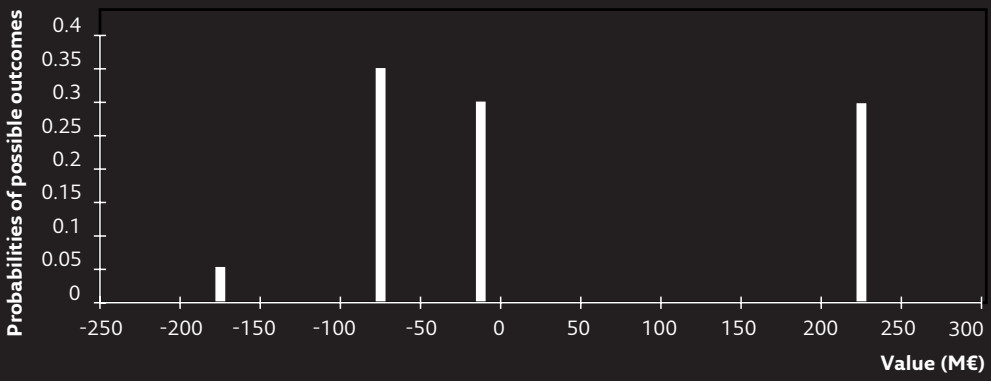


FIGURE 2 **Risk profile of a classical drug development program**



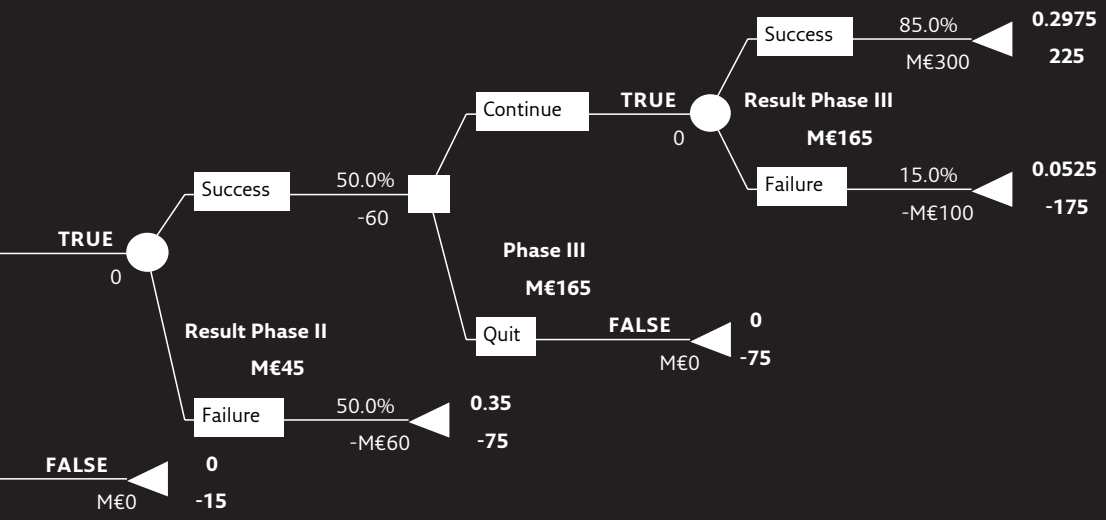
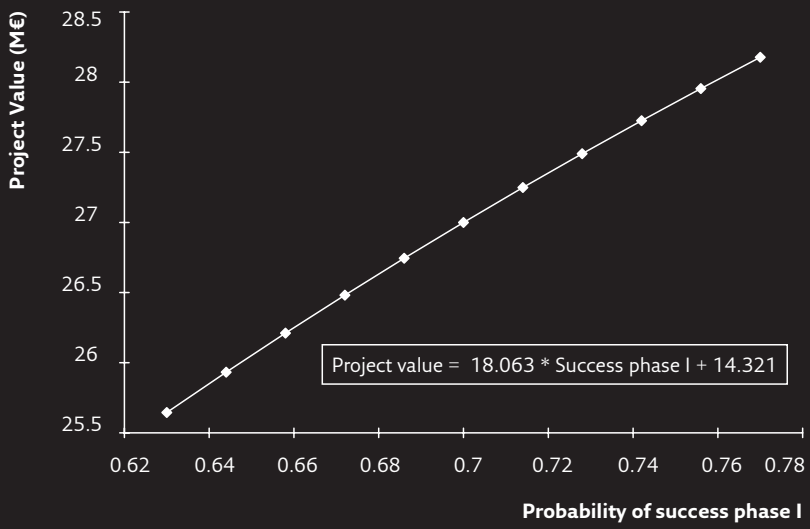


FIGURE 3 **Sensitivity analysis of the success in phase I probability on the project value**



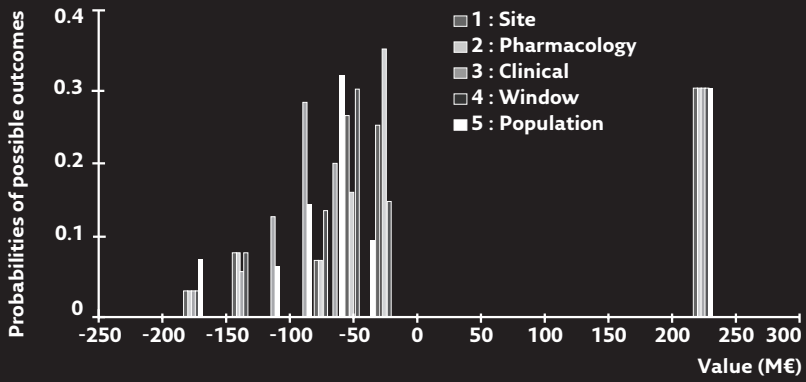
A similar decision tree can be constructed using the question-based approach. The same overall initial values are used (i.e. the probability the hypothetical compound will reach market launch remains 30%, the overall costs remain M€175 and the market value payoff remains M€ 400; table 3). The combination of the probabilities and costs are chosen in a way that the project value estimate is M€ 27 (similar to the 'classic' phase development project value). The estimated probabilities that the drug will successfully answer the question is assumed to be based on consensus between experts and are valid for a new CNS active drug (e.g. a new antipsychotic or anxiolytic or antidepressant) [8]. The purpose of replacing all the phases by questions is to try and clarify the real issues that need to be solved throughout the development (see introduction).

TABLE 3 **Hypothetical input parameters question based development plan**

Parameter	Value
Success action site	75%
Success pharmacological effect	65%
Success clinical efficacy	80%
Success therapeutic window	85%
Success population	90%
Costs action site	M€ 25
Costs Pharmacological effect	M€ 25
Costs Clinical effect	M€ 60
Costs Clinical window	M€ 30
Costs Population	M€ 35
Estimated marketvalue	M€ 400

By creating this question-based decision tree (QBD tree), a new factor is introduced in drug development optimisation: sequence. In contrast to the phase oriented development plan, the sequence in which the questions will be addressed is variable. The five questions can be arranged in 5! (=5*4*3*2*1) ways. The decision tree therefore consists of 120 different sequence options of successful drug development. The fact that the sequence is introduced as an extra variable is reflected in the risk profile of the QBD tree. Analysis shows that the first question addressed determines the risk profile of the development program. The risk profile of all first options is represented in Figure 4.

FIGURE 4 **Risk profile for the question based development tree for the first choices only**



This graph shows the first decision has a substantial effect on the risk profile of the development plan. All first choices have different negative outcome values if the drug is abandoned at some later stage. If all questions are successfully answered, there is always a 30% probability the profit will be M€ 225 independent of the sequence. However (similar to the example shown in table 2), the expected project value also incorporates the probabilities and costs if the drug development is eventually abandoned. Obviously, this reckoning varies for each sequence. For instance, table 4 compares the possible outcomes for the (optimal) sequences starting with 'pharmacology' and 'clinical'.

TABLE 4 **Comparison of the possible outcomes (M€) in the question based development for the first questions 'pharmacology' and 'clinical'**

1st question: Pharmacology			**1st question: Clinical**		
Outcome	**Probability**	**Outcome *Probability**	**Outcome**	**Probability**	**Outcome* Probability**
-175	3%	-6	-175	3%	-6
-140	8%	-12	-140	6%	-8
-80	7%	-6	-110	13%	-14
-50	16%	-8	-85	28%	-24
-25	35%	-9	-60	20%	-12
225	30%	67	225	30%	67
	Total:	**27**		**Total:**	**3**

This table shows that if the first question is 'pharmacology', the estimated optimal project value is much higher than the estimated optimal project value if the first question is 'clinical'. Similarly, the optimal choice of the remaining questions can be analysed. From the risk profiles and decision analysis on all sequence options the unique optimal sequence of events can be determined which is given in table 5. This table represents the optimal path among the 120 different successful options of the all-sequence-decision tree and the only sequence with an estimated project value of M€ 27. All other sequences have lower estimated project values.

TABLE 5 **Optimal path in the question based development tree**

Priority ranking	Question
1	Pharmacology
2	Site
3	Window
4	Clinical
5	Population

Subsequent sensitivity analyses of the success probabilities on the estimated project value highlight the impact of changed success probabilities on the project value of the question based development tree (Figure 5). Furthermore, sensitivity analyses can serve as a check of the initial model estimations by showing their relevant contributions to the project value.

The reviews presented in this section affect both the 'clinical' and 'pharmacology' question by increasing the knowledge on available biomarkers to answer these questions in the development of new drugs. Therefore, two-way sensitivity analyses were performed to show how the project value changes with combined changes in the success probabilities for these questions (Figure 6). The data used to construct Figure 6 is represented in the break-even table 6. This table shows the estimated project value for different sets of success probability estimations.

This break-even table and the 2-way sensitivity graph show that, for each 1% increase in the probability of success on 'pharmacology', the project value increases by M€ 0.8. In other words, introduction of additional costs up to M€ 0.8 on research that increases the probability of successfully answering the 'pharmacology'

TABLE 6 **Break-even table for changes in the estimated success probability for 'clinical' and 'pharmacology' on the project value (M€)**

	Success clinical										
Success pharmacology	**72%**	**74%**	**75%**	**77%**	**78%**	**80%**	**82%**	**83%**	**85%**	**86%**	**88%**
59%	12	14	16	18	20	22	24	26	28	30	32
60%	13	15	17	19	21	23	25	27	29	31	33
61%	14	16	18	20	22	24	26	28	30	32	35
62%	15	17	19	21	23	25	27	29	31	34	36
64%	15	18	20	22	24	26	28	30	33	35	37
65%	16	18	21	23	25	27	29	31	34	36	39
66%	17	19	21	24	26	28	30	32	35	37	40
68%	18	20	22	25	27	29	31	34	36	39	41
69%	19	21	23	26	28	30	32	35	37	40	42
70%	20	22	24	27	29	31	33	36	38	41	44
72%	20	23	25	27	30	32	35	37	40	42	45

question by 1% does not negatively affect the overall project value. Similarly, for each 1% increase in the estimated success on the 'clinical' question, M€ 1.4 is gained in project value for each review.

Assuming that the knowledge obtained on the available methods for evaluating three CNS drugs presented in the three reviews each increase the probability of success on both 'pharmacology' and 'clinical' with only 1%, the increase in project value in each case is M€ 0.8 + M€ 1.4 = M€ 2.2. If an antipsychotic drug, a benzodiazepine and an antidepressant are developed using the question-based approach, the estimation of success probabilities and required costs for answering the questions yields one unique question priority list with an optimal project value of M€ 27. If the three reviews each increase the probability of success on both 'pharmacology' and 'clinical' by 1%, the combined increase in project value for the three drugs amounts to M€ 6.7. Furthermore, the probability will be reduced that a method will be selected which will not show an effect at a therapeutically relevant dose of an efficacious drug. This approach can prevent studies that may produce conflicting or misleading results.

FIGURE 5 **Spider graph after sensitivity analysis on the success probabilities in the question based drug development program**

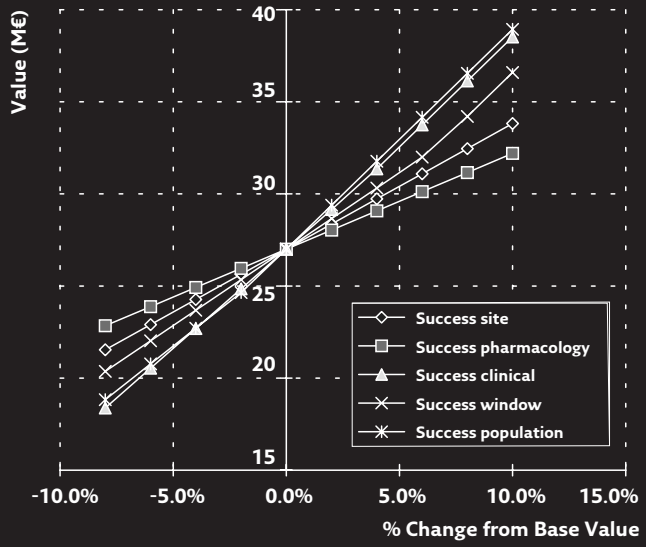
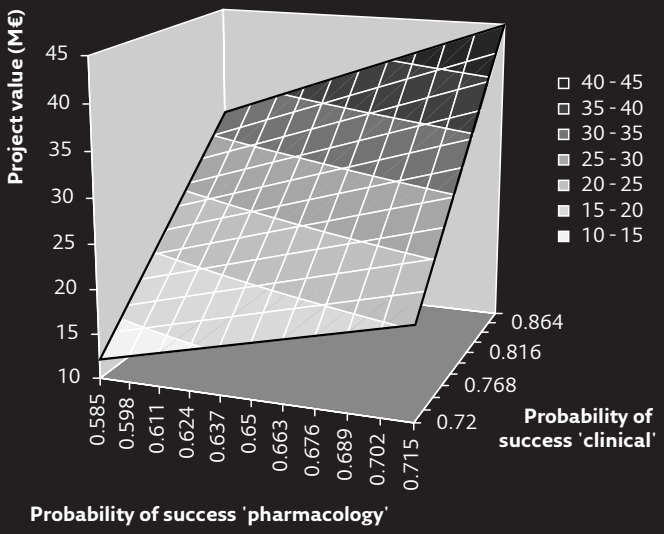


FIGURE 6 **Two-way sensitivity analysis of the success of 'pharmacology', probability and success of 'clinical', on the project value**



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SECTION 1

SECTION 2

SECTION 3

SECTION 4

Literature evaluation

Developing a new formulation

Bridging the gap to Japan

Market advantage

CHAPTER 5

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Comparison of an oral solution and an oral sustained release formulation of rilmenidine in eight healthy volunteers and correlation with *in vitro* sustained release properties

Abstract

RATIONALE Rilmenidine is a centrally acting antihypertensive. At the present time, the dosage for rilmenidine is 1 mg once a day, which in some patients needs to be increased to 1 mg twice a day. In order to increase the duration of the effect without increasing the occurrence of peak-dose related side effects, a sustained release (SR) formulation has been developed at a dose of 2 mg. This study aimed to investigate the relationship between *in vitro* and *in vivo* characteristics of dissolution of the slow release formulation. Secondly, the clinical effects and pharmacokinetics of this formulation of rilmenidine compared to a solution in healthy volunteers were investigated.

METHODS This was a double-dummy, double blind, randomised, two-way cross-over study in four healthy male and four healthy female volunteers with a six days washout between administrations. Rilmenidine was administered either as a 1 mg solution or a 2 mg SR tablet. Blood samples were taken prior to dosing and at various times up to 36 hours after administration and plasma analysed for unchanged rilmenidine. Deconvolution was used to determine the *in vivo* dissolution of the tablet, which was compared to the *in vitro* dissolution using linear regression. In order to estimate the prediction error of this correlation, the observed *in vivo* results were compared with the predicted *in vivo* kinetics according to the appropriate Food and Drug Administration (FDA) guideline. The clinical effects were evaluated by blood pressure, heart rate and visual analogue scales (VAS) of alertness, mood and calmness.

RESULTS The slope of the mean *in vitro-in vivo* dissolution correlation was 1.1 with a range from 0.71 to 1.7. The average predicted area under the curve (AUC) and maximum observed concentrations (C_{max}) deviated 6.7% and 12% from the observed values. The mean absolute average internal prediction errors of the *in vitro-in vivo* correlation (IVIVC) were 32% for AUC and 14% for C_{max}. C_{max} values were 3.7 ± 0.77 ng·ml^{-1} with the solution, and 2.6 ± 0.32 ng·ml^{-1} after the tablet, normalised to a 1 mg dose. These concentrations were reached later for the sr formulation than for the solution (5.4 ± 0.52 h compared with 2.1 ± 0.79 h). The time during which the concentration was greater than 75% of C_{max} (t_{75}) was 3.4 h longer for the tablet than for the solution (95% confidence interval: 0.5, 6.3 h). The relative bioavailability of the tablet compared to the solution was 126 ± 54% (coefficient of variation 43%). Both preparations showed similar treatment effects on blood pressure and alertness VAS, with a significantly earlier maximum for the solution (around 3½ hrs) than for the slow release tablet (about 5-6 hours).

CONCLUSION Although the internal prediction errors of the *in vitro-in vivo* correlation exceeded FDA guideline values, the *in vitro* dissolution kinetics are predictive of the *in vivo* dissolution kinetics. However, the pharmacokinetic properties of rilmenidine appear to be highly variable as illustrated by the high variability in relative bioavailability. The clinical effects of the rilmenidine 2 mg tablet and the 1 mg solution were not statistically significantly different.

Introduction

Rilmenidine (2-(dicyclopropylmethyl)-amino-2-oxazoline) is registered as an anti-hypertensive drug in several European countries. Rilmenidine is a centrally acting drug with binding selectivity to I_1 imidazoline receptors over α_2-adrenoceptors. Early clinical studies have indicated that after single administration the drug has a dose-dependent blood pressure lowering effect at doses of 0.5 mg or higher. The maximal effect occurs between 2-3 hrs after drug administration and lasts a minimum of 12 hours. Comparative studies in hypertensive patients have shown that the drug effectively lowers blood pressure compared to congeners like clonidine at equipotent doses. In the same dose range, mild sedation and reduced salivary flow have been reported, although these side effects are considerably less than for the nonspecific α_2 agonists. The drug is commonly prescribed in a dose of 1 mg orally once daily, but some patients require twice daily dosing. In order to increase the duration of the effect without increasing the occurrence of peak-dose related side effects, a 2 mg tablet has been developed which has sustained release (SR) properties *in vitro*. This formulation is intended to provide around-the-clock therapeutic drug concentrations after a once daily administration. To aid in the optimisation of the sustained release profile of novel formulations, the dissolution characteristics were compared *in vitro* and *in vivo* for a 2 mg tablet. Deconvolution techniques were used to determine the *in vivo* sustained release dissolution profile, and a 1 mg solution was used as an 'immediate release' form to correct for the absorption of rilmenidine. The SR dissolution profile was subsequently compared to the *in vitro* characteristics of the new tablet. This study aimed to investigate the relationship between *in vitro* and *in vivo* characteristics of dissolution of the slow release formulation. Secondly, the clinical acceptability and pharmacokinetics of this formulation of rilmenidine compared to a solution in healthy volunteers were investigated.

ref. 1-3

ref. 4-7

ref. 8

Methods

Study design

This was a double-dummy, double blind, randomised, two-way cross-over study in eight healthy volunteers with a washout between administration of at least six days.

Subjects

Subjects were male or female subjects, healthy as determined during screening, who gave signed informed consent. The study was approved by the Medical Ethics Review Board of Leiden University Medical Center, and performed according to the principles of the Helsinki Declaration. Eight (4 males, 4 females) subjects completed the study.

Drug administration

All subjects received a sustained release formulation of rilmenidine, 2 mg (active treatment) with the solution vehicle as placebo or a rilmenidine solution, 1 mg (active treatment) with sustained release placebo tablet. The sustained release formulation and placebo tablets were produced by Servier, Gidy France. Servier also produced a rilmenidine 1 mg/ml solution, according to GMP procedures. One ml of this solution was further diluted by adding 150 ml water. This final dilution was prepared the afternoon before dispensing and placed in a refrigerator (4°C) overnight.

Sampling

Subjects were studied after an overnight fast (with the exception of occasional water). Alcohol and xanthine containing food and beverages were not allowed from 12 hours before until 36 hours after dosing. A cannula was inserted in a forearm vein to facilitate repeated blood sampling. Samples were collected pre-dose and ½, 1, 1½, 2, 2½, 3, 4, 5, 6, 8, 10, 12, 14, 18, 24, and 36 hours after drug administration in heparin-containing polypropylene tubes (Sarstedt ®) for rilmenidine assay. The tubes were immediately centrifuged for 10 minutes at 4°C and 1500 g and plasma was subsequently divided into two aliquots, frozen and stored at -20°C until analysis. Four

hours after drug administration a standardised lunch was provided and a dinner was given after ten hours. Subjects went home after 24 hours and returned to the research unit 36 hours after drug administration for final measurements. The same procedure was repeated in the second study period.

Drug concentration analysis

Rilmenidine concentrations were determined using gas chromatography-mass spectrometry (GC-MS) following liquid-liquid extraction according to the method described by Ung et al.

ref. 9

In vitro dissolution

SR tablets containing 2 mg rilmenidine were placed into a 37 °C medium of 0.05 M phosphate buffer at pH 6.8 (according to the FDA guideline) using USP apparatus II (paddles). Samples (10 ml) were taken at 0, 1, 2, 4, 8, 12 and 16 hours. After filtration of the samples through a 10 µm polypropylene filter, an aliquot (5 µl) was injected onto the HPLC column (Nucleosil 100-3 C 18 (Macherey Nagel), 150 x 4.6 mm). The concentration of rilmenidine was determined spectrophotometrically at 205 nm by reference to a calibration curve.

Pharmacokinetic analysis

A non-compartmental pharmacokinetic analysis was performed for each subject and each treatment. The estimated parameters were the maximum observed concentration (C_{max}; normalised to a 1 mg dose assuming linear kinetics, $C_{max,norm}$) and corresponding t_{max} as well as the last measurable concentration (C_{last}). The area under the concentration versus time curve from 0 to C_{last} was calculated using the linear trapezoidal rule for rising or static concentrations and the logarithmic trapezoidal rule for declining levels (AUC_t; normalised to a 1 mg dose assuming linear kinetics, $AUC_{t,norm}$). The terminal half life ($t_{½}$) was estimated using the slope of the elimination phase. The concentration 24 hours after dosing was also determined (C_{24}). Additionally, the time interval between administration and first measurable concentrations (t_{lag}) and the time during which the concentration was equal to or greater than 75% of the C_{max} (t_{75}) were estimated. The relative

bioavailability of the SR tablet compared to the solution was estimated according to the following equation:

$$F_{rel} = 100 \cdot \frac{D_{sol} \cdot AUC_{tab}}{D_{tab} \cdot AUC_{sol}}$$

were D_{sol} and D_{tab} are the doses for the solution and tablet, and AUC_{sol} and AUC_{tab} are the AUC_t values for the solution and the tablet respectively.

Compartmental modelling was carried out for the solution data of each subject using WinNonlin software version 3.1 (Pharsight Corp, Mountain View, CA) in order to provide parameters for the numerical deconvolution. A mono- or bi-exponential model, with or without lag time was fitted to the data and the best model fit assessed by comparison of the value of the Akaike Information Criterion (AIC). Coefficients and exponentials from the model fit with the lowest value for AIC were used for the subsequent deconvolution analysis.

Deconvolution analysis

Numerical deconvolution was performed using PCDCON software version 3.0 (William R. Gillespie, Ph.D., The University of Texas at Austin) according to the method described by Gillespie et al. The model-fitted coefficients and exponentials for the solution were used to describe the unit impulse response. The input of rilmenidine was deconvolved from these two profiles to provide a percentage cumulative amount dissolved (equivalent to the *in vivo* dissolution). The *in vivo* dissolution profile for each individual subject was related to the mean *in vitro* dissolution profile for the SR tablet using linear regression. In addition, an average *in vivo* dissolution profile from all subjects was related to the *in vitro* dissolution profile, yielding a predicted *in vivo* dissolution profile.

ref. 10

In order to estimate the predictive value of this *in vivo-in vitro* correlation, internal prediction errors were assessed according to the appropriate FDA guideline. The predicted *in vivo* dissolution of the tablet was convolved with the individual solution concentration profiles. This resulted in predicted AUC and C_{max} values for the tablet that were compared to actually observed values, and absolute percent prediction errors were calculated for both individual and mean group values.

ref. 11

Pharmacodynamic determinations

Blood pressure and heart rate were measured immediately before drug sampling (except at 18 hours), and at ¼ and ¾ hours after the drug administration. These vital signs were measured after the subject had been sitting in a semi-recumbent position for at least 5 minutes. An automated blood pressure monitor (MPV1072, Nihon Kohden, Japan) was used, which displays an average value for two duplicate measurements at each time point. Visual analogue lines as originally described by Norris were also used in this study. The subjects were asked to indicate with vertical marks on 16 horizontal 100-mm lines how he/she felt at that moment. The 16 categories were (Dutch translations of): Alert/Drowsy, Calm/Excited, Strong/Feeble, Confused/Clear-headed, Well-coordinated/Clumsy, Lethargic/Energetic, Contented/Discontented, Troubled/Tranquil, Mentally slow/Quick-witted, Tense/Relaxed, Attentive/Dreamy, Incompetent/Proficient, Happy/Sad, Antagonistic/Amicable, Interested/Bored and Withdrawn/Gregarious. From this set of lines three factors were derived as identified by Bond and Lader, corresponding to alertness, mood and calmness. These factors were used to quantify subjective central nervous system effects. Visual analogue scores were recorded at ½, 1, 2, 4, 6, 8, 10, 12, 14, 24 and 36 hours. Reports of adverse events were elicited by the question "How do you feel" and by recording spontaneous reports.

ref. 12

ref. 13

Statistical analysis

Pharmacodynamic measurements were characterised by calculating the time of maximum effect (t_{max}) and the corresponding measurement (E_{max}), and the area under the curve (AUEC) over the 0-12 hours time period. These AUECs were subsequently divided by the corresponding time span resulting in a weighted average value. Measures were compared between treatments using paired t-tests. Calculations were performed using SPSS for Windows V10.0.7 (SPSS, Inc., Chicago, IL).

Results

Subject demographics

All subjects completed both study occasions. No serious adverse events occurred during the study. Subjects were 23 years of age (range 18-27 years),

with an average weight of 69.8 kg (range 49.1-87.7 kg) and height of 177 cm (range 161-191 cm). Average pre-dose blood pressures were (systolic/diastolic) 114/62 mmHg (range 97-139/52-72 mmHg) and a heart rate of 65 bpm (range 48-85 bpm).

Pharmacokinetic parameters

The main pharmacokinetic parameters are represented in Table 1. The mean plasma concentrations after both solution and SR tablet are represented in Figure 1.

T_{max} was reached on average 3.3h later for the SR formulation than for the solution (95% CI: 2.5, 4.0 h). $C_{max,norm}$ for the SR tablet was on average 1.1 ng/ml lower for the tablet than for the solution (95% CI: 0.6, 1.6 ng/ml). The time during which the concentration was greater than 75% of C_{max} (t_{75}) was 3.4 h longer for the tablet than for the solution (95% CI: 0.5, 6.3 h). Average normalised AUC was similar for both treatments but more variable for the solution than for the tablet (CV of 37% and 17% respectively).

The relative bioavailability of the SR tablet compared to the solution (F_{rel}) was 126 ± 54 % (Mean ± standard deviation). $T_{½}$ was similar for both treatments but more variable for the solution than for the tablet (CV of 68% and 43% respectively).

TABLE 1 **Average pharmacokinetic parameters after oral administration of a 1 mg rilmenidine solution and a 2 mg sustained release tablet including P-values for the difference**

Parameter	Solution			SR Tablet			
	Mean	SD	CV (%)	Mean	SD	CV (%)	P-value
C_{max} (ng/ml)	3.7	0.77	21	5.2	0.64	12	0.000
$C_{max, norm}$ (ng/ml)	3.7	0.77	21	2.6	0.32	12	0.001
t_{max} (h)	2.1	0.79	37	5.4	0.52	10	0.000
t_{75} (h)	2.8	1.9	67	6.2	3.258	53	0.028
t_{lag} (h)	0.56	0.18	31	0.75	0.27	36	0.197
AUC_t (ng·h/ml)	33	12	37	73	12	17	0.000
$AUC_{t, norm}$ (ng·h/ml)	33	12	37	37	6	17	0.470
C_{24} (ng/ml)	0.59	0.22	37	1.12	0.62	56	0.126
$t_{½z}$ (h)	10.1	6.9	68	8.0	3.5	43	0.288
F_{rel} (%)	-	-	-	126	54	43	

FIGURE 1 **Average rilmenidine plasma concentration after oral administration of a 1 mg solution (●; solid line) and a 2 mg sustained release tablet (▲; dashed line)**

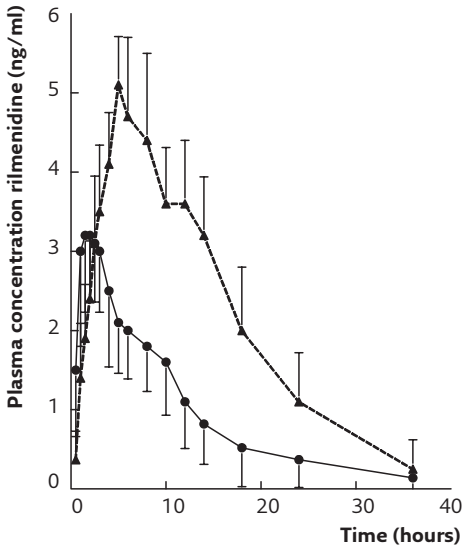
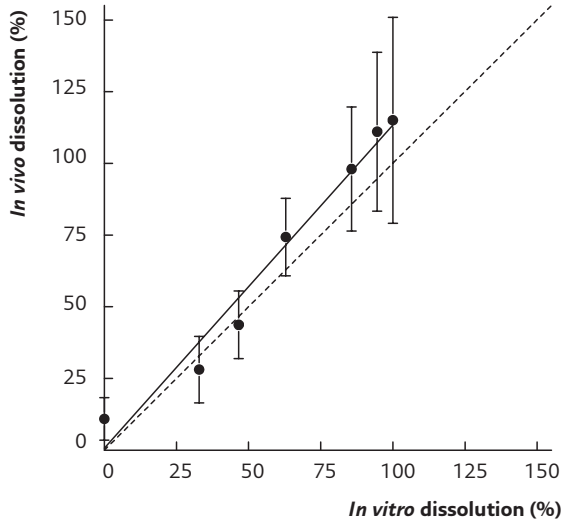


FIGURE 2 **Relationship between *in vitro* and *in vivo* dissolution of the 2 mg sustained release tablet (●; solid line). y=1.126x + 0.692, R^2=0.99, CV=44%. Dashed line is the line of identity**



In vitro – in vivo correlation of the dissolution

The relationship between the *in vitro* and mean *in vivo* dissolution is given in Figure 2. The slope of the mean *in vitro – in vivo* correlation was 1.12. The variability in the *in vivo* dissolution was considerable with a range of the individual slopes of 0.71 to 1.67.

The overall difference between the average predicted values and observed values was 6.8% for the AUC and 11.9% for the C_{max}. However, the means of the individual absolute percent prediction errors (the internal prediction errors) were 32.4% for the AUC and 13.6% for the C_{max}.

Pharmacodynamic parameters

BLOOD PRESSURE AND HEART RATE The main results on the average pharmacodynamic parameters are listed in Table 2. The mean time-effect curve for diastolic blood pressure is represented in Figure 3. Blood pressures dropped during treatment: the maximum decrease (systolic / diastolic) was 15.6/10.6 mmHg with the rilmenidine solution (from 111/60 at baseline), and 22.1/14.2 mmHg with the tablet (from 117/63 at baseline). The difference between the two preparations was not statistically significant (95% confidence interval (95% CI) -1.5, 14.4 mmHg systolic, and –0.6, 7.7 mmHg diastolic). The differences in average response (AUEC over 12 hours) were also not significant (Table 2).

The maximum effect of the tablet (Table 2) occurred on average 2.9 h (95% CI 0.5, 5.3 h) later for systolic, and 2.5 h (95% CI 1.0, 4.0 h) later for diastolic blood pressure, compared to the solution.
A significantly higher increase in E_{max} of heart rate of 4.6 bpm was observed for the solution (95% CI: 1.2, 7.9 bpm). The time of maximal effect for heart rate was similar for the two preparations.

VISUAL ANALOGUE SCORES Clear differences in times of maximal effect were noted for VAS alertness as represented in Table 2. T_{max} occurred on average at 3.5 h after the administration of the solution, and 5.4 h after the ingestion of the sustained release tablet resulting in an average difference of 1.9 h (95% CI 0.1, 3.7 h) between the two treatments.
There were no differences in the other VAS factors (mood/calmness).

TABLE 2 **Average pharmacodynamic parameters after oral administration of a 1 mg rilmenidine solution and a 2 mg sustained release tablet including P-values for the difference**

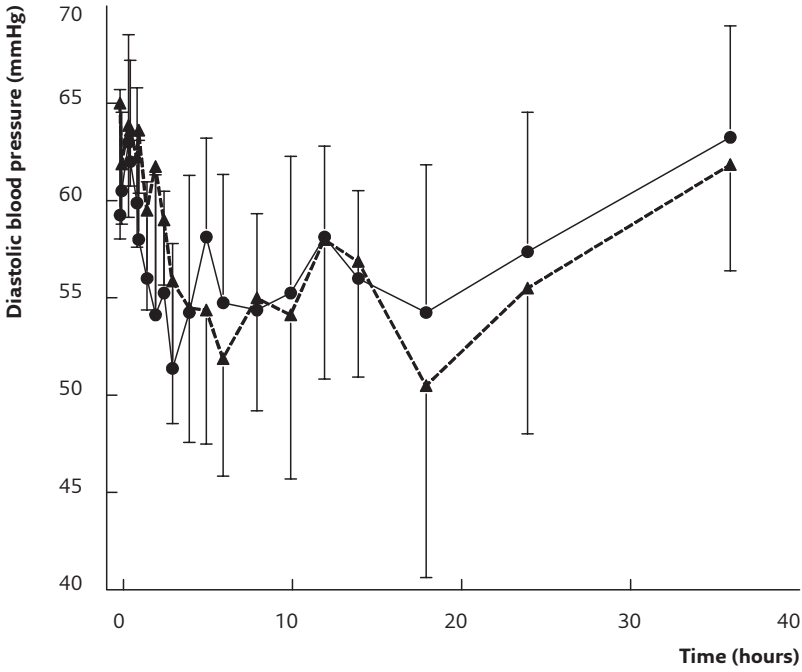
Parameter	Solution		SR Tablet		
	Mean	SD	Mean	SD	P-value
	Systolic blood pressure				
t_{max} (h)	3.3	1.9	6.2	2.9	0.026
E_{max} (mmHg)	15.6	6.2	22.1	9.4	0.097
$AUEC_{0\text{-}12h}$ (mmHg)	105.0	8.7	104.8	9.3	0.908
	Diastolic blood pressure				
t_{max}(h)	3.4	1.9	5.9	2.1	0.005
E_{max} (mmHg)	10.6	3.6	14.2	5.2	0.083
$AUEC_{0\text{-}12h}$ (mmHg)	55.7	4.9	56.1	5.2	0.521
	Heart rate				
t_{max} (h)	5.0	3.1	5.3	1.1	0.846
E_{max} (bpm)	12.3	5.4	7.8	2.6	0.014
$AUEC_{0\text{-}12h}$ (bpm)	65.6	6.3	66.2	5.3	0.574
	VAS Alertness				
t_{max} (h)	3.5	1.8	5.4	0.9	0.044
E_{max} (mm)	16.4	14.4	25.4	10.1	0.163
$AUEC_{0\text{-}12h}$ (mm)	63.6	17.1	63.0	14.8	0.815

Discussion

This study shows that sustained release has been achieved for the tablet: maximum levels were lower, levels above 75% of the C_{max} were maintained for longer periods, and concentrations were two-fold higher at 24 hours after ingestion. The dose-normalised C_{max} for the SR tablet was lower than for the solution, while the corresponding t_{max} and t_{75} were much longer for the SR tablet. The relative bioavailability of the tablet was 126%, indicating that a slow release formulation could have a favourable absorption profile compared to the solution.

Deconvolution assumes that the only difference between the two modes of drug administration for a subject lies in the *in vivo* dissolution of the tablet. All other pharmacokinetic parameters and processes are assumed identical. Therefore the (between subject) variability in the plasma AUC after tablet administration must be equal to or higher than the AUC after the solution. Dissolution of the tablet can be the only source of additional variability.

FIGURE 3 **Average diastolic blood pressure after oral administration of a 1 mg solution (●; solid line) and a 2 mg sustained release tablet (▲; dashed line)**



However, we found that the AUC is more variable after the solution (CV=37%) than after the SR tablet (CV=17%). This can only be attributed to other sources of variability, for instance due to differences in the absorption process. Additional evidence is provided by the fact that average relative bioavailability is larger than 100% which must be due to either differences in the relative absorption process or high variability in pharmacokinetic parameters within a subject. These arguments imply that the basic pharmacokinetic behaviour (absorption, distribution, elimination) is not identical for the two occasions resulting in high variability in the *in vitro – in vivo* correlation. As a result, the internal prediction errors were higher than 10% for AUC and C_{max}, thus exceeding the stringent criteria mentioned in the guideline for evaluating the predictability of a level A *in vitro - in vivo* correlation (deconvolution followed by comparison of the fraction of drug absorbed to the fraction of drug dissolved). Nevertheless, the percent difference between the mean observed and predicted AUC and C_{max} were relatively low, indicating that the average *in vitro* dissolution kinetics of the SR tablet is predictive of its *in vivo* characteristics.

Despite a two-fold difference in exposure, no significant difference was observed for the $AUEC_{0\text{-}12h}$ between the rilmenidine slow release formulation and solution on blood pressure, heart rate and visual analogue scores. However, the maximum effects occurred significantly later after ingestion of the tablet compared to the solution. Although diurnal influences cannot be excluded without use of a placebo, it seems very likely that these different time effects are due to the 'slow release' profile of the tablet, compared to the 'immediate release' profile of the solution. The data from this study can be used to optimise the dissolution characteristics of a sustained release preparation. Such a preparation could prolong the antihypertensive activity, while reducing peak-concentration related side effects of rilmenidine.

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CHAPTER 6

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Concentration-effect relationships of two infusion rates of the imidazoline antihypertensive agent rilmenidine for blood pressure and development of side-effects in healthy subjects

Abstract

OBJECTIVES The aim of this study was to compare the effect profiles of *iv* administered rilmenidine aimed at the same target plasma concentration, but attained with different rates.

METHODS The study was placebo controlled, randomised, double-blind, double-dummy, three-way, cross-over in nine healthy volunteers. All subjects randomly received either a fast infusion, a slow infusion or a placebo infusion. The target concentration was set at 8 ng/ml with a five-fold difference in the time to reach the maximum concentration. Saccadic eye movements, electroencephalography (eeg), blood pressure and heart rate were measured every half hour. Drug plasma concentrations, adverse events, salivary flow and visual analogue scales were obtained every hour.

RESULTS Changes in systolic/diastolic blood pressure at the end of the infusion were (mean ± SD) 13.3 ± 16.4/ 7.9 ± 7.5 mmHg with the fast infusion and 16.3 ± 12.7/10.2 ± 7.9 mmHg during the slow infusion, compared to 0.0 ± 13.2/1.3 ± 8.3 mmHg for the placebo occasion. Decrease of saccadic peak velocity (SPV) at the end of the infusion was 18.0 ± 5.2% during the fast infusion, 23.0 ± 7.0% with the slow infusion, and 6.1 ± 5.2% with placebo. PK/PD analysis showed similar concentration-dependent linear reduction in SPV and blood pressure up to the maximum observed rilmenidine plasma level for both the fast and the slow infusion.

CONCLUSIONS The rate of infusion of rilmenidine in healthy volunteers does not influence the PK/PD relationship of saccadic eye movements and blood pressure up to the maximum observed rilmenidine plasma concentrations. Rilmenidine causes clear concentration-dependent blood pressure reductions in healthy volunteers.

Introduction

Rilmenidine (2-(dicyclopropylmethyl)-amino-2-oxazoline) is a centrally acting anti-hypertensive drug with binding selectivity to newly described I_1 imidazoline receptors (identified in the lateral reticular nucleus in the brainstem and proximal tubular cells in the kidney) over α_2-adrenoceptors. It has dose-dependent blood pressure lowering effects above 0.5 mg in both healthy and hypertensive subjects. Rilmenidine is registered in several European countries as 1 mg tablets, and the most frequently used dose is

ref. 1-5

1 tablet once daily. Its concentration-related side-effects (sedation, xerostomia) are less important in comparison to congeners (*e.g.* clonidine), and are probably mediated by α_2-adrenoceptor stimulation. Recently, the central nervous system effects of rilmenidine 1 and 2 mg *po* were studied using saccadic eye movements, electroencephalography (EEG) and auditory evoked responses (AER). Rilmenidine caused a dose-dependent reduction of saccadic peak velocity (SPV), but little change in the other parameters. These effects were clearly smaller than with lorazepam 2.5 mg. Characterisation of concentration-effect relationships for dry mouth and sedation (which were not determined in the previous study) may be helpful in optimising the clinical dose.

ref. 6

In addition to the plasma concentrations, the rate of increase of concentration may also influence the effect. The classic example is provided by Kleinbloesem *et al* who demonstrated that a high rate of increase of nifedipine concentrations did not lead to a blood pressure reduction in healthy volunteers, contrary to a low rate of increase of nifedipine concentrations. The current study was therefore designed to compare the effect profiles of *iv* administered rilmenidine aimed at the same target plasma concentration, but attained with different rates. The target concentration was set at high concentrations of 8 $ng \cdot ml^{-1}$, *i.e.* above the therapeutic range. The *iv* infusions were programmed to yield a five-fold difference in the time to reach the maximum concentration.

ref. 7

ref. 8

Methods

Design

This was a placebo controlled, randomised, double-blind, double-dummy, three-way, cross-over, monocentric study in nine healthy volunteers, with a one-week wash-out period.

Subjects

Subjects were male or female, healthy as determined during screening, who gave signed informed consent. The study was approved by the Medical Ethics Review Board of Leiden University Medical Center, and performed according to the principles of the Helsinki Declaration.

Treatments and measurement times

Infusions were performed with a volumetric infusion pump (Sigma 6000+, Stöpler Instrumenten & Apparaten B.V., Utrecht, The Netherlands) with a constant infusion rate of 0.25 ml/min over the first 4 hours followed by a constant infusion rate of 1.0 ml/min over 1 hour. The total volume infused was 120 ml. Infusion rates were determined after simulation using PK data from previous studies.

ref. 17

All subjects randomly received the following treatments:
Fast infusion: NaCl 0.9% over 4 hours followed by rilmenidine infusion (1.8 mg/hr) over 1 hour resulting in a total administered dose of 1.8 mg.
Slow infusion: rilmenidine (0.52 mg/hr) over 5 hours resulting in a total administered dose of 2.6 mg.
Placebo infusion: NaCl 0.9% over 5 hours.
Blood samples were obtained hourly for the first six hours, with more frequent measurements around the end of the infusion (seven samples in the 4-6 hour period), and at increasing time intervals for 24 hours. Pharmacodynamic measurements were performed at half-hour intervals for six hours and at decreasing frequency after the end of the infusion.

Blood Pressure

Blood pressure and heart rate were measured with an automated blood pressure monitor (MPV1072, Nihon Kohden, Japan), which displays an average value for two sequential (duplicate) measurements at each time point. All measurements were made after the subject had been sitting in a semi-recumbent position for at least 5 minutes.

Saccadic Eye Movements

ref. 6
ref. 9-13

Saccadic eye movements are a sensitive measure for central nervous system effects of rilmenidine and clonidine. Saccadic eye movements were recorded as described previously using a micro-computer-based system for data recording (Cambridge Electronics Design, Cambridge, UK), Nihon Kohden equipment for stimulus display, signal collection and amplification (Nihon Kohden Corporation, Tokyo, Japan), and disposable surface electrodes (Medicotest N-00-S, Olstykke, Denmark). Average values of latency (= reaction time), peak saccadic velocity and inaccuracy

(difference between stimulus angle and corresponding saccade in %) were calculated for all artifact-free saccades.

ElectroEncephaloGraphy (EEG)

EEG-registrations have been used previously to quantify drug effects of rilmenidine and clonidine. EEG registrations of total power for Delta- (<4Hz), Theta- (4-7.5 Hz), Alpha- (7.5-12.5 Hz) and Beta- (12.5-30 Hz) activity were obtained at leads Fz-Cz and Pz-Oz., as described previously.

ref. 7

ref. 10-13

Visual Analogue Scales

Visual analogue scales as originally described by Norris were previously used to quantify subjective effects of benzodiazepines. From the set of sixteen lines three factors were derived as described by Bond and Lader, corresponding to alertness, mood and calmness. These factors were used to quantify subjective drug effects.

ref. 14

ref. 9

ref. 15

Salivary Flow

Salivary production was estimated by measuring the weight increase of three dental rolls put into the oral cavity over a period of 3 minutes. Salivary production was assessed regularly up to eleven hours after start of the infusion with an additional 24-hour measurement. The dental rolls and accompanying collection tubes used for this measurement were Sarstedt neutral Salivettes7 (Sarstedt, Etten Leur, The Netherlands).

Analyses

PHARMACOKINETICS Rilmenidine plasma levels were measured using gas chromatography/mass spectrometry. The limit of detection was 0.3 ng/ml and the linearity of the assay has been checked over a range of 0.3 - 2 ng/ml. The pharmacokinetics were described using NONMEM version V (NONMEM Project group, UCSF, San Francisco, CA), applying the first order conditional estimation (FOCE) method with the interaction option. Intra-individual error was modelled using a combination a constant (small) standard deviation and a constant coefficient of variation error model.

ref. 16

PHARMACOKINETIC/PHARMACODYNAMIC RELATIONSHIPS The observed pharmacodynamic effects were plotted against the predicted rilmenidine concentrations for each individual. PK/PD modelling was performed using nonlinear mixed effect modelling as implemented in NONMEM. Rilmenidine pharmacokinetics was described using empirical Bayes estimates for the pharmacokinetic parameters. First order conditional estimation was used with an additive residual error. Models with and without a hypothetical effect compartment were used. Concentration-effect parameters for the fast infusion were determined along with parameters estimating the difference between slow and fast infusion. Additive inter-individual variability was used on paired (within-subject) data. Only PD data obtained during and after the actual infusion of rilmenidine were used. Nested models were compared on the basis of the change in the minimum value of the objective function. A change of 3.8 (associated with 1 degree of freedom) was considered significant at p=0.05. Contrast parameters between the slow and fast infusion are reported with approximate 95% confidence intervals calculated using two times the reported standard error.

STATISTICS Changes from baseline at the end of the infusion were calculated for the pharmacodynamic parameters and compared between treatments using paired Student t-tests. Differences are reported with 95% confidence intervals (95% CI). Data are shown as average with standard deviation (M ± SD) unless indicated otherwise. Calculations were performed using SPSS for Windows V9.0.1 (SPSS, Inc., Chicago, IL).

Results

Demographics

All nine (5 males, 4 females) subjects completed the study. Subjects were on average 22.2 years of age (range: 19-26 years). No serious adverse events occurred during the study.

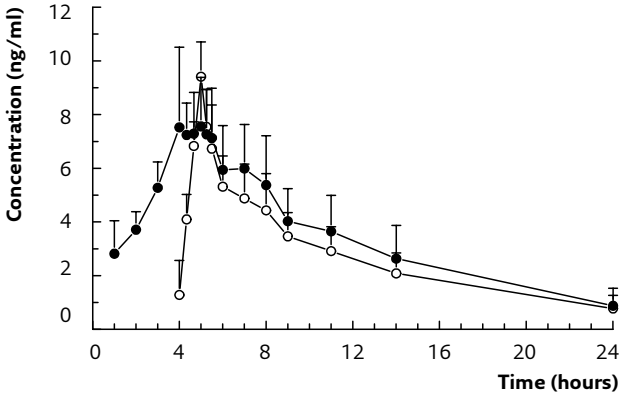
Pharmacokinetics

The time-concentration profiles for the two infusion regimens are shown in Figure 1. The maximal concentrations were 9.6 ± 1.0 ng/ml with the fast rate infusion, and 8.1 ± 2.4 ng/ml after the slow rate infusion. Prior experience with rilmenidine and visual inspection of the individual data clearly indicated

ref. 17

the need for a two-compartment model. The average pharmacokinetic parameters with inter-individual variation coefficients (cv) were: clearance of 0.53 L/min (cv 31%), initial half-life of 15.7 min (cv 21%), terminal half-life of 361 min (cv 23%), and central volume of distribution of 129 L (cv 33%). These results correspond to the pharmacokinetics found in previous healthy volunteer studies after single dose administration. ref. 17

FIGURE 1 **Time-concentration profiles (Mean + SD) for slow- (●) and fast- (○) rilmenidine infusions**



Pharmacodynamics

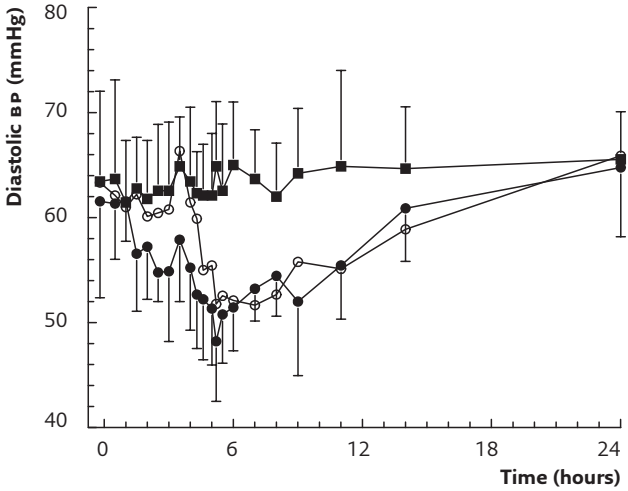
Table 1 presents differences between the three treatments in changes from baseline at the end of the infusion for pharmacodynamic parameters.

BLOOD PRESSURE AND HEART RATE The average time effect curves for diastolic blood pressure are shown in Figure 2, and similar profiles were seen for systolic blood pressure. At baseline, blood pressure was similar for all three treatments. Changes in systolic/diastolic blood pressure at the end of the infusion were 13.3±16.4/ 7.9±7.5 mmHg with the fast infusion and 16.3±12.7/10.2±7.9 mmHg during the slow infusion, compared to 0.0±13.2/1.3±8.3 mmHg for the placebo occasion. Blood pressure decreased significantly compared to placebo, but no significant differences were observed between the slow and fast infusions (Table 1). Heart rate decreased slightly during slow but not fast rilmenidine infusion. At the end of the rilmenidine infusions, blood pressures slowly returned to normal, without apparent differences between the fast and slow rate infusions.

TABLE 1 **Placebo corrected changes from baseline (95% confidence intervals) in pharmacodynamic parameters at the end of the infusion**

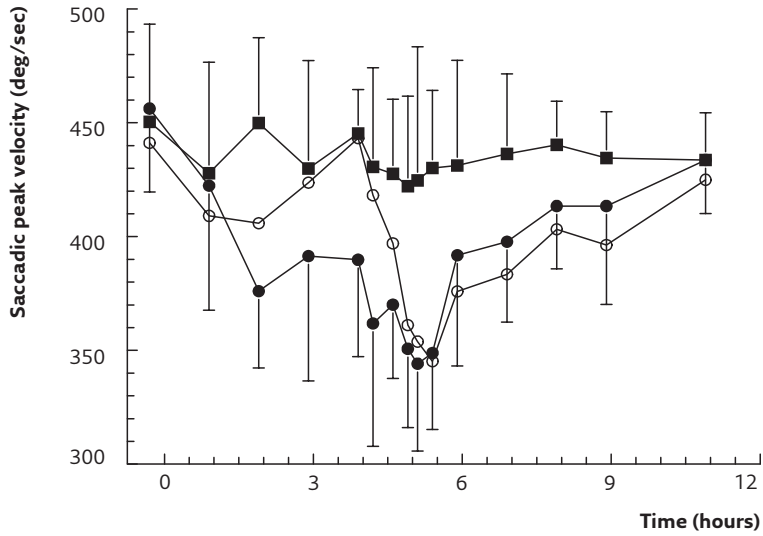
	Fast Infusion		Slow Infusion		Fast minus Slow Infusion	
Systolic BP (mmHg)	-13.3	(-23.3/-3.3)	-16.3	(-24.3/-8.4)	3.0	(-9.6/15.6)
Diastolic BP (mmHg)	-6.6	(-11.8/-1.3)	-8.9	(-15.0/-2.8)	2.3	(-5.8/10.5)
Heart rate (bpm)	-4.0	(-13.1/5.1)	-7.2	(-13.0/-1.5)	3.2	(-3.9/10.4)
Saccadic Peak Velocity (% change)	-11.9	(-17.5/-6.2)	-16.9	(-22.6/-11.1)	5.0	(-2.2/12.2)
EEG Delta Pz-Oz (% change)	28.8	(11.4/46.1)	28.7	(16.0/41.3)	-3.3	(-21.9/15.4)
EEG Beta Fz-Cz (% change)	21.5	(-1.5/44.4)	15.6	(-1.0/32.1)	0.8	(-26.0/27.7)
VAS alertness (% change)	-11.9	(-29.0/5.1)	-13.9	(-29.4/1.6)	2.0	(-13.5/17.4)
Salivary flow (% change)	-78.8	(-120.5/-37.1)	-82.3	(-122.4/-42.2)	3.5	(-16.0/23.1)

FIGURE 2 **Time-effect profiles (Mean + SD) for diastolic blood pressure for placebo- (■), slow- (●) and fast- (○) rilmenidine infusions**



SACCADIC EYE MOVEMENT The average time profiles for the saccadic peak velocity (SPV) is shown in Figure 3. Decrease at the end of the infusion was 18.0±5.2% during the fast infusion, 23.0±7.0% with the slow infusion, and 6.1±5.2% with placebo (Table 1). No significant differences were found in saccadic inaccuracy (as defined in the method section) and saccadic latency between the two active treatments.

FIGURE 3 **Time-effect profiles (Mean + SD) for saccadic peak velocity for placebo- (■), slow- (●) and fast- (○) rilmenidine infusions**

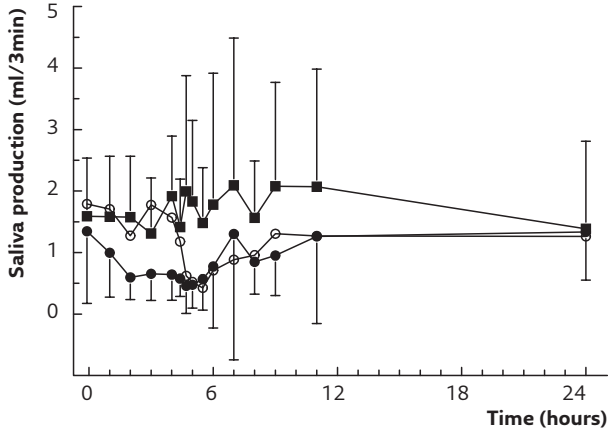


ELECTROENCEPHALOGRAPHY (EEG) Delta Pz-Oz activity increased significantly during both slow and fast rilmenidine infusion compared to placebo, with a similar trend for EEG Beta Fz-Cz power (Table 1). There were no significant differences between the two active treatments. No significant differences were observed for any of the other EEG parameters.

VISUAL ANALOGUE SCALES (VAS) Decrease in VAS scores of alertness at the end of the infusion was 13.6±21.5% during the fast infusion and 15.6±12.6% with the slow rate infusion, compared to 1.7±11.9% with placebo (Table 1). After both infusion regimens, VAS-alertness returned to baseline within about 6 hours. There were no significant effects for VAS scores of calmness and mood between the three treatments.

SALIVARY FLOW Saliva production decreased significantly to similar levels during both rilmenidine infusions (Table 1, Figure 4). At the end of the infusion, salivary flow had decreased 60.7±13.2% during the fast infusion and 64.2±19.9% during the slow infusion, compared to an increase of 18.1±49.0% with placebo. The production of saliva slowly returned to normal baseline values after the infusions were stopped. No differences between slow and fast infusions were observed.

FIGURE 4 **Time-effect profiles (Mean + SD) for saliva production for placebo- (■), slow- (●) and fast- (○) rilmenidine infusions**



Pharmacokinetic/pharmacodynamic relationships (PK/PD)

Concentration effect relationships between rilmenidine, saccadic peak velocity and diastolic blood pressure were examined. NONMEM parameters of the final models are presented in Table 2. For saccadic peak velocity, a linear concentration-effect model was used with a hypothetical effect compartment. Inclusion of this effect compartment resulted in a significant improvement in fit. Visual inspection of the individual concentration-effect graphs did not suggest the need for a more complex concentration-effect model. For diastolic blood pressure, a linear concentration-effect model was used without a hypothetical effect compartment. Inclusion of this effect compartment did not result in a significant improvement in fit, indicating that no hysteresis loop was observed. Visual inspection of the individual concentration-effect graphs did not suggest the need for a more complex concentration-effect model.

Both slopes and intercepts for saccadic peak velocity and diastolic blood pressure did not differ between the slow and fast infusion (Table 2). However, the slope of the concentration-effect curves for diastolic blood pressure after the slow infusion seems to be larger than after the fast infusion. This is supported by the slightly lower concentrations and higher maximum effect on diastolic blood pressure after the slow infusion. The lack of significance could be due to the small population size.

TABLE 2 **Mean PK/PD parameters for saccadic peak velocity and diastolic blood pressure for fast infusion and difference slow-fast infusion**

	Mean	95% CI	inter-individual variability (SD)
Saccadic peak velocity[1]			
$t_{1/2}$ K_{e0} (min)	5.6	1.5/9.7	0.0 (fixed)
Intercept fast infusion	445	418/472	32
Difference in intercept (slow-fast)	11.8	-6.9/30.5	16
Slope fast infusion	-11.3	-14.0/-8.6	1.3
Difference in slope (slow-fast)	-1.1	-3.1/0.8	0.0
Residual error (SD)	31.2		
Diastolic blood pressure[2]			
Intercept fast infusion	61.4	58.2/64.6	3.1
Difference in intercept (slow-fast)	2.2	-0.9/5.3	0.9
Slope fast infusion	-1.20	-1.68/-0.72	0.13
Difference in slope (slow-fast)	-0.48	-1.12/0.16	0.29
Residual error (SD)	4.29		

1. units for intercepts in deg/sec; units for slopes in (deg/sec)/(ng/ml)

2. units for intercepts in mmHg; units for slopes in (mmHg)/(ng/ml)

Discussion

The current study showed clear relationships between the rilmendine concentration and reduction of blood pressure, saccadic peak velocity and salivary flow in normotensive subjects. Concentration-effect relationships were not affected by the rate of infusion *per se*, contrary to the effects of the calcium channel blocker nifedipine, where blood pressure reduction is larger with slow rate than with fast rate infusion. This difference could be due to the different modes of action of the two antihypertensive agents. Nifedipine acts peripherally, and fast blood pressure reductions are probably rapidly counteracted by the baroreceptor reflex (with tachycardia), whereas slow increases in plasma concentrations allow the baroreceptor reflex to reset downward. Rilmenidine on the other hand acts centrally, possibly by reducing the level around which blood pressures fluctuate (without changes in heart rate), and may therefore evade counter-regulatory mechanisms. The central nervous system (CNS) effects of rilmenidine on saccadic peak velocity (SPV) and EEG-Delta- and -Beta-power were also not affected by the infusion rate. Another study where infusion rate-effects were evaluated on the same pharmacodynamic endpoints, was performed with temazepam. This benzodiazepine caused a larger average increase of EEG-Beta activity

ref. 7

ref. 18

ref. 13

after fast- than after slow-rate infusion, but this rate-effect was marginal and not observed for SPV or any other CNS-parameter. Thus, CNS-effects of temazepam and rilmenidine were unaffected by infusion rates.

ref. 6

In the current study, rilmenidine reduced SPV, similar to previous reports. The average SPV-reductions at the end of the rilmenidine infusions were 18.0-23.0%. However, it is difficult to interpret these changes clinically. The concomitant occurrence of (statistically non-significant) reductions in visual analogue scales of alertness suggests that the SPV-reduction reflects sedation. However, the clinical relevance of these changes is difficult to determine, since saccadic eye movements have not been quantitatively related to clinical effects of centrally acting antihypertensive agents. For benzodiazepines, the clinical correlates of such SPV-changes are well validated. Isolated SPV-effects at low benzodiazepine levels reflect a larger sensitivity of this biomarker to CNS-depression, than other pharmacodynamic or subjective measures. Other CNS-effects only occur at higher benzodiazepine doses. The clinical relevance of SPV-reductions is not only determined by the size but also by the duration of the effect. The impact of short-lasting effects on daily life is subject to a wide inter-subject variability, and strongly influenced by external factors like activities (*e.g.* demanding circumstances), day time, coffee-intake, *etc*. Also, the duration of treatment is relevant, since patients may develop tolerance to or become imperceptive of the sedative properties of drugs during prolonged treatment. The current study showed clear concentration-dependent effects of rilmenidine on blood pressure and on the central nervous system. The clinical relevance of these effects remains to be demonstrated during prolonged treatment.

ref. 14

ref. 9-10

ref. 12

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CHAPTER 7

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Concentration-effect relationships of two rilmenidine single-dose infusion rates in hypertensive patients

Abstract

OBJECTIVES The current study was designed to assess the concentration-effect relationships for the anti-hypertensive effects of rilmenidine in patients to aid in the design of an optimised concentration profile of a sustained release formulation.

METHODS This was a placebo controlled, randomised, double-blind, two-way partial cross-over, study in subjects with hypertension. Twenty-six patients were randomised to receive two of three possible 12-hour infusion regimens, each consisting of a loading phase (2 h) and a maintenance phase (10 h): Low Profile infusion (total dose of rilmenidine 1.45 mg), high profile (total dose 3.3 mg) or placebo. Drug plasma concentrations, adverse events, blood pressure and heart rate, and visual analogue scales were measured frequently up to 24 hours after dosing. Salivary flow was determined up to 15 hours.

RESULTS The high concentration profile was well tolerated and still produced a significant blood pressure reduction of 10.4/5.8 mmHg after 24-hours. After 24 hours, the low concentration profile showed no significant effects on blood pressure compared to placebo. Decreases in salivary flow were -36% for the high infusion and -20% for the low profile compared to placebo. Pharmacokinetic-pharmacodynamic analyses show infusion rate independent linear concentration-dependent reductions in DBP and salivary flow up to the maximum observed rilmenidine concentration for both infusions.

CONCLUSIONS The high concentration profile was well tolerated and still produced a significant blood pressure reduction after 24-hours. Pharmacokinetic-pharmacodynamic relationships were linear and unaffected by the rate of infusion. These results should aid in the design of an optimal slow release profile.

Introduction

Rilmenidine (2-(dicyclopropylmethyl)-amino-2-oxazoline) is a centrally acting anti-hypertensive with binding selectivity to the I_1 imidazoline receptors over α_2-adreno-ceptors. It has dose (concentration)-dependent blood pressure lowering effects above 0.5 mg in both healthy and hypertensive subjects. Rilmenidine is registered in several European countries

ref. 1-4

at a recommended dose of 1 tablet of 1 milligram once or twice daily. Clinical experience indicates that with 1 mg dosing blood pressure control might not be maintained for 24 hours per day in all patients. Twenty-four hour monitoring of the effects of 1 mg rilmenidine in 80 hypertensive patients after 4 weeks treatment with this dosage suggested a significant duration of action
ref. 5 of 14 hours. In a study of 146 patients with hypertension (95 < diastolic blood pressure [DBP] < 115mmHg), trough level blood pressure control was consi-
ref. 6 dered inadequate in 56% of subjects after 4 weeks of treatment. An unspecified number of these patients became adequately controlled after increasing the dosing frequency to 1 mg twice daily. This dosage regimen is less acceptable during chronic treatment, while on the other hand elevating the dose of once-daily administration may increase the incidence of peak concentration-
ref. 7 related side-effects, such as sedation and dry mouth (xerostomia).

A sustained release formulation of the drug could maintain plasma levels in between a minimum effective (anti-hypertensive) concentration and a maximum non-sedative peak level. In addition to the plasma concentrations, the rate of increase of concentration may also influence the effect. The
ref. 8 classic example is provided by Kleinbloesem et al who demonstrated that a high rate of increase of nifedipine concentrations did not lead to a blood pressure reduction in healthy volunteers, contrary to a low rate of increase of nifedipine concentrations. However, a previously performed study showed no influence of the rate of infusion of rilmenidine on both blood pressure and
ref. 9 development of side-effects. The current study was part of several investi-
ref. 9-10 gations aimed at the design of an optimal slow release profile. The present study aimed to establish minimum anti-hypertensive 24 hour trough concentrations in hypertension, and to identify pharmacokinetic / pharmacodynamics relationships that could aid in the design of an optimal controlled release concentration profile. The low profile had a plateau phase with estimated minimum effective concentrations. The high profile was designed to reach a plateau phase with estimated maximum tolerated concentrations, and a minimum effective trough level.

Methods

Design

This was a placebo controlled, randomised, double-blind, double-dummy, single-dose, two-way partial cross-over, monocentric study in subjects with hypertension, with a minimum washout period of four days.

Subjects

Hypertensive subjects, treated with a maximum of two different anti-hypertensive drugs, gave signed informed consent to participate in this non-therapeutic study. Patients were included after their treating physicians were informed. After a general health screen (during which relevant additional conditions were excluded, including causes for secondary hypertension or hypertensive complications) all anti-hypertensive agents were withdrawn (gradually in the case of ß blockers), while blood pressure was monitored regularly. After return of diastolic blood pressure (DBP) to values between 95-115 mmHg, patients were scheduled for their first study occasion within five days following the detection of hypertension. Subjects who remained normotensive or whose blood pressure were higher than 115 mmHg on two consecutive occasions or once above 120 mmHg, were referred to their treating physicians, and excluded from further participation. The study was approved by the Medical Ethics Review Board of Leiden University Medical Center, and performed according to the principles of the Helsinki Declaration.

Treatments

Hypertensive patients were randomised to receive two of three possible 12-hour infusion regimens, each consisting of a loading phase (2 h) and a maintenance phase (10 h):

Low Profile infusion: (estimated minimum effective plateau phase of 2-3 µg/L): a constant rate infusion (14 mL/h) of a 25.3 mg/L rilmenidine solution, 0.35 mg/h over 2 hours followed by a ten hour constant rate infusion (3.0 mL/h) of the 25.3 mg/L rilmenidine solution, 0.075 mg/h. The total dose of rilmenidine infused was 1.45 mg. The infusion regimen was modelled, based on the maintenance of an estimated minimum effective peak level of approximately 2.9 µg/L for ten hours, leading to a trough concentration of approximately 1.0 µg/L after 24 h.

High Profile infusion: (estimated maximum tolerated plateau phase of 6-7 µg/L): a constant rate infusion (14 mL/h) of a 56.6 mg/L rilmenidine solution, 0.8 mg/h over 2 hours followed by a ten hour constant rate infusion (3.0 mL/h) of the 56.6 mg/L rilmenidine solution, 0.17 mg/h. The total dose of rilmenidine infused was 3.3 mg. The infusion regimen was modelled, based on the maintenance of an estimated maximum tolerated peak level of approximately 6.5 µg/L for ten hours, and an estimated minimum effective trough concentration of approximately 2.3 µg/L after 24 h.

Placebo infusion: a constant rate infusion (14 mL/h) of sodium chloride, 0.9% over 2 hours followed by a ten hour constant rate infusion (3.0 mL/h) of NaCl 0.9%. The total volume of NaCl 0.9% infused per subject was 58 ml.

A syringe infusion pump (Harvard, model 22 Harvard Electronics, Southnatick, Mass, USA) was used to infuse one syringe of 40 ml during the loading phase. During this time, a one-lead telemetric ECG-recording was obtained. Subsequently, a volumetric infusion pump (Sigma 6000+, Stöpler Instrumenten & Apparaten B.V., Utrecht, The Netherlands) was used to administer during the maintenance phase from 2 to 12 hours. The infusion syringes and bottles were connected to the iv cannula via a line that was primed with the rilmenidine solution prior to the start of the infusion.

Hæmodynamics

Blood pressure and heart rate were measured with an automated blood pressure monitor (MPV1072, Nihon Kohden, Japan), which displays an average value for two sequential (duplicate) measurements at each time point. All measurements were made after the subject had been in a semi-recumbent position for at least 5 minutes.

Visual Analogue Scales

ref. 11
ref. 12
ref. 13

Visual analogue scales (VAS) as originally described by Norris were previously used to quantify subjective effects of benzodiazepines. From the set of sixteen lines three factors were derived as described by Bond and Lader, corresponding to alertness, mood and calmness. These factors were used to quantify subjective drug effects.

Salivary Flow

Saliva flow was estimated by measuring the weight increase of three dental rolls put into the oral cavity over a period of 3 minutes. The dental rolls and accompanying collection tubes used for this measurement were Sarstedt neutral Salivettes® (Sarstedt, Etten Leur, The Netherlands). For each measurement three dental rolls and a collection tube were weighed together. Subsequently, one roll was placed sublingually and the other two rolls were

positioned between each lower gum and cheek. After 3 minutes, the dental rolls were immediately put in their collection tubes and weighed later on the same day.

Telemetric electrocardiography

During the first hour of the infusion, a one-lead telemetric ECG-monitoring was performed using the Nihon Kohden Lifescope II telemetric recording system (Nihon Kohden Europe, Amsterdam, The Netherlands).

Measurement times

For the first fifteen hours after the start of the infusion and 23 and 24 hours, drug plasma concentrations, adverse events, blood pressure and heart rate, and visual analogue scales were measured every hour. An additional blood sample was obtained after 33 h. Salivary flow was determined at 0, 1, 2, 4, 8, 12 and 15 hours.

Analyses

PHARMACODYNAMICS Pharmacodynamic parameters were compared between treatments by calculating the area under the effect curve over 0-15hrs (using the linear trapezoidal rule on protocol times) and dividing this area by the corresponding time interval. The result is a weighted average response.
These areas-under-the-effect-curves (AUECS) were compared between treatments using an analysis of variance for cross-over design taking into account treatment, period and subject effects. Carryover was assumed absent because of the sufficiently long washout period. Treatment response was quantified using Least Square Means with associated standard errors. Contrasts between the treatments were calculated within the ANOVA model and are reported with 95% confidence intervals. Calculations were performed using SAS for Windows V6.10 (SAS Institute, Inc., Cary, NC, USA) and SPSS for Windows V10.0.7 (SPSS, Inc., Chicago, IL, USA).

PHARMACOKINETICS Rilmenidine plasma levels were measured by using a gas chromatographic/mass spectrometric method. The limit of detection was 0.3 ng/ml and the linearity of the assay has been checked over

ref. 14

a range of 0.3 - 2 ng/ml. The average assay precision (coefficient of variation) is approximately 7% while the average assay accuracy (percentage of error) is 4%. The rilmenidine pharmacokinetics were described using a two-compartment model with constant coefficient of variation intra-individual error, using NONMEM version V (GloboMax LLC, Hanover, Md), applying the first order conditional estimation (FOCE) method with the 'interaction' option.

PHARMACOKINETICS/PHARMACODYNAMICS (PK/PD)

PK/PD modelling with SBP, DBP and salivary flow as effect measures was performed. The average placebo profiles for these measures indicated a clear placebo response. Placebo correction was therefore implemented by subtracting the average placebo profile from the active treatment profiles at corresponding timepoints. The empirical Bayes estimates from the PK analysis were used to generate predicted rilmenidine concentrations. For each timepoint the estimated rilmenidine concentration was plotted against the placebo corrected pharmacodynamic response. A linear concentration-effect model was estimated because individual and average graphs did not suggest any other model. Hysteresis was not apparent and therefore a direct concentration-effect relationship was assumed. Estimates were obtained for slopes and intercepts for the low treatment with difference estimates for the high treatment. A common additive between subject variability was assumed for both treatments. Residual variability was also assumed additive. First-order conditional estimation (FOCE) was used and 95% confidence intervals (95% CI) for the difference estimates between low and high treatment were calculated using population mean $\pm$ 2 times the approximate standard error as obtained from the analysis. Data management was performed using SPSS for Windows V10.0.7 (SPSS, Inc., Chicago, IL, USA).

Results

Subjects

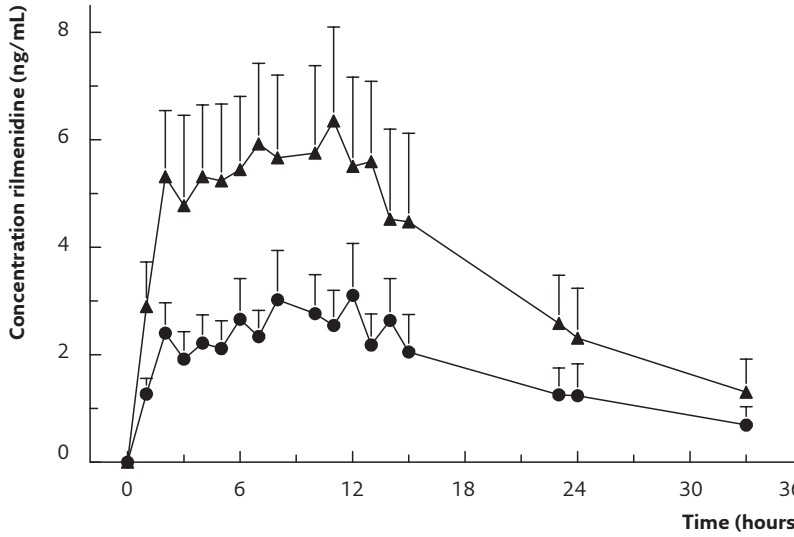
Forty-five subjects gave written informed consent for participation in the study. Six subjects did not comply with the screening criteria: five for obesity and one for use of more than two anti-hypertensives. After antihypertensive withdrawal, eleven subjects did not comply with the inclusion criteria: seven kept DBP below 95 mmHg, one withdrew for personal reasons, and three developed DBP above 120 mmHg. Twenty-eight Caucasian subjects were included in the study (18 males, 10 females) after withdrawal of co-medication for 1-6 weeks. These subjects were 53.7 years of age (range 38-65

years), with an average weight of 83.4 kg (range 59-104.5 kg) and an average height of 173.9 cm (range 161-190.4 cm). The average blood pressure at inclusion was 175/102 mmHg (ranges SBP 155-192, DBP 96-114 mmHg). From the 28 included patients, two subjects dropped out. One subject took disallowed concomitant medication throughout the study and the other subject withdrew for personal reasons. The study population therefore comprised twenty-six (26) completed and analysed subjects.

Pharmacokinetics

The time-concentration profiles for the two infusions are shown in Figure 1. Average clearance was 23.7 L/hr with a standard error of the mean (SEM) of 1.25 L/hr and an inter-individual variability as coefficient of variation (IICV) of 33%. Central volume was estimated as 213 L (SEM 33.3 L, IICV 27%) and the peripheral volume was 104 (SEM 39.4 L, IICV 49%). The residual error was 11.6%.

FIGURE 1 **Time-concentration profiles (Mean + SD) for low- (●) and high profile (▲) rilmenidine infusions**



Hæmodynamics

The overall responses of the hæmodynamic parameters represented by time-corrected AUECS for the 0-15h period and contrasts between the three treatments are shown in table 1. Average time effect curves are shown in figure 2. No significant effects were observed for heart rate.

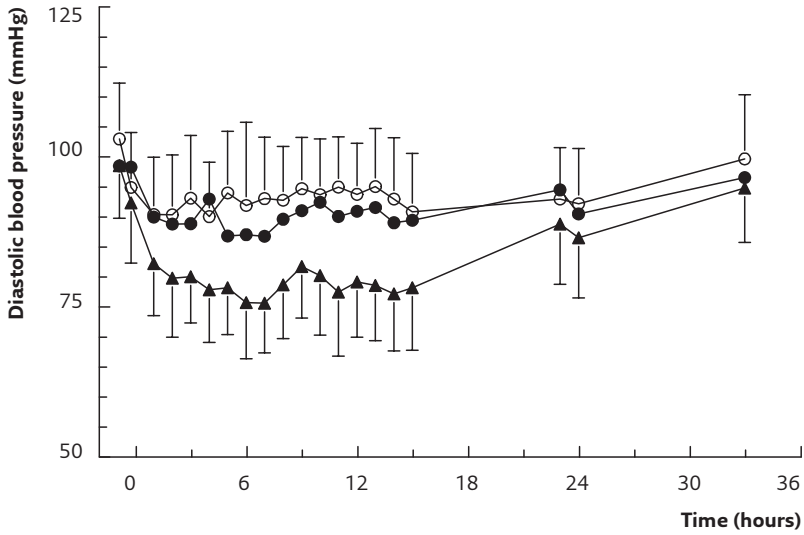
TABLE 1 **Time-corrected AUECS (0-15 h) for all pharmacodynamic parameters: Least mean square means (LSM), standard errors (SE) and contrasts (Delta) with 95% confidence intervals (95% CI) between treatments (* p<0.05)**

Parameter	Low infusion (n=19)		High infusion (n=20)		Placebo (n=13)		High vs Placebo	Low vs Placebo	High vs Low
	LSM	SE	LSM	SE	LSM	SE	Delta (95% CI)	Delta (95% CI)	Delta (95% CI)
Systolic BP (mmHg)	155.1	1.63	138.6	1.57	161.3	2.14	-22.7 * (-28.5, -16.9)	-6.2 * (-12.1, -0.3)	-16.6 * (-21.3, -11.8)
Diastolic BP (mmHg)	89.5	0.94	79.7	0.91	92.8	1.23	-13.1 * (-16.5, -9.8)	-3.3 (-6.7, 0.1)	-9.8 * (-12.6, -7.1)
HR (bpm)	66.7	0.86	64.2	0.83	65.4	1.12	-1.2 (-4.2, 1.9)	1.3 (-1.9, 4.4)	-2.4 (-5.0, 0.1)
VAS alertness (mm)	69.8	1.54	70.1	1.48	72.1	2.01	-2.0 (-7.4, 3.4)	-2.2 (-7.8, 3.3)	0.2 (-4.3, 4.7)
VAS mood (mm)	76.8	1.24	79.1	1.20	78.8	1.62	0.3 (-4.1, 4.7)	-2.0 (-6.5, 2.5)	2.2 (-1.4, 5.9)
VAS calmness (mm)	78.1	1.16	79.9	1.12	78.7	1.52	1.1 (-3.0, 5.2)	-0.6 (-4.8, 3.6)	1.7 (-1.7, 5.1)
Salivary flow ($mL \cdot 3min^{-1}$)	1.89	0.11	1.50	0.11	2.37	0.15	-0.87 * (-1.27, -0.48)	-0.48 * (-0.89, -0.07)	-0.39 * (-0.72,-0.07)

The average baseline blood pressures were hypertensive and quite similar among the three treatment groups. At baseline, the average SBP/DBP varied between 161.5/92.6 and 168.3/98.3 mmHg. Overall blood pressures (time-corrected AUECS 0-15h) diminished slightly during placebo, to average values of 161.3 ± 2.1 mmHg systolic, and 92.8 ± 1.2 mmHg diastolic (least square means and standard errors). Slightly stronger overall blood pressure reductions occurred with the low profile infusion, to 155.1 ± 1.6 mmHg for systolic and 89.5 ± 0.9 mmHg for diastolic blood pressure. The effects of the high profile infusion regimen were much stronger: the average systolic values over the 0-15h period dropped to 138.6 ± 1.6 mmHg, and the diastolic pressure to 79.7 ± 0.9 mmHg. The contrasts between the different treatments showed

statistically significant differences between placebo on the one hand and the low and the high profile rilmenidine treatments on the other; and between the two active treatments. No differences in heart rate response were found between the three treatments.

FIGURE 2 **Time-effect profiles (Mean + SD) for diastolic blood pressure for placebo- (○), low- (●) and high profile (▲) rilmenidine infusions**



The high profile infusion regimen resulted in good blood pressure control over the 0-15h period, but blood pressure slowly increased while the subjects remained in the research unit over the next nine hours. As shown in figure 2, the average blood pressures were still reduced twenty-four hours after the start of the placebo infusion, compared to the baseline values: systolic values were 162.8 ± 3.2 mmHg and diastolic pressure was 92.6 ± 2.0 mmHg (least square mean and standard error). The effect of the high profile infusion regimen after 24 hours differed significantly from placebo: blood pressure was 152.4 ± 2.5 mmHg systolic and 86.8 ± 1.5 mmHg diastolic, and the contrast with placebo was 10.4 (1.4, 19.4) mmHg and 5.8 (0.3, 11.3) mmHg, respectively (difference and 95% confidence interval). The average blood pressure at 24 hours after the start of the low profile infusion was 157.2 ± 2.4 mmHg systolic and 89.8 ± 1.5 diastolic, which did not differ significantly from the two other treatments. The difference from placebo was 5.7 (-3.3, 14.6) mmHg systolic and 2.8 (-2.7, 8.3) mmHg diastolic.

Pharmacokinetics/pharmacodynamics (PK/PD)

PK/PD parameter estimations for SBP, DBP and salivary flow are represented in table 2. The average placebo-corrected PK/PD relationships are presented in figure 3 for DBP and figure 4 for salivary flow. All three parameters (SBP, DBP and salivary flow) showed linear concentration-effect relationships. No significant differences were observed between the low and the high infusion for both slopes and intercepts for all effect parameters.

TABLE 2 **PK/PD parameters using empirical Bayes estimates vs placebo corrected diastolic blood pressure and salivary flow. Population average (Mean), standard error of the population average (SEM), 95% confidence intervals (95% CI) and inter-individual variability as standard deviation (IISD)**

Parameter		Mean	SEM	95% CI	p	IISD
Systolic blood pressure						
Intercept (mmHg)	Low	3.85				11.9
	High	0.23				11.9
	Difference	-3.62	4.71	-13.1, 5.8	NS	
Slope (mmHg)/(ng/mL)	Low	-3.01				2.17
	High	-4.27				2.17
	Difference	-1.26	1.28	-3.8, 1.3	NS	
Residual error (SD; mmHg)	11.3					
Diastolic blood pressure						
Intercept (mmHg)	Low	0.243				
	High	-2.38				1.15
	Difference	-2.62	2.77	-8.2, 2.9	NS	1.15
Slope (mmHg)/(ng/mL)	Low	-1.39				
	High	-2.23				0.00
	Difference	-0.837	0.736	-2.3, 0.6	NS	0.00
Residual error (SD; mmHg)	6.69					
Salivary flow						
Intercept (mL/3min)	Low	-0.011				
	High	-0.173				
	Difference	-0.162	0.559	-1.3, 1.0	NS	
Slope (mL/3min)/(ng/mL)	Low	-0.247				
	High	-0.152				
	Difference	0.0948	0.105	-0.12, 0.30	NS	
Residual error (SD; mL/3min)	0.682					

FIGURE 3 **Concentration rilmenidine-diastolic blood pressure profiles (Mean + SD) for low- (●) and high profile (▲) rilmenidine infusions**

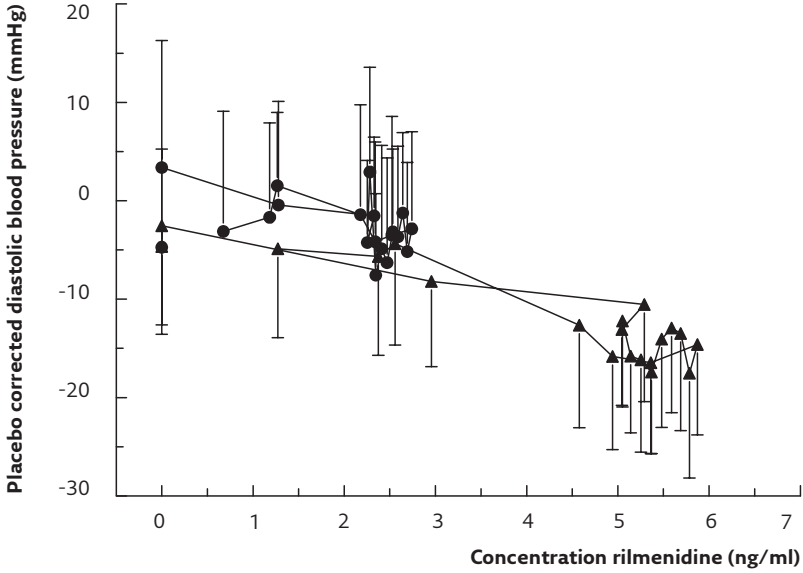
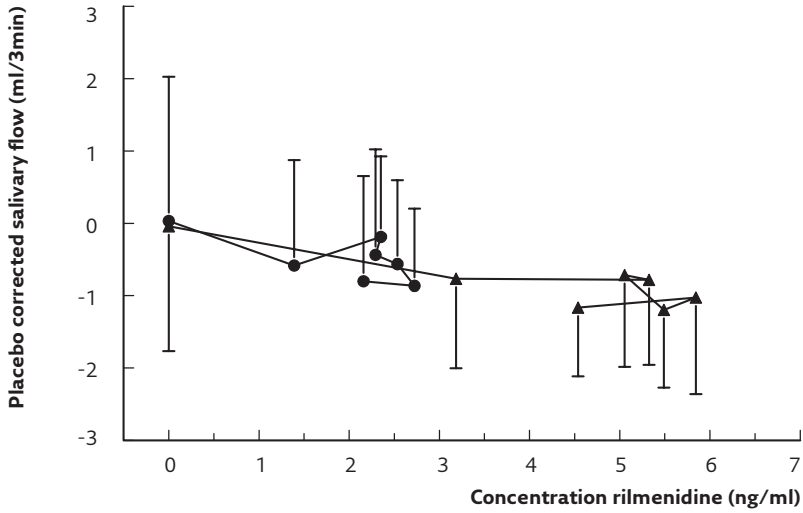


FIGURE 4 **Concentration rilmenidine-saliva production profiles (Mean + SD), low- (●) and high profile (▲) rilmenidine infusions**



Safety

No serious adverse events occurred during the study.

SEDATION, ALERTNESS, MOOD OR CALMNESS The contrasts between the different treatments on visual analogue scales are shown in table 1. There were no significant differences between the placebo-, the low profile- or the high profile infusions for the three different scores derived from the visual analogue scales. The visual analogue scale scores during the placebo-infusion were quite variable, which precluded the demonstration of any effects of rilmenidine. Sedation was reported by 21 of the 26 subjects during any stage of the study since selection. Mild sedation was reported by 4/13 subjects during the placebo occasion (31%), and by 13/19 and 13/20 subjects during the low and high profile rilmenidine treatments (65-68%). Seven subjects reported sedation on both rilmenidine occasions. Three subjects reported sedation during one of the rilmenidine infusions and placebo infusion (one during the low profile infusion and placebo infusion, one during the high profile infusion and the placebo infusion and one during the high profile infusion, the placebo infusion and the pre-dosage period). Nine subjects reported sedation on one of the two rilmenidine occasions (five subjects during the low profile infusion and four subjects during the high profile infusion).

DRY MOUTH The overall responses on the salivary flow represented by time-corrected areas-under-the-effect-curves (AUECS) for the 0-15h period and contrasts between the three treatments are shown in table 1. The contrast between the different treatments indicated that the average saliva production differed significantly between the placebo infusion, the low profile infusion and the high profile. The average saliva production over the 0-15h period was 1.50 ± 0.11 ml·3min^{-1} with the high profile (-36%), and 1.89 ± 0.11 ml·3min^{-1} with the low profile infusion (-20%), compared to 2.37 ± 0.15 ml·3min^{-1} with placebo (mean $\pm$ standard error). Spontaneous reports of dry mouth (xerostomia) were only slightly more prevalent with rilmenidine (on 4/19 low and 5/20 high profile occasions, 21 and 25%, respectively) than with placebo (on 2/13 occasions, 15%).

HEADACHES Headaches occurred frequently during all stages of the study. Three subjects had headaches during the placebo occasion, but two of these also had headaches during the pre-treatment and washout periods. During the low profile infusion, 13 subjects developed headache, three of whom had similar complaints before and after treatment, and one only

during the washout period. Five patients had headaches with the high profile treatment, two of whom also complained of headache during either the pre-treatment or the washout period.

TELEMETRIC ELECTROCARDIOGRAPHY No clinically significant ECG-changes or heart rate changes were noted by telemetric ECG monitoring in any patient, during the first hour of the rilmenidine- or placebo-infusions.

Discussion

Both rilmenidine treatments produced demonstrable anti-hypertensive effects. The low infusion profile was aimed to maintain a 10-hour plateau at estimated minimum effective concentrations for around 10 hours. Even so, a mean hypotensive effect of 5.7/2.8 mmHg still existed twelve hours after the low dose infusion was stopped (24 hours after the start of the infusion), although the differences form placebo were not statistically significant. These results are difficult to compare to the clinical effects of anti-hypertensive agents, because clinical trials usually deal with prolonged treatment, and blood pressure reduction may gradually develop over time. The Veteran Affairs Cooperative Study Group on Anti-hypertensive Agents for instance reported systolic/diastolic changes from baseline in the order or 11-16/10-12 mmHg after six weeks of treatment with a variety of anti-hypertensive drugs. Compared to a blood pressure reduction of 5/3 mmHg with placebo in the same study, this would indicate a net chronic anti-hypertensive effect in the order of 6-11/7-9 mmHg, compared to an acute reduction of 6.2/3.3 mmHg (on average over 24 hours), found in the present study after short-term infusion of a low dose of rilmenidine.

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The effects of the high profile rilmenidine infusion were more pronounced. Average blood pressures of 138.6/79.7 over the fifteen-hour period after start of the infusion are virtually normotensive. Compared with the effects of placebo, an average anti-hypertensive effect of 10.4/5.8 mmHg still existed 12 hours after infusion was stopped (24 hours after the start of the infusion) and continued to be statistically significant. The average blood pressure of 152.4/86.8 mmHg at this time was borderline hypertensive on systolic blood pressure, and 5/19 patients were still normotensive (defined as a blood pressure below 140/90), compared to 1/13 with placebo and 2/19 after the low rilmenidine profile infusion. Assuming that blood pressure would improve further with prolonged treatment, as seen with most anti-hypertensives, it seems that the high profile dose of 3.3 mg of rilmenidine

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may be efficacious in most patients. This dose is higher than the currently recommended dose of 1-2 mg/day. Obviously, this would need to be confirmed in clinical studies, because the effects may still increase during prolonged treatment.

The occurrence of side effects could pose limitations on the administration of higher doses of rilmenidine treatment. Nevertheless, salivary flow displayed a dose-related reduction, and the time-effect profiles corresponded linearly to the average predicted time-concentration profiles as shown in figure 4. The clinical relevance of these findings is uncertain, since spontaneous reports of dry mouth were only slightly more prevalent. Mild sedation was more often reported during the low and high profile rilmenidine treatments compared to placebo but this difference between placebo and rilmenidine did not recur in the VAS scores measuring alertness, mood or calmness. This was largely attributable to a larger than expected variability of VAS-scores, as shown by the wide variety in responses during placebo treatment. All subjects received standardised instructions about the visual analogue scales, but there may have been a partial lack of understanding of the purpose of the scales. This instrument has been developed for drug studies in young healthy volunteers, who form a generally well-educated, co-operative and homogeneous group. Patients not only differ widely in social and educational background, but also in their perception of the study. The patients' preoccupation with the therapeutic effects of the drug during the study is likely to have influenced their capability and motivation to adopt the self-reflective attitude needed to fill in the visual analogue lines. The influence of circumstances on the sensitivity of visual analogue scales is well-known: healthy subjects readily reported the sedative effects of diphenhydramine 25 mg on visual analogue scales in the laboratory, whereas the same subjects did not indicate any sedation on the scales with doses below 100 mg during a driving test. At least for α_2-adrenoceptor agonists like clonidine, this apparent methodological discrepancy may be due to the fact that the attenuation of attention under resting condition caused by clonidine is overcome by arousal. Methods other than subjective assessments, such as saccadic eye movements where circumstances are kept more constant, are more sensitive to sedation than visual analogue lines, and showed linear concentration-effect relationship up to concentrations of 8.43 ng/ml in healthy volunteers. However, the influence of arousal on attention could also indicate that reduction of saccadic peak velocity under laboratory conditions would overestimate the level of sedation during clinical treatment, *i.e.* under every-day circumstances. Also, tolerance to sedation may develop during chronic treatment, which was not assessed in this single repeated dose study.

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The results of this study combined with additional investigations of the effects of different infusion rates on blood pressure, salivary flow and saccadic peak velocity in healthy volunteers and the evaluation of a sustained release profile *in vivo* are very helpful in assessing the benefits of a controlled release formulation in an early stage of drug development. The data from the current study suggest that a sustained mean plasma concentration of rilmenidine circa 4-6 ng/mL will be needed to permit once daily monotherapy with rilmenidine and to achieve modern therapeutic goals of SBP<140 and DBP<80 mmHg. Further multiple dose studies of oral sustained release tablets in patients should be performed to confirm adequate sustained blood pressure control. Furthermore, potential tolerance to side effects over an extended treatment period should be investigated to define the optimal therapeutic window of a new sustained release formulation.

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CHAPTER 8

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Pharmacokinetic / pharmacodynamic assessment of tolerance to central nervous system effects of a 3 mg sustained release tablet of rilmenidine in hypertensive patients

Abstract

AIMS Previous single dose studies showed clear blood pressure lowering effects of a potential sustained release profile rilmenidine, with concentration dependent effects on the central nervous system. The aim of this study was to evaluate potential changes in concentration-effect-relationships for these central nervous system effects during a 4-week treatment period with an experimental sustained release (sr) formulation of rilmenidine 3 mg once daily in mild to moderate hypertensive patients.

METHODS Fifteen mild to moderate hypertensive patients were withdrawn from their own anti-hypertensive treatment (gradually in the case of beta-blockers) and switched immediately to a 4 week rilmenidine SR treatment. The central nervous system effects of the treatment were evaluated using saccadic eye movements for sedative effects and visual analogue scales for subjective effects on alertness, mood and calmness. Measurements for pharmacokinetic (PK) and pharmacodynamic (PD) evaluations were performed on the first day of the treatment period and repeated after one week and four weeks of treatment.

RESULTS No serious or severe adverse events were reported. Blood pressure control remained adequate. Drug concentrations increased during the study, whereas treatment related reductions in saccadic peak velocity (SPV) remained similar on all three study days. The slopes of the concentration-effect-curves for SPV remained unchanged throughout the study, while the intercepts tended to increase as a result of increased pre-dose values. Similar effects were observed for visual analogue scales for alertness: pre-dose values increased significantly during the study, while the size of the treatment responses (slopes) remained unaltered.

CONCLUSIONS Four-week treatment with rilmenidine SR 3 mg OD produced slight adaptations to drug-induced cns-effects. The reasons for these adaptations cannot be determined but may include drug tolerance and habituations to study procedures. Blood pressure control remained stable and adequate throughout the study.

Introduction

Rilmenidine (2-(dicyclopropylmethyl)-amino-2-oxazoline) is a centrally acting anti-hypertensive with binding selectivity to the I1 imidazoline receptors over α2-adrenoceptors. It has dose (concentration)-dependent blood pressure lowering effects above 0.5 mg in both healthy and hypertensive subjects. Rilmenidine is registered in several European countries at a recommended dose of 1 tablet of 1 milligram once or twice daily. Clinical experience indicates that with 1 mg dosing blood pressure control might not be maintained for 24 hours per day in all patients. In a study of 146 patients with hypertension (95<DBP<115mmHg), trough level blood pressure control was considered inadequate in 56% of subjects after 4 weeks of treatment. An unspecified number of these patients became adequately controlled after increasing the dosing frequency to 1 mg twice daily. This dosage regimen is less acceptable during chronic treatment, while on the other hand elevating the dose of once-daily administration may increase the incidence of peak concentration-related side-effects, such as sedation and dry mouth (xerostomia).

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A sustained release formulation of the drug could maintain plasma levels in between a minimum effective (anti-hypertensive) concentration and a maximum non-sedative peak level. In addition to the plasma concentrations, the rate of increase of concentration may also influence the effect. The classic example is provided by Kleinbloesem et al who demonstrated that a high rate of increase of nifedipine concentrations did not lead to a blood pressure reduction in healthy volunteers, contrary to a low rate of increase of nifedipine concentrations. However, a previously performed study showed no influence of the rate of infusion of rilmenidine on both blood pressure and central nervous system effects (visual analogue scales and saccadic eye movements). The current study was performed after several investigations aimed at the design of an optimal slow release profile. From these studies, it was concluded that a 3 mg sustained release formulation would have the optimal profile for adequate blood pressure control with an improved side-effect profile. Since many centrally active drugs show some tolerance development to side effects during prolonged treatment, the current study aimed to investigate the effects of four-week treatment with a 3 mg sustained release formulation on the PK/PD relationships between rilmenidine plasma concentrations and central nervous system effects (saccadic eye movements and visual analogue scales).

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Methods

Design

This was a phase II single centre open non-controlled study without direct individual benefit for patients. Screening assessment took place within 17 days prior to the rilmenidine treatment. Patients were acquainted with the experimental methods and conditions in a short training session taking place within one week prior to rilmenidine treatment. Eligible patients were then withdrawn from their own anti-hypertensive treatment and switched directly into a 4-week 3 mg o.d. rilmenidine SR treatment. The withdrawal was gradual for beta-blockers, and immediate for other anti-hypertensive agents, but in all cases as short as possible in order to prevent loss of blood pressure control. Measurements for PK/PD evaluation were performed on the first day (D1-D2) of the rilmenidine treatment period and repeated after one week (D8) and four weeks (D29) of treatment. At the end of the 4-week rilmenidine treatment period, patients were re-allocated to their own anti-hypertensive treatment.

Subjects

Mild to moderately hypertensive subjects (males and females), treated with a maximum of two different anti-hypertensive drugs, gave signed informed consent to participate in this study. After a general health screen (during which relevant additional conditions were excluded, including causes for secondary hypertension) eligible patients were enrolled in the study.

Treatments

Rilmenidine Sustained Release (SR) was presented as white round-shaped film coated tablets containing 3 mg of active medication. Patients were requested to take one tablet of rilmenidine every morning under fasting conditions, with approximately 150 ml of water, 30 minutes before breakfast time. Patients were instructed to take their study medication regularly (between 07:00 and 09:00 h in the morning). Patients were instructed to maintain a diary, where intake of study medication was to be recorded.

Hæmodynamics

Blood pressures were measured after the patient had been sitting quietly for at least 10 minutes, pre-dose and repeatedly post-dose on each of the three study days. All measurements were carried out with an automated sphygmomanometer Nihon Kohden MPV 1072.

Visual Analogue Scales

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Visual analogue scales as originally described by Norris have been used previously to quantify subjective effects of benzodiazepines. From these scales, three factors can be derived as described by Bond and Lader corresponding to alertness, mood and calmness. These visual analogue scales were practiced at a training session (three times), and measured pre-dose and every hour for twelve hours after dosing, on each of the three study days.

Saccadic eye movements

ref 8, 14

Saccadic eye movements have been used previously to quantify drug effects of rilmenidine and clonidine. Saccadic eye movements were practiced at a training session (three times), and measured pre-dose and every hour for twelve hours after dosing, on each of the three study days, with an additional measurement after 24 hours for the first dosing. Recording of eye movements was performed in a quiet room with ambient illumination. There was only one patient per session in the same room. Recording and analysis of saccadic eye movements was conducted with a microcomputer-based

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system for sampling and analysis of eye movements. The equipment used for stimulus display, signal collection and amplification was from Nihon Kohden (Nihon Kohden Corporation, Tokyo, Japan). Disposable silver-silver chloride electrodes (Medicotest N-00-s, Olstykke, Denmark) were applied on the forehead and beside the lateral canthi of both eyes of the patient for registration of the electro-oculographic signals. Skin resistance was reduced to less than 5 kOhm before application of the electrodes. Head movements were restrained using a fixed head support. The target consisted of an array of light emitting diodes on a bar, fixed at 50 cm in front of the head support. Saccadic eye movements were recorded for stimulus amplitudes of ± 15 degrees to either side. Fifteen saccades were recorded for each stimulus amplitude with interstimulus intervals varying randomly between 3 and 6

seconds. Average values of latency (i.e. reaction time), saccadic peak velocity and inaccuracy of all artifact-free saccades were used as parameters. Saccadic inaccuracy was calculated as the absolute value of the difference between the stimulus angle and the corresponding saccade, expressed as a percentage of the stimulus angle.

Blood sampling

Patients were randomly allocated to one of the following investigation schedules for pk/pd evaluation:

Days 1-2:
- Schedule 1: pre-dose and 1, 4, 7, 10 and 24 h after dosing or
- Schedule 2: pre-dose and 2, 5, 8, 11 and 24 h after dosing or
- Schedule 3: pre-dose and 3, 6, 9, 12 and 24 h after dosing.

Day 8 and Day 29
- Schedule 1: pre-dose and 1, 4, 7 and 10 h after dosing or
- Schedule 2: pre-dose and 2, 5, 8 and 11 h after dosing or
- Schedule 3: pre-dose and 3, 6, 9 and 12 h after dosing.

Blood samples for rilmenidine assay (9 ml) were obtained in lithium heparin-containing polypropylene tubes. Blood samples were drawn from an IV cannula (inserted into the arm opposite to the one where blood pressure was measured) which was kept patent using a heparin-NaCl solution. Blood samples will be taken after discarding the contents of the cannula. At the 24 hour time point on day 2, blood was collected using a vacuette with a venapuncture.

Analyses

ref 16

PHARMACOKINETICS Rilmenidine plasma levels were measured by using a gas chromatographic/mass spectrometric method. Q Rilmenidine PK was modelled using a one-compartment model with first order absorption and a lag-time using NONMEM Version V software (NONMEM Project Group, UCSF, San Francisco, CA, USA) using the first order conditional estimation method with interaction. Residual error was modelled as a combination of a constant coefficient of variation component and an additive component. Individual empirical Bayes estimates for absorption half-life, elimination half-

life, clearance and lag-time were determined for all occasions separately, and predicted individual rilmenidine concentration profiles were obtained using these estimates.

PHARMACODYNAMICS Areas under the curve were calculated for saccadic eye movement data and visual analogue scale scores using the linear trapezoidal rule on (expected) protocol times. These AUECS were subsequently divided by the corresponding time span resulting in a weighted average response. Additionally, the minimum measurement was determined with the associated actual time point for parameters with a clear response. In the case of multiple minima, the first occurrence was taken. No corrections for baseline response were implemented for either AUECS or E_{min}.

Response measurements (AUEC, E_{min}, T_{min}) were compared between the 3 days using paired Student's t-tests without correction for multiple comparisons because of the limited number (3) of contrasts and because all contrasts are sensible and clearly address the main objectives of the study.

PHARMACOKINETICS/PHARMACODYNAMICS (PK/PD) Using the predicted rilmenidine concentrations, a linear concentration-effect model with additive residual error was applied to saccadic peak velocity and VAS Alertness scores without use of an effect compartment, because individual graphs did not indicate the need for a more complex model (*eg* delay or non-linearity in the concentration-effect relationship). Parameter estimates for slopes and intercepts were obtained using NONMEM with the first order conditional estimation method. Estimates were obtained for the parameters on day 1 and changes were estimated from the day 1 value to the day 8 value and from the day 1 value to the day 29 value. Significance of changes was assessed by calculating 95% confidence intervals for the difference estimates using 2 times the reported standard error of the estimates.

Data management and additional calculations were performed using SAS for Windows V8.2 (SAS Institute, Inc., Cary, NC, USA).

Results

Subjects

Fifteen (7 male, 8 female) hypertensive patients were randomised to one of the blood sampling schedules. The age ranged from 41 to 65 years with a

mean of 51.3 (SD 7.2) years. All patients had mild to moderate essential hypertension, and received one or two anti-hypertensive agents (ACE inhibitors 33.3%, beta-blockers 26.7%, diuretics 26.7 %, angotensin II inhibitors 26.7 %, calcium antagonists 6.7 %, combined form (beta-blocker and diuretic) 6.7 % of the study population). Two patients suffered from arthrosis and these patients used allowed concomitant medication (ibuprofen 400 mg prn).

Safety assessments

No serious or severe adverse events were reported. The most frequently reported adverse events were sleepiness, dry mouth and headache, which occurred in 93%, 60% and 46.7% of the patients, respectively. Most adverse events were of a mild intensity. No clinically significant abnormalities were found for any of the safety laboratory measurements.

Pharmacokinetics

The concentration-time profile on days 1, 8 and 29 are represented in figure 1. The following population mean (approximate standard error of population mean (SEM)) pharmacokinetic parameters were estimated: elimination half life 567 min (72.0 min, inter-individual coefficient of variation (IICV) 65%), absorption half life 270 min (44.7 min, IICV 81%), clearance 0.457 L/min (0.0292, IICV 40%) and a lag time of 165 min (4.61, IICV 0%).

Hæmodynamics

After patients switched rapidly from their own antihypertensive treatment(s) to rilmenidine. The mean (SD) pre-dose blood pressures on day 1 were 131.7/76.6 (SD 12.1/7.9) mmHg. On day 29, the average (SD) pre-dose systolic/diastolic blood pressure was 140.5/80.6 (SD 21.0/10.3) mmHg.

Saccadic eye movements

The effects of prolonged treatment on the AUECS of saccadic peak velocity (SPV), reaction time (RT) and inaccuracy are presented in Table 1. The average curves for saccadic peak velocity (SPV) on days 1, 8 and 29 are presented in

Figure 2. The primary endpoint of saccadic peak velocity (SPV AUEC) showed no significant changes during four weeks of treatment with rilmenidine SR 3.0 mg OD. The minimum SPV values during days 1, 8 and 29 of treatment were comparable, with average (SD) values of 413.2 (48.2), 415.4 (53.7) and 401.5 (63.0) deg/sec, respectively. Hence, these data provide no indications for tolerance development. There are clear indications for a treatment effect that is comparable among the three treatment days. The average minimum values (E_{min}) on the three treatment days all represent decreases in excess of 15%, which is well over the level of clinical significance of 10% below baseline, associated with a decrease in SPV observed after the loss of one night of sleep. The other two parameters (reaction time and inaccuracy) did not show any significant effects except for a decrease in AUEC inaccuracy for day 8 compared to day 1.

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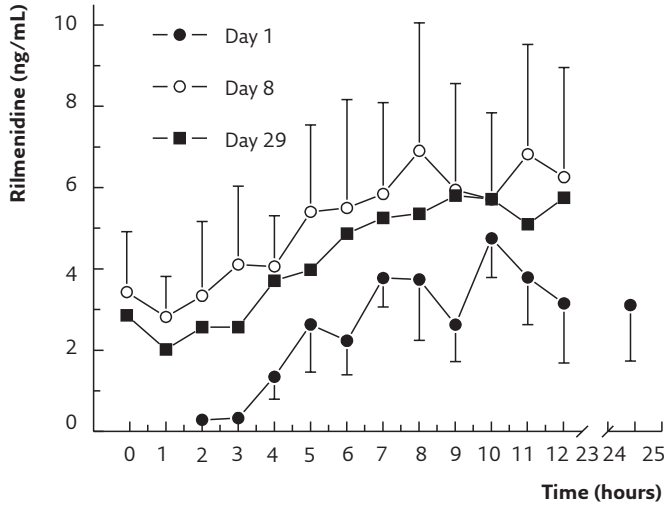
FIGURE 1 **Average rilmenidine concentration-time profiles at day 1, 8 and 29 (mean + SD)**



Visual analogue scales

The AUECS of VAS alertness, mood and calmness are represented in Table 1. The average curves for visual analogue scale alertness on days 1, 8 and 29 are presented in Figure 3. All subjective scales showed significant increases from day 1 to day 8 and from day 1 to day 29. No significant changes were observed from day 8 to day 29. The VAS baseline values all increased from day 1 to day 8 and from day 1 to day 29.

TABLE 1 **Time-corrected AUECS (0-12 h) for all pharmacodynamic parameters: Mean, standard deviations (SD) and contrasts with 95% confidence intervals (95% CI) between treatment days (* p<0.05)**

	Day 1 Mean (SD)	Day 8 Mean (SD)	Day 29 Mean (SD)	Day 1 - 8 Mean (95% CI)	Day 1 - 29 Mean (95% CI)	Day 8 - 29 Mean (95% CI)
Saccadic peak velocity (deg/sec)	458.9 (41.7)	458.9 (44.4)	456.5 (48.1)	0.0 (-12.1, 12.1)	2.4 (-17.1, 21.9)	2.4 (-13.6, 18.4)
Saccadic reaction time (msec)	236 (21)	229 (21)	231 (22)	7.1 (-0.5, 14.7)	4.7 (-3.7, 13.1)	-2.4 (-9.0, 4.2)
Saccadic inaccuracy (%)	9.46 (3.19)	8.39 (2.62)	8.56 (2.86)	1.06 (0.38, 1.75) *	0.89 (0.10, 1.69) *	-0.17 (-0.65, 0.31)
VAS alertness (mm)	72.1 (13.5)	77.4 (12.6)	78.1 (12.7)	-5.36 (-8.16, -2.56) *	-6.04 (-9.18,-2.89)*	-0.68 (-3.43, 2.08)
VAS mood (mm)	78.9 (12.0)	81.4 (10.9)	82.4 (11.7)	-2.45 (-4.23, -0.66) *	-3.52 (-5.60,-1.44)*	-1.07 (-2.82, 0.68)
VAS calmness (mm)	79.9 (7.6)	83.9 (7.4)	84.5 (6.9)	-3.95 (-5.92, -1.99) *	-4.61 (-7.25,-1.96)*	-0.65 (-2.69, 1.39)

Pharmacokinetics/pharmacodynamics (PK/PD)

PK/PD parameter estimations for VAS alertness and SPV are represented in table 2. The average PK/PD relationships are presented in figure 4 for SPV and figure 5 for VAS alertness. Both parameters (VAS and SPV) showed linear concentration-effect relationships. No significant changes in slopes between days were observed for VAS or SPV, indicating that the CNS-effect of rilmenidine per unit concentration remained unaltered. The intercept for the SPV PK/PD relationships did not change significantly from day 1 to either day 8 or day 29. The intercept of the VAS alertness scale increased significantly after day 1: the difference between day 8 and day 1 was 12.5 (4.5, 20.5) mm and the difference between day 29 and day 1 was 13.0 (2.9, 23.1) mm.

Discussion

This study was part of a series of investigations, designed for the development of an optimal controlled release formulation of the centrally active antihypertensive agent rilmenidine. Previous studies showed clear concentration dependent effects on blood pressure and the central nervous system of a potential sustained release profile of rilmenidine. Furthermore, these studies suggested that the optimal therapeutic window would be

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FIGURE 2 **Average saccadic peak velocity-time profiles at day 1, 8 and 29**

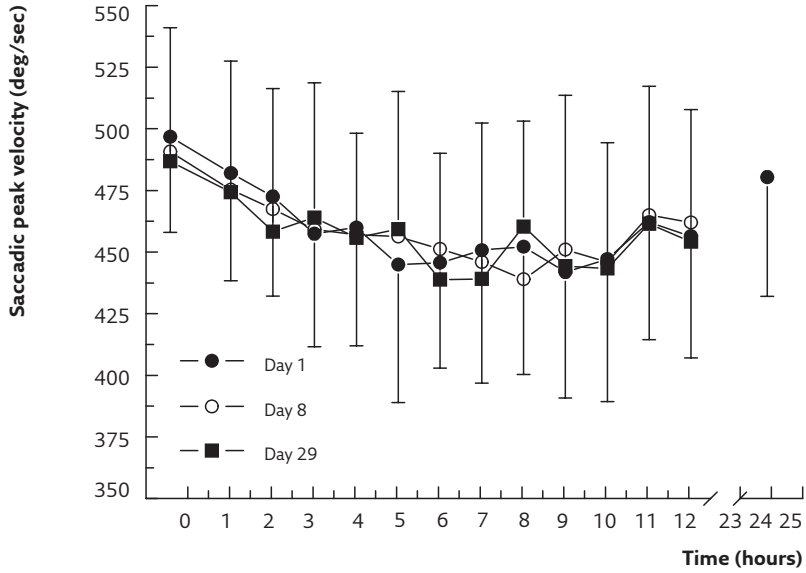


FIGURE 3 **Average visual analogue scale alertness-time profiles at day 1, 8 and 29**

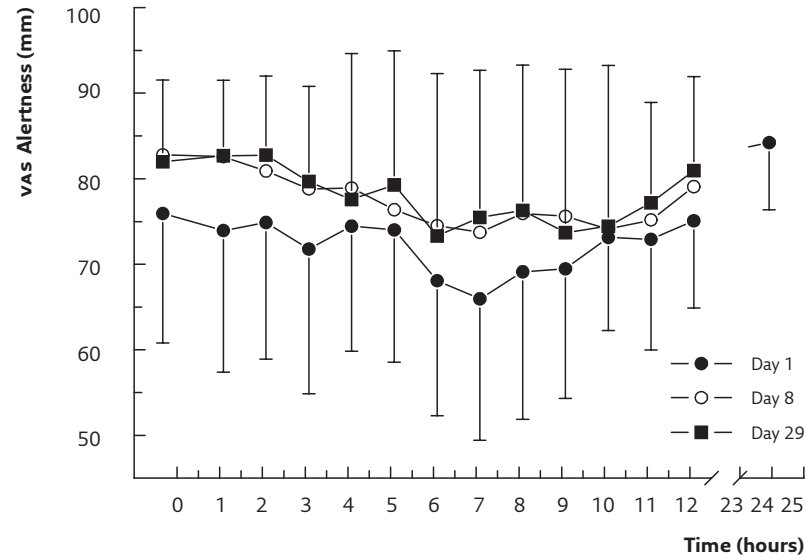


TABLE 2 **PK/PD parameters using empirical Bayes estimates for saccadic peak velocity and VAS alertness. Population average, standard error of the population average (Mean), 95% confidence intervals (95% CI) and inter-individual variability as standard deviation (IISD)**

	Mean	SEM	95% CI	IISD
Saccadic peak velocity				
Slope day 1 (deg.sec-1.ng-1.mL)	-6.66	1.63	-9.9 / -3.4	4.77
Change to day 8	-0.0870	2.35	-4.9 / 4.6	
Change to day 29	-0.286	2.96	-6.2 / 5.6	
Intercept day 1 (deg.sec-1)	476	9.69	457 / 495	37.5
Change to day 8	16.2	12.6	-9.0 / 41.4	
Change to day 29	9.34	18.4	-27.5 / 46.1	
Residual variability (SD)	26.1			
VAS alertness				
Slope day 1 (mm.ng-1.mL)	-0.828	0.519	-1.9 / 0.2	2.06
Change to day 8	-1.37	1.00	-3.4 / 0.6	
Change to day 29	-1.32	1.08	-3.5 / 0.8	
Intercept day 1 (mm)	75.5	3.47	68.6 / 82.4	9.42
Change to day 8	12.5	4.02	4.5 / 20.5	
Change to day 29	13.0	5.06	2.9 / 23.1	
Residual variability (SD)	7.17			

would be 4-6 ng/ml. Although effective, these concentrations have shown to produce some changes in saccadic eye movements and visual analogue scales effects, which could be consistent with the clinical phenomenon of sedation. These effects could become less pronounced during prolonged treatment, due to tolerance development. The aim of this study was to evaluate potential changes in pharmacokinetic/pharmacodynamic (PK/PD)-relationships for these central nervous system effects during a 4-week treatment period with rilmenidine SR 3 mg OD.

ref 12
ref 17

The design of the study was based on two assumptions. First, a rapid switch from prestudy antihypertensives to rilmenidine was considered unlikely to affect the central nervous system effects. A rapid switch could affect the blood pressure control, which soon after the switch would still be partly affected by the interrupted prestudy treatment and would not be individually optimised. However, adequate long-term blood pressure control has already been established with rilmenidine, and this was not the aim of the study. The second assumption was that PK/PD-analyses reduce the need for a placebo-control. Any major placebo-response would dilute the relationship

ref 18

FIGURE 4 **Average PK/PD relationship between predicted rilmenidine concentrations and saccadic peak velocity**

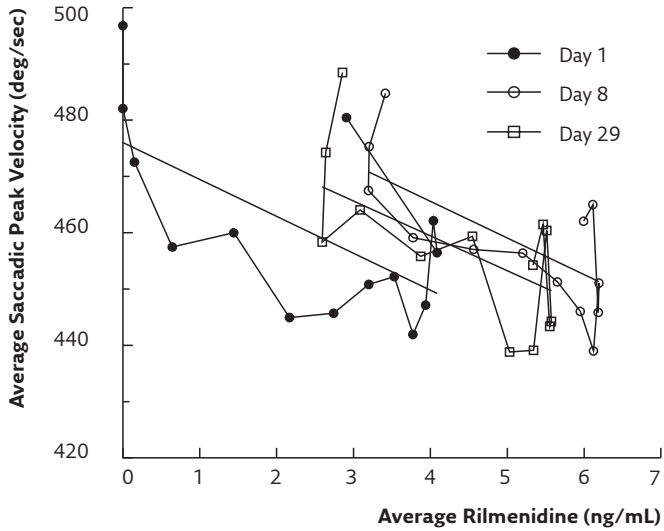
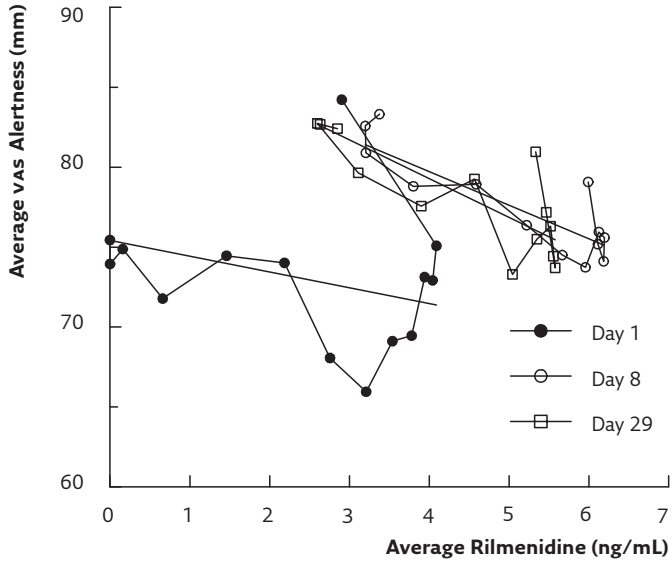


FIGURE 5 **Average PK/PD relationship between predicted rilmenidine concentrations and VAS alertness**



between the drug concentration and the pharmacodynamic parameter. Hence, a clear concentration-effect-relationship was considered a strong argument for drug-dependency of the parameter.

PK/PD-analyses were essential for the aims of the study, because they can be used to quantify changes in sensitivity to the drug and the development of tolerance. For linear concentration-effect-relationships, changes can occur in the slope and/or the intercept of the concentration-effect-curve. A decreased slope signifies that the same concentration range produces a less pronounced response. In this case, the effect at the highest observed concentration is decreased, for instance, due to desensitisation or dynamic counter-regulation. An increase in the intercept signifies that the entire concentration-effect-curve is right-shifted. Elevated pre-dose values will usually lead to an increased intercept of the concentration-effect curve.

The accumulation of rilmenidine during the four week period did not lead to an increase in CNS-effects. This was due to shifts in the concentration-effect-relationships for SPV and VAS Alertness scores. There were no changes in the slopes of the PK/PD-relationships from day 1 to day 8 or 29, whereas the intercept tended to increase. This net effect of these changes was that subjects became less sensitive to rilmenidine's SPV-effects. This finding was corroborated by the subjective measures of sedation. The AUEC of the visual analogue scales (VAS) for alertness increased on days 8 and 29 compared to day 1. These elevated AUECS could be largely attributed to an increase in predose alertness. The treatment responses remained unchanged, with rising rilmenidine concentrations. As a result, the PK/PD-relationship with VAS alertness showed a similar pattern as the PK/PD-relationship with SPV: slopes remained unaffected during multiple dosing, while intercepts increased. Compared to day 1, VAS alertness increased statistically significantly on both day 8 and 29.

The mechanisms behind these changes are unclear. The main PK/PD-changes for VAS and SPV are increases in the intercepts of the concentration-effect relations. Thus, a higher concentration range produces a similar treatment effect. Adaptation phenomena can cause an increased pre-dose effect (*eg* rebound after drug withdrawal), but this has not been reported for rilmenidine, and is particularly unlikely considering the accumulation of the drug with this sustained release profile. Pharmacological tolerance to the central nervous system effects of rilmenidine would primarily (or at least additionally) be expected to cause reductions in slopes of the concentration-effect relations, which were not observed in this study.

Therefore, explanations for the observed alterations do not seem to be purely pharmacological. In addition, methodological causes can be considered, related to learning effects or habituation to the study procedures. If causes for the adaptations would be methodological, this would also be expected in a placebo group. However, a recent four-week placebo-controlled trial with the imidazoline antihypertensive moxonidine did not reveal any changes in the placebo-treated group. The concentration-effect-relationships in the moxonidine-group displayed clear changes in intercepts but not in slopes - quite comparable to the findings of the current study. Although the reasons for these habituation processes cannot be determined exactly, it is clear that the subjective predose assessment of alertness (and mood and calmness) improved slightly during the study, while adequate blood pressure control was observed throughout the four-week treatment period.

ref 19

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The value of timing additional studies

In this section, four studies were presented that investigated what the improved profile of a new sustained release formulation would be and what its effects are in patients with hypertension. Using blood pressure and the most sensitive marker for the side-effect sedation (saccadic peak velocity), the therapeutic window of the new formulation was improved. Furthermore, it was possible to correlate the *in vivo* with the *in vitro* dissolution of a new formulation. Combined, these studies helped in designing an improved sustained release formulation for an existing drug with adequate clinical effects at tolerated levels. Because the drug has been on the market for quite some time, the development of the original formulation apparently did not optimally answer these questions. Now additional studies had to be performed at a post-registration stage. In this chapter it is investigated what the value of these additional studies have if they are performed at an early stage versus later on in the development.

TABLE 1 **Input parameters of the retrospective question-based approach for the development of rilmenidine**

Parameter	Input
Success action site	85.00%
Success pharmacological effect	85.00%
Success clinical efficacy	75.00%
Success therapeutic window	70.00%
Success population	80.00%
Estimated market value	400
Costs action site	30
Costs pharmacological effect	35
Costs clinical effect	40
Costs clinical window	35
Costs population	35

The first step in the analysis consists of estimation of the success probabilities and the accompanying costs. Obviously, this is done retrospectively since both the original and the new formulations have already been developed. The set of input parameters used in the QBD tree is shown in table 1. The overall probability of

success, the cumulative costs and the final payoff equal the values in the previous section and are based on historical data. It can be argued that for this compound the window question will have the relatively lowest probability of success with this compound. The clinical question would also be relatively difficult to answer and would introduce substantial costs since this would involve large patient studies. The penetration to the site of action, and the pharmacological effects the drug would exert, could have higher probabilities of success.
The optimal priority list of this question-based approach for the development of rilmenidine yields the optimal sequence of questions represented in table 2.

TABLE 2 **Optimal question sequence in the development of rilmenidine**

Priority ranking	Question
1	Window
2	Clinical
3	Population
4	Site
5	Pharmacology

However, the actual development of rilmenidine apparently did not fully answer the "window" and "clinical" question. This deficiency is reflected in the fact that in the actual development of rilmenidine, a sustained release formulation was developed after registration of the original rilmenidine formulation. Rilmenidine is currently available in several European countries, but was not registered in, for instance, the USA and the Netherlands. The development of a formulation with sustained release properties could allow introduction of the drug in these countries. In this section, a comparison of the optimal development plan with the actual development sequence was made. The studies presented here all address the "window" and "clinical" question and were performed late in the development (assuming that worldwide registration is the endpoint). This approach yields the sequence represented in table 3.
The project value of the question-based development of rilmenidine is (as demonstrated in the previous sction) the sum of all possible outcomes weighted for the probability that a particular outcome will happen. Calculation of the project value of the optimal sequence with the input parameters presented in table 2 yields an estimated value of M€ 14.9. However, the project value of the actual development

program (with the same overall success probability and costs) is only M€ 2.2.
In order to investigate how this substantial drop in value is created, risk analyses were performed that show all possible outcomes and the probability this will happen. The results of these analyses are represented in figure 1.

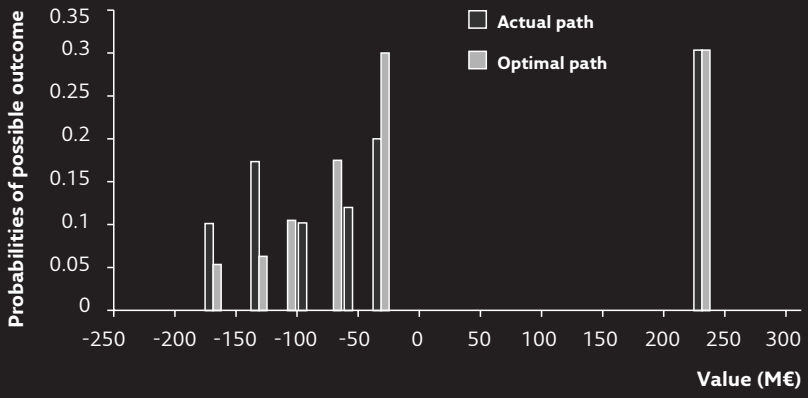
TABLE 3 **Actual question sequence in the development of rilmenidine**

Priority ranking	Question
1	Population
2	Site
3	Pharmacology
4	Window
5	Clinical

This analysis shows substantial differences in the two different priority sequences. Obviously, if all questions are answered successfully, no difference exists between the two options; both have a payoff of M€ 225 and a probability of 30% this will happen. However, if the product is abandoned somewhere in the process, the probabilities of the subsequent negative outcomes differ. In the optimal path, the higher the negative outcome is, the lower the corresponding probability is. The actual situation shows relatively high probabilities the outcome will be M€ -137.5 (17,3% vs. 6,3%) or even M€ -175 (10,1% vs. 5.4%). M€ -137.5 will be the outcome if the product is abandoned after answering "window" in the sequence presented in table 3 and M€ -175 if the product is abandoned after the last question ("clinical"). By moving these questions to early in the development, the risk of late abandonment is reduced in the optimal sequence.
The analysis shows that without reducing the probability of failure, the project value can be increased simply by rearranging the sequence of studies. The increased value allows additional studies to be performed that, in the NPV approach, would only introduce additional costs and development time. The studies presented in this section are an example of additional studies that combined solves the "window" and "clinical" question. While the NPV would decrease, the question-based approach adequately describes the impact of these studies.
The impact could have been even more valuable if the studies were performed at an early stage in the original development of rilmenidine. The inclusion of these studies at an early stage in the original development of rilmenidine would have

allowed the introduction of the improved formulation for this compound and thereby reducing the additional costs of having to introduce a new formulation. Remarkably, the NPV of this hypothetical development plan would be lower than the one actually used due to additional costs and development time. However, the result would be a formulation that would potentially meet a larger market demand (e.g. registration in the USA and other countries where rilmenidine is currently not registered) and the revenues could therefore have been even higher.

FIGURE 1 **Risk profiles of the QBD programs of rilmenidine**



From a business perspective, there is quite another view, which basically says 'introduce the product into the market, if it is basically satisfactory, as soon as possible. Additional useful information will be obtained from market experience and then consider what might be accomplished with an improved formulation'. This approach has been adopted in the nifedipine case, where after introduction of the original product, an improved formulation was successfully developed based on the discovery of a novel pharmacodynamic property of the drug. The improved product after the launch of the original product is sometimes referred to as a 2nd-cycle product. Clearly, critical evaluation of emerging new post-launch data is always necessary and additional investigations sometimes lead to highly successful new products, as proven by the nifedipine case. However, the starting point for the first cycle of development should be to develop the best possible treatment.

SECTION 1

SECTION 2

SECTION 3

SECTION 4

Literature evaluation

Developing a new formulation

Bridging the gap to Japan

Market advantage

CHAPTER 9

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Pharmacodynamics and pharmacokinetics of a single oral dose of nitrazepam in healthy male and female volunteers. An interethnic comparative study between Japanese and Caucasian volunteers

Abstract

BACKGROUND Potential interethnic differences in drug disposition and effects between Japanese and Caucasians hamper the registration in Japan of medications already used in Western countries. A systematic comparison of potential mechanisms of differences in drug response between racial groups can facilitate the transition of drugs between ethnic groups.

OBJECTIVES To compare the pharmacodynamics and pharmacokinetics of a single oral dose of nitrazepam (5 mg) in 8 Japanese and 8 Caucasian matched healthy males and females, in a double-blind, placebo-controlled, cross-over study.

METHODS The study was performed in a Japanese and a Dutch centre, using the same methods and study design. Japanese and Caucasian subjects were individually matched for gender, age and body stature. Drug effects were measured with saccadic and smooth pursuit eye movements and visual analogue lines obtained from the scales of Bond and Lader.

RESULTS There were no pharmacokinetic differences between the Japanese and Caucasian subjects. Clearance was 0.91±0.165 and 1.17 ±0.492 ml/min/kg, and $t_{1/2}$ was 22.1±4.96 and 21.5±7.51 hr, respectively. Pharmacokinetic parameters showed no significant correlation with age, height or weight. The average time-effect-curves for the different parameters were comparable between the groups. Compared to placebo, both groups showed similar significant reductions in average peak velocity (-75±40 vs -85±24 °/sec; M ± SD), and increases in saccadic inaccuracy (2.3±2.1 vs 2.1±0.9%) and reaction time (17.1±13.3 vs 18.2±14.4 msec). Visual analogue scores showed clear sedation in the Caucasians, but non-significant effects in the Japanese subjects. Smooth pursuit did not change significantly in either group. Slopes and intercepts of concentration-effect relationships for saccadic peak velocity showed considerable intersubject variability, but no clear differences between the two groups.

CONCLUSIONS The pharmacokinetics and pharmacodynamics of nitrazepam are similar in healthy Japanese and matched Caucasian subjects. Interethnic comparative studies are feasible and provide meaningful information about potential racial differences in disposition and action of drugs. Such studies can form a rational basis for comparative clinical trials.

Introduction

Drug responsiveness may differ among racial and ethnic groups. For some compounds and drugs, like alcohol, propanolol and debrisoquin, these differences are caused by genetic diversity in factors such as drug
ref. 1 metabolism or pharmacodynamic sensitivity. In other cases, interethnic differences may not be predictable. This may obstruct the transition of drug information from the ethnic group in which they have been developed to another. The possibility of interracial differences in drug disposition and effect has contributed to the policy of the Japanese Ministry of Health and Welfare, Koseisho, to require the repetition of a major part of the drug development programme in Japanese subjects, before an agent used abroad
ref. 2 can also be registered in Japan. Considering the difficulties in organising
ref. 3 clinical trials in Japan, it would in many instances be more rational to identify the factors that systematically determine interindividual and interethnic differences in drug responsiveness, and to adapt subsequent clinical trials or treatment regimens accordingly.

Several systematic differences in determinants of drug responsiveness can be distinguished. Average body stature differs between Japanese and Caucasians, and this is an important determinant of the disposition of many drugs. Genetic diversity in drug metabolism or action are other potential sources of variability in drug response between the Caucasian and Japanese populations. The identification of potential interethnic differences in the disposition or action of a drug that has already been widely studied in the Western market, and is considered for registration in Japan, could therefore start with a comparative study of Caucasian and Japanese subjects. Systematic comparative 'bridging' studies are in line with the increasing globalisation of drug development, that is brought about by the implementation of the International Conference on Harmonisation Guidelines on Good Clinical Practice (ICH-GCP), in numerous countries including Japan. 'Bridging' studies are advocated in the draft ICH guidelines on ethic factors
ref. 4 in the acceptability of foreign clinical data. In such comparative studies, research methods would have to be identical and subjects would have to be carefully matched for demographic factors that are known to influence drug disposition. The present study is aimed to test the feasibility of such an approach, by studying the pharmacokinetics and pharmacodynamics of the benzodiazepine nitrazepam in Caucasian subjects, carefully matched to the participants of a study in Japan, using the same methods and study design. The Japanese trial was performed prior to the Caucasian part of the study,
ref. 5 which allowed the matching of subjects and methods among the two

studies. The eye movement methods used in this study were carefully matched during a mutual exchange programme among investigators of the two research centres, but the studies were otherwise performed independently.

Methods

Subjects

Caucasian subjects were individually matched with Japanese volunteers of a previous study, for gender (four males, four females), age (intended allowed difference up to 5 yrs), height (up to 5 cm) and weight (up to 5%). The study was performed according to the Declaration of Helsinki and approved by the Medical Ethics Review Board of Leiden University Medical Center and the Institutional Review Board of Showa University.

Study design and group size estimation

The study was designed as a double-blind, randomised, placebo-controlled cross-over trial with a two-week washout period. The study was independently performed in two research institutes in The Netherlands and in Japan. The sample size of four male and four female healthy volunteers was based upon the size of the Japanese study group. Based on the extensive previous experience with CNS-effects of benzodiazepines, this sample size was expected to allow the detection of nitrazepam effects using saccadic peak velocity. The sample size would allow the detection of a 24% difference in Clearance/kg between the two groups, with a power of 80% using an ½ of 5%. The power was less for the pharmacodynamic parameters.

ref. 6

Drugs

Nitrazepam was administered as 5 mg capsules, at which it is registered as a hypnotic in the Netherlands and in Japan. The nitrazepam capsules and identical appearing placebo capsules were from the same batch for the Japanese and the Caucasian study (Shionogi Co, Tokyo, Japan), and the same randomisation order was used. The drugs were given orally, with a glass of water (with the subject in a comfortable sitting position), under fasted conditions, followed by a lunch after 4 hours. On all occasions, the

time of ingestion of the trial medication was between 09:00 and 10:00 AM local time. Other medications, illicit drugs, alcohol, tobacco, or xanthine-containing foods and beverages were not allowed during the study, except oral contraceptives and occasional paracetamol.

Pharmacodynamic Determination

The primary pharmacodynamic parameters were determined from saccadic eye movements, which have been shown to be highly sensitive to benzodiazepines. In addition, smooth pursuit eye movements and visual analogue scales of sedation and unsteadiness were assessed. The conditions and methods for these analyses were the same for the Japanese and the Caucasian part of the study. Subjects were instructed not to use alcoholic or xanthine-containing products within 24 hours before drug administration, and to limit the consumption of these products and maintain a regular diurnal rhythm for one week before each study day. Subjects were acquainted with the procedures during a training session, prior to the first study occasion.

ref. 6

EYE MOVEMENTS Saccadic eye movements and smooth pursuit eye movements were recorded 10 min before drug intake and at 30- 45- 60- 75- 90- 105- 120- 135- 150- 165- 180- 240- 360- and 480 min after administration. Recordings of eye movements were performed in a quiet room with ambient illumination. Recordings and analyses of saccadic eye movements and smooth pursuit eye movements were conducted with a microcomputer-based system for sampling and analysis of eye movements, and were performed as described previously with adaptions as described below. In both centres, the same equipment was used for stimulus display, signal collection and amplification (Nihon Kohden Corporation, Tokyo, Japan). Sampling, analogue-to-digital conversion and analysis of eye movement signals were also performed with the same equipment and software (Cambridge Electronics Design, Cambridge, UK). Disposable silver-silver chloride electrodes were applied on the forehead and beside the lateral canthi of both eyes of the subject for registration of the electro-oculographic signals. Skin resistance was reduced to less than 5 kOhm before application of the electrodes. Head movements were restrained using a fixed head support. The target consisted of an array of light emitting diodes on a bar, fixed at 50 cm in front of the head support. Saccadic eye movements were recorded for stimulus amplitudes of: 10, 15 and 20 degrees to either side. Eleven saccades were recorded for each stimulus amplitude with interstimulus intervals varying randomly between 3 and 6 seconds. Average peak saccadic

ref. 6-7

velocity of all artifact-free saccades were used as the primary parameter. For smooth pursuit eye movements the target moved sinusoidally at frequencies ranging from 0.3 to 1.1 Hz in steps of 0.1 Hz. The amplitude of target displacement corresponded to 20 degrees eyeball rotation to both sides. Four cycles are recorded for each stimulus frequency. The time in which the eyes were in smooth pursuit of the target were calculated for each frequency and expressed as a percentage of stimulus duration. The average percentage of smooth pursuit for all stimulus frequencies was used as efficacy parameter.

VISUAL ANALOGUE SCALES Subjective drug effects are often quantified with a set of 10 cm visual analogue scales as described by Norris, and Bond and Lader. These visual analogue scales have not been validate for Japanese subjects. Therefore, the six indicators most relative to sedation were translated into Japanese, to assess three domains of sedation with two indicators each: mental sedation (Alert-Drowsy and Muzzy-Clear headed), tranquillisation (Calm-Excited and Tense-Relaxed) and physical sedation (Well coordinated-Clumsy and Lethargic-Energetic). These indicators corresponded to numbers 1,2,4,5,6,12 in the scales of Norris, and numbers 1,2,4,5,6,10 in the Bond and Lader scales. Visual analogue scales were completed at the times of blood sampling: before administration and at 30- 60- 90- 120- 150- 180- 240- 360- 480- and 1440 min (24 hours) after drug administration.

ref. 7
ref. 8

Blood sampling

Before drug intake, a cannula was inserted into a forearm vein, kept patent with saline after each blood sample. Blood samples (9 ml) were collected in Li heparin tubes. Plasma concentrations of nitrazepam were measured in blood samples taken at the following times: two just before drug administration (one extra for HPLC calibration) and at 30- 60- 90- 120- 150- 180- 240- 360- 480- and 1440 min (24 hours) after administration.

Analyses

DRUG CONCENTRATION ANALYSIS Plasma concentrations of nitrazepam were analysed separately in both centres, using the same HPLC-method.

ref. 9-10

PHARMACOKINETIC, PHARMACODYNAMIC AND STATISTICAL ANALYSES The pharmacokinetic and statistical analyses were performed in a single centre, using the original Japanese and Caucasian raw data. Eye movement parameters were analysed as areas under the effect curve calculated using the linear trapezoidal rule over 0-240 min divided by 240 min to obtain a weighted average response. For each group (Japanese/ Caucasian) separately, placebo results were compared to nitrazepam using paired Student t-tests. Groups were compared by analysing the difference compared to placebo using unpaired Student t-tests. Differences between groups are presented with 95% confidence intervals (95% CI). Model independent nitrazepam pharmacokinetic parameters (C_{max}, t_{max}, $AUC_{(0\text{-}24h)}$, $AUC_{(0\text{-}\infty)}$, Cl_{sys}, Cl_{sys}/kg and $t_{½}$) were calculated using WinNonlin (V1.1; Scientific Consulting, Inc., Apex, NC, USA) using automatic detection of the terminal part of the curve to be used for log-linear regression. Parameters were compared between groups using unpaired t-tests. $AUC_{(0\text{-}\infty)}$ and C_{max} were analysed after log-transformation, and the resulting difference was back-transformed, yielding an estimate of percentage increase with the associated 95% confidence interval (95% CI).

Correlations between anthropometric measures (age, height, weight) and PK parameters were performed using Pearson's and Spearman's correlation coefficients. Statistical analysis and calculations were performed using SPSS for Windows V6.1.2 (SPSS, Inc., Chicago, IL). Areas under the effect curve were calculated using BMDP/Dynamic V7.0 (BMDP Statistical Solutions Ltd, Cork, Ireland).

Results

Subjects

The eight Caucasian subjects had and average age of 23.3 (range 19-27) years, a weight of 58.3 (48.0- 82.0) kg and a height of 167.0 (157.0-179.5) cm. The Japanese demographic characteristics were 22.8 (range 18-26) years, 56.4 (45.0- 78.0) kg and 164.1 (155-178) cm. Differences between some matched subjects slightly exceeded the intended maximal values: age differed by 6 yrs in two cases, weight by 10% in one and 6.5% in two cases (but less than 5 kg in each), and height by 6 cm in one case.

Pharmacodynamic Determinations

SACCADIC EYE MOVEMENT The average time-corrected area-under-the-effect-curves (AUECS) for saccadic and smooth pursuit eye movements after placebo- and nitrazepam-treatment in the two groups are presented in Table 1. In both groups, saccadic peak eye movements showed clear treatment effects, as shown in Table 2. The average placebo-corrected time curves of saccadic peak velocity for the Japanese and Caucasian subjects were comparable between the two groups (Figure 1). Comparison of the two groups showed no significant differences in any of these parameters (Table 2).

TABLE 1 **Eye movement AUECS (0-240min) for Japanese and Caucasian subjects**

	JAPANESE			CAUCASIAN		
Parameter	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**
Nitrazepam						
Inaccuracy (%)	10.3	2.1	8	8.2	3.0	8
Peak velocity (°/sec)	301.5	33.2	8	315.8	33.4	8
Reaction time (ms)	221.2	18.7	8	219.3	10.0	8
Smooth pursuit (%)	59.0	12.2	7	48.9	14.9	8
Placebo						
Inaccuracy (%)	8.5	2.2	7	6.1	2.2	8
Peak velocity (°/sec)	375.3	48.4	7	400.7	49.8	8
Reaction time (ms)	204.9	17.2	7	201.1	15.5	8
Smooth pursuit (%)	60.5	14.0	8	50.4	16.7	8

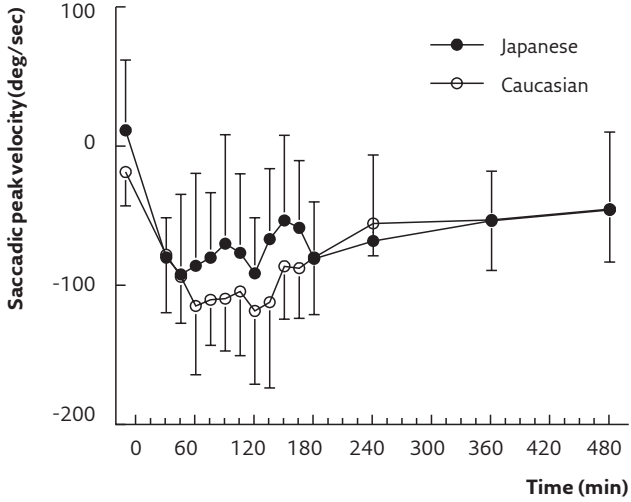
TABLE 2 **Treatment effects on eye movements for Japanese and Caucasian subjects**

	JAPANESE		CAUCASIAN		DIFFERENCE	
Parameter	**Mean**	**95 % CI**	**Mean**	**95 % CI**	**Mean**	**95 % CI**
Nitrazepam-Placebo						
Inaccuracy (%)	2.3	(0.3, 4.2)	2.1	(1.3, 2.8)	0.2	(-1.8, 2.2)
Peak velocity (°/sec)	-74.5	(-111.4, -37.6)	-85.0	(-105.2, -64.8)	10.5	(-28.3, 49.3)
Reaction time (ms)	17.1	(4.8, 29.4)	18.2	(6.1, 30.2)	-1.1	(-16.5, 14.4)
Smooth pursuit (%)	-2.4	(-8.6, 3.8)	-1.5	(-7.7, 4.7)	-0.9	(-8.8, 7.1)

SMOOTH PURSUIT The smooth eye movement AUEC data for the Japanese and Caucasian subjects are represented in Table 1. Both groups failed to show significant nitrazepam effects, as shown in Table 2. Also, no differences in treatment effects were detected between the two ethnic groups.

VISUAL ANALOGUE SCALES The individual time-effect curves for visual analogue scores showed clear sedative effects in the Caucasians. In contrast, the effects were non-significant in the Japanese subjects, and often the reverse of the Caucasian effect. It was concluded that the two ethnic groups had interpreted the visual analogue scales differently, and no comparative analysis was made.

FIGURE 1 **Average (+ SD) saccadic peak velocity-time curves for Japanese (●) and Caucasian (○) subjects, shown as difference from placebo**



Pharmacokinetic determinations

The mean nitrazepam concentration-time profiles for both Japanese and Caucasian subjects are presented in Figure 2. The pharmacokinetic parameters are shown in Table 3. Clearance (M±SD) was 0.91±0.165 and 1.17±0.492 ml/min/kg and $t_{½}$ was 22.1±4.96 and 21.5±7.51 hr, in the Japanese and Caucasian groups, respectively.

The analysis of $AUC_{(0\text{-}24h)}$, $AUC_{(0\text{-}\infty)}$ and C_{max} after log-transformation and back-transformation of the resulting difference showed that Japanese $AUC_{(0\text{-}24h)}$ was 33.6 % higher (95% CI -1.1, 80.7 %), Japanese $AUC_{(0\text{-}\infty)}$ was 26.3 % higher (95% CI -5.8, 69.4 %) and Japanese C_{max} was 15.9 % higher (95% CI -18.0, 63.7 %). Similar interracial comparisons of clearance and

clearance/kg showed that Caucasian clearance was 26 % higher (95% CI -6, 99 %) and Caucasian clearance/kg was 22 % higher (95% CI -10, 67 %). None of these pharmacokinetic differences reached statistical significance between the two racial groups after paired -or unpaired comparison. The correlations between anthropometric measures (age, weight, height) and pharmacokinetic parameters were not statistically significant.

FIGURE 2 **Average (+ SD) nitrazepam concentration-time curves for Japanese (●) and Caucasian (○) subjects**

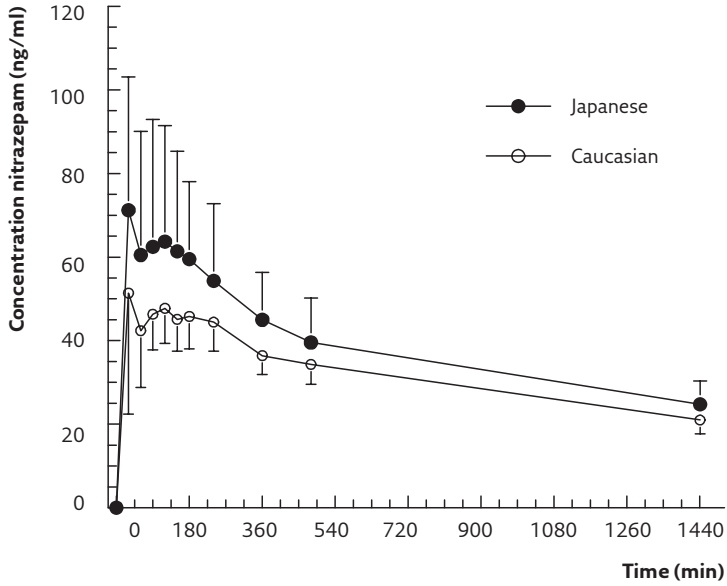


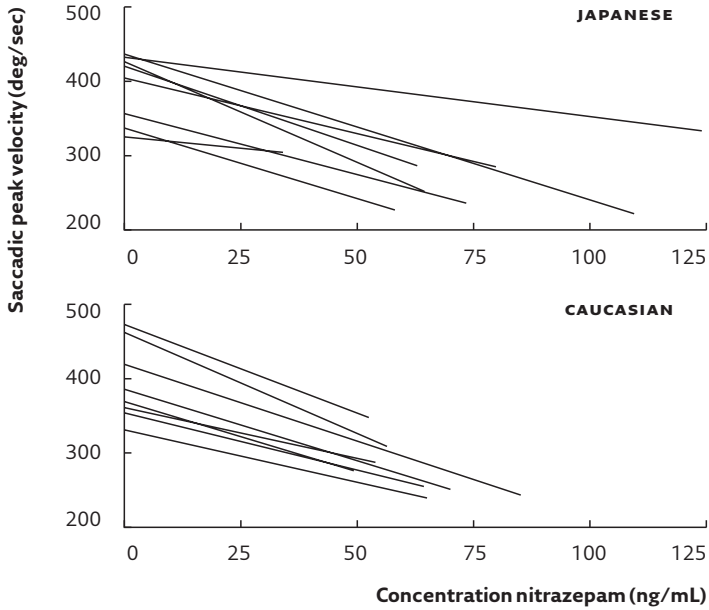
TABLE 3 **Pharmacokinetic parameters for Japanese and Caucasian subjects, obtained by model independent analysis**

	JAPANESE			CAUCASIAN			DIFFERENCE	
Parameter	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**95% CI**
$t_{½}$ (min)	1329	298	8	1292	450	8	36.5	(-378.9, 451.9)
t_{max} (min)	93.8	74.2	8	101.5	71.4	8	-7.75	(-85.89, 70.39)
C_{max} (ng/ml)	75.8	28.9	8	62.1	11.7	8	13.7	(-11.1, 38.5)
Clearance (ml/min)	50.8	11.4	8	65.6	22.6	8	-14.8	(-34.6, 5.1)
Clearance/kg (ml/min/kg)	0.91	0.17	8	1.17	0.49	8	-0.26	(-0.68, 0.16)

Concentration-effect relationships

The individual nitrazepam concentration-time profiles could not be adequately described using standard pharmacokinetic models. Individual plots of saccadic peak velocity against concomitant (linearly interpolated) nitrazepam concentration revealed possible hysteresis in some subjects, possible proteresis in others and no sign of either in the rest. No indications were present to consider a concentration-effect model that was more complex than a simple linear association. Figure 3 shows the plots using standard linear regression for both Japanese and Caucasian subjects.
The mean slopes of the plots of the Japanese subjects were 0.26 (95% CI -0.32, 0.84)° $sec^{-1}ml{\cdot}ng^{-1}$ less steep than the corresponding slopes for the Caucasian subjects (-1.66±0.69 vs -1.91±0.48° $sec^{-1}ml{\cdot}ng^{-1}$). The intercepts for the Japanese subjects were 2.0 (95% CI -43.2, 39.2)°/sec lower than for Caucasian subjects (392.5±45.6 vs 394.5±51.9°/sec). The Caucasian slopes were 24.2 (95% CI -20.2, 93.2) % steeper than the Japanese. Both the slopes and the intercepts were not statistically different by paired and unpaired comparison.

FIGURE 3 **Slopes of linear relationships between nitrazepam concentrations and saccadic peak velocities for Japanese and Caucasian subjects**



Discussion

The objectives of the study were to compare the pharmacokinetics and CNS-pharmacodynamics of a single oral dose of nitrazepam 5 mg between Caucasian and Japanese male and female volunteers, matched for gender, age and body stature. Nitrazepam was chosen because it is a widely used benzodiazepine in both regions, but the pharmacokinetics in the Japanese population have not been reported. In addition, nitrazepam is partly metabolised in the liver by enzymes that exhibit polymorphism subject to interracial differences. The study did not represent a formal 'bridging' study for registration purposes, but was intended to assess the feasibility of such studies as international enterprises.

ref. 11-12
ref. 4

Nitrazepam is metabolised by reduction of the nitro group to the corresponding amine, followed by acetylation to the acetamido-compound. The first step is catalysed by cytochrome P450 isoenzymes, which show considerable interethnic variation, *e.g.* of the CYP2E1 and CYP2C sub-families, such as CYP2C19. Polymorphism of these isoenzymes could theoretically lead to differences in nitrazepam effects. The second metabolic step is catalysed by N-acetyltransferase, which is also polymorphic and potentially subject to interracial differences. Based on the activity of N-acetyltransferase (NAT2), individuals can be distinguished as fast or slow acetylators. Ninety percent of the Japanese people are fast acetylators, compared to less than 50% of the Europeans. More rapid metabolism in Japanese subjects could theoretically reduce the duration of action of the parent compound relative to Caucasians; both NAT2 metabolites are inactive. Demographic factors may also contribute to interethnic pharmacokinetic differences. Body stature may influence the disposition of drugs like nitrazepam, while gender and age can cause differences in drug metabolising enzymes.

ref. 13
ref. 14-15
ref. 16-17
ref. 18
ref. 19
ref. 18
ref. 20

Most of these pharmacokinetic factors, which can contribute to interracial variability in drug action, can be determined in the ethnic group where the drug was initially developed. The identification and quantification of such 'ethnically sensitive' factors can thus provide a rational basis for subsequent designs of clinical trials or treatment regimens in other populations, if the factors affecting drug disposition are also known in the new population. However, pharmacodynamic differences remain difficult to predict, and comparative studies such as the current one may elucidate unexpected interethnic differences in drug action. The design and size of such studies obviously depend on the drug in question, but identification of 'ethnically

sensitive' compounds should ideally be performed during the early phases of drug development. The current study was a phase I-type investigation, intended to test the feasibility and informativeness of interregional interethnic pharmacokinetic/ pharmacodynamic studies. The draft ICH guidelines on ethnic factors indicate that comparative studies can be performed within the original region, in a population representative of the new region, *e.g.* immigrants. However, this may eliminate only part of the ethnic variability between the two regions, since many habits of culture, feeding *etc.* which may influence drug effects, will have been adopted by the immigrants. Therefore, comparative studies are best performed in the original populations, which however necessitates careful matching of subjects, study designs and methodology.

ref. 4

ref. 4

In the current study, subjects were matched for gender, age and body stature, but the study was not designed to characterise the heterogeneity of acetylator status or cytochrome P450 activity in the Japanese and Caucasian populations. This would have required the determination of genetic polymorphism and careful identification of nitrazepam metabolites in much larger samples. In the current study however, relevant differences in drug metabolism would have become evident from differences in pharmacokinetics between the two groups. The results showed no significant pharmacokinetic differences between the two groups, although the 95% confidence intervals were too wide to confirm bioequivalence. Therefore, subtle interethnic differences in drug metabolism cannot be excluded, but these are certainly not as important as the interindividual variability within each ethnic group. Theoretically, sampling errors in this small study could have obscured the detection of ethnic differences in drug disposition. In the Japanese group however this is unlikely, since the most relevant 'ethnically sensitive' metabolic factor in this study, NAT2-activity, is present in 90% of the Japanese population. The Caucasian study group also seems to be representative of the European population, because their pharmacokinetic results agree well with textbook data on nitrazepam ($t_{½}$ 20-28h, t_{max} 45-240 min, clearance 0.86 ± 0.12 ml·min^{-1}·kg^{-1}; *cf.* Table 3).

ref. 21-22

Apart from the mentioned drug-dependent differences, there are also ethnic differences in methodology. An example is the difference in visual analogue scales between the two study groups. Visual analogue scores showed clear sedative effects in the Caucasians, but the majority of Japanese subjects scored a value of approximately zero throughout the study day. It is likely that young Japanese immigrants would have responded more like Caucasians, illustrating the point that interethnic comparative studies are best performed

in the original populations. This requires a thorough validation in particular of subjective methods, such as descriptions of adverse events, visual analogue scales, questionnaires *etc.,* before they can be applied from one population to another. The literal translation of relevant visual analogue scales performed in this study did not take sufficient account of differences in instructions and in sociocultural factors such as subject-investigator-relationships. Validation requirements are more easily met for objective methods, which are therefore more suitable to perform interethnic comparative studies. The eye movement methodology used in this study was carefully adjusted during an intensive mutual exchange programme between the Japanese and Dutch research centres.

Although concentration-effect-relationships could be established only crudely, no significant interethnic variations in pharmacokinetic / pharmacodynamic relationships of nitrazepam were found. Intersubject variability was much larger than interracial variation. However, the results did not meet formal criteria for equivalence. Therefore, they would need confirmation, repetition in special populations (elderly) and extension to some specific situations (drug interactions). If similarity in drug disposition and effects among the ethnic groups is confirmed, data from previous studies of nitrazepam in Caucasian subjects can be translated to the Japanese situation, leading to rational dose adaptations for treatment regimens. This could reduce the need for repetition of large scale studies and would rationalise the dose selection for the ones that are still necessary. Thus, systematic comparative studies could ease the burden for clinical reseach in Japan, where resources that are fully up to the requirements of ICH-GCP are currently limited. The present study shows that interethnic comparative pharmacokinetic/ pharmacodynamic investigations with rigorous similarity in design, subjects and methodology are feasible across different regions. Such studies can make a useful addition to *in vitro* assessment of 'ethnically sensitive' pharmacokinetic differences, not only for global drug development but also in multiethnic societies.

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CHAPTER 10

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Contraception 2003, accepted

Pharmacokinetic differences between Caucasian and Japanese subjects after single and multiple doses of a potential combined oral contraceptive (Org 30659 and EE)

Abstract

OBJECTIVES To compare the pharmacokinetic parameters and safety of the progestagen Org 30659 ((17α)-17-hydroxy-11-methylene-19-norpregna-4,15-dien-20-yn-3-one) and ethinyl estradiol (EE) in Caucasian and Japanese women after single and multiple doses.

METHODS This was an open-label parallel design of a single dose followed by a multiple dose period in healthy young Japanese and Caucasian subjects.

RESULTS The AUC of Org 30659 after single dosing was increased by a factor 1.75 (90% CI: 1.48-2.08) in Japanese women compared to Caucasian women. At steady-state this difference increased to a factor 1.90 (90% confidence interval [CI]: 1.60-2.25). The AUC of EE after single dosing was similar in Caucasian and Japanese women but at steady-state it was increased by a factor 1.38 (90% CI: 1.15-1.64) in the Japanese. Weight normalisation reduced but did not remove all the observed differences. Sex Hormone Binding Globulin (SHBG) played no significant role in the differences between Caucasian and Japanese subjects. Both the single and multiple dose treatment with Org 30659/EE were generally well tolerated by all subjects. The Japanese population reported more and different treatment related adverse events than the Caucasian population.

CONCLUSIONS The peak concentration and extent of exposure of Org 30659 and to a lesser extent of EE in Japanese women are higher than in Caucasian women. Furthermore, the peak concentration and extent of exposure at steady-state of Org 30659 and to a lesser extent of EE are higher than would be predicted assuming linear pharmacokinetics over time. No major safety issues were observed.

Introduction

The main goal of combined oral contraceptives is prevention of pregnancy by inhibiting ovulation without jeopardising safety. Because the early combined oral contraceptives (COCS) had a relatively high steroid content, the preparations showed good contraceptive efficacy but also produced a great variety of adverse effects, including some serious ones. In the late 1960s, various reports raised concern about venous thromboembolic events occurring in

ref. 1 COC users, which were attributed to the estrogen administered with the pill. Therefore, the estrogen content of newly developed COCs has been reduced. Howeve, concern continued when in the late 1970s also the progestagenic
ref. 2 component became suspected in causing arterial thromboembolic accidents (particularly, myocardial infarction). Thus, development of new oral contraceptives has been directed towards designing regimens with aminimal effective dose of both the estrogen and the progestagen. However, this carries a number of drawbacks. Firstly, reducing the steroid dosage may jeopardise contraceptive efficacy in certain subgroups of women, for example, women with a low steroid bioavailability. Secondly, low steroid doses may lead to an increase in the incidence of irregular bleeding. Thirdly, as the dose is reduced in an effort to eliminate the negative effects, several of the positive properties associated with oral contraceptives (OCS), like reduced
ref. 3-10 risk of fibrocystic tumors of the breasts and endometrial and ovarian cancer, could also be eliminated.

In addition to the desired progestagenic activity, currently available progesta-
ref. 11 gens have a varying degree of intrinsic androgenicity. When these progestagens are used at a high a dose, typical androgen-associated side effects such as acne and hirsutism may become more frequent. Furthermore, androgenic progestagens are associated with more pronounced metabolic disturbances (*e.g.* in lipid metabolism and carbohydrate metabolism) than non-androgenic
ref. 12 progestagens. Research has been directed towards the development of progestagens showing a strong dissociation between progestagenic and androgenic activity like desogestrel and etonogestrel, in order to minimise metabolic side effects. The new progestagen Org 30659 ((17α)-17-hydroxy-
ref. 13 11-methylene-19-norpregna-4,15-dien-20-yn-3-one) is a novel selective progestagenic steroid with low intrinsic androgenic activity.

Modern low-dose OCS were launched in Japan in 1999, following the lifting of a ban on their licensing and sale. Ethnic differences could occur in the pharmacological or pharmacokinetic properties of investigational new drugs. Therefore, before a new drug is introduced in Japan sufficient data should be presented on the effects of the drug in a Japanese population. In some cases a comparison between the 'western' population and the Japanese population can be used to extrapolate the data from 'western' studies to the Japanese (especially when no differences are observed). In the current study, the pharmacokinetics and safety of Org 30659 and ethinyl estradiol (EE) in Caucasian and Japanese women were compared after single and multiple dose administration of tablets containing a combination of 200 µg Org 30659 and 20 µg EE.

Methods

Design

This was a two-centre, open-label, parallel design of a single dose (SD) followed by a multiple dose (MD) period in 18 Japanese and 18 Caucasian healthy females. Japanese subjects were studied in Tokyo, Japan and Caucasian subjects concurrently in Leiden, The Netherlands using the same study protocol. Subjects not using oral contraception were to start treatment (administration of SD) between 3 and 8 days after the first day of their last menstrual cycle. Subjects using oral contraception were to start treatment (administration of SD) between 28 and 30 days after they had stopped taking their last oral contraceptive pill. All subjects enrolled in this study were instructed to use adequate non-hormonal contraceptive measures throughout the study period.

Subjects

Japanese (both parents Japanese) and Caucasian (both parents Caucasian) female subjects were healthy as determined during screening. The study was approved by the Medical Ethics Review Board of Leiden University Medical Center and approved by the Institutional Review Board of Obara hospital, and performed according to the principles of ICH-GCP, the Helsinki Declaration and Dutch and Japanese law.

Treatments

All subjects received treatments from the same batch of study medication. A single oral administration of Org 30659/EE (1 tablet containing 200 μg Org 30659 and 20 μg EE) was followed by a 72 h pharmacokinetic (PK) sampling period (SD). The single dose was followed by a washout period of 5 to 7 days, before the start of the multiple dose period. This consisted of once daily oral intake of Org 30659/EE (1 tablet containing 200 μg Org 30659 and 20 μg EE) for 24 days, followed by a 72 h PK sampling period. Treatment administration was supervised on the first dose and at the multiple doses on days 2, 4, 6, 15 and 24. Additionally, compliance with the dosing regimen was checked on days 9, 12, 18 and 20. Furthermore, subjects were instructed to record the exact time of drug administration on a diary provided by the centre. At each visit to the trial centre, a pill count was performed by the site's staff.

Blood Sampling and Measurements

Testing for drugs of abuse (cocaine, morphine and tetrahydrocannabinoid) was performed during screening and before administration of the single dose. For all subjects a qualitative, colour immuno- urine pregnancy test was performed during screening, pre-dose SD period, pre-dose MD period and pre-dose on MD days 6, 15 and 24. Upon arrival on Day –1 a breath test to detect the use of alcohol was performed.

A 12-lead electrocardiogram was obtained regularly throughout the study period. At regular time intervals, systolic and diastolic blood pressure (BP, mm Hg) and pulse rate (PR, BPM) were measured using an automated blood pressure monitor. Vital signs were recorded before any other procedures, took place. The subject was sitting or semi-recumbent for at least 5 minutes prior to start of the measurement.

Blood samples for routine laboratory safety were drawn pre-dose and 24 hours after SD and pre-dose on days 6, 15 and 24 after the start of the MD period and at post study screening. From these samples, Sex Hormone Binding Globulin (SHBG) was also determined. Analysis of the laboratory safety blood samples was performed at the two centres except for SHBG, which was analysed at the Leiden University Medical Centre using immunometric assay and luminescentic detection (Immulite, DCP, Los Angeles, CA, USA).

Blood was collected and processed to Potassium-EthyleneDiamineTetra-Acetic acid (K-EDTA) plasma for the assessment of Org 30659 concentrations and to serum for the assessment of EE concentrations. Org 30659 concentrations were determined at the Department of Drug Metabolism and Kinetics, NV Organon, Oss, the Netherlands using a validated gas chromatographic assay with mass spectrometric detection. The range for the Org 30659 assay was 0.05 - 25 ng/mL plasma. EE concentrations were measured at PPD Pharmaco, Richmond, Virginia, USA using a validated radioimmunoassay after high performance liquid chromatography isolation of EE. The range for the EE assay was 2.25 – 48 pg/mL serum. Blood samples for Org 30659 and EE determination were drawn pre-dose and ¼, ½, ¾, 1, 1½, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48 and 72 hours after SD and after the last MD. Furthermore, pre-dose blood samples were taken at days 2, 4, 6 and 15.

ref. 14

Pharmacokinetic analysis

Based on the Org 30659 concentrations in plasma and the EE concentrations in serum, the following (non-compartmental) pharmacokinetic parameters

were calculated, both after the first single dose and at steady-state after the final dose: the peak concentration (C_{max}) and its time of occurrence (t_{max}), the elimination half-life ($t_{½}$), the area under the concentration-versus-time curve ($AUC_{0-\infty}$ and $AUC_{0-\tau}$), the apparent clearance (CL_{app}) and the apparent volume of distribution ($V_{z,app}$). From the steady-state wash-out curve after the final dose (dosing interval, τ=24h), the following additional pharmacokinetic parameters were calculated: the peak concentration at steady-state corrected for pre-dose concentration ($C_{max,corr}$), the average concentration at steady-state (C_{av}) and the degree of fluctuation (DF). Also weight-normalised (wn) pharmacokinetic parameters were calculated. Two types of accumulation ratios were calculated from the single-dose and steady-state parameters: R_{A1} (=$C_{ss,max}/C_{sd,max}$) and R_{A2} (=$AUC_{ss,0-\tau}/AUC_{sd,0-\tau}$). Based on the pre-dose concentrations during the multiple-dose treatment, the mean pre-dose concentration at steady-state ($C_{ss,min,av}$) and the mean time of attainment of steady-state (t_{ss}) were obtained. All calculations were performed using SAS V6.12 (SAS Institute, Inc., Cary, NC).

Statistical analysis

Analysis of Variance (ANOVA) was performed on the PK parameters using a mixed model with fixed factors 'Ethnic group' (Japanese versus Caucasian) and 'Regimen' (steady-state versus single dosing), interaction 'Ethnic group'-by-'Regimen' and random factor 'Subject' nested within 'Ethnic group'. The primary PK parameters were (wn-)AUC and (wn-)C_{max}: for single dose $AUC_{0-\infty}$ and C_{max} were used and for steady-state AUC_{0-24} and $C_{max,corr}$, respectively. For the factor 'Ethnic group', point estimates and 90% confidence intervals for the ratio of Japanese over Caucasian means were calculated, both at steady-state and after single dosing. Similar pharmacokinetics between Japanese and Caucasian subjects was to be concluded when the 90% confidence intervals for the primary PK parameters were fully contained within the acceptance range of 0.70-1.43.
For the factor 'Regimen', point estimates and 95% confidence intervals for the ratio of steady-state over single-dose means were calculated, both for Japanese and Caucasian subjects. When the 95% confidence intervals for the primary PK parameters contained a ratio of one (indicating non-significance at the 5% level of significance), the absence of a regimen effect on the pharmacokinetics was to be concluded (i.e. time independent pharmacokinetics). To study the influence of SHBG on the primary pharmacokinetic parameters of Org 30659 and EE, additional ANOVAs were performed using the same model extended with pre-dose SHBG value as covariate (after logarithmic

transformation). For the single-dose PK parameters the pre-dose SHBG value from single-dose period day 1 was used and for the steady-state PK parameters the pre-dose SHBG value from multiple-dose period day 24 was used.
For all other (supportive) pharmacokinetic parameters, point estimates and 95% confidence intervals for the ratio of ethnic group means and for the ratio of regimen means were calculated using the same ANOVA model as for the primary PK parameters. Conclusions for these parameters were based on classical hypothesis testing (at the 5% level of significance).
Log-transformed values of the parameters were used in the ANOVAS except for t_{max} for which an ANOVA on ranks was performed.
All analyses were performed using SAS V6.12 (SAS Institute, Inc., Cary, NC).

Data management

Data from working copies of case report forms, obtained from ProMaSys (Protocol Management System v3.2, CHDR, The Netherlands), were directly entered into the ProMaSys. The Japanese site had access to this database system using an Internet connection.

Results

Demographics

In the Netherlands, a total of 19 subjects were included in this trial. One subject dropped-out due to difficulty in blood sampling immediately after the first dose and was subsequently replaced. In Japan, a total of 18 subjects were included in this trial. Mean (SD) age, weight, height and BMI of the Caucasian subjects were 25.4 (4.5) years, 69.4 (8.8) kg, 172 (6.4) cm and 23.4 (2.3) kg/m^2, respectively. Mean (SD) age, weight, height and BMI of the Japanese subjects were 27.8 (6.1) years, 53.6 (5.4) kg, 158 (5.7) cm and 21.5 (2.3) kg/m^2, respectively.

Comparison of single-dose and steady-state pharmacokinetics

The single-dose and steady-state pharmacokinetic parameters for both ethnic groups are summarised in Tables 1 and 2. The average time-effect profiles from 0-8 hours after dosing are displayed in Figure 1. The results of

the ANOVAs for the statistical comparison of single-dose and steady-state PK parameters are given in Table 3. For two subjects, both after single dosing and at steady-state, several plasma concentrations of Org 30659 around t_{max} were unreportable and therefore no PK parameters for Org 30659 were reported for these subjects.

TABLE 1 **Single-dose and steady-state pharmacokinetics of Org 30659 in Caucasian and Japanese subjects. Based on n=34 subjects for Org 30659. Presented are median (min-max) for t_{max}; geometric mean (geometric CV%) for other PK parameters**

	Org 30659			
	CAUCASIAN WOMEN		**JAPANESE WOMEN**	
Parameter (unit)	**Single Dose**	**Steady-state**	**Single Dose**	**Steady-state**
C_{max} (ng/mL)	1.78 (32.8)	3.99 (32.7)	2.85 (34.1)	7.87 (28.8)
t_{max} (h)	0.75 (0.50-1.02)	0.50 (0.45-1.50)	0.767 (0.75-1.50)	0.583 (0.50-1.00)
$t_{½}$ (h)	10.4 (47.8)	13.3 (26.5)	14.4 (31.9)	12.1 (25.6)
AUC* (ng(h/mL)	6.23 (27.4)	13.6 (29.5)	10.9 (31.3)	25.4 (30.1)
wn-CL_{app} (L/h/kg)	0.469 (28.2)	0.213 (30.3)	0.346 (27.1)	0.149 (29.3)
wn-$V_{z,app}$ (L/kg)	6.80 (43.8)	4.30 (44.7)	7.22 (47.5)	2.60 (43.4)
C_{av} (ng/mL)	-	0.567 (29.5)	-	1.06 (30.1)
DF (%)	-	674 (23.2)	-	713 (18.6)
$C_{min,av}$ (ng/mL)	-	0.157 (48.7)	-	0.319 (43.2)
R_{A1}	-	2.17 (23.1)	-	2.76 (34.2)
R_{A2}	-	2.52 (21.9)	-	2.93 (36.4)

*: $AUC_{0-\infty}$ for single dose and AUC_{0-24} for steady-state; -: not applicable.

Steady-state was reached for Org 30659 after 24 days of multiple dosing and for EE after 15 days of multiple dosing. The pharmacokinetics of Org 30659 and EE are time-dependent, both in Caucasian and in Japanese women, after administration of this combination drug. In general, C_{max} and AUC at steady-state are higher than would be predicted assuming linear pharmacokinetics over time.

Assuming linear pharmacokinetics over time for Org 30659 there would have been no significant differences in C_{max} and AUC between steady-state and single dosing. In Table 3 it can be read that the C_{max} at steady-state is 111% higher than after single dosing in Caucasian women (in Japanese women 165%). The daily AUC_{0-24} at steady-state is 115% higher than the total $AU_{0-\infty}$ after single dosing in Caucasian women (in Japanese women 132%).

For EE, the C_{max} at steady-state is 37% higher than after single dosing in Caucasian women (in Japanese women 53%). The AUC_{0-24} at steady-state is 11% lower than the total $AUC_{0-\infty}$ after single dosing in Caucasian women, whereas in Japanese women the AUC is 24% higher at steady-state than after single dosing.

TABLE 2 **Single-dose and steady-state pharmacokinetics of EE in Caucasian and Japanese subjects. Presented are median (min-max) for t_{max}; geometric mean (geometric CV%) for other PK parameters**

	EE			
	CAUCASIAN WOMEN		JAPANESE WOMEN	
Parameter (unit)	**Single Dose**	**Steady-state**	**Single Dose**	**Steady-state**
C_{max} (pg/mL)	49.2 (37.7)	80.6 (31.2)	74.5 (34.0)	128 (37.0)
t_{max} (h)	2.00 (0.75-4.02)	1.50 (0.50-2.02)	1.50 (1.00-2.00)	1.50 (0.50-2.02)
$t_{½}$ (h)	27.9 (48.9)	19.8 (24.2)	19.8 (46.8)	17.4 (25.3)
AUC* (pg·h/mL)	747 (42.8)	664 (26.2)	741 (33.5)	912 (25.7)
wn-CL_{app} (L/h/kg)	0.389 (40.2)	0.438 (26.2)	0.511 (33.2)	0.411 (25.8)
wn-$V_{z,app}$ (L/kg)	15.7 (38.8)	12.5 (29.4)	14.6 (40.6)	10.3 (25.7)
C_{av} (pg/mL)	-	27.6 (26.2)	-	38.0 (25.7)
DF (%)	-	241 (20.6)	-	296 (19.2)
$C_{min,av}$ (pg/mL)	-	13.2 (42.6)	-	15.1 (40.1)
R_{A1}	-	1.64 (23.5)	-	1.73 (28.4)
R_{A2}	-	1.70 (28.6)	-	1.96 (22.9)

*: $AUC_{0-\infty}$ for single dose and AUC_{0-24} for steady-state; -: not applicable.

TABLE 3 **Comparison of single-dose and steady-state pharmacokinetic parameters for both Caucasian and Japanese subjects. Point estimates are ratios of geometric least-squares (LS) means. Based on n=34 subjects for Org 30659 and n=36 subjects for EE. Time-independent pharmacokinetics if Regimen effect was not significant**

Compound	Ethnic group	Parameter	Point Estimate μ(ss)/μ(sd)	95% Confidence Interval	Significance
Org 30659	Caucasian	C_{max}	2.11	1.82-2.45	yes
		AUC	2.15	1.87-2.47	yes
		$t_{½}$	1.28	1.08-1.52	yes
	Japanese	C_{max}	2.65	2.30-3.05	yes
		AUC	2.32	2.03-2.65	yes
		$t_{½}$	0.84	0.71-1.00	yes
EE	Caucasian	C_{max}	1.37	1.21-1.54	yes
		AUC	0.89	0.76-1.04	no
		$t_{½}$	0.71	0.56-0.90	yes
	Japanese	C_{max}	1.53	1.34-1.74	yes
		AUC	1.24	1.05-1.45	yes
		$t_{½}$	0.88	0.68-1.13	no

FIGURE 1 **Average (+ SD) concentration-time profiles (from 0-8 hours after dosing) of Org 30659 and EE after single dose (○) and at steady state (●) for both Caucasian and Japanese subjects**

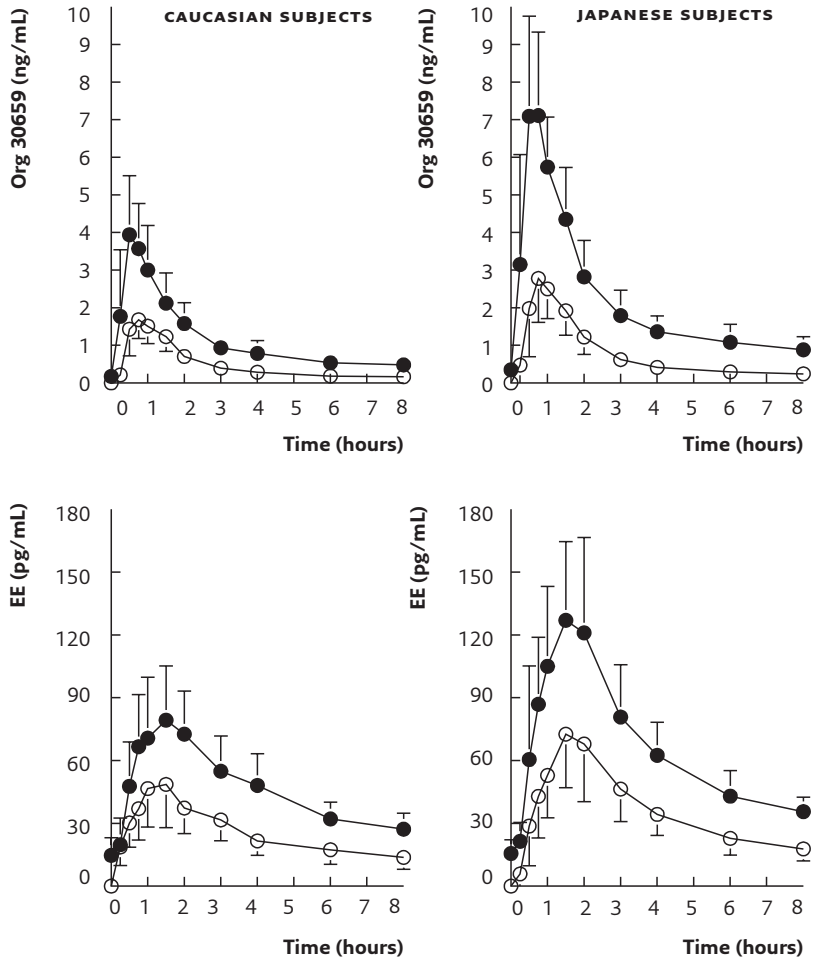


Table 3 shows that the elimination half-life of Org 30659 in Caucasian subjects is significantly longer at steady-state than after single dosing, whereas in Japanese subjects the half-life is significantly shorter at steady-state. For EE, the half-life at steady-state in Caucasians is significantly shorter than after single dosing.

For Org 30659, the median t_{max} at steady-state (0.5 h) was found to be significantly shorter than the median t_{max} after single dosing (0.75 h). Also for EE, the median t_{max} at steady-state was somewhat shorter than after single dosing.

Comparison of pharmacokinetics in Caucasian and Japanese women

The results of the ANOVAs for the statistical comparison of primary PK parameters between Caucasian and Japanese women are given in Table 4.

TABLE 4 **Comparison of Caucasian and Japanese pharmacokinetic parameters after single dose and at steady state. Point estimates are ratios of geometric least-squares (LS) means. Based on n=34 subjects for Org 30659 and n=36 subjects for EE. Similar pharmacokinetics if 90% Confidence Interval contained within acceptance range 0.70-1.43**

Compound	Regimen	Parameter	Point Estimate μ(Jap)/μ(Cauc)	90% Confidence Interval	Conclusion kinetics
Org 30659	Single dose	C_{max}	1.60	1.33-1.93	not similar
		wn-C_{max}	1.24	1.04-1.48	not similar
		AUC	1.75	1.48-2.08	not similar
		wn-AUC	1.36	1.15-1.60	not similar
	Steady state	C_{max}	2.01	1.67-2.43	not similar
		wn-C_{max}	1.55	1.29-1.86	not similar
		AUC	1.90	1.60-2.25	not similar
		wn-AUC	1.46	1.23-1.72	not similar
EE	Single dose	C_{max}	1.51	1.24-1.84	not similar
		wn-C_{max}	1.17	0.97-1.40	similar
		AUC	0.99	0.82-1.19	similar
		wn-AUC	0.76	0.64-0.91	not similar
	Steady state	C_{max}	1.69	1.39-2.05	not similar
		wn-C_{max}	1.31	1.09-1.57	not similar
		AUC	1.38	1.15-1.64	not similar
		wn-AUC	1.07	0.89-1.27	similar

Pharmacokinetics of Org 30659 in Japanese and Caucasian women are not similar after administration of this combination drug. The C_{max} of Org 30659 after single dosing is 60% higher in Japanese women than in Caucasian women. At steady-state this difference mounts up to 101%. Corrected for

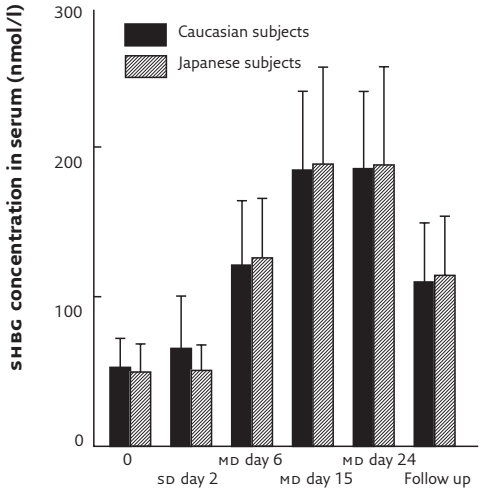
differences in body weight between Caucasian and Japanese women, the weight normalised-C_{max} of Org 30659 after single dosing is 24% and at steady-state 55% higher in Japanese women than in Caucasian women. The AUC of Org 30659 after single dosing is 75% higher in Japanese women than in Caucasian women. At steady-state this difference mounts up to 90%. Corrected for differences in body weight between Caucasian and Japanese women, the wn-AUC of Org 30659 after single dosing is 36% and at steady-state 46% higher in Japanese women than in Caucasian women. No overall significant differences in elimination half-life or t_{max} were observed between the two ethnic groups.

The C_{max} of EE follows the same pattern as the C_{max} of Org 30659. The C_{max} of EE after single dosing is 51 % higher in Japanese women than in Caucasian women. At steady-state this difference mounts up to 69%. After weight-normalisation the difference disappears for the single dose (17% higher, but not significantly different). At steady-state the wn-C_{max} is still 31% (statistically significant) higher in Japanese women. The AUC of EE after single dosing is similar in Caucasian and Japanese women. After weight normalisation, the wn-AUC is 24% lower in Japanese women. The AUC of EE at steady-state is 38% higher in Japanese women than in Caucasian women. Corrected for differences in body weight, this difference is reduced to 7%. The mean $t_{1/2}$ after single dosing for Caucasians was significantly longer than the $t_{1/2}$ after single dosing for Japanese women (see Table 2). There were no significant differences in t_{max} between Caucasian and Japanese women.

Sex hormone binding globulin

Averaged sex hormone binding globulin (SHBG) values are represented in Figure 2. SHBG increased during the trial for all subjects. At follow up all SHBG values had decreased but were still above the baseline values. After inclusion of SHBG into the PK model of Org 30659, the ratio of geometric AUC means for steady-state over single dose decreased from 2.15 to 1.12 for Caucasian subjects and from 2.32 to 1.20 for Japanese subjects. The statistical analysis showed that SHBG played no significant role in the comparison of the pharmacokinetics between Caucasian and Japanese subjects. However, the increase in SHBG after multiple dosing provided an explanation for the observed differences in pharmacokinetics of Org 30659 between single dose and steady-state.

FIGURE 2 **Average (+ SD) Sex Hormone Binding Globulin concentrations for both Caucasian and Japanese subjects. (Follow-up was performed 7-15 days after last intake of study medication)**



Safety parameters

There were neither serious adverse events reported nor any deaths. None of the reported adverse events led to the drop-out of any subject from the trial. One reported severe adverse event was in fact an exacerbation of pre-existing shoulder pain. All the other adverse events were of mild or moderate intensity. The most frequently reported drug related adverse events included bleeding irregularities. Adverse events in this trial were somewhat differently reported between the two ethnic groups, particularly regarding the reproductive disorder class. Adverse events in the reproductive disorder class were reported by Caucasian subjects as abdominal pain associated with bleeding irregularities (n=6 and n=15 respectively) while the Japanese subjects reported this as dysmenorrhoea with bleeding irregularities (n=3 and n=18 respectively). Breast enlargement (n=5), peripheral oedema (n=9), treatment related hot flushes, somnolence and malaise (n=4) were reported only by Japanese. In general more treatment related adverse events were reported by the Japanese population compared to the Caucasian population (82 and 45 respectively). No clinically relevant abnormalities were found in vital signs, nor were there any consistent changes observed in ECG parameters and other safety measures. No other clear difference in any of the safety measurements was noted between the two ethnic groups.

Discussion

The primary objectives of this trial were to compare the pharmacokinetic parameters and safety of Org 30659 and EE in Caucasian and Japanese women after single and multiple dose administration and to compare the single dose and steady-state pharmacokinetics of Org 30659 and EE.

The pharmacokinetics of Org 30659 and to a lesser extent EE were significantly different between the two ethnic groups following the single dose administration of the tablet containing both 200 µg Org 30659 and 20 µg EE. This difference in the kinetics was further increased after steady-state was reached, following multiple administration of these tablets. Weight normalisation reduced but did not diminish the observed differences. SHBG concentrations could not explain these findings either. Therefore, other factors like phenotypic differences (including dietary or environmental factors) and genetic differences in absorption and/or metabolism could account for the observed kinetic differences between the two ethnic groups.

Ethnic differences in the pharmacokinetics of EE have been reported previously. EE is extensively metabolised by UDP-gluceronosyltransferase (UGT) 1A1, which also catalyses the glucuronidation of bilirubin and xenobiotic phenols and some steroids. Mutations of UGT1A1 cause the unconjugated hyperbilirubinemias known as Crigler-Najjar syndrome and Gilbert's syndrome. The frequencies of individual UGT1A1 polymorphisms show extensive variability across ethnic groups. The incidence of nonphysiologic neonatal hyperbilirubinemia is twice as high in East Asians as in whites. Furthermore, consistent inter-individual and ethnic differences have been reported in the degree of oxidative metabolism of EE that could attribute to the differences observed in the current study.

ref. 15-17
ref. 18-22
ref. 23
ref. 24
ref. 25-26

Phase II metabolism, and in particular conjugation with glucuronic acid is also suggested to be the major metabolic route for Org 30659 *in vivo*. UGT2B7 is suggested to play an important role in this conjugation. Large differences in polymorphism between Asians and Caucasians have been suggested. It is reported that only 9.4% of the investigated Asian population are UGT2B7(Y^{268}) homozygous compared with 29.2% of Caucasians. Asians had a higher prevalence of the homozygous UGT2B7(H^{268}) compared to the Caucasian population: 56.2% vs 21.8%. The corresponding phenotypical differences of these polymorphisms have not been determined. The data from this study suggests that the homozygous UGT2B7(Y^{268}) genotype might be associated with higher metabolic activity compared to the homozygous UGT2B7(H^{268}) genotype.

ref. 27
ref. 22

The apparent accumulation of Org 30659 after multiple dosing is probably related to the strong increase in SHBG levels induced by EE. The reduced clearance at steady-state could be a reflection of the binding of Org 30659 to proteins or change in metabolism at steady-state. However, the accumulation of EE cannot be explained by increased SHBG levels because EE binds for 98% to albumin and has no affinity for SHBG. The accumulation of EE is notably less than of Org 30659.

ref. 28

Adverse events in this study were somewhat differently reported between the two ethnic groups. More treatment related adverse events were reported by the Japanese population compared to the Caucasian population. The clear difference in pharmacokinetics between the two groups (higher Org 30659 concentrations and (peak) EE concentrations in the Japanese population) could contribute to these apparent differences. However, another factor that may explain these differences could be the number of OC-users before the start of the trial. None of the Japanese subjects were OC-users, while all but five of the Caucasian subjects were OC-users. This difference in experience with the use of COC may have contributed to the difference in AE reporting. No consistent changes were found in vital signs, ECG and clinical laboratory data and no clear differences for these safety measures were noted between the two ethnic groups, supporting that the combination of Org 30659 and EE was safe.

This study showed that it is possible to adequately perform a bridging study between Japanese subjects studied in Japan and Caucasian volunteers studied in the Netherlands using a single protocol and data management system. This type of study can be performed at an early stage in the drug development and can provide essential information for the future development of western drugs in Japan. There appear to be differences in the pharmacokinetic handling of both EE and a new synthetic progestagen between Japanese and Caucasian women. The clinical consequences of these differences require further investigation.

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Added value of bridging studies

Japanese people are considered a special target patient population by the Japanese drug registration authorities. Introduction of a western drug on the Japanese market requires proving efficacy and safety in the Japanese population, which often requires repetition of most clinical trials in Japan. A way to improve this time and cost consuming process can be to perform bridging studies between typical Caucasian and typical Japanese subjects to compare the characteristics that could potentially differ between the two populations. According to IMS health, multinational pharmaceutical companies are looking to aggressively expand their drug sales in Japan. From 1996 to 2000, the proportion of the Japanese market held by foreign companies increased from around 20% to almost 28%. Some of the biggest names in the industry, including Pfizer, AstraZeneca, Aventis and Eli Lilly, have recently made clear their intent to target the world's second-largest drug market (www.IMShealth.com). Their interest is being driven by the huge potential gains if they could match their global market shares in the Japanese market. This expansion could translate into billions of dollars in extra revenues. The task is becoming more readily achievable, as regulations overseeing drug development are harmonised between Japan, the USA and Europe, and as changes in healthcare delivery and finance foster new sales and marketing approaches.

Obviously, racial differences could occur in the pharmacological properties of investigational new drugs. Therefore, before a new drug is introduced in Japan sufficient data should be presented on the effects of the drug in a Japanese population. In some cases a comparison between the 'western' population and the Japanese population can be used to extrapolate the data from 'western' studies to the Japanese (especially when no differences are observed). Extrapolation of data could save the costs of additional studies in Japan. Therefore, it can be very rewarding to perform such a comparative (or 'bridging') study. Two examples of comparative studies are presented in this thesis.

The first example shows a study on the effects of nitrazepam, a registered drug for the treatment of anxiety. This study was first performed in Japan and afterwards repeated in matched Caucasian subjects in the Netherlands using the same protocol. The study was designed to explore the possibility and feasibility to perform

bridging studies in two study centres using one protocol. Once the infrastructure was established and both study sites' procedures were harmonised in the nitrazepam study, a bridging study for a potentially new oral contraceptive was performed as presented in chapter 10.
The Japanese registration authorities often require repetition of most clinical trials in Japanese subjects before registration. However, in some cases, a comparative trial can show that the complete repetition of all the clinical trials is not necessary. The 'population' question should nevertheless be adequately answered. Potential differences in ethnopharmacological factors would require additional trials in the Japanese population. Therefore, early comparative studies between Caucasians and Japanese have intrinsic value to the drug development program.

The comparative trial can be performed early in the development or late in the development depending on the estimated probability the study will show similar pharmacokinetics and/or drug effects and the costs associated with the study. The study presented in chapter 10 is performed early in the development and showed significant differences in pharmacokinetics. This finding requires parallel development in Japan in order to launch the product world-wide. If this study was performed at a later stage in the development, subsequent development in Japan would be delayed resulting in a loss of market value due to patent expiry and loss of revenues. It can be argued that the success probabilities for development in Japan would be higher in this case because development in the Caucasian population already produced significant knowledge about the drug.

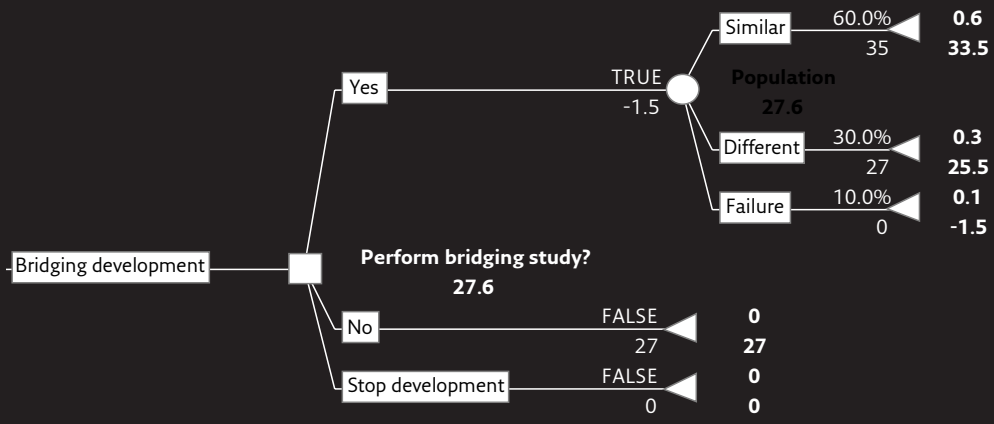
For the development of a (hypothetical) new drug a bridging scenario is considered. The drug development team has assumed that the worldwide development of the drug can be accomplished in three ways;

1. an early bridging study is performed that shows the two ethnic groups are 'similar' (this probability is estimated to be 60%)
2. an early bridging study is performed that shows the two ethnic groups are 'different' but the drug is developed in parallel in the two groups (this probability is estimated to be 30%)
3. the drug is developed in parallel in the two groups

In order to decide whether or not it is rewarding to perform the bridging study, which will introduce additional costs of M€ 1.5, a decision tree is constructed.

The risk adjusted project value after the bridging study is estimated at M€ 35 for the first option. The second option is estimated lower because the costs will be doubled (the drug will have to be developed in both groups in parallel). However, because the bridging study will enhance the success probability of the drug, the risk adjusted project value is not estimated at half the project value of the first option but at M€ 27. Similarly, if the bridging study is not performed but the drug is successfully developed according to the last option, the 'risk adjusted' project value is also set at M€ 27 but obviously, the costs of the bridging study have not been made. The constructed decision tree is represented in figure 1.

FIGURE 1 **Decision tree for a new drug, which is used to determine the feasibility of a bridging study**

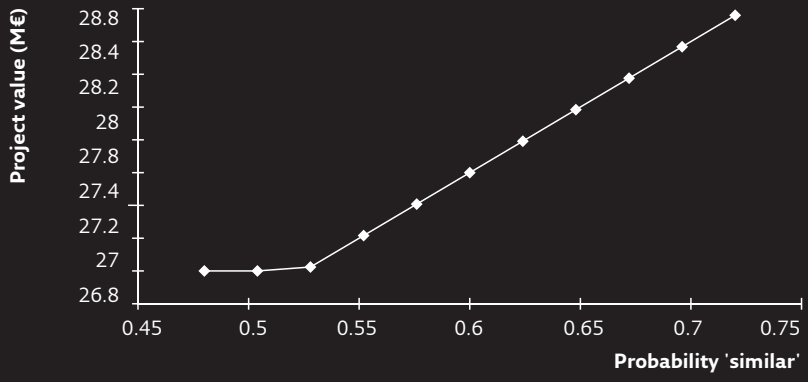


The decision tree clearly shows that the additional bridging study enhances the estimated risk adjusted project value with the current input parameters. In order to find out when the bridging scenario is no longer favourable, sensitivity analysis on the probability the two ethnic groups are 'similar' is performed. The result of this analysis is shown in Figure 2.

From this graph, it can be concluded that the probability the two ethnic groups are 'similar' may be estimated as low as 53% and still the bridging study will produce additional value despite the introduction of M€ 1.5. If the probability drops below

the 53%, it is more sensible to skip the bridging study and immediately start parallel development in both groups. The latter option produces the estimated risk adjusted project value of M€ 27.

FIGURE 2 **Sensitivity analysis on the probability that the bridging study will show the two ethnic groups are ´similar´**



However, the example of the potential oral contraceptive illustrates that it is crucial to take all questions into account when constructing the drug development decision tree. Even if one identifies the 'population' question as an important question, the other questions can have more significant impact on the optimal development strategy. In the presented case, the probability the drug would prove to have an improved side-effect profile would have to be estimated very low. The side effects associated with registered oral contraceptives are relatively rare and the exact mechanism of these effects is unknown and still subject to discussion. It would require additional evidence on the mechanism leading to these side effects to convince registration authorities that the new mechanism of action of the drug will lead to lower incidence of the side effects. Subsequent proof of improved side effect profile over existing medication would be very difficult (and expensive) due to the already relatively rare side effects.

Therefore, the probability of successfully answering the 'clinical' question would have to be estimated relatively low and the associated costs very high. Using both this low probability and high cost for the 'clinical' question in the QBD tree yields

a negative estimated risk adjusted project value instead of the M€ 27 used in the decision analysis represented in figure 1. As a consequence, the drug was discontinued after the comparative study presented in chapter 10 was completed. Adequate estimation of the costs and probabilities of success using the QBD-approach would have saved at least the presented comparative trial.
The construction of a decision tree for this drug would have revealed that the 'clinical' question far outweighs the 'population' question and abandoning the development of this drug would have been the best decision.

SECTION 1

SECTION 2

SECTION 3

SECTION 4

Literature evaluation

Developing a new formulation

Bridging the gap to Japan

Market advantage

CHAPTER 11

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SUBMITTED

Pharmacodynamic and pharmacokinetic effects of MRLA023, a $GABA_A$ $\alpha_{2,3}$ subtype selective agonist, compared to lorazepam and placebo in healthy volunteers

Abstract

AIM This study investigated the effects of two doses (0.5 mg and 1.5 mg) of MRLA023, which is a $GABA_A$ $\alpha_{2,3}$ subtype selective partial agonist. This compound MRLA023 is expected to result in comparable anxiolytic efficacy as clinically used benzodiazepines with an improved tolerability profile. It is hypothesised to be less sedating because it lacks efficacy at the α_1 subtype, the subtype believed to mediate the sedative effects of benzodiazepines.

MATERIAL AND METHODS Twelve healthy male volunteers participated in this placebo controlled, double-blind, double-dummy, four-way, cross-over study. As a positive control, the non-selective benzodiazepine lorazepam (2mg) was used in a therapeutic anxiolytic dose. The Central Nervous System (CNS) effects of the two doses of MRLA023 and lorazepam were compared with placebo. Saccadic Eye Movements (SEM) and Visual Analogue Scales (VAS) were used to assess the sedative properties of MRLA023. The psychomotor and cognitive effects were assessed using body sway and a standardised battery of neurophysiological memory tests.

RESULTS Lorazepam caused sedation (maximum SEM reduction of 89 deg/sec and a VAS alertness score maximum effect of 4.5 mm) and impairment of memory and body stability (three of the four memory tests studied showed significant memory impairment and body sway with eyes closed was increased by 28%). MRLA023 had significant dose dependent effects on SEM (85 deg/sec maximum reduction at the higher dose, approximating that of lorazepam) but not on VAS score of alertness. No changes were observed in saccadic latency and saccadic inaccuracy for either doses of MRLA023, in contrast to significant increases with lorazepam. Contrary to lorazepam, MRLA023 caused no detectable memory impairment or postural imbalance.

CONCLUSION These results show that the effect profile of MRLA023 differs from that of lorazepam, at doses that were equipotent with regard to SEM -effects. Therapeutic equipotency cannot be proven at this stage, because the clinical effects of MRLA023 have not yet been determined. The differentiation between pharmacodynamic effects for the selective $GABA_A$ agonist MRLA023 may be related to selectivity for different $GABA_A$ receptor subtypes. Further studies will show whether this pharmacological selectivity is associated with an improved side effect profile.

Introduction

Generalised anxiety disorder (GAD) is a severe, chronic, and distressing illness that often requires long-term management. The lifetime prevalence is approximately 4 to 6 percent in the general population and is more common in women than in men. Benzodiazepines are the most frequently prescribed pharmacological treatment for GAD. Benzodiazepines possess several advantages over other anxiolytics, including rapid action, ease of use and a wide margin of safety. Although benzodiazepines are relatively safe drugs and are widely used in the treatment of anxiety, they may produce untoward side effects such as sedation, memory impairment and muscle relaxation.

ref. 1

MRLA023 is a $GABA_A$ $\alpha_{2,3}$ subtype selective partial agonist, which is expected to result in comparable anxiolytic efficacy as clinically used benzodiazepines with an improved tolerability profile based upon pre-clinical animal models. In particular, MRLA023 has the potential to be an effective, non-sedating anxiolytic. It is believed to be non-sedating because it lacks efficacy at the α_1-subtype, the subtype believed to mediate the sedative effects of benzodiazepines.

ref. 2-6

Based on tolerability findings in healthy volunteers, two doses of MRLA023 were selected for this study: 0.5 mg and 1.5 mg. The highest dose was chosen to evaluate the sedative, cognitive, and motor effects that could be expected with doses at the upper end of the anticipated dose range to be evaluated in further studies. The lower dose of MRLA023 was tested in order to establish the pharmacodynamic effects expected at a dose that might still demonstrate anxiolytic efficacy. For lorazepam, a dose of 2 mg was selected, which is known to be both therapeutically relevant and sedating.

ref. 7-8

The current study was designed to compare the central nervous system (CNS) effects of two dose levels of MRLA023 with those of placebo and 2 mg lorazepam in healthy male subjects. Saccadic eye movements and visual analogue scales were used to assess the sedative properties. According to a recent literature review of non-selective benzodiazepine anxiolytics, effects on saccadic eye movements are also closely correlated with therapeutic effects. In addition, the psychomotor and cognitive effects of MRLA023 were compared with those of 2 mg lorazepam and placebo, using body sway and a standardised psychometric battery. Finally, the plasma levels of MRLA023 were correlated with statistically significant pharmacodynamic effects.

ref. 9

Methods

Design

This study was a placebo controlled, randomised, double-blind, double-dummy, four-way, cross-over, monocentric study in twelve healthy male volunteers, with a five-day washout period.

Subjects

Twelve healthy non-smoking volunteers were recruited from the CHDR database. All volunteers received a full medical examination and gave written informed consent before entry to the study. Subjects were asked not to drink alcohol 48 hours prior to the study, abstain from caffeine-containing products 8 hours prior to the study and from grapefruit, grapefruit juice and St. John's Wort for at least 2 weeks prior to the study until the completion of the study. The study was approved by the Medical Ethics Review Board of Leiden University Medical Center, and performed according to the principles of the Helsinki Declaration and GCP.

Treatments

Subjects received each single oral dose MRLA023 0.5mg, MRLA023 1.5mg, lorazepam 2 mg or placebo administered with 250 ml of water in a fasting state at approximately 8 to 9 AM on day 1 of each study period. Subjects always received 3 tablets of MRLA023 or matching placebo and 2 capsules of lorazepam or matching placebo. The treatment sequences were determined using 4x4 Latin Squares, balanced for 1st order carry-over.

Safety

Adverse events, ECG, blood pressure and heart rate measurements were assessed throughout the study. ECGs were assessed with a Cardiofax, equipped with ECAPS12 analysis program (Nihon Kohden, Japan). Blood pressure and heart rate were measured with an automated blood pressure monitor (MPV1072, Nihon Kohden, Japan), which displays an average value for two sequential (duplicate) measurements at each time point. All ECG, blood pressure and heart rate measurements were made after the subject had been sitting in a semi-recumbent position for at least 5 minutes.

Pharmacokinetics

Blood samples were drawn on day 1 of each occasion day predose (within 30 minutes prior to dosing) and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10 and 24 hours postdose and were processed to obtain plasma for assay of MRLA023 and lorazepam concentrations. All blood samples were protected from exposure to direct light throughout the sample handling procedures. Blood samples (5 ml) drawn from an intravenous catheter were collected into sodium heparinised tubes. Blood samples were inverted 6 times and immediately centrifuged in a refrigerated centrifuge at 2000 gs for 10 minutes at 4°C. Plasma was transferred to a 4.5 cc Nunc cryotube and stored at −20°C within 30 minutes. Plasma samples collected following each MRLA023 dose were assayed for MRLA023. Analysis was accomplished by solid phase extraction of the analyte and an internal standard from plasma using a 96-well plate format followed by reversed phase HPLC and MS/MS detection. Plasma samples collected following the lorazepam dose were assayed for lorazepam. Lorazepam and its stable-isotoped labelled internal standard were extracted from basified plasma into methyl-t-butyl ether with an automated procedure using a Tomtec Quadra 96 Model 320. Extracts were evaporated under nitrogen, reconstituted and analyzed by LC/MS/MS using positive ion Turbo Ionspray with multiple reaction monitoring.

Pharmacodynamics

Pharmacodynamic measurements were performed predose (within 30 minutes prior to dosing) and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8 and 10 hours postdose. Pharmacodynamic tests were perfomed in a quiet room with ambient illumination with only 1 subject in the same room per session. Each session consisted of the following sequence of tests: saccadic eye movements; body sway eyes open; body sway eyes closed; VAS. Cognitive function tests were performed in the 1-3 hours-postdose period between the other measurements.

SACCADIC EYE MOVEMENTS Saccadic eye movements were recorded using a micro-computer-based system for data recording (Cambridge Electronics Design, Cambridge, UK), Nihon Kohden equipment for stimulus display, signal collection and amplification (Nihon Kohden Corporation, Tokyo, Japan), and disposable surface electrodes (Medicotest N-00-S, Olstykke, Denmark). Average values of latency (= reaction time), peak saccadic velocity and inaccuracy (difference between stimulus angle and corresponding saccade in %) were calculated for all artifact-free saccades. Saccadic peak velocity has been validated as the most sensitive measure for the sedative effects of benzodiazepines.

ref. 10

ref. 11-13

VISUAL ANALOGUE SCALE Visual analogue scales as originally described by Norris were previously used to quantify subjective effects of benzodiazepines. From the set of sixteen scales three composite factors were derived as described by Bond and Lader, corresponding to alertness, mood and calmness. These factors were used to quantify subjective drug effects.

ref. 14 ref. 13 ref. 15

BODY SWAY Body sway was measured with an apparatus similar to the Wright ataxiameter, which integrates the amplitude of unidirectional body movement transferred through a string attached to the subject's waist. Two-minute measurements were made in the antero-posterior direction with eyes open and eyes closed, with subjects standing comfortably on a firm surface with their feet slightly apart.

ref. 16

COGNITIVE FUNCTION TESTS The neurophysiological memory tests were performed using FePsy (The Iron Psyche), an automated system containing a battery of computerised tests for cognitive (neuropsychological) functions. The recognition and recall test and the Corsi block tapping task were included in this study. The Corsi block tapping test was constructed according the principles of the original Corsi block tapping task. This task assessed the nonverbal memory span.

ref. 17 ref. 18

Analysis

PHARMACOKINETICS The pharmacokinetics of MRLA023 was investigated using non-linear mixed effect modeling as implemented in NONMEM version V software (NONMEM Project Group, University of California, San Francisco, CA), applying the first order conditional estimation (FOCE) method with the 'interaction' option. A series of PK models was attempted and compared using the likelihood ratio test. Ultimately, a two-compartment model with first-order absorption and a lag-time was used to describe the pharmacokinetics of MRLA023. Intra-individual error was modelled using a constant coefficient of variation error model. No pharmacokinetic parameters were calculated for lorazepam.

ref. 19

PHARMACOKINETIC/PHARMACODYNAMIC RELATIONSHIPS The observed pharmacodynamic effects were plotted against the predicted MRLA023 concentrations for each individual. Because the average placebo profile for saccadic peak velocity was not flat, the average placebo profile was subtracted from all saccadic peak velocity data at corresponding protocol time points and the result was subjected to PK/PD analysis. PK/PD modelling

was performed using non-linear mixed effect modelling as implemented in NONMEM. Empirical Bayes pharmacokinetics estimates were generated and used to describe the concentration profile for investigation of the PK/PD relationship between MRLA023 and saccadic peak velocity. A linear concentration-effect model was estimated without an effect compartment. Individual graphs indicated that no improvement could be obtained using either a more complex concentration-effect model or an effect compartment and further analysis was not attempted.

STATISTICS Treatment response was characterised for continuously measured variables by calculating the area under the effect curve (AUEC) relative to baseline over 6 hours. The pre-values were averaged and set at time = 0 hr. Change from average pre-value (delta) was calculated. The AUECS were calculated using the linear trapezoidal rule up to 6 hours on the basis of protocol (planned) time points and were subsequently divided by the corresponding time span resulting in weighted average change from pre-value. All variables were analysed untransformed except for body sway because only body sway clearly indicated an increase in variability in response with an increase in average response. As cognitive function test results were assessed only once for each treatment, raw scores were analysed. Statistical analysis was initially performed using analysis of variance with factors treatment (4 levels) subject (12 levels) occasion (4 levels) and carry-over (5 levels, coded as the treatment preceding the current treatment, including 'no preceding treatment'). If the carry-over effect was found to be non-significant, the analysis was rerun without the carry-over factor. The four treatments were compared within the ANOVA model using the following contrasts: placebo - MRLA023 0.5mg, placebo - MRLA023 1.5mg, lorazepam 2mg - MRLA023 1.5mg and placebo - lorazepam 2mg. Overall p-value for the treatment effect was reported along with the specified contrasts with 95% confidence intervals and p-values. All calculations were performed using SAS for Windows V8.1 (SAS Institute, Inc., Cary, NC, USA).

Results

Subjects

Twelve subjects, judged to be in good health on the basis of medical history, physical examination and routine laboratory data, participated in the study after giving written informed consent. Two subjects dropped out; one was repeatedly unable to swallow the capsules and another withdrew after the

second occasion for personal reasons. These two subjects were replaced by two other healthy male subjects, using the same randomisation sequence. Twelve subjects completed study. Subjects were on average 25 years of age (range 20-29), average weight of 82.3 kg (range 74.6-87.7 kg) and average height of 184.1 cm (range 177.6-191.9 cm).

Clinical observations

No serious adverse reactions occurred following any of the treatments. The most frequently reported adverse event was sedation by eight, five, zero and two subjects after administration of lorazepam, the high and low doses of MRLA023 and placebo, respectively. Other reported adverse events were drowsiness after MRLA023 0.5mg administration (three subjects), dizziness after MRLA023 1.5mg administration (four subjects), sleepiness and headache after lorazepam 2mg administration (seven and three subjects, respectively) and fatigue and headache after placebo administration (six and five subjects, respectively). ECG, blood pressure and heart rate measurements demonstrated no clinically significant effects for both doses of MRLA023, lorazepam and placebo.

Pharmacokinetics

The average plasma concentration-time curves for both doses of MRLA023 and lorazepam are shown in figure 1. Both doses of MRLA023 and lorazepam showed maximum concentrations after approximately 2 hours. The average pharmacokinetic parameters (with interindividual variation coefficients (CV) of MRLA023 were: clearance/bioavailability of 246 mL min^{-1} (CV 29%), initial half-life of 142 min (CV 6%), terminal half-life of 437 min (CV 0%, fixed), central volume of distribution/bioavailability of 71.1 L (CV 20%), absorption half-life of 33.6 min (CV 39%) and a lag-time of 27.4 min (CV 19%).

Pharmacodynamics

SACCADIC EYE MOVEMENTS Saccadic peak velocity (SPV), which was used to assess sedative properties, demonstrated significant effects with lorazepam and both doses of MRLA023 (figure 2 and table 1). There was a dose-dependent increase of SPV with MRLA023 0.5 and 1.5 mg ($AUEC_{0\text{-}6hr}$ decrease of 22 deg/sec and 45 deg/sec). No changes were observed in

saccadic latency and saccadic inaccuracy for either doses of MRLA023, in contrast to the significant increases with lorazepam. The high dose of MRLA023 and lorazepam caused similar average maximum effects on SPV relative to baseline. However, the effects of lorazepam lasted slightly longer, leading to a significant difference in time-corrected $AUEC_{0\text{-}6hr}$ (table 1).

FIGURE 1 **Average drug concentration profiles (mean + SD) of MRLA023 0.5mg (□), MRLA023 1.5mg (○) and lorazepam 2mg (Δ) after oral administration**

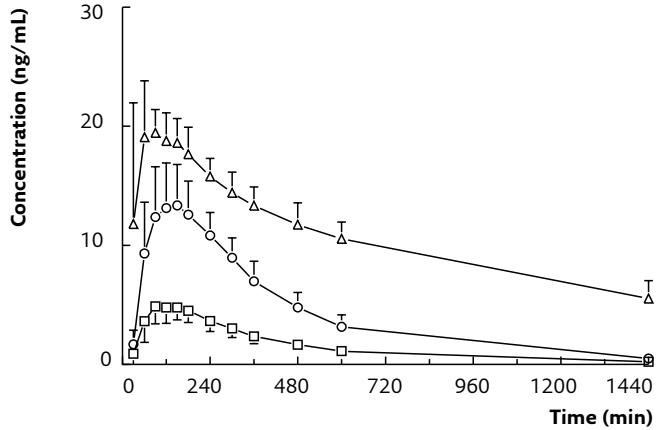
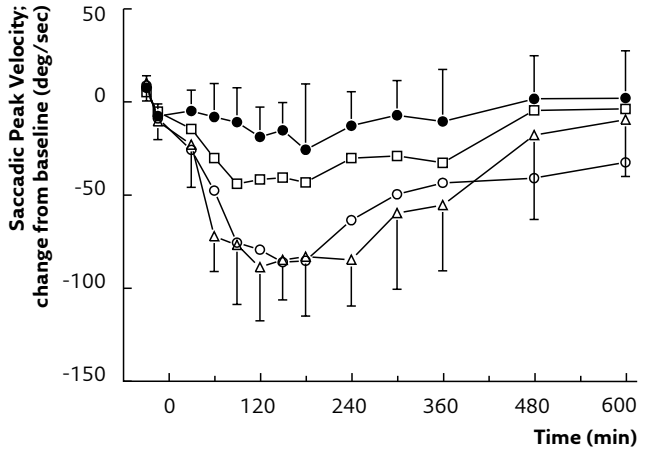


FIGURE 2 **Average time profile (mean + SD) of Saccadic Peak Velocity (change from baseline) after oral administration of placebo (●), MRLA023 0.5mg (□), MRLA023 1.5mg (○) and lorazepam 2mg (Δ)**

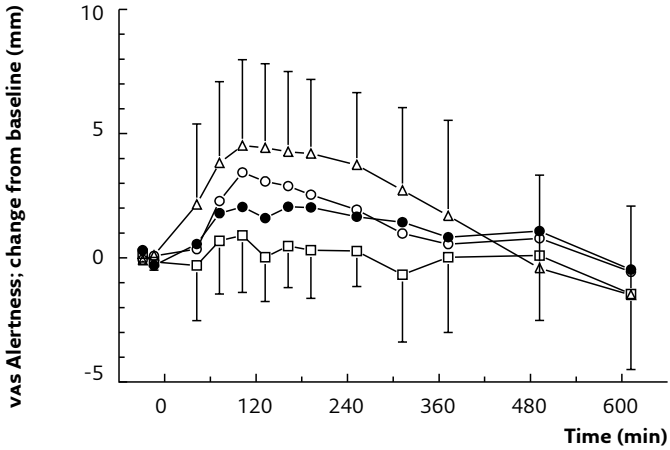


VISUAL ANALOGUE SCALE The VAS score of alertness, which was used to estimate subjective sedative effects, only showed a significant average effect after lorazepam (table 1). The average curve for the high dose of MRLA023 was in between the average curves of lorazepam and placebo (figure 3), and consequently, the $AUC_{0\text{-}6hr}$ of the high dose of MRLA023 did not differ significantly from either lorazepam or placebo. Subjective calmness was reduced after the high dose of MRLA023, while none of the other treatments showed any effect. No significant effects were observed for the VAS contentedness subscale.

TABLE 1 **Pharmacodynamic measurements $AUC_{0\text{-}6hr}$ relative to baseline: Saccadic Eye Movements, Visual Analogue Scales and Body Sway; ANOVA results (contrast, 95% CI, p-value)**

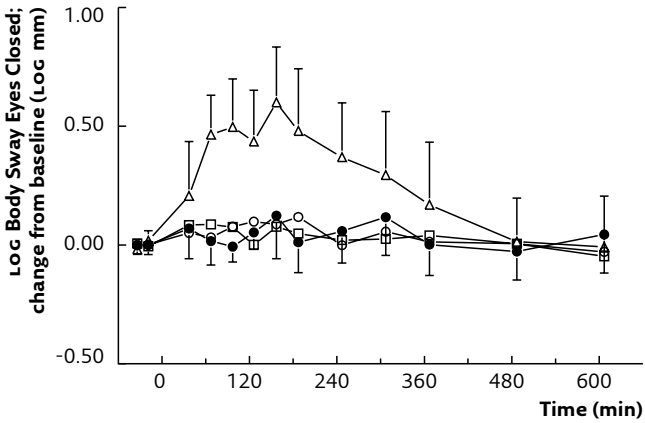
Variable	Overall treatment effect (p-value)	Placebo MRLA023 0.5mg	Placebo MRLA023 1.5mg	Lorazepam 2mg MRLA023 1.5mg	Placebo Lorazepam 2mg
Saccadic Peak Velocity (deg/sec)	<.0001	21.58 (8.40 / 34.76) p = 0.002	45.24 (32.06 / 58.42) p < 0.001	-13.99 (-27.17 / -0.81) p = 0.038	59.23 (46.05 / 72.41) p < 0.001
Saccadic Latency (sec)	0.0003	-0.002 (-0.014 / 0.009) p = 0.672	-0.009 (-0.021 / 0.002) p = 0.116	0.017 (0.006 / 0.029) p = 0.005	-0.027 (-0.039 /-0.015) p < 0.001
Saccadic Inaccuracy (%)	0.0008	-0.09 (-1.27 / 1.08) p = 0.874	-0.03 (-1.21 / 1.14) p = 0.954	2.21 (1.03 / 3.38) p < 0.001	-2.24 (-3.42 / -1.07) p < 0.001
VAS Alertness (ln mm)	0.0082	1.35 (-0.37 / 3.08) p = 0.119	-0.33 (-2.05 / 1.39) p = 0.698	1.47 (-0.25 / 3.19) p = 0.092	-1.80 (-3.52 /-0.08) p = 0.041
VAS Contentedness (ln mm)	0.2630	-0.25 (-0.97 / 0.48) p = 0.492	-0.71 (-1.44 / 0.02) p = 0.055	-0.47 (-1.20 / 0.25) p = 0.193	-0.24 (-0.96 / 0.49) p = 0.510
VAS Calmness (ln mm)	0.0097	-0.14 (-0.46 / 0.17) p = 0.355	-0.53 (-0.84 /-0.22) p = 0.002	-0.43 (-0.74 /-0.12) p = 0.009	-0.10 (-0.41 / 0.22) p = 0.529
Log Body Sway Eyes Closed (log mm)	<.0001	0.009 (-0.087 / 0.106) p = 0.849	-0.001 (-0.098 / 0.095) p = 0.976	0.310 (0.214 / 0.407) p < 0.001	-0.312 (-0.408 /-0.215) p < 0.001
Log Body Sway Eyes Open (log mm)	<.0001	-0.026 (-0.102 / 0.050) p = 0.487	-0.021 (-0.097 / 0.055) p = 0.575	0.267 (0.192 / 0.343) p < 0.001	-0.288 (-0.364 /-0.213) p < 0.001

FIGURE 3 **Average time profile (mean + SD) of VAS Alertness (change from baseline) after oral administration of placebo (●), MRLA023 0.5mg (□), MRLA023 1.5mg (○) and lorazepam 2mg (Δ)**



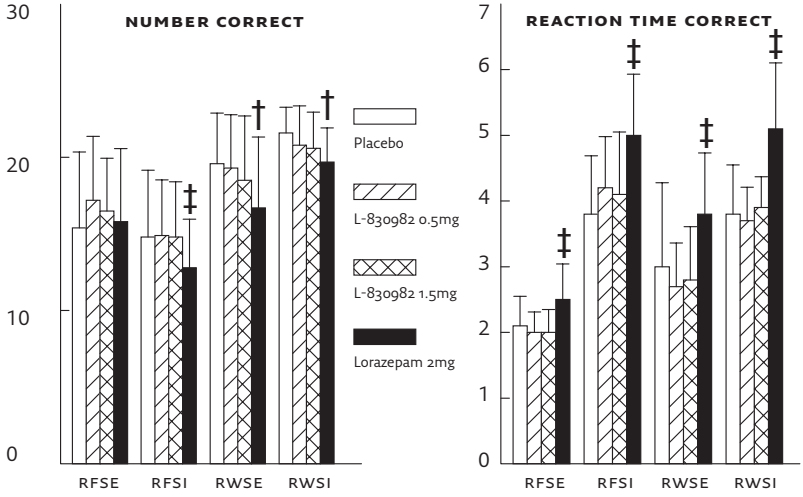
BODY SWAY No body instability was observed after either dose of MRLA023 compared to placebo (figure 4). Lorazepam, however, caused a profound and highly significant increase in body sway (table 1).

FIGURE 4 **Average time profile (mean + SD) of LOG Body Sway Eyes Closed (change from baseline) after oral administration of placebo (●), MRLA023 0.5mg (□), MRLA023 1.5mg (○) and lorazepam 2mg (Δ)**



COGNITIVE FUNCTION TESTS AND CORSI BLOCK TAPPING TASK Three of the four recognition tests revealed that lorazepam caused significant memory impairment, compared to placebo (figure 5). In contrast, neither dose of MRLA023 showed any significant effect on memory. Aside from the effects of lorazepam on the ability to answer correctly, it also significantly increased the reaction times to the correct answers of all memory tests with a range of 0.5-1.3 sec from placebo (figure 5). These significantly higher reaction times were not found with MRLA023. No treatment effects were observed on the Corsi block tapping task.

FIGURE 5 **Effects on cognitive function tests (mean + SD). RFSE = Recognition Figures Serial; RFSI = Recognition Figures Simultaneous; RWSE = Recognition Words Serial; RWSI = Recognition Words Simultaneous. †: p<0.05 compared to placebo, ‡: p<0.05 compared to placebo and MRLA023 1.5mg**

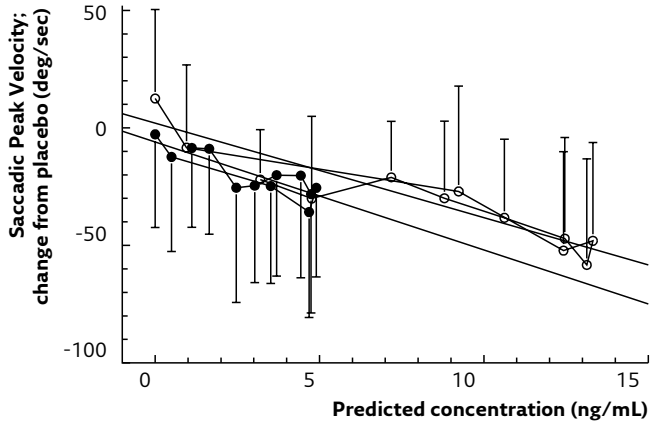


Pharmacokinetic/pharmacodynamic relationships (PK/PD)

Concentration-effect-relationships were only determined for statistically significant pharmacodynamic effects of MRLA023 (*ie* only for SPV). The average PK/PD relationship between the changes in SPV from baseline and the predicted concentration for both doses of MRLA023 is represented in figure 6. A linear concentration-effect model was estimated without an effect

compartment for both doses of MRLA023. Both slope and intercept for SPV did not differ significantly between the two doses of MRLA023. There were no obvious signs of hysteresis or maximum effects. Individual graphs indicated that no improvement could be obtained using either a more complex concentration-effect model or an effect compartment.

FIGURE 6 **Concentration-effect profiles for Saccadic Peak Velocity of MRLA023 0.5mg (●) and MRLA023 1.5mg (○)**



Discussion

The current placebo-controlled study in healthy male volunteers investigated the effects of two doses of MRLA023, a $GABA_A$ $\alpha_{2,3}$ subtype selective partial agonist. The benzodiazepine lorazepam was used in a therapeutic anxiolytic dose as a positive control. As expected, lorazepam caused sedation (shown by SPV-decreases and VAS-effects), impairment of memory and body sway. These effects are typical for benzodiazepines, and are often used as indicators of the drug's effects. MRLA023 caused dose dependent SPV-effects of a similar magnitude as lorazepam, but MRLA023 had no detectable effects on VAS alertness score, memory or postural stability.

ref. 12-13

The results from the current study suggest that the clinical effects of MRLA023 may differ from those of lorazepam, but this relies on the assumption that equipotent therapeutic doses were used. Therapeutic equipotency cannot be proven at this stage, because the clinical effects

of MRLA023 have not yet been determined in patients with anxiety. However, lorazepam 2 mg and the highest dose of MRLA023 caused similar reductions in Saccadic Peak Velocity (SPV). Although a decrease in SPV is usually viewed as a biomarker for sedation, a recent review showed that the SPV-effect is also closely linked to therapeutic levels of benzodiazepines. If the clear SPV-reductions with MRLA023 and lorazepam are indicative of the therapeutic dose, MRLA023 would be expected to have fewer of the adverse effects in patients that are typically associated with non selective benzodiazepines like lorazepam. Clinical studies are needed to confirm that MRLA023 has similar efficacy but fewer adverse effects than lorazepam in patients with generalised anxiety disorder. In any case, the CNS-effect profile of the selective partial GABA-agonist MRLA023 in healthy humans differs from that of the non-selective agonist lorazepam.

ref. 20
ref. 9

Although direct comparative studies are rare, non-selective benzodiazepines, like diazepam, zopiclone, flurazepam, lormetazepam, triazolam, temazepam and lorazepam, all show comparable effects on memory, alertness and postural stability. This suggests that the differentiation between MRLA023 and lorazepam, observed in the current study, could be related to the differences in GABA-subtype (non-) selectivity. It is reported that the effects of benzodiazepines are mediated by specific $GABA_A$ receptor subtypes. The α_1 sub-unit is believed to primarily mediate the sedative properties and as a consequence, potentially mediate memory properties of $GABA_A$ agonists. The selective α_1-agonist zolpidem causes clear SPV-reductions and is used as a hypnotic agent. The current study supports the link between the α_1 sub-unit and sedation. MRLA023 is a $GABA_A$ partial $\alpha_{2,3}$ agonist devoid of α_1-activity in pre-clinical experiments. MRLA023 causes no memory impairment and less subjective sedation than the non-selective full benzodiazepine-agonist lorazepam. However, preclinical evidence also suggests that the α_2,α_3 and α_5 sub-units mediate the therapeutic, myorelaxation and motor impairment properties of $GABA_A$ agonists. In view of this, MRLA023 shows a surprising lack of motor impairment. There are several explanations. It could reflect the partial agonist character of the MRLA023. The purported α_2,α_3-effect of MRLA023 may become more apparent at higher doses, not evaluated in this study. Finally, the pre-clinical subselectivity may not always show the same pattern in humans.

ref. 21-26
ref. 4
ref. 27

Previous studies with other partial $GABA_A$ agonists showed less differentiating effects than MRLA023. However, these agents show no subtype selectivity. Bretazenil, for example, showed differences from placebo in saccadic peak velocity, body sway and the VAS score of alertness. This could be due to

ref. 21

the difference in selectivity for different subtypes compared with MRLA023, because bretazenil is generally less potent on all α-subtypes than a full agonist like diazepam. Ro 41-3696, reported to be a partial agonist, induced fewer effects on psychomotor performance and memory than 10 mg zolpidem at 1.5 h after intake but the effects were still significantly greater than placebo. Moreover, it is unknown whether these doses were equipotent, an important requisite for comparison of partial agonism and subtype selectivity.

ref. 28

ref. 29

In conclusion, this study showed a clear differentiation in pharmacodynamic effects for the selective $GABA_A$ agonist MRLA023, which was not found for the non-selective benzodiazepine lorazepam. This differentiation may be related to selectivity for different $GABA_A$ receptor subtypes, which may be associated with fewer side effects at therapeutically effective doses in patients.

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The value of determining critical questions early

This section presents a study in the development of a potential new drug for the treatment of generalised anxiety disorder (GAD). The development of a partially selective GABA-a agonist could have a therapeutic advantage over existing anxiolytics (existing benzodiazepines in particular). The main issue for these kinds of new drugs is that, after reaching the site of action (*i.e.* it must pass the blood-brain-barrier), the proposed mechanism of action points to a differentiation of the effects. The presented study indeed showed differentiation of effects.

The input parameters for the question-based development tree of this compound are presented in table 1. These input parameters are based on a classic drug development program of this type of compound (*i.e.* the parameters are estimated without the impact of the presented study).

TABLE 1 **Input parameters for the question based development plan of mr1a023 without the presented study**

Parameter	Value
Success action site	75%
Success pharmacological effect	65%
Success clinical efficacy	80%
Success therapeutic window	85%
Success population	90%
Costs action site	M€ 25
Costs Pharmacological effect	M€ 25
Costs Clinical effect	M€ 60
Costs Clinical window	M€ 30
Costs Population	M€ 35
Estimated market value	M€ 400

Similar to the value estimation of section 1, an optimal question sequence is determined by decision analysis. This priority list is represented in table 2. The estimated risk adjusted project value of this optimal sequence is M€ 27.

TABLE 2 **Optimal path in the question based development tree of MRLA023**

Priority ranking	Question
1	Pharmacology
2	Site
3	Window
4	Clinical
5	Population

According to the priority list presented in table 2, the two most important questions for this novel drug are 'pharmacology' and 'site'. In the classic NPV approach, performance of an additional cross-over study to determine the pharmacodynamic effects on sedation, body stability and memory compared to the existing market leader introduces extra development costs and maybe extra time. However, because of the early evaluation, a less sedative dose can be selected to examine the efficacy in patients. This maximum dose with a differential effect profile is crucial because it is the core of the market advantage over the existing benzodiazepines. Furthermore, the study prevents late failure created by an unwitting selection of a dose set too high. With this study, a maximum dose can be selected that is less sedating without any effects on memory, therewith providing important information regarding the 'window' question. Furthermore, the pharmacokinetic parameters of the drug, as estimated in the presented study, provide a first indication of what the optimal dosing regimen could be. The 'site' question is not fully answered, but the effects observed in the presented study are indicative of CNS penetration.

In order to estimate the impact of the additional study presented in this section, a new set of input parameters can be estimated that incorporate the knowledge and costs obtained in the presented study. Since an important part of the 'window' question is resolved, the probability of success on 'window' is now at least 86%. The probability of successfully answering the 'pharmacology' question is raised to at least 66% because of the demonstrated differential profile on the pharmacodynamic measures in the presented study. It remains uncertain if the differential profile is observed in patients. The indications for central activity observed in this study enhance the probability of success on 'site' to at least 76%. The costs of the study are added by elevating both 'window' and 'pharmacology' costs with M€ 1 (this includes the introduction of preparatory expenses, sponsor resources/time etc.). These updated input parameters are listed in table 3.

TABLE 3 **Input parameters for the question based development plan of MRLA023 after the presented study**

Parameter	Value
Success action site	76%
Success pharmacological effect	66%
Success clinical efficacy	80%
Success therapeutic window	86%
Success population	90%
Costs action site	M€ 25
Costs Pharmacological effect	M€ 26
Costs Clinical effect	M€ 60
Costs Clinical window	M€ 31
Costs Population	M€ 35
Estimated market value	M€ 400

With this new set of parameters the estimated risk-adjusted project value is estimated at M€ 28.2. With the NPV approach, the inclusion of this additional early pharmacology study would decrease the project value because it increases the costs of development without increasing the value of the drug. In other words, the knowledge obtained in this relatively inexpensive study is less likely to be adequately valued using NPV analysis. Question-based development shows that the introduction of extra costs can increase the project value by increasing the probabilities of success at a later stage in the development of this drug.

The negative impact of the additional study presented in this section could have led to a more classical approach to the development of this drug. This 'classical approach' could consist of a single ascending dose study in healthy volunteers to investigate safety and tolerability, followed by a multiple dose study and a food interaction study. Thereafter, the drug would enter phase II where in small groups of patients the efficacy would have to be established and the less sedating dose selected. This approach introduces substantially more costs.

The investigation of new centrally active drugs in patients is severely hampered by several factors. The heterogeneity of the clinical population, the differences in drug responses, and the methodology available to investigate sedative effects in patients

would require a substantial increase in the number of patients needed to show a statistically significant effect. Furthermore, the costs of investigating patients instead of healthy volunteers would further increase the costs of the development. Combined, a study in more patients than the twelve healthy volunteers used in this study would have a considerable impact on the project value.

CHAPTER 12

Summary and Conclusions

Introduction

The development of new medicines is an expensive and time-consuming process. It takes an average of 12-15 years to discover and develop a new medicine. Most of that time is spent testing the safety of the drug. The average cost of bringing one new medicine to market in 1990 was estimated at
ref. 1 $500 million. The Tufts University Center for the Study of Drug Development found that the time from synthesis of a new drug to marketing approval has
ref. 2 increased over time. While in the 1960s the approximate time from first synthesis to approval was about 8 years, this time period has increased to 14.2 years in the 1990s. Most of this increase in time is due to the prolonged period from first administration to humans to submission for registration, which increased from 3.1 to 8.6 years.

The exact costs of drug development today are difficult to determine. Over $500 million is supposedly spent to introduce one single new drug on
ref. 3 the market. About 30% of these costs ($150 million) are spent on the clinical
ref. 1 development phases I to III. Therefore, stopping as soon as possible the development of drugs that will fail to reach registration is highly rewarding. Growing research & development (R&D) expenditures have fuelled the development of hundreds of new medicines over the past half-century by the pharmaceutical industry. As illustrated before, drug development is both high-cost and high-risk. It was, for instance, estimated that in the period between 1980 and 1984 only three of every 10 NCE's had returns
ref. 4 higher than average after-tax R&D costs. Many attempts have been made to optimise the development of new medicines, both by improving the drug target and the process of development.

Target optimisation

Computer aided drug design has made it possible to identify many compounds that adequately bind or fit onto the target site. The synthesis of new biologically active compounds has been facilitated by the use of combinatorial chemistry. The most active compound on receptor level is selected from the wide range of new compounds by comparing hundreds of compounds at once using high throughput screening. By sequencing the human genome, many new potential targets for new drugs have been identified. Unfortunately, the relationship between a modification of a certain biological target and the improvement of a clinical endpoint is often unknown, particularly for multifactorial diseases. There are probably 100's of genes associated

with the potential of a breast cancer to metastasise but it remains unknown which one(s) or which combination to block yet. Developing an inhibitor for each target is prohibitively expensive, so biological knowledge will have to precede chemical synthesis.

Process optimisation

The cost of development and the increased number of lead compounds have made adequate selection of compounds to enter the clinical phase crucial. Mergers and take-overs of pharmaceutical companies to form bigger companies with larger pipelines reflect attempts of procedural optimisation. The obvious goal is to have more investigational compounds so that more drugs will be successful and spread the risk of failure. Because the selection of compounds has become so important, more efforts are aimed at early discontinuation of failures (*i.e.* compounds that will not reach registration). Therefore, there is a growing pressure on the drug development process to enhance the relevance of studies at all stages. Traditionally, phase 1 studies were mainly concerned with kinetics and tolerability of a new compound in healthy volunteers, but efforts are now made to include potential biomarkers of clinical endpoints.

In order to compare and select the compounds that will enter the clinical phase of the drug development process, the Net Present Value (NPV) is often used. NPV is commonly used because of its ability to discount present and future cash flows and to provide an estimate of the total (financial) value of a project. NPV uses a discount rate to convert a stream of future cash flows to a single value today. In the calculation of a project's NPV, a comparison is made between the situations that arise if the project is continued or abandoned. NPVs greater than zero indicate the amount the organisation will earn in excess of traditional financial investment of the outgoing cash flow. The NPV can then be used to compare different projects using the same factors. The value of future cash flows is predominantly dependent on the factor time (spending money as late as possible and generating revenues as early as possible). Therefore, the most influential parameters for NPV calculations of a drug development project are costs of development (as low as possible), revenues (as high as possible) and time to introduction on the market (as early as possible).

The difficulty of development of useful treatments is not solved by the described target -and process optimisation efforts. A structured approach

ref. 5-6

that combines and optimises both knowledge and procedural aspects of drug development may improve success rates and/or reduce some of the expensive failures of development.

Classic development phases

Classically, the clinical development program of a drug is divided in four phases:

- Phase I: Research using small groups of healthy volunteers. Traditionally, this phase mainly focuses on if the human body tolerates the new drug and on finding a dose where the level of tolerance is acceptable. Furthermore, it examines how the drug enters and leaves the human body. In general, this phase takes about 1 to 2 years.
- Phase II: Research on a group of patients where the first proof for efficacy is established. More characteristics of the NCE are determined and a safe and well-tolerated dose is determined where the drug is efficacious.
- Phase III: The potential new drug is tested on thousands of patients in multi-centre research projects to investigate the side effects of the drug at a set dose in more detail. Furthermore, the efficacy of the drug at the determined dose is compared to existing medication. Further research is conducted to investigate possible side effects after long-term treatment and development of the drug for different indications is investigated.
- Phase IV: The registered drug is monitored closely to examine the occurrence of unexpected side-effects and interactions with other drugs.

The different phases of the research and development process are represented in figure 1. The description of these phases is typically process oriented and contains very little information about which scientific aspects are actually covered during the development.

Generic question groups

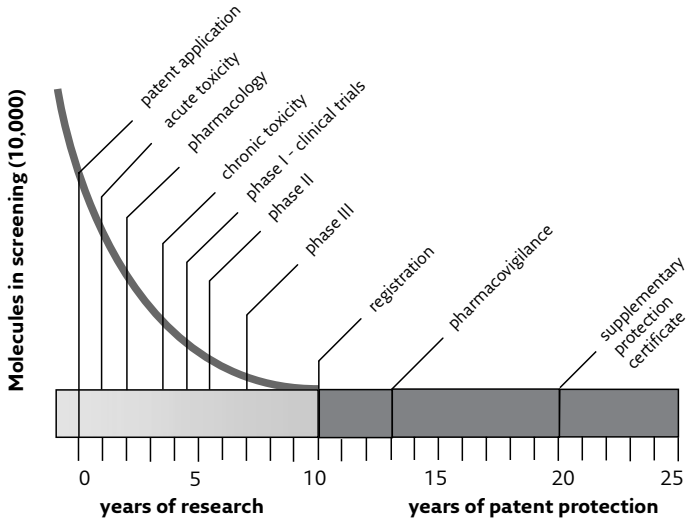
During these phases a number of generic questions are generally answered. Posing these questions throughout the development of a drug is in agreement with the learn-confirm view described by Sheiner. ref. 7 The main generic question groups are:

- Does the biologically active compound/active metabolites get to the site of action?

Related issues: Absorption, route of administration, bioavailability, distribution, tissue distribution, accumulation, action site penetration, metabolism, active metabolites, metabolic routes, excretion: hepatic/renal, clearance, half-life

- Does the compound cause its intended pharmacological/functional effect(s)?
  Related issues: Effects related to mechanism of action, additional effects of primary pharmacological activity, effects of secondary pharmacological activity, other, undesirable effects
- Does the compound have beneficial effects on the disease or its clinical pathophysiology?
  Related issues: Effects on relevant physiological systems, effects on disease, undesirable clinical effects
- What is the therapeutic window of the new drug?
  Relevant issues: Clinical effects at tolerated dose, dose regimens/intervals, controlled drug delivery, forgiveness
- How do the sources of variability in drug response in the target population affect the development of the product?
  Relevant issues: Compliance, pharmacogenomics, ethnic differences, concomitant medication, variability in pharmacokinetics, pharmacodynamics and disease state

FIGURE 1 **Pharmaceutical research and development process for a new product (http://www.fda.gov/cder/)**



Each of these questions has a probability to be successfully answered but answering these questions will introduce development costs. The set of probabilities and costs varies from drug to drug and is therefore unique. For one drug it can be very difficult to successfully answer the 'site' question whereas it can be relatively easy for another compound. The unique set of probabilities and costs defines the optimal development strategy for each compound. Addressing a question with low probability of success at an early phase can be highly rewarding. NPV analysis merely shows that these type of additional studies only introduce additional costs and development time and the NPV of the project will decrease. Therefore, the NPV of development projects does not adequately reflect the value of additional knowledge on a compound, which requires a different value estimation method. The option-based theory takes into consideration the fact that projects can also be discontinued at various stages of the development program. The early discontinuation of drugs that will be unsuccessful is desirable and the value of early evaluation studies on relevant questions can be incorporated in the option-based theory. However, the option -based method is rarely used and is often defined using the classical phase I-III description of the process as decision knots in the decision tree. These phases are not relevant as targets in the development process but merely a classification based on the type of study and the number of patients involved in the trial.

This thesis introduces a question-based approach to drug development which uses decision knots that are relevant for the development of new drugs: generic questions that are really answered throughout the development program. The resulting question-based decision tree reflects the true risks and uncertainties that are faced in the development of an individual drug. Furthermore, the question-based approach shows how the project value can increase by performing an additional early phase evaluation study that helps to adequately answer a question later on. These studies can help in preventing unsuccessful compounds to enter late stages of development after substantial costs have been incurred. The early discontinuation can substantially reduce the costs of drug development. By defining the costs and probabilities of success and constructing the decision tree for a new compound, the bottleneck in the development of each individual drug will be identified. In four sections, several examples are presented to illustrate the use and impact of the question-based development plan.

Value of research on biomarkers

In Section 1, three methodological reviews on potential biomarkers for the effects of drugs in healthy volunteers are presented. The proposed generic questions can be answered in several studies (e.g. the FTIH can provide an indication of 'pharmacology' and 'clinical'). To adequately answer questions, appropriate methodology to show effects is needed. For some questions/drugs this selection of the appropriate methodology is easy: e.g. 'site' or 'pharmacology' for a peripherally acting antihypertensive agent (plasma levels of the drug and blood pressure, respectively). But for drugs indicated for diseases such as depression, schizophrenia and anxiety this selection of the appropriate methodology is more difficult. The probability of successfully answering the question is therefore linked to the available methodology. Increased knowledge about the specificity and selectivity of the available methodology allows better selection of methods in clinical trials, and therefore, the probability of successfully answering the question will grow. The added value can be obtained by an increased probability of success but also on an increased probability a failure will be discontinued early (reduced costs). So knowledge of methodology (and research on methodology) has intrinsic value which should be included in the project value of a new drug.

NPV shows that additional research costs money (including time) and therefore, the NPV will drop if research on biomarkers is included in the project value of a drug. However, the question-based approach assumes the method factor to be incorporated in the probability factor of the compounds potential to successfully answer the relevant question. The reviews presented in section 1 all address the available methods for answering the 'pharmacology' and 'clinical' questions.

Currently, no validated biomarkers for the effects of antipsychotics, benzodiazepines or antidepressants in healthy volunteers are available, but a useful marker should meet the following requirements:

1. a clear, consistent response across studies (from different groups) and drugs
2. a clear response of the biomarker to a therapeutic dose of the drug
3. a dose (concentration)-response relationship
4. a plausible relationship between the biomarker, the pharmacology of the drug and the pathogenesis of the disease.

If these basic requirements are used as a filter on all described methodology in healthy volunteers, most methods are not very useful biomarkers. Chapter 2 describes that only prolactin response to antipsychotic agents fulfils the

requirements of a useful biomarker. The same goes for saccadic peak velocity as biomarker for the effects of benzodiazepines in healthy volunteers, as described in Chapter 3. Chapter 4 shows that REM sleep characteristics are of limited value as biomarkers for antidepressant effects in healthy volunteers, but this observation can be caused by inadequate modelling of the complex structure of human sleep. Sensitivity analyses on the probability of success on 'pharmacology' versus project value according to the question-based development approach allows construction of a 'break-even' table. This table shows how much project value is gained by increasing the method factor for each percent. The question-based development approach using historical data input (probabilities and costs) shows that every % increase in success probability of the 'pharmacology' question (by increased knowledge on the available methods) causes an increase in project value of M€ 0.8. A similar analysis of the 'clinical' question shows an increase in project value of M€ 1.4 for each % increase in success probability. Furthermore, the probability a method will be selected that will not show an effect at a therapeutically relevant dose of an efficacious drug will be reduced thereby preventing useless studies and the use of volunteers/resources.

The value of timing additional studies

Section 2 showed that with a relatively small number of volunteers the question "Can a new formulation improve the therapeutic window and clinical effects of an existing drug?" could be answered. Using blood pressure and the most sensitive marker for the side effect of sedation (saccadic peak velocity), it was possible to identify the optimal therapeutic window of a drug. Furthermore, it was possible to correlate the *in vivo* with the *in vitro* dissolution of the new formulation. Combined, these studies helped in designing a sustained release formulation for an existing drug with adequate clinical effects at tolerated levels. Because the drug has been on the market for quite some time, the development of the original formulation apparently did not optimally answer these questions. Now additional studies were performed at a post-registration stage. The followed strategy was apparently to bring the original product to the market as soon as possible and use additional market-experience to consider a different (hopefully patentable) formulation. Additional useful information was obtained from market experience. This approach has also been adopted in the nifedipine case, where after introduction of the original product, an improved formulation was successfully developed based on the discovery of a novel pharmacodynamic property of the drug. The improved product after the launch of the

original product is sometimes referred to as a 2nd-cycle product. Another possible strategy could have been early inclusion of additional studies to evaluate the effects of a different formulation. The inclusion of these studies at an early stage in the original development of rilmenidine would have allowed the introduction of the sustained release formulation for rilmenidine and thereby reducing the additional costs of having to introduce a new formulation. The NPV of such a hypothetical development plan would probably have been lower than the one actually used, but the result would be a formulation that would meet a larger market demand and the revenues could therefore have been higher. These studies increased the knowledge on rilmenidine which would not be adequately valued using NPV, in contrast with the question-based approach. The rating of the success probabilities would yield a suggested policy to examine the 'window' and 'clinical' questions early in the development.

Added value of bridging studies

Two comparative studies between Japanese and Caucasian subjects are presented in Section 3. The introduction of new drugs in Japan is increasingly interesting for western pharmaceutical companies. Japan has a large population and the availability of western drugs is rare. The Japanese registration authorities often require repetition of most clinical trials in Japanese subjects before registration. However, in some cases, a comparative trial can show that the complete repetition of all the clinical trials is not necessary. The 'population' question should nevertheless be adequately answered. If there is a difference in ethnopharmacological factors, additional trials in the Japanese population are required. Therefore, early comparative studies between Caucasians and Japanese have intrinsic value to the drug development program. The added value can be caused by two options: one option is that the comparative studies show that there are no differences in drug response that affect dose. Similar drug responses in both groups would allow extrapolation of the western data to the Japanese population and hence prevent additional trials in Japan. Another option would be the timing of these comparative studies. If the probability the drug response is similar is high, the QBD tree shows that one should decide to answer the 'population' question late in development (there are more important questions to ask). But especially if there is a real probability the drug response will be different between the ethnic groups, an early evaluation study can add substantially to the project value of the new drug. The example in Chapter 10 of the potential oral contraceptive illustrates that it is important to take all

questions into account when constructing the drug development decision tree. Even if one identifies the 'population' question as tangible, the other questions can have more significant impact on the optimal development strategy. In the presented case, the probability the drug would prove to have an improved side-effect profile would have to be estimated very low. As a consequence, the drug was discontinued after the comparative study presented in this thesis had been completed. Adequate estimation of the probabilities of success using the QBD-approach would have saved at least the presented comparative trial. The construction of a decision tree for the oral contraceptive would have revealed that the 'clinical' question far outweighs the 'population' question.

The value of determining critical questions early

In Section 4, a study in the development of a potential new drug for the treatment of generalised anxiety disorder (GAD) is presented. The development of a selective GABA-a partial agonist could have a therapeutic advantage over existing anxiolytics. The main issue for these kinds of new drugs is that the proposed mechanism of action indeed shows a differentiation of the effects. Also, the drug or active metabolites must reach the site of action (*i.e.* it must pass the blood-brain-barrier). Therefore, the two most important questions for the novel drug are 'pharmacology' and 'site'. In the classic NPV approach, performance of an additional cross-over study to determine the pharmacodynamic effects on sedation, body stability and memory compared to the existing market leader introduces extra development costs and maybe time. However, because of the early evaluation, the study showed indications that there are *in vivo* differentiation of effects and a less sedative dose can be selected to examine the efficacy in patients. The maximum dose is crucial because it is the core of the market advantage over the existing drugs. Therewith, the study attempts to prevents late failure because of relative over dosage. The 'site' question is not fully answered but the effects observed in the presented study are indicative for central penetration.

Conclusions

The use of NPV analysis in drug development does not adequately reflect the additional value of knowledge. Similar to other efforts, NPV analysis is

an attempt to deal with the uncertainties and risks of drug development in a procedural approach. A question-based approach to drug development seems more rational and better incorporates the alteration of success probabilities. Furthermore, the question-based approach has implications for the execution of the drug development project and the selection of new biologically active compounds. It is beyond the scope of this thesis to present a universal model for drug selection and development. Based on several examples this thesis illustrates that the combination of success probabilities and accompanying costs to answer the questions are a unique data set that can vary with different compounds. Even if the overall probability of success and the overall costs are the same, these unique sets dictate an optimal development strategy. The sequence of relevant questions should serve as a priority list in the development of new drugs throughout the program. Regular updates of all probabilities and costs will optimally direct the development process. Another advantage of the question-based approach is that experts of different company departments all involved in the development of new drugs discuss and agree on the chances and threats in the development of new drugs.

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CHAPTER 13

Samenvatting en Conclusies

Inleiding

De ontwikkeling van nieuwe geneesmiddelen is een duur en tijdrovend proces. Het duurt gemiddeld 12-15 jaar om een potentieel geneesmiddel te ontwikkelen nadat het molecuul ontdekt is. De meeste tijd hiervan wordt besteed aan het testen van de veiligheid van het middel. De gemiddelde kosten die gemaakt worden om één geneesmiddel op de markt te brengen werd
ref. 1 in 1990 geschat op $500 miljoen. De Tufts University Center for the Study of Drug Development heeft gevonden dat de tijd van synthese van het middel tot de goedkeuring van het middel voor de markt toegenomen is in de loop
ref. 2 der tijd. Terwijl in de zestiger jaren de tijd van synthese tot goedkeuring nog ongeveer 8 jaar was, is deze periode geschat op 14.2 jaar in de jaren negentig. Het merendeel van deze toename in tijd is te wijten aan een toename in tijd van eerste toediening aan de mens tot indiening van het dossier voor goedkeuring (de klinische fase van het geneesmiddel ontwikkelingsproces). Deze periode nam in de bestudeerde periode toe van 3.1 tot 8.6 jaar.

De exacte kosten van het huidige geneesmiddel ontwikkelingsproces zijn moeilijk vast te stellen. Er wordt verondersteld dat meer dan $500 miljoen wordt uitgegeven om een nieuw geneesmiddel te kunnen introduceren op de
ref. 3 markt. Ongeveer 30% van deze kosten ($150 miljoen) wordt uitgegeven aan
ref. 1 de klinische ontwikkelingsfasen I tot III. Het is dan ook lonend om zo snel mogelijk te stoppen met de ontwikkeling van middelen die uiteindelijk niet geregistreerd zullen worden. Toenemende investeringen in onderzoek en ontwikkeling hebben geleid tot de ontwikkeling van honderden nieuwe geneesmiddelen in de laatste 50 jaar. Zoals eerder beschreven is geneesmiddel ontwikkeling zowel een duur als riskant proces. Het is bijvoorbeeld geschat dat in de periode tussen 1980 en 1984 maar 3 van de 10 potentiële geneesmiddelen hogere opbrengsten hadden dan gemiddelde onderzoek–
ref. 4 en ontwikkelingskosten. Er zijn vele pogingen gedaan om de ontwikkeling van geneesmiddelen te optimaliseren, zowel door het verbeteren van de aangrijpingspunten van stoffen als het proces van ontwikkelen.

Optimalisatie van het aangrijpingspunt

Door het in kaart brengen van het menselijk genoom zijn vele nieuwe potentiële aangrijpingspunten voor nieuwe geneesmiddelen geïdentificeerd. Met behulp van computersimulatie kunnen stoffen worden ontworpen die in theorie effectief zijn op de geïdentificeerde moleculaire aangrijpingspunten (receptoren). Het synthetiseren van een optimaal biologisch actief molecuul

(ligand) voor de receptor wordt vergemakkelijkt door gebruik te maken van 'combinatorial chemistry' (een techniek waarbij verschillende biologisch actieve delen van het molecuul op alle mogelijke manieren aan elkaar worden gezet). De daarna noodzakelijke selectie van het beste ligand wordt geoptimaliseerd door gebruik te maken van 'high throughput screening' op receptor niveau. Hierbij wordt gebruik gemaakt van cellen die de betreffende receptor tot expressie brengen en waarbij receptor activiteit bijvoorbeeld gepaard gaat met het aanmaken van een fluorescerende marker. Helaas is de relatie tussen het modificeren van een bepaald biologisch aangrijpingspunt en de verbetering van een klinisch eindpunt vaak onbekend, vooral voor multifactoriële ziekten.

Proces optimalisatie

De selectie van potentiële geneesmiddelen is steeds belangrijker geworden waardoor de farmaceutische industrie vaak overgaat tot schaalvergroting. De fusies en overnames binnen deze industrie hebben vaak tot doel meer potentiële geneesmiddelen in de portfolio te krijgen waardoor de risico's zoveel mogelijk gespreid worden. Verder heeft deze schaalvergroting vaak optimalisatie van personeel en investeringen tot doel. Doordat de selectie van potentiële geneesmiddelen steeds belangrijker is wordt er ook geprobeerd zo vroeg mogelijk in de ontwikkeling te stoppen met stoffen die niet succesvol zullen zijn. Daartoe wordt steeds vaker geprobeerd biomarkers voor effecten bij patiënten al te meten in de vroege fase van het geneesmiddel ontwikkelingsproces. Waar vroeger studies naar de effecten van nieuwe stoffen in gezonde vrijwilligers vaak gericht waren op de veiligheid en verdraagzaamheid van de nieuwe stoffen, wordt tegenwoordig steeds meer geprobeerd om te voorspellen wat de effecten van geneesmiddelen in patiënten zal zijn.

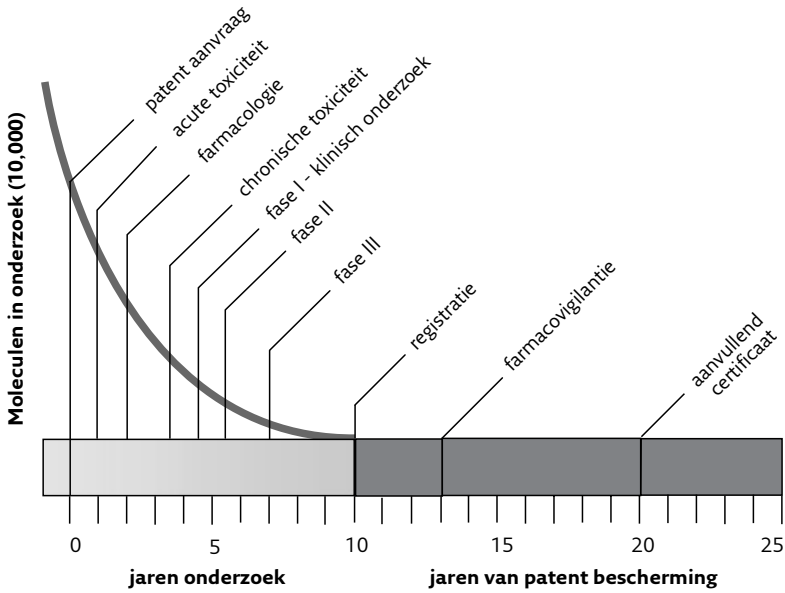
Om te selecteren welke stoffen in ontwikkeling zullen worden genomen wordt vaak gebruik gemaakt van een bedrijfskundige maat: de netto contante waarde, of 'Net Present Value' (NPV). De NPV van een project kan worden bepaald door de huidige waarde van de verwachte toekomstige opbrengsten te berekenen en ze vervolgens op te tellen waarna van het gevonden getal de initiële investering wordt afgetrokken. Bij de berekening van de NPV van een project wordt een vergelijk gemaakt tussen de situatie die ontstaat als het project wel en niet wordt uitgevoerd. De waarde van toekomstige geld stromen is voornamelijk afhankelijk van de factor tijd (zo laat mogelijk geld uitgeven en zo snel mogelijk geld binnen krijgen). Hierdoor zijn de belangrijkste

parameters die gebruikt worden om de NPV van een project te optimaliseren: lage ontwikkelingskosten, hoge opbrengsten bij introductie op de markt en korte tijd tot introductie op de markt.

De moeilijkheid om bruikbare therapieën te ontwikkelen wordt niet opgelost door de beschreven pogingen tot optimalisatie van aangrijpingspunt en het ontwikkelingsproces. Daarom zou een structurele aanpak waarbij zowel inhoud als procedures geoptimaliseerd worden mogelijk de succes kans kunnen vergroten en/of de kosten van niet-succesvolle stoffen kunnen reduceren.

ref. 5-6

FIGURE 1 **Ontwikkelings proces van een nieuw geneesmiddel (http://www.fda.gov/cder/)**



Traditionele ontwikkelingsfases

Het klinische ontwikkelingsprogramma van een geneesmiddel wordt traditioneel ingedeeld in 4 fases;

- Fase I: onderzoek in kleine groepen gezonde vrijwilligers. Traditioneel wordt in deze fase onderzocht bij welke dosering het geneesmiddel goed verdragen wordt en hoe het geneesmiddel wordt opgenomen in het lichaam en weer uitgescheiden wordt.

- Fase II: in deze fase wordt in beperkte patiënten groepen het eerste bewijs geleverd voor de effectiviteit van het middel. Hierna wordt een dosis geselecteerd die zowel veilig als effectief is.
- Fase III: de dosering van het geneesmiddel zoals geselecteerd in de fase II studies wordt in detail getest op bijwerkingen en effectiviteit in duizenden patiënten in grote onderzoeksprojecten waar meerdere ziekenhuizen en instellingen aan meewerken. Verder wordt in deze fase de effectiviteit van het middel vaak vergeleken met die van bestaande geneesmiddelen. Het betreft hier langdurige projecten en de effecten van het middel worden onderzocht na langdurige behandeling. Als deze fase afgerond is wordt het geneesmiddel geregistreerd en op de markt gebracht.
- Fase IV: van het geregistreerde geneesmiddel wordt bijgehouden of er onverwachte bijwerkingen of onverwachte interacties met andere geneesmiddelen optreden.

De verschillende fases van het ontwikkelingsproces zijn weergegeven in figuur 1. De beschrijving van deze fasen is typisch proces georiënteerd en bevat weinig informatie over de wetenschappelijke aspecten van het middel die eigenlijk worden onderzocht tijdens de ontwikkeling

Generieke hoofdvragen

Tijdens deze fasen moet eigenlijk een aantal generieke vragen worden beantwoord. Het stellen van deze vragen gedurende de ontwikkeling van een middel kom overeen met de Leer-Bevestig theorie zoals beschreven door Sheiner. De belangrijkste generieke hoofdvragen zijn: ref. 7

- Komt het middel op de plaats waar het zijn activiteit heeft?
  Gerelateerde onderwerpen: absorptie, administratie route, biologische beschikbaarheid, distributie, weefsel distributie, accumulatie, action site penetratie, metabolisme, actieve metabolieten, metabolisme routes, excretie: hepatisch/renaal, klaring, halfwaarde tijd.
- Veroorzaakt het middel het bedoelde farmacologisch / functioneel effect?
  Gerelateerde onderwerpen: effecten gerelateerd aan het werkingsmechanisme, additionele effecten van de primaire farmacologische activiteit, effecten van de secondaire farmacologische activiteit, andere, ongewenste effecten.
- Heeft het geneesmiddel gunstige effecten op de klinische patofysiologie van de ziekte?
  Gerelateerde onderwerpen: effecten op relevante fysiologische systemen, effecten op de ziekte, ongewenste klinische effecten.

- Wat zijn de therapeutische grenzen van het middel?
  Gerelateerde onderwerpen: klinische effecten bij een tolereerbare dosering, doseringschema/interval, gecontroleerde geneesmiddel afgifte, effecten van het vergeten van medicatie binnen het doseringschema.
- Hoe beïnvloeden de bronnen van variabiliteit in geneesmiddel respons in de patiënt populatie de ontwikkeling van het product?
  Gerelateerde onderwerpen: Therapietrouw, farmacogenetica, etnische verschillen, co-medicatie, variabiliteit in farmacokinetiek, farmacodynamiek en ziekte status.

Elk van deze vragen heeft een bepaalde kans om succesvol beantwoord te worden, maar het beantwoorden van elk van deze vragen zal gepaard gaan met bepaalde kosten. Deze set van kansen en kosten kan variëren per middel. Wat voor het ene middel een moeilijk te beantwoorde vraag is kan relatief eenvoudig zijn voor een ander geneesmiddel. Hierdoor heeft elk middel een optimale ontwikkel strategie. Het in een vroeg stadium adequaat beantwoorden van de meest relevante vragen is dus erg lucratief. NPV analyse laat zien dat het doen van deze additionele vroege fase studies naar de meest relevante vragen alleen maar meer tijd en kosten introduceren waardoor de totale NPV van het project daalt. De NPV van geneesmiddel ontwikkelingsprojecten reflecteert dus niet de waarde van toegevoegde kennis van het middel. Een andere waardebepaling zou eigenlijk gebruikt moeten worden. De optie gebaseerde theorie gaat uit van het feit dat een project in de verschillende fases ook gestopt kan worden waardoor het belangrijk is zo vroeg mogelijk te stoppen met de ontwikkeling van niet-succesvolle middelen. Hierdoor worden de additionele waarde van vroege evaluatie studies en de kansen adequaat geïncorporeerd in de waarde van het project. Deze methode wordt echter zelden gebruikt in de praktijk en opties worden meestal gedefinieerd als de klassieke beschrijving van het ontwikkelingstraject: de fasen I, II en III worden weergegeven als beslispunten in een beslisboom. Deze fasen zijn echter niet relevant als doel op zich in het ontwikkelingsproces maar slechts een classificatie gebaseerd op het type studie en het aantal patiënten dat betrokken is bij de studies.

In dit proefschrift wordt een vraag gebaseerde aanpak van geneesmiddelontwikkeling gepostuleerd dat gebruik maakt van beslispunten die relevant zijn voor de ontwikkeling van het geneesmiddel namelijk de vragen die eerder zijn gedefinieerd. Deze vraag gebaseerde aanpak reflecteert de werkelijke onzekerheden en risico's die genomen worden bij de ontwikkeling van een nieuw middel. Verder kan deze methode aantonen dat de waarde van een project toe kan nemen door het uitvoeren van een extra vroege fase studie die de

relevante vragen helpt op te helderen. Deze studies kunnen voorkomen dat niet-succesvolle middelen pas laat in de ontwikkeling worden gediscontinueerd en daardoor aanzienlijk bijdragen aan de kosten van geneesmiddel ontwikkeling. Als de kansen en kosten behorend bij de verschillende ontwikkelingsvragen worden gedefinieerd kan met behulp van beslisboom analyse worden aangetoond waar de knelpunten zitten in de ontwikkeling van het betreffende middel. In vier secties worden in dit proefschrift voorbeelden van het gebruik en de impact van de vraag gebaseerde aanpak gegeven.

Waarde van biomarker onderzoek

In Sectie 1 worden drie methodologische reviews over mogelijke biomarkers voor de effecten van geneesmiddelen in gezonde vrijwilligers beschreven. De kans dat een vraag adequaat kan worden beantwoord is afhankelijk van het middel en de beschikbare methoden om de vraag te kunnen beantwoorden. De NPV analyse laat zien dat onderzoek naar en reviewen van biomarkers en methoden additionele kosten (en wellicht extra tijd) met zich mee brengt. Daarmee wordt de totale projectwaarde lager. De vraag gebaseerde aanpak laat juist zien dat de kennis van de beschikbare methode toegevoegde waarde heeft voor de projectwaarde. De toegevoegde waarde kan behaald worden doordat de kans dat de vraag succesvol wordt beantwoord vergroot wordt of doordat een niet succesvol geneesmiddel vroeg uit ontwikkeling kan worden genomen. Er zijn momenteel geen gevalideerde biomarkers voor antipsychotica in gezonde vrijwilligers. Een bruikbare biomarker moet in ieder geval:

- Een consistente respons geven na toediening van verschillende middelen die hetzelfde therapeutisch effect hebben
- Een consistente respons geven bij therapeutische doseringen van het middel
- Een relatie tussen de dosering en de respons hebben
- Een plausibel verband hebben tussen de biomarker, de farmacologie van het middel en/of de pathofysiologie van de ziekte

Wanneer deze vereisten als filter worden toegepast op alle beschreven methoden, dan blijkt dat er vele methoden niet erg bruikbaar zijn als biomarker. In hoofdstuk 2 wordt beschreven dat alleen prolactine respons na antipsychotische middelen aan alle vereisten van bruikbare biomarkers voldoet. In hoofdstuk 3 geldt hetzelfde voor maximale saccadische oogsnelheid als biomarker voor de effecten van benzodiazepines in gezonde vrijwilligers. Hoofdstuk 4 laat zien dat REM slaap als biomarker voor antidepressiva momenteel van beperkte waarde is maar dat dit mogelijk komt door de

inadequate modelering van de complexe structuur van de slaap. De review gepresenteerd in deze sectie hebben allemaal invloed op de 'pharmacology' vraag. Sensitiviteitsanalyse van de kans op succesvol beantwoording van deze vraag op de totale projectwaarde laat zien hoeveel de impact van deze reviews is op de projectwaarde van een gemiddeld nieuw middel. Voorts wordt aangetoond dat kennis over de beschikbare methoden de kans vermindert dat een methode wordt geselecteerd dat geen effect laat zien van therapeutisch relevante doseringen van een effectief middel. Daarmee kan worden voorkomen dat er onnodige studies worden uitgevoerd bij mensen en resources bespaard kunnen worden.

Waarde van timing additionele studies

Sectie 2 beschrijft een aantal studies bij een relatief kleine groep vrijwilligers die gecombineerd adequaat antwoord geven op de vraag of een nieuwe formulering van een bestaand middel het therapeutisch window en de klinische effecten kan verbeteren. Gebruikmakend van bloeddruk en de meest gevoelige biomarker voor de bijwerking sedatie (saccadische topsnelheid) bleek het mogelijk om het therapeutisch window van de nieuwe formulering te bepalen. Het uiteenvallen van de vertraagd afgifte tablet *in vitro* (in een bekerglas) kon worden gecorreleerd aan de situatie *in vivo* (in het menselijk lichaam). Gecombineerd bepaalden deze studies het optimale profiel van een nieuwe formulering met betrekking tot therapeutische klinische werking bij verdraagbare concentraties van het middel. Aangezien dit middel reeds een poos op de markt is bleek de oorspronkelijke ontwikkeling geen adequaat antwoord te hebben gegeven op deze vraag. Daarom zijn deze additionele studies na registratie nodig geweest. De inclusie van deze studies in een vroeg stadium van de oorspronkelijke ontwikkeling van rilmenidine zou hebben geleid tot introductie van de optimale vertraagd afgifte tablet. Daarmee zouden de extra kosten die gepaard gaan met de introductie van de nieuwe formulering bespaard kunnen worden. De NPV van dit hypothetisch ontwikkelingsplan zou waarschijnlijk lager zijn dan degene die daadwerkelijk is gebruikt, maar het resultaat zou een formulering zijn die een groter marktsegment zou kunnen bereiken. De waarde van de vergrootte kennis van het middel door middel van deze studies zijn niet adequaat weer te geven in een NPV analyse in tegenstelling tot de vraag gebaseerde aanpak. Het inschatten van de kansen op succesvolle beantwoording van de 'window' en 'clinical' vragen zou resulteren in een ontwikkelingsplan waarbij deze vragen vroeg in de ontwikkeling opgehelderd zouden moeten worden.

Waarde van brug studies

De introductie van nieuwe geneesmiddelen in Japan is steeds interessanter voor westerse farmaceutische industrieën. Japan heeft een grote populatie en de beschikbaarheid van westerse geneesmiddelen is relatief schaars. De Japanse registratie autoriteiten eisen vaak dat de klinische ontwikkelings studies worden herhaald in Japanse vrijwilligers. In sommige gevallen zou een vergelijkende studie tussen Japanse en westerse (Caucasische) vrijwilligers / patiënten de noodzaak voor volledige herhaling van het ontwikkelingsprogramma kunnen voorkomen. Deze Sectie 3 beschrijft twee vergelijkende studies tussen de beide etnische groepen. De 'population' vraag zal niettemin adequaat beantwoord moeten worden. Als er een verschil is in etnofarmacologische factoren tussen Japanners en Caucasiërs, zal dit extra studies in de Japanse populatie noodzakelijk maken. Daarom hebben vergelijkende studies tussen de beide etnische groepen intrinsieke waarde. Deze toegevoegde waarde kan tot uiting komen in twee opties: één optie is dat uit de vergelijkende studie blijkt dat geen verschillen bestaan die doseringsaanpassing nodig maken. Dit zou extrapolatie van de westerse data naar de Japanse populatie mogelijk maken en daarmee extra studies in Japan voorkomen. Een andere optie is de timing van de vergelijkende studies. Als de kans groot is dat reactie op het middel vergelijkbaar is dan zal de vraag gebaseerde beslisboom laten zien dat het wijs is deze vraag laat in de ontwikkeling aan bod te laten komen (er zijn immers meer cruciale vragen voor een dergelijk middel). Als daarentegen de kans groot is dat een doseringsaanpassing nodig is in de Japanse populatie dan zal de vroege vergelijkende studie aanzienlijk bijdragen in de projectwaarde van het nieuwe geneesmiddel. Het voorbeeld in deze sectie van het nieuwe anticonceptiemiddel illustreert dat het belangrijk is alle vragen in ogenschouw te nemen bij het ontwerpen van het geneesmiddel ontwikkelingsprogramma. Ook al wordt de 'population' vraag belangrijk ingeschat, dan nog kan het zijn dat andere vragen belangrijker zijn en daarmee meer impact op de ontwikkeling hebben. In dit voorbeeld zou de kans dat aangetoond kan worden dat het middel een verbeterd bijwerkingsprofiel heeft ten opzichte van bestaande middelen uit dezelfde klasse laag ingeschat moeten worden. Daarmee is deze vraag belangrijker geworden dan de 'population' vraag. Het middel uit het voorbeeld is dan ook gediscontinueerd om deze reden. Het inschatten van de kansen volgens de vraag gebaseerde aanpak zou er toe hebben geleid dat dit van tevoren aan het licht was gekomen en zou hebben voorkomen dat de vergelijkende studie uitgevoerd werd.

Waarde van vroeg beantwoorden kritische vragen

In de laatste Sectie 4 staat de ontwikkeling van een nieuw middel tegen angststoornissen met een nieuw werkingsmechanisme centraal. Het idee achter het nieuwe werkingsmechanisme is dat, in tegenstelling tot bestaande middelen uit deze klasse, een differentiatie van de klinische effecten kan worden bereikt. Bestaande middelen zijn weliswaar effectief, maar veroorzaken ook bijwerkingen met name slaperigheid, vermindering van het geheugen en duizeligheid. Het nieuwe werkingsmechanisme veronderstelt dat het middel even effectief kan zijn met weinig of geen van de genoemde bijwerkingen. Om deze differentiatie te weeg te kunnen brengen moet het middel uiteraard eerst op de plaats komen waar het zijn werking heeft; het brein. Hierdoor zijn 'pharmacology' en 'site' de belangrijkste vragen voor dit nieuwe middel. De gepresenteerde studie waarin de effecten op sedatie, geheugen en lichaamsstabiliteit worden vergeleken met de marktleider uit deze klasse zou in de NPV analyse extra ontwikkelingskosten en wellicht tijd introduceren. De studie toonde aan dat de differentiatie in effecten van het middel ook in proefpersonen waarneembaar is. Op basis van deze studie kon een minder sederende dosering kon geselecteerd worden om de effectiviteit in patiënten te onderzoeken. Deze maximale dosering is cruciaal want het betreft de kern van het marktvoordeel dat het middel kan hebben boven de bestaande middelen. Door deze studie werd voorkomen dat de ontwikkeling van het middel gestaakt wordt in een laat stadium omdat de verkeerde dosering was gekozen. De 'site' vraag is niet volledig beantwoord maar de effecten geobserveerd in deze studie zijn indicatief voor penetratie van het middel in het brein.

Conclusies

Het gebruik van Net Present Value berekeningen geeft niet adequaat de toegevoegde waarde van kennis weer. Het is, net als diverse andere benaderingen, een poging om procedureel optimaliserend om te gaan met de onzekerheid die vast zit aan het ontwikkelen van nieuwe geneesmiddelen voor complexe ziekten. Een vraag gebaseerde aanpak van dit probleem lijkt rationeler en tot beter inzicht te leiden in de inhoudelijke kant van het geneesmiddel ontwikkelingsproces. Daarnaast heeft deze aanpak implicaties voor de uitvoering van het project en de selectie van de mogelijke nieuwe biologisch actieve stoffen. Het valt buiten het doel van dit proefschrift een een-

duidig allesomvattend model te presenteren. Aan de hand van diverse voorbeelden wordt geïllustreerd dat de combinatie van kansen op succes en bijbehorende kosten een unieke set oplevert die per middel kan verschillen. Daarnaast wordt aangetoond dat, zelfs bij gelijk blijvende overall kansen en kosten, deze unieke set een optimale volgorde dicteert. Deze volgorde zou dan ook moeten dienen als prioriteitenlijst door het gehele ontwikkelingsprogramma. Voorts heeft de vraag gebaseerde aanpak als voordeel dat experts van de verschillende afdelingen die betrokken zijn bij de geneesmiddelontwikkeling samen overeenstemming krijgen over de kansen en bedreigingen van hun nieuwe middel en daarmee mogelijke hiaten in kennis over de stof tijdig kunnen aanvullen.

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Curriculum Vitæ

Samuel Jacob (Saco) de Visser was born January 9, 1975 in Haarlem (the Netherlands). In 1993 he obtained his Athenæum diploma (VWO) at the Dr W.A. Visser 't Hooft Lyceum in Leiden. He started studying chemistry and pharmacochemistry at the Free University of Amsterdam with internships at the Centre for Human Drug Research / SERVIER R&D (clinical pharmacology) and at the ministry of Health Welfare and Sports (management, policy and life sciences). He graduated in 1998 with a specialisation in molecular toxicology. Subsequently, he travelled around Australia, New Zealand and Indonesia for nine months. Since 1999 he holds a position as clinical scientist at the Centre for Human Drug Research.

STELLINGEN BEHOREND BIJ HET PROEFSCHRIFT: 'A QUESTION BASED APPROACH TO DRUG DEVELOPMENT'

1 The true risks of innovative drug development can be assessed better with a question-based approach than with Net Present Value (NPV) calculations. *(this thesis)*

2 In contrast to more traditional approaches, adding an early evaluation study to a drug development program can increase (rather than decrease) the project value. *(this thesis)*

3 Any attempt to integrate scientific knowledge rather than just operational aspects into a model for drug development will reduce the enormous costs of the development of new drugs. *(this thesis)*

4 The number of tests used in human psychopharmacology is excessive and reduction of the number of tests as well as further evaluation and validation is long overdue. *(this thesis)*

5 Although the quest for a non-sedative anxiolytic compound apparently continues, it remains to be demonstrated that sedation is not the primary mechanism of action of anxiolytic agents in most patients.

6 Homeopathy cannot be viewed as an evidence-based form of therapy but is effective for some patients. *(Ernst, Br J Clin Pharmacol 54;6, 577-582, 2002)*

7 Functional deficits of recreational MDMA ('Ecstasy') use may remain long after drug use has ceased and are consistent with serotonergic axonal loss in higher brain regions. *(Parrott et al, Pharmacol Biochem Behav. 71;4, 837-44, 2002)*

8 Motilin does not play a primary role in the pathophysiology of functional dyspepsia. *(Kamerling et al, Am J Physiol Gastrointest Liver Physiol 284, G776-G781, 2003)*

9 If institutions and sponsors fail to ensure publication of the knowledge obtained from the research, they arguably fail to honour their implicit commitment to the subjects who volunteered for the study. *(Schulman et al, New Engl J Med, 347;17, 1335-1341, 2002)*

10 If you want to know what a man's like, take a good look at how he treats his inferiors, not his equals. *(Sirius Black, Harry Potter and the goblet of fire)*

11 The saddest aspect of life right now is that science gathers knowledge faster than society gathers wisdom. *(Isaac Asimov)*

12 A good traveller has no fixed plans, and is not intent on arriving. *(Lao Tzu)*

Saco de Visser, 10 september 2003, Leiden