CHAPTER 1

Introduction

BACKGROUND

Gabaergic systems

The inhibitory neurotransmission in the vertebrate central nervous system (CNS) is primarily mediated by y-aminobutyric acid (GABA). It is estimated that depending on the brain region about 20 to 50% of all central synapses use GABA as their transmitter [1]. The enhancement of neuronal inhibition by GABA is one of the most powerful therapeutic strategies for the treatment of diseases in which some form of CNS over-activation seems to play a role, such as generalized anxiety disorders, sleep disturbances, muscle spasms and seizure disorders (see table 1). Historically the $GABA_A$ receptor has been the target of many drug treatments. The earliest compounds were ions like bromide, then came barbiturates, and finally, from 1960s onwards, a number of benzodiazepines. Existing treatments are efficient but are often hampered by the presence of side effects. At present, the $GABA_A$ receptor is still a drug target of interest, and involved in the development of many novel treatments for various diseases, with an improved efficacy and a reduced adverse event profile. In this thesis, several studies are presented, which are devoted to various aspects of different GABAergic drugs. A range of methodologies have been used to describe relevant characteristics of GABAergic agents in different stages of development.

GABA and its receptor

The action of GABA is mostly mediated by two classes of receptors, GABA type A ($GABA_A$) and type B ($GABA_B$) receptors. In contrast to the $GABA_A$ receptor, the $GABA_B$ receptor is a metabotropic receptor that is present on pre- and postsynaptic neurons. The GABA type C receptors, which are comprised of proteins that are related to $GABA_A$ receptor subunits [2], are found primarily in the retina [3]. $GABA_B$ and $GABA_C$ will not be discussed further here. $GABA_A$ receptors are ligand-gated chloride ion channels which are not only stimulated by GABA but also by pharmacologically and clinically important drugs, such as benzodiazepines, barbiturates, steroids, anaesthetics, and anticonvulsants [4]. The $GABA_A$ receptor is a pentameric structure composed of five distinct glycoprotein subunits that span a lipid bilayer and form a cylindrical structure whose center constitutes an ion channel. Binding of GABA to its recognition sites on the receptor results in conformational changes that can lead to opening of the channel with a resulting influx of chloride into the cell [3,5]. The resulting hyperpolarisation of the post-synaptic cell membrane increases the inhibitory tone. Benzodiazepines do not independently activate this process but rather facilitate the action of GABA by increasing the frequency of ion channel opening [2]. Other psychoactive drugs, including barbiturates, anaesthetic steroids and alcohol allosterically modify the receptor at different sites, and have the same effect of enhancing the neuronal inhibition [6] (see figure 1). Binding of an inverse agonist to the GABA receptor reduces the chloride flux in the absence of GABA [7] and decreases the inhibitory effects of GABA. Furthermore, there is a spectrum of efficacies that range from full-agonists, through partial agonist, antagonist and partial inverse agonist to full inverse agonist [8] (see figure 2).

Many possibilities in the pentameric composition of the GABA receptor are possible because of the heterogeneity of subunits [4,9]. There are several molecular families of mammalian subunits (α1-α6, β1- β4, y1- y4, δ, ε, π, ρ1- ρ3) [2] and the most receptors seem to be composed of two of four α subunits (1, 2, 3, or 5), two β subunits (2 or 3) and one y subunit [5]. Benzodiazepines only bind to $GABA_A$ receptors that include the α1, α2, α3 or α5 and not the α4 or α6 subunit. The benzodiazepine site is located at the interface between the α and y2 subunit. Both the affinity and efficacy of benzodiazepines is determined by the type of α and y subunits that are present in the receptor [5].

GABAERGIC DRUG DEVELOPMENT

Pharmacokinetic modification

As so many treatments involve the GABA receptor, the pharmacokinetic properties of a compound often determine the indication of the drug. For example, the differential use of benzodiazepines as muscle relaxants, hypnotics or anxiolytics is largely determined by the pharmacokinetic characteristics, like the dose, route of administration, effect compartment half-life and formation of active metabolites. In the prevention of epileptic seizures and anti-anxiety treatment, continuous treatment is pursued, so that compounds with long elimination half-lives of parent drug or active metabolites are of advantage. If on the other hand a benzodiazepine is taken as a hypnotic, the concentration should be high enough to cause sleep and the duration of action should be restricted to the night; hence a compound with a short elimination half-life is preferred. Benzodiazepines for induction of anesthesia or interruption of status epilepticus should have pharmacokinetic properties that are compatible with high CNS-concentrations, a rapid onset and a limited duration of action. The method of changing the pharmacokinetic

properties among benzodiazepines has shown to be an effective approach, to adapt drugs with a similar pharmacological activity to different therapeutic indications.

Primarily based on their diverse pharmacokinetic properties, benzodiazepines have been in widespread use for more than 40 years, as drugs for conditions like anxiety, epilepsy, sleep disorders, mania, muscle spasms and anesthesia [10]. Benzodiazepines have a safer mechanism of action compared to earlier $GABA_A$-agonists like barbiturates and bromide derivatives, since they only enhance the action of GABA while barbiturates can directly activate the $GABA_A$ receptor in the absence of GABA, making them less safe in case of an overdose. The disadvantages of benzodiazepines are the side effects, like sedation, postural instability, memory impairment and the potential development of tolerance, abuse and dependence after long-term use. Depending on the clinical setting, the various pharmacological attributes of the benzodiazepines may be either beneficial or a liability. For example, the myorelaxant and cognitive impairing properties may be beneficial when they are used as premedication for anesthesia [11,12], but are clearly disadvantageous for everyday activities when given for other indications. The sedative/hypnotic properties are useful for treating sleep disorders, but are undesirable for an anxiolytic [11]. Patients with anxiety disorders, who are a large part of the benzodiazepine consumers, are particularly prone to experience side effects [13]. Benzodiazepines are also often used in the elderly population as hypnotics or tranquilizers, while particularly in this group the side effects are associated with higher incidences of falls [14] and cognitive impairment [15,16]. Therefore, a clear medical need remains for the development of improved therapies that are more efficacious, easy to use, and better tolerated than those already marketed. There is a limit to how this can be achieved with modifications of the pharmacokinetic properties of benzodiazepines. Consequently, $GABA_A$-ergic drugs with distinct pharmacological characteristics have been designed.

Pharmacological modification

As there was still need for more therapeutic selectivity and a larger therapeutic window, more GABAergic agents were developed to improve the side effect profile.

In the late 1980s and the early 1990s, non-selective, partial agonists were launched with equivalent affinity for all $GABA_A$ subtypes but lower efficacies [17]. Their development was based on the assumption that neurons mediating anticonvulsant and anti-anxiety effects have a higher receptor reserve than neurons mediating other unwanted effects. Pre-clinical profiles showed that they all demonstrated a margin between doses that produce anxiolysis and sedation that is superior to that associated with the non-selective full agonists such as diazepam [18]. For some of these agents, like bretazenil and pazinaclone, the sedative effects could not be differentiated from anxiolytic effects [19-22]. For other non-selective partial agonists, the development fate is unknown [8].

The development of GABAergic compounds has not been limited to partial agonists but also other compounds that directly or indirectly affect GABA or its receptor.

Vigabatrin elevates brain GABA levels by inhibiting the enzyme GABA transaminase which is responsible for intracellular GABA catabolism [23]. In contrast, tiagabine elevates synaptic GABA levels by inhibiting the GABA uptake transporter, GAT1, and preventing the uptake of GABA into neurons and glia [23]. Neuroactive steroids allosterically modulate the $GABA_A$ receptor and were seen as a potential therapeutic use in neurological and psychiatric disorders [24]. So far, ganaxolone has shown to be effective in the treatment of epilepsy [25]. Ethanol also modulates the $GABA_A$ receptor and elicits, in a dose-dependent manner, an array of central depressant effects.

Recently, several GABA analogues have been synthesized, but interestingly none of these actually influence the GABA-binding site on the GABA receptor. Tiagabine affects the $GABA_A$ receptor by inhibiting GAT1. GABApentin and pregabalin are chemically related lipophilic GABA-analogues, which do not mimic GABA at $GABA_A$ or $GABA_B$ receptors, nor do they augment $GABA_A$ responses like benzodiazepines or barbiturates [26]. Pregabalin rather seems to bind primarily to the $\alpha 2\delta$ subunit of voltage-gated calcium channels in the CNS. Binding to these channels induces release of neurotransmitters at many sites in the CNS to attenuate abnormal hyperexcitability and abnormal synchronization of neuronal networks, thereby providing anticonvulsant and analgesic effects [27]. The details of the mechanism of reducing the neurotransmitters remain to be defined. Pregabalin was originally launched for the treatment of neuropathic pain and epilepsy, and has recently also been registered as an anxiolytic [28]. In this thesis, possible sleep improving properties of pregabalin are investigated.

All these agents were developed to affect the action of GABA or its receptor using different approaches to improve the side effect profile. This thesis describes several ways to show how the pharmacological improvements are reflected in potential therapeutic advantages in humans. It is shown that studies in healthy volunteers can demonstrate distinctive pharmacodynamic characteristics of novel GABAergic drugs in comparison to existing treatments. In the early

stages of development, the clinical relevance of these improved pharmacological characteristics is not always clear, particularly if the pathophysiology and the involvement of GABAergic systems are incompletely understood. In such cases, studies in patients can explore the potential therapeutic usefulness of innovative GABAergic agents, and the role of GABAergic mechanism in the disease. This thesis describes how these different strategies were explored in a range of studies with different GABAergic or GABA-like drugs in healthy volunteers and patients.

THE ASSESSMENT OF PHARMACODYNAMIC EFFECTS OF NEWLY DESIGNED $GABA_A$-ERGIC AGENTS IN EARLY PHASE DRUG DEVELOPMENT

New development of subtype selective (partial) agonists

The insights into the complexity of the $GABA_A$ receptor family and the identification of the subtypes modulated by benzodiazepines raised the possibility that some of the clinical properties of benzodiazepines might be mediated through different receptor subtypes. The different receptor subtypes are located at diverse brain areas with the $\alpha 1$ subtype present in most brain areas and $\alpha 5$ only in the hippocampus. Several preclinical studies were undertaken to elucidate the different pharmacological effects of the discrete $GABA_A$ receptor subtypes. The anxiolytic effect of benzodiazepines is thought to be mediated by $GABA_A$ $\alpha 2$ receptors [29,30], and recently more emphasis is given to $GABA_A$ $\alpha 3$ [8,31,32]. These two subtypes are also believed to be associated with muscle relaxation [33,34]. The widespread $\alpha 1$ subtype appears to be involved in the sedative effects of generalized CNS-depression [30,35-38]. The hippocampal $\alpha 5$ subtypes could have a role in memory [39]. These findings have stimulated the development of compounds that are selective for a certain subtype to cause specific pharmacological effects; or conversely don't bind to subtypes to avoid undesirable effects. This selectivity could be achieved by selective affinity or efficacy for the receptor subtype involved with a certain function (see figure 3). Zolpidem and zaleplon are examples of compounds with a higher affinity for the $\alpha 1$ subtype, and both are registered as selective hypnotics [40]. Additionally, imidazo[1,2-a] pyrimidines with selectivity for the $\alpha 2,3$ subtype have been developed as anxiolytics with putatively reduced sedative properties [41].

Pharmacodynamic measurements in early drug development

An important question is whether the pre-clinical differentiating pharmacological characteristics of these novel agents are reflected by a similar distinctive profile in humans. Unfortunately, the functional relevance of the different $GABA_A$ receptor subtypes has not yet been determined in human health and disease, which thwarts the direct evaluation of pharmacological properties of subtype-selective $GABA_A$-agonists in early clinical development. Benzodiazepines have shown effects on a wide range of pharmacodynamic measurements including saccadic eye movements, smooth pursuit performance, body sway, adaptive tracking, memory testing and Visual Analogue Scales (VAS) of alertness, contentedness and calmness [19,42-45]. It is not unreasonable to assume that these rather diverse effects of benzodiazepines in some way reflect the variations in $GABA_A$ receptor subtypes. By inference, it seems plausible that subjective alertness and impairment of body sway in humans are related to $\alpha 1$-stimulation. Reduction of saccadic peak velocity has been shown to be closely related to the anxiolytic potencies of benzodiazepines [46], and could thus reflect $\alpha 2,3$-activity. Memory effects could be related to $\alpha 5$-receptor subtypes. The effects of different compounds with different binding and efficacy profiles on this CNS-test battery could therefore provide an accurate impression of their selectivity. Knowledge about the pharmacodynamic profile of these selective agents is primarily helpful in the prediction of side effects. Secondly, measurement of pharmacodynamic parameters might be useful in the determination of a biomarker for the therapeutic efficacy. In this thesis, the pharmacodynamic profile of four different $GABA_A$ subtype selective agents has been investigated. Chapter 2, 3 and 4 of this thesis describe studies that have been performed with $\alpha 2,3$ selective (partial) $GABA_A$ agonists TPA023, MK-0343 and SL65.1498 that showed promising differential effects in the pre-clinical phase. In these studies, the pharmacodynamic effects have been determined and compared to the effects of the full agonist lorazepam in healthy volunteers. Another selective compound in this thesis is the hypnotic zolpidem, which is selective for the $\alpha 1$ subtype. Its pharmacodynamic and pharmacokinetic/pharmacodynamic effects are described in Chapter 5. One subject developed florid pseudo-hallucinations during this study. A comparison of the detailed pharmacokinetic and pharmacodynamic profiles of the selective $\alpha 1$-agonist between this subject and the other healthy volunteers, allowed us to describe several aspects of zolpidem-induced pseudo-hallucinations in Chapter 6.

Search for biomarkers to predict pharmacological selectivity

In preclinical research, different animal models are used to quantify various effects of GABAergic drugs on memory, sedation, anxiety and muscle tension. These studies are used to predict the functional selectivity of novel compounds in drug development [35,47,48]. Clearly, such an approach would also be very helpful in the early clinical phases of development. However, no clear a priori hypothesis can be formulated, to predict the anticipated effect profile for a certain subtype-selective $GABA_A$ agonist. The different studies described in Chapters 2, 3 and 4 and previous CHDR-studies with benzodiazepines allowed us to evaluate the relationships between the pharmacological characteristics of different GABAergic compounds, and their distinctive CNS-effect profiles. The relationships between body sway and visual analogue scales (VAS) of alertness relative to saccadic peak velocity (SPV) were compared among different GABAergic drugs. SPV was chosen because in clinical studies, this eye movement parameter has been shown to be closely associated with anxiolytic and sedative effects of benzodiazepines [46] and sedative effects of other drugs and circumstances [43,44,49]. VAS alertness and body sway reflect other functional aspects of GABAergic stimulation (subjective sedation and postural instability). Chapter 7 describes how the relative effect relationships differed among GABAergic compounds with distinct pharmacological characteristics. This provided a first step in the charting of selective CNS-biomarkers for $GABA_A$ receptor subtypes in healthy humans.

THE EXPLORATION OF PHARMACODYNAMIC EFFECTS TO IDENTIFY NOVEL INDICATIONS

The studies presented in Chapters 2, 3 and 4 suggest that the selectivity for certain $GABA_A$ receptor subtypes is also present in humans. These pharmacological properties can be demonstrated in healthy volunteers, but such studies provide limited indications for the therapeutic relevance of subtype selectivity. Several studies were performed in patients, to explore potential therapeutic effects of novel GABAergic or GABA-like compounds.

As described in the previous section, the $\alpha 2,3$ subtypes are associated with both anxiolysis and muscle relaxation [33]. It was decided to investigate the clinical effects of TPA023 in essential tremor, a neurological condition that increases with anxiety and improves with muscle relaxation. Essential tremor (ET) typically shows a postural and kinetic tremor between 4-12 Hz [50]. Benzodiazepines, barbiturates (primidone) and alcohol –all GABAergic compounds [51] have a well-determined therapeutic efficacy on ET [52], which is limited by a partial response and by side effects. Although the pathophysiology of ET is unknown, the clear effects of various GABAergic drugs suggest that certain $GABA_A$ receptor subtypes may be involved. Chapter 8 describes the effect of the $\alpha 2,3$ selective partial $GABA_A$ agonists TPA023 on essential tremor in comparison to that of ethanol, of which the activity is largely mediated by the $GABA_A$ receptor [53]. Laboratory tremography was used to determine the effects on tremor and pharmacodynamic CNS effects were also assessed in this patient group.

Giving a subunit-selective agent to this patient group could reveal the role of the different $GABA_A$ receptor subtypes in attenuating this type of tremor and consequently provide a new class of successful drugs for this disorder with potential fewer side effects.

Pregabalin was originally launched for the treatment of neuropathic pain and epilepsy, and has currently also been registered as an anxiolytic [26]. Clinical studies showed that pregabalin did not only seem to improve neuropathic pain but also affected sleep interference scores that were part of these studies. This raised the question whether pregabalin, besides the indirect effect of sleep improvement as a consequence of pain relief, might have a direct sleep-modulating effect [54]. This possibly novel finding and consequently novel indication of the drug was a serendipitous discovery that was not based on pre-clinical assumptions as for the compounds described in the previous section. Subsequently, new studies in animals, healthy volunteers and patients with disturbed sleep were set up to verify the effects of pregabalin on sleep. The last part of this thesis describes efforts to identify a new potential indication for pregabalin, and to explore its effects on sleep disorders in patients with partial epilepsy.

As pregabalin was in development as adjuvant therapy in patients with partial epilepsy, it was thought that pregabalin could have beneficial effects on sleep in patients with partial epilepsy. However, the prevalence of sleep disturbance and the need for a sleep-improving agent in this patient group was unknown. A small number of articles about epilepsy and sleep had been published [55-59] which resulted in studies in which the effects of antiepileptic drugs on sleep were investigated [60]. However, before studies with pregabalin and sleep disturbed epilepsy patients were initiated, it was necessary to investigate the incidence of the problem and its effect on daily life in this patient group. Therefore, an inquiry study

was performed to investigate the prevalence of sleep disturbance in patients with partial epilepsy and its effects on quality of life. This study is described in Chapter 9 of this thesis. Based on the results of this inquiry study, a study to determine the effects of pregabalin on sleep disturbance seemed useful. Polysomnographic registrations and sleep questionnaires were used to determine the effects of pregabalin in patients with partial epilepsy, which is described in Chapter 10.

SUMMARY

This thesis describes different ways of exploring the pharmacological and therapeutic effects of novel GABAergic and GABA-like agents in humans. Systematic pharmacodynamic evaluations, using well-characterised positive controls, can confirm or refute the unique pharmacological properties of $GABA_A$ subtype selective drugs in healthy volunteers. Such studies can help to predict dosing regimens and therapeutic advantages of these drugs. The distribution of different $GABA_A$ receptor subtypes provides clues for their functional relevance. This knowledge can be used to optimise the desirable and undesirable effect profiles of selective GABAergic drugs. Very little is still known about the pathophysiological relevance of GABA-systems in CNS-disorders, although GABAergic treatments are in use for a wide range of clinical conditions. The availability of novel compounds with well defined pharmacological characteristics can clarify the involvement of these mechanisms in normal or abnormal physiology. This thesis hopes to show that carefully designed studies, using a range of CNS-measurement that reflect different GABAergic systems, can aid in the development of new GABAergic drugs, and help to unravel the role of the different GABAergic systems in health and disease.

Table 1 Overview of different GABA-receptor binding places, its ligands and indication of treatment

Direct GABA-receptor binding		
BENZODIAZEPINE BINDING PLACE	Benzodiazepines	Anxiety disorder
		Epilepsy
		Sleep disturbance
		Neuropathic pain
		Muscle spasm
		Essential tremor
		Anaesthesia
		Alcohol withdrawal
	Flumazenil	Benzodiazepine overdose
NEUROSTEROID BINDING PLACE	Ganaxolone	Epilepsy
ETHANOL BINDING PLACE	Ethanol	Essential tremor
BARBITURATE BINDING PLACE	Barbiturates	Anxiety disorder
		Epilepsy
		Sleep disturbance
		Anaesthesia
Indirect GABA-receptor activation		
BINDING $\alpha 2\delta$-SUBUNIT CA-CHANNEL	Pregabalin	Epilepsy
		Generalized Anxiety disorder
		Neuropathic pain
	Gabapentin	Epilepsy
		Neuropathic pain
PRESYNAPTIC GAT-1 TRANSPORTER BLOCKADE	Tiagabine	Epilepsy
GABA-TRANSAMINASE DESTRUCTION	Vigabatrin	Epilepsy
OPENING K-CHANNEL	Retigabine	Epilepsy
DECREASES GLUTAMATE RELEASE	Lamotrigine	Epilepsy

Figure 1 Different binding places of a GABA$_A$ receptor.

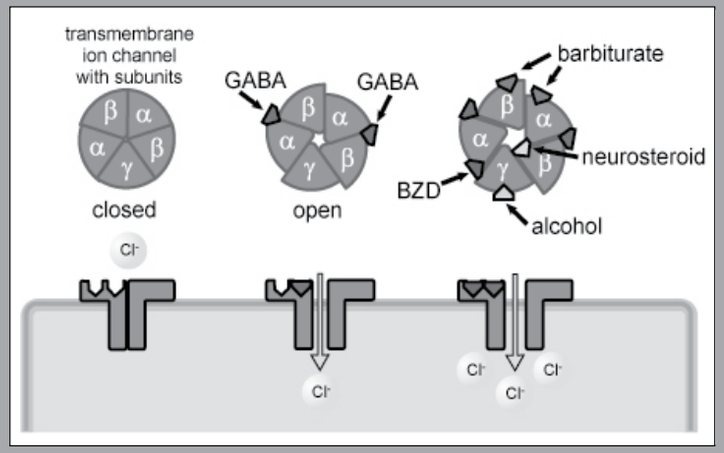


Figure 2 Schematic representation of the modulatory effects on GABA-mediated CL flux of BZ site with differing intrinsic efficacies.

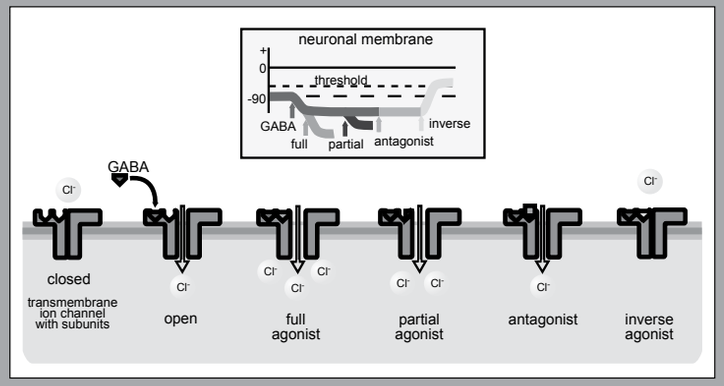
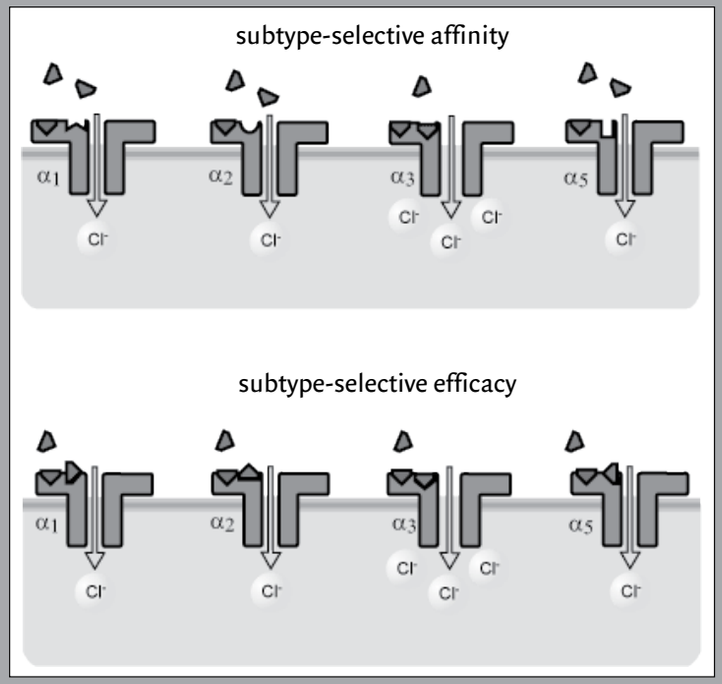


Figure 3 Strategies for developing subtype-selective compounds acting at the BZ site of the GABA$_A$ receptor.



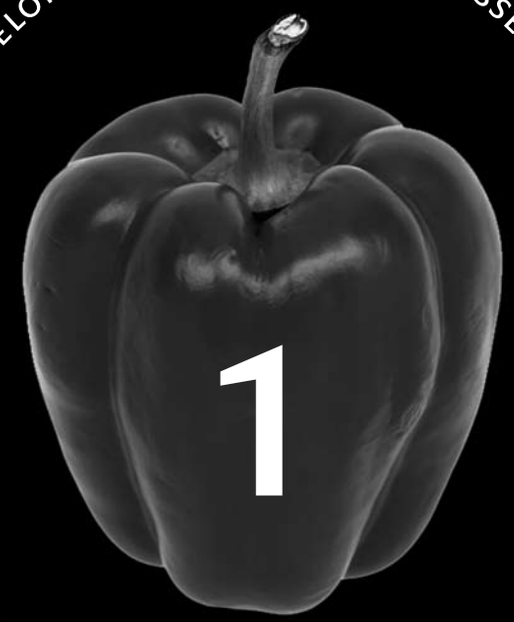
A. Subtype selective affinity: a compound binds selectively to a particular receptor subtype, but not to other subtypes. In this example, the compound shows specific affinity and agonist efficacy for the α3-subtype, but because it can not bind to the other subtypes, will not alter GABA function at the α1-, α2-, α5-subtypes. B. Absolute subtype-selective efficacy: a compound binds to all four GABA$_A$ subtypes with equal affinity, but only shows efficacy at one particular subtype. In this example, the compound is a full agonist at the α3-subtype, a partial agonist at the α1-, α2- and α5-subtypes. TPA023 (not shown in Figure 3) is an antagonist at the α1-subtype and a partial agonist at α2- and α3-subtypes.

REFERENCES

1 Bloom FE, Iversen LL. Localizing 3H-GABA in nerve terminals of rat cerebral cortex by electron microscopic autoradiography. Nature 1971; 229: 628-630.
2 Barnard EA, Skolnick P, Olsen RW, Mohler H, Sieghart W, Biggio G, Braestrup C, Bateson AN, Langer SZ. International Union of Pharmacology. XV. Subtypes of gamma-aminobutyric acidA receptors: classification on the basis of subunit structure and receptor function. Pharmacol Rev 1998; 50: 291-313.
3 Bormann J. The 'ABC' of GABA receptors. Trends Pharmacol Sci 2000; 21: 16-19.
4 Sieghart W. Structure and pharmacology of gamma-aminobutyric acidA receptor subtypes. Pharmacol Rev 1995; 47: 181-234.
5 Mohler H, Crestani F, Rudolph U. GABA(A)-receptor subtypes: a new pharmacology. Curr Opin Pharmacol 2001; 1: 22-25.
6 Roy-Byrne PP. The GABA-benzodiazepine receptor complex: structure, function, and role in anxiety. J Clin Psychiatry 2005; 66 Suppl 2: 14-20.
7 Ueno S, Bracamontes J, Zorumski C, Weiss DS, Steinbach JH. Bicuculline and gabazine are allosteric inhibitors of channel opening of the $GABA_A$ receptor. J Neurosci 1997; 17: 625-634.
8 Atack JR. The benzodiazepine binding site of GABA(A) receptors as a target for the development of novel anxiolytics. Expert Opin Investig Drugs 2005; 14: 601-618.
9 Whiting PJ. The GABA-A receptor gene family: new targets for therapeutic intervention. Neurochem Int 1999; 34: 387-390.
10 Dawson GR, Collinson N, Atack JR. Development of subtype selective $GABA_A$ modulators. CNS Spectr 2005; 10: 21-27.
11 Williams TJ, Bowie PE. Midazolam sedation to produce complete amnesia for bronchoscopy: 2 years' experience at a district general hospital. Respir Med 1999; 93: 361-365.
12 Buffett-Jerrott SE, Stewart SH. Cognitive and sedative effects of benzodiazepine use. Curr Pharm Des 2002; 8: 45-58.
13 Nutt DJ. Overview of diagnosis and drug treatments of anxiety disorders. CNS Spectr 2005; 10: 49-56.
14 Ray WA, Thapa PB, Gideon P. Benzodiazepines and the risk of falls in nursing home residents. J Am Geriatr Soc 2000; 48: 682-685.
15 Paterniti S, Dufouil C, Alperovitch A. Long-term benzodiazepine use and cognitive decline in the elderly: the Epidemiology of Vascular Aging Study. J Clin Psychopharmacol 2002; 22:285-293.
16 Madhusoodanan S, Bogunovic OJ. Safety of benzodiazepines in the geriatric population. Expert Opin Drug Saf 2004; 3: 485-493.
17 Haefely W, Martin JR, Schoch P. Novel anxiolytics that act as partial agonists at benzodiazepine receptors. Trends Pharmacol Sci 1990; 11: 452-456.
18 Atack JR. Anxioselective compounds acting at the GABA(A) receptor benzodiazepine binding site. Curr Drug Targets CNS Neurol Disord 2003; 2: 213-232.
19 van Steveninck AL, Gieschke R, Schoemaker RC, Roncari G, Tuk B, Pieters MS, Breimer DD, Cohen AF. Pharmacokinetic and pharmacodynamic interactions of bretazenil and diazepam with alcohol. Br J Clin Pharmacol 1996; 41: 565-573.
20 Delini-Stula A, Berdah-Tordjman D. Antipsychotic effects of bretazenil, a partial benzodiazepine agonist in acute schizophrenia--a study group report. J Psychiatr Res 1996; 30: 239-250.
21 Evans SM, Foltin RW, Levin FR, Fischman MW. Behavioral and subjective effects of DN-2327 (pazinaclone) and alprazolam in normal volunteers. Behav Pharmacol 1995; 6: 176-186.
22 Linden M, Hadler D, Hofmann S. Randomized, double-blind, placebo-controlled trial of the efficacy and tolerability of a new isoindoline derivative (DN-2327) in generalized anxiety. Human Psychopharmacology-Clinical and Experimental 1997; 12: 445-452.
23 Leach JP, Brodie MJ. New antiepileptic drugs--an explosion of activity. Seizure 1995; 4: 5-17.
24 Gasior M, Carter RB, Witkin JM. Neuroactive steroids: potential therapeutic use in neurological and psychiatric disorders. Trends Pharmacol Sci 1999; 20: 107-112.
25 Nohria V, Giller E. Ganaxolone. Neurotherapeutics 2007; 4: 102-105.
26 Taylor CP, Angelotti T, Fauman E. Pharmacology and mechanism of action of pregabalin: The calcium channel alpha(2)-delta (alpha(2)-delta) subunit as a target for antiepileptic drug discovery. Epilepsy Res 2007; 73: 137-150.
27 Dooley DJ, Taylor CP, Donevan S, Feltner D. Ca2+ channel alpha2delta ligands: novel modulators of neurotransmission. Trends Pharmacol Sci 2007; 28: 75-82.
28 Tassone DM, Boyce E, Guyer J, Nuzum D. Pregabalin: A novel gamma-aminobutyric acid analogue in the treatment of neuropathic pain, partial-onset seizures, and anxiety disorders. Clin Ther 2007; 29: 26-48.
29 Low K, Crestani F, Keist R, Benke D, Brunig I, Benson JA, Fritschy JM, Rulicke T, Bluethmann H, Mohler H, Rudolph U. Molecular and neuronal substrate for the selective attenuation of anxiety. Science 2000; 290: 131-134.
30 Rudolph U, Crestani F, Mohler H. GABA(A) receptor subtypes: dissecting their pharmacological functions. Trends Pharmacol Sci 2001; 22: 188-194.
31 Atack JR, Hutson PH, Collinson N, Marshall G, Bentley G, Moyes C, Cook SM, Collins I, Wafford K, McKernan RM, Dawson GR. Anxiogenic properties of an inverse agonist selective for alpha3 subunit-containing GABA A receptors. Br J Pharmacol 2005; 144: 357-366.
32 Dias R, Sheppard WF, Fradley RL, Garrett EM, Stanley JL, Tye SJ, Goodacre S, Lincoln RJ, Cook SM, Conley R, Hallett D, Humphries AC, Thompson SA, Wafford KA, Street LJ, Castro JL, Whiting PJ, Rosahl TW, Atack JR, McKernan RM, Dawson GR, Reynolds DS. Evidence for a significant role of alpha3-containing $GABA_A$ receptors in mediating the anxiolytic effects of benzodiazepines. J Neurosci 2005; 25: 10682-10688.
33 Rowlett JK, Platt DM, Lelas S, Atack JR, Dawson GR. Different $GABA_A$ receptor subtypes mediate the anxiolytic, abuse-related, and motor effects of benzodiazepine-like drugs in primates. Proc Natl Acad Sci usa 2005; 102: 915-920.
34 Crestani F, Low K, Keist R, Mandelli M, Mohler H, Rudolph U. Molecular targets for the myorelaxant action of diazepam. Mol Pharmacol 2001; 59: 442-445.
35 Griebel G, Perrault G, Simiand J, Cohen C, Granger P, Decobert M, Francon D, Avenet P, Depoortere H, Tan S, Oblin A, Schoemaker H, Evanno Y, Sevrin M, George P, Scatton B. SL651498: an anxioselective compound with functional selectivity for alpha2- and alpha3-containing gamma-aminobutyric acid(A) (gaba(A)) receptors. J Pharmacol Exp Ther 2001; 298: 753-768.
36 McKernan RM, Rosahl TW, Reynolds DS, Sur C, Wafford KA, Atack JR, Farrar S, Myers J, Cook G, Ferris P, Garrett L, Bristow L, Marshall G, Macaulay A, Brown N, Howell O, Moore KW, Carling RW, Street LJ, Castro JL, Ragan CI, Dawson GR, Whiting PJ. Sedative but not anxiolytic properties of benzodiazepines are mediated by the GABA(A) receptor alpha1 subtype. Nat Neurosci 2000; 3: 587-592.
37 Rudolph U, Crestani F, Benke D, Brunig I, Benson JA, Fritschy JM, Martin JR, Bluethmann H, Mohler H. Benzodiazepine actions mediated by specific gamma-aminobutyric acid(A) receptor subtypes. Nature 1999; 401: 796-800.
38 Tobler I, Kopp C, Deboer T, Rudolph U. Diazepam-induced changes in sleep: role of the alpha 1 GABA(A) receptor subtype. Proc Natl Acad Sci usa 2001; 98: 6464-6469.
39 Collinson N, Kuenzi FM, Jarolimek W, Maubach KA, Cothliff R, Sur C, Smith A, Otu FM, Howell O, Atack JR, McKernan RM, Seabrook GR, Dawson GR, Whiting PJ, Rosahl TW. Enhanced learning and memory and altered gabaergic synaptic transmission in mice lacking the alpha 5 subunit of the $GABA_A$ receptor. J Neurosci 2002; 22: 5572-5580.
40 Crestani F, Martin JR, Mohler H, Rudolph U. Mechanism of action of the hypnotic zolpidem in vivo. Br J Pharmacol 2000; 131: 1251-1254.
41 Goodacre SC, Street LJ, Hallett DJ, Crawforth JM, Kelly S, Owens AP, Blackaby WP, Lewis RT, Stanley J, Smith AJ, Ferris P, Sohal B, Cook SM, Pike A, Brown N, Wafford KA, Marshall G, Castro JL, Atack JR. Imidazo[1,2-a]pyrimidines as functionally selective and orally bioavailable GABA(A)alpha2/alpha3 binding site agonists for the treatment of anxiety disorders. J Med Chem 2006; 49: 35-38.
42 van Gerven JM, Roncari G, Schoemaker RC, Massarella J, Keesmaat P, Kooyman H, Heizmann P, Zell M, Cohen AF, Dingemanse J. Integrated pharmacokinetics and pharmacodynamics of Ro 48-8684, a new benzodiazepine, in comparison with midazolam during first administration to healthy male subjects. Br J Clin Pharmacol 1997; 44: 487-493.
43 van Steveninck AL, Schoemaker HC, Pieters MS, Kroon R, Breimer DD, Cohen AF. A comparison of the sensitivities of adaptive tracking, eye movement analysis and visual analog lines to the effects of incremental doses of temazepam in healthy volunteers. Clin Pharmacol Ther 1991; 50: 172-180.
44 van Steveninck AL, Verver S, Schoemaker HC, Pieters MS, Kroon R, Breimer DD, Cohen AF. Effects of temazepam on saccadic eye movements: concentration-effect relationships in individual volunteers. Clin Pharmacol Ther 1992; 52: 402-408.
45 van Steveninck AL, Mandema JW, Tuk B, Van Dijk JG, Schoemaker HC, Danhof M, Cohen AF. A comparison of the concentration-effect relationships of midazolam for EEG-derived parameters and saccadic peak velocity. Br J Clin Pharmacol 1993; 36: 109-115.
46 de Visser SJ, van der Post JP, de Waal PP, Cornet F, Cohen AF, van Gerven JM. Biomarkers for the effects of benzodiazepines in healthy volunteers. Br J Clin Pharmacol 2003; 55: 39-50.

47 Griebel G, Perrault G, Simiand J, Cohen C, Granger P, Depoortere H, Francon D, Avenet P, Schoemaker H, Evanno Y, Sevrin M, George P, Scatton B. SL651498, a $GABA_A$ receptor agonist with subtype-selective efficacy, as a potential treatment for generalized anxiety disorder and muscle spasms. CNS Drug Rev 2003; 9: 3-20.
48 Atack JR, Wafford KA, Tye SJ, Cook SM, Sohal B, Pike A, Sur C, Melillo D, Bristow L, Bromidge F, Ragan I, Kerby J, Street L, Carling R, Castro JL, Whiting P, Dawson GR, McKernan RM. TPA023 [7-(1,1-Dimethylethyl)-6-(2-ethyl-2H-1,2,4-triazol-3-ylmethoxy)-3-(2-fluor ophenyl)-1,2,4-triazolo[4,3-b]pyridazine], an Agonist Selective for {alpha}2- and {alpha}3-Containing $GABA_A$ Receptors, Is a Nonsedating Anxiolytic in Rodents and Primates. J Pharmacol Exp Ther 2006; 316: 410-422.
49 van Steveninck AL, van Berckel BN, Schoemaker RC, Breimer DD, van Gerven JM, Cohen AF. The sensitivity of pharmacodynamic tests for the central nervous system effects of drugs on the effects of sleep deprivation. J Psychopharmacol 1999; 13: 10-17.
50 Deuschl G, Bain P, Brin M. Consensus statement of the Movement Disorder Society on Tremor. Ad Hoc Scientific Committee. Mov Disord 1998; 13 Suppl 3: 2-23.
51 Louis ED. A new twist for stopping the shakes? Revisiting GABAergic therapy for essential tremor. Arch Neurol 1999; 56: 807-808.
52 Chen JJ, Swope DM. Essential tremor: diagnosis and treatment. Pharmacotherapy 2003; 23: 1105-1122.
53 Kulonen E. Ethanol and GABA. Med Biol 1983; 61: 147-167.
54 Kubota T, Fang J, Meltzer LT, Krueger JM. Pregabalin enhances nonrapid eye movement sleep. J Pharmacol Exp Ther 2001; 299: 1095-1105.
55 Gottesmann C. GABA mechanisms and sleep. Neuroscience 2002; 111: 231-239.
56 Nutt DJ, Malizia AL. New insights into the role of the GABA(A)-benzodiazepine receptor in psychiatric disorder. Br J Psychiatry 2001; 179: 390-396.
57 Brown N, Kerby J, Bonnert TP, Whiting PJ, Wafford KA. Pharmacological characterization of a novel cell line expressing human alpha(4) beta(3)delta GABA(A) receptors. Br J Pharmacol 2002; 136: 965-974.
58 Puia G, Ducic I, Vicini S, Costa E. Molecular mechanisms of the partial allosteric modulatory effects of bretazenil at gamma-aminobutyric acid type A receptor. Proc Natl Acad Sci usa 1992; 89: 3620-3624.
59 Ator NA. Selectivity in generalization to gabaergic drugs in midazolam-trained baboons. Pharmacol Biochem Behav 2003; 75: 435-445.
60 Drover DR. Comparative pharmacokinetics and pharmacodynamics of short-acting hypnosedatives : zaleplon, zolpidem and zopiclone. Clin Pharmacokinet 2004; 43: 227-238.

SECTION 1 THE ASSESSMENT OF PHARMACODYNAMIC EFFECTS OF NEWLY DESIGNED GABAERGIC AGENTS IN EARLY PHASE DRUG DEVELOPMENT



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CHAPTER 2

Pharmacodynamic and pharmacokinetic effects of TPA023, a $GABA_A$ α2,3 subtype-selective agonist, compared to lorazepam and placebo in healthy volunteers

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ABSTRACT

TPA023, a $GABA_A$ α2,3 subtype-selective partial agonist, is expected to have comparable anxiolytic efficacy as benzodiazepines with reduced sedating effects. The compound lacks efficacy at the α1 subtype, which is believed to mediate these effects. This study investigated the effects of 0.5 and 1.5 mg TPA023 and compared them with placebo and lorazepam 2mg (therapeutic anxiolytic dose). Twelve healthy male volunteers participated in this placebo controlled, double blind, double dummy, four-way, crossover study. Saccadic Eye Movements and Visual Analogue Scales (VAS) were used to assess the sedative properties of TPA023. The effects on postural stability and cognition were assessed using body sway and a standardised battery of neurophysiological memory tests. Lorazepam caused a significant reduction in saccadic peak velocity (SPV), the VAS alertness score and impairment of memory and body sway. TPA023 had significant dose dependent effects on saccadic peak velocity (85 deg/sec maximum reduction at the higher dose) that approximated the effects of lorazepam. In contrast to lorazepam, TPA023 had no detectable effects on saccadic latency or inaccuracy. Also unlike lorazepam, TPA023 did not affect VAS alertness, memory or body sway. These results show that the effect profile of TPA023 differs markedly from that of lorazepam, at doses that were equipotent with regard to effects on saccadic peak velocity. Contrary to lorazepam, TPA023 caused no detectable memory impairment or postural imbalance. These differences reflect the selectivity of TPA023 for different $GABA_A$ receptor subtypes.

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 [1]. Benzodiazepines are the most frequently prescribed pharmacological treatment for GAD [2,3]. Although benzodiazepines are relatively safe drugs and are widely used in the treatment of anxiety, they may produce untoward side effects such as memory impairment, sedation, and muscle relaxation. Particularly in the elderly, these adverse effects are associated with higher incidences of falls [4] and cognitive impairment [5,6].

The anxiolytic effect of benzodiazepines is thought to be mediated by $GABA_A$ α2 receptors [7,8], although more recently more emphasis is given to $GABA_A$ α3 [9-11]. TPA023 is a $GABA_A$ α2,3 subtype-selective partial agonist with higher efficacy at the α2 and α3 subtypes, compared to antagonist efficacy at the α1 and α5 subtype [12,13]. The α1 subtype appears to be involved in the sedative effects [8,14-17]. TPA023 is therefore expected to result in comparable anxiolytic efficacy as clinically used benzodiazepines, with reduced sedation at therapeutically equivalent dosages. Pre-clinical studies in rodents and primates have already shown that TPA023 has anxiolytic effects without showing sedation [12,13]. Based on tolerability findings in healthy volunteers, two doses of TPA023 were selected for this study: 0.5 mg and 1.5 mg. Both doses were within the range expected to be anxiolytic. Lorazepam 2 mg, which is known to be therapeutically relevant [18,19], was chosen for comparison. Benzodiazepines typically impair memory, alertness and postural stability [20-23]. It is expected that therapeutic doses of partial subtype-selective $GABA_A$ agonists will not show these side effects to the same extent. The aims of this study were to identify the side effect profiles of a TPA023-dose that was expected to be anxiolytic, and compare them to those of a therapeutic dose of lorazepam. It was hypothesised that for at least one of the two dose levels of TPA023 administered, the sedating effects of a single oral dose in healthy male subjects would be similar to placebo.

METHODS

Design

This study was a placebo controlled, randomised, double blind, double-dummy, four-way, crossover, single-centre study in twelve healthy male volunteers. Subjects visited the research unit in the

morning of each study period and stayed at the site until ten hours postdose. The next morning they visited the unit again for the last measurements.

Subjects

Twelve healthy non-smoking volunteers were recruited from the Centre for Human Drug Research database. All volunteers gave written informed consent and were medically screened 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 (juice) and St John's Wort at least 2 weeks prior to study start until completion of the study. The study was approved by the Medical Ethics Review Board of Leiden University medical Centre, and performed according to the principles of the Helsinki Declaration and the International Conference on Harmonisation/Good Clinical Practice (ICH/GCP).

Treatments

Each subject received a single oral dose TPA023 0.5mg, TPA023 1.5mg, lorazepam 2mg and placebo in a randomized order with at least a five-day washout period. Medication was administered with 250 ml of water in a fasted state at approximately 8 to 9 AM. As it was a double-dummy study, subjects always received three tablets of TPA023 or matching placebo and two capsules of lorazepam or matching placebo. The treatment sequences were determined using 4x4 Latin Squares, balanced for first 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 (5ml) were taken during each study period within 30 minutes predose 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 TPA023 and lorazepam concentrations.

Plasma was separated from heparinized blood samples by centrifugation (2000 gs, 10 min, 4°C) to 4.5 cc Nunc cryotubes and stored at -20°C within 30 minutes after sampling. TPA023 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. Lorazepam and its stable-isotoped labeled 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 performed in a quiet room with ambient illumination with only one 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) [24]. Average values of latency (= reaction time), peak saccadic velocity and inaccuracy (difference between stimulus angle and corresponding saccade in %) were calculated for all arte-fact-free saccades. Saccadic peak velocity has been validated as the most sensitive measure for the sedative effects of benzodiazepines [25-28].

Visual Analogue Scale

Visual analogue scales as originally described by Norris [29] were previously used to quantify subjective effects of benzodiazepines [27]. From the set of sixteen scales three composite factors were derived as described by Bond and Lader [30], corresponding to alertness, mood

and calmness. A higher score on these scales indicates a negative effect (sedation, excitation and decrease in mood respectively). These factors were used to quantify subjective drug effects.

Body Sway

Body sway was measured with an apparatus similar to the Wright ataxia meter [31], 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.

Cognitive function tests

Memory testing was performed using the validated FePsy program (The Iron Psyche), an automated system containing a battery of computerised tests for cognitive (neuropsychological) functions [32,33]. Word and picture recognition and recall tests were performed to assess reaction time and number of correct and incorrect answers. The Corsi block tapping test, constructed according the principles of the original Corsi block tapping task [34], assessed the nonverbal memory span.

ANALYSIS

Pharmacokinetics

The pharmacokinetics of TPA023 were investigated using non-linear mixed effect modelling 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 [35]. Ultimately, a two-compartment model with first-order absorption and a lag-time was used to describe the pharmacokinetics of TPA023. Intra-individual error was modelled using a constant coefficient of variation error model. No pharmacokinetic parameters were calculated for lorazepam.

Pharmacokinetic/pharmacodynamic relationships

The observed pharmacodynamic effects were plotted against the predicted TPA023 concentrations for each individual. Because the average placebo profile for saccadic peak velocity showed a small diurnal decrease, 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 TPA023 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 - TPA023 0.5mg, placebo - TPA023 1.5mg, lorazepam 2mg - TPA023 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. The current study had a >0.99 a-priori probability (α=0.05, two-tailed, MSE=331), in a sample size of 12 subjects, to detect a larger than 45 deg/sec difference in average saccadic peak velocity between the treatments and placebo. A previous study showed that this difference corresponds to the average change after one night of sleep deprivation [25]. There was a 0.80 a-priori probability to detect a 21 deg/sec mean difference between the treatments. 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. Two other healthy male subjects, using the same randomisation sequence, replaced these two subjects. Twelve subjects therefore completed the study. Subjects were on average 25 years of age (range 20-29 yrs), average weight of 82 kg (range 75-90 kg) and average height of 184 cm (range 178-192 cm).

Clinical observations

No serious adverse reactions occurred following any of the treatments. The most frequently reported adverse event after administration of lorazepam, the high and low doses of TPA023 and placebo were sedation (including drowsiness) by eight, five, three and two subjects respectively. Other reported adverse events were, dizziness after TPA023 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).

Pharmacokinetics

The average plasma concentration-time curves for both doses of TPA023 and lorazepam are shown in figure 1. Both doses of TPA023 and lorazepam showed maximum concentrations after approximately 2 hours. The average pharmacokinetic model based parameters (with inter-individual variation coefficients (CV) of TPA023 were: apparent clearance (clearance divided by bioavailability) of 246 mL/min (CV 29%), initial half-life of 142 min (CV 6%), terminal half-life of 437min (CV 0%, fixed), apparent central volume of distribution (volume divided by 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 for benzodiazepines relates to sedative and anxiolytic properties [28], demonstrated significant effects with lorazepam and both doses of TPA023 (figure 2 and table 1). There was a dose-dependent increase of SPV with TPA023 0.5 and 1.5 mg (AUEC0-6hr decrease of 22 deg/sec and 45 deg/sec). No changes were observed in saccadic latency and saccadic inaccuracy for either doses of TPA023, in contrast to the significant increases with lorazepam. The high dose of TPA023 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 AUEC0-6hr (table 1).

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 lower dose of TPA023 did not show any effects on any of the subscales. The average curve for the high dose of TPA023 was in between the average curves of lorazepam and placebo (figure 3), and consequently, the AUC 0-6hr of the high dose of TPA023 did not differ significantly from either lorazepam or placebo. Subjective calmness was reduced after the high dose of TPA023, while none of the other treatments showed any effect. No significant effects were observed for the VAS contentedness subscale.

Body Sway

No postural instability was observed after either dose of TPA023 compared to placebo (figure 4). Lorazepam, however, caused a profound and highly significant increase in body sway (table 1).

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 TPA023 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 TPA023. No treatment effects were observed on the Corsi block tapping task.

Pharmacokinetic/pharmacodynamic relationships (PK/PD)

Concentration-effect-relationships were only determined for statistically significant pharmacodynamic effects of TPA023 (ie only for SPV). The average PK/PD relationship between the changes in SPV from baseline and the predicted concentration for both doses

of TPA023 is represented in figure 6. A linear concentration-effect model was estimated without an effect compartment for both doses of TPA023. Both slope and intercept for SPV did not differ significantly between the two doses of TPA023. 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.

DISCUSSION

The current placebo-controlled study in healthy male volunteers investigated the effects of two doses of TPA023, a $GABA_A$ α2,3 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), and impairments of memory and postural stability. These effects are typical for benzodiazepines, and are often used as indicators for the drugs' effects [26,27]. TPA023 caused dose dependent SPV-effects of a similar magnitude as lorazepam, but TPA023 had no detectable effects on VAS alertness score, memory or postural stability. A comparison between the two drugs is dependent on the relative efficacies of the used therapeutic equipotency. This cannot be proven at this stage, because the clinical effects of TPA023 have not yet been determined in patients with anxiety. However, lorazepam 2 mg and the highest dose of TPA023 caused similar reductions in SPV, and in this respect the two treatments were equipotent. At these SPV-equipotent doses, effects on VAS alertness, body sway and cognitive function differed markedly between both drugs. These differences may have implications for the pharmacological activities of the two drugs, and their therapeutic effect profiles.

The question arises, how the effect selectivity of TPA023 was observed in this study, relates to the preclinical binding profile to the different α subunit subtypes [8,16,36]. In pre-clinical experiments, TPA023 is a $GABA_A$ partial α2,3 agonist and an antagonist at the α1and α5 subtype. The α1 subunit is believed to primarily mediate the sedative properties and as a consequence, to contribute to memory impairment caused by non-selective $GABA_A$ agonists. Alpha-2 and more recently also α3 activity is held responsible for the anxiolytic effects [7-11]. Preclinical evidence also suggests that the α2, α3 and α5 subunits mediate myorelaxation and motor impairment. If both the anxiolytic and motor effects of TPA023 are attributed to α2 efficacy, the compound shows a surprising lack of motor impairment in healthy volunteers. There are several explanations. First, the preclinical binding profile to the different α-subunit subtypes is characterized by maximal activity, not by measures of sensitivity. The maximal effects of TPA023 were not determined in the current study. Thus, different subtypes may show differences in sensitivity, and motor impairment may become more apparent at higher TPA023-doses that were not evaluated in this study. The preclinical binding profile would predict that even high TPA023-concentrations would still cause less body sway than a full agonist. Alternatively, receptor subtype selectivity may show different patterns in humans than in preclinical models. In this case, different studies with a variety of subtype-selective $GABA_A$ agonists would be needed to define distinct effect profiles that are predictive for the different desired and undesired effects of this new drug class. Finally, the results of this study may be chance findings. However, this is unlikely, because effect profiles as different as for TPA023 are not found among full-agonist benzodiazepines.

Although direct comparative studies are rare, non-selective benzodiazepines, like diazepam [37,38], zopiclone [39], flurazepam [39], lormetazepam [39], triazolam [39], temazepam [40] and lorazepam [19,40,41], usually show comparable effects on memory, alertness and postural stability. Other GABAergic anxiolytic agents, that are non-selective partial agonists at all $GABA_A$ receptor subtypes, also show less differentiating effects than TPA023 [42,43]. Bretazenil, which is less potent on all α-subtypes compared to a full agonist like diazepam [44], showed little evidence of a dissociation between sedative effects and effects on VAS alertness and saccadic eye movements at a dose of 0.5 mg [38]. 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 [45], but the effects were still significantly larger than after placebo. Abecarnil, another non-selective partial agonist, also did not show significant effects compared to placebo [46,47]. However, it is unknown whether these doses were equipotent, an important requisite for comparison of partial agonism and subtype selectivity. True subtype-selective agonists are novel agents and mostly still experimental. For compounds like L-838417 [15], NGD 91-2, NGD 91-3 [43], quinolone 'compound 4' [42,48] and SL-651498 [14,49], no clinical data are available. Only for SL-651498 it was reported that different Phase IIa/b trials for GAD and muscle spasms were conducted with this compound [43], but results have not been provided. Comparative studies with full agonists have not been published. Thus, experience suggest that non-selective $GABA_A$ and benzodiazepine agonists cause a general depression of alertness, memory and motor stability, although the overall level of these reductions is dose-dependent, and probably different between full and partial agonists.

Many biomarkers of 'alertness' are used in healthy volunteer studies, and although there are differences in sensitivity, these markers usually show comparable effects of different sedative drugs or circumstances [25-28]. Previous studies have shown that a decrease in SPV is a highly sensitive indicator of sedation, not only caused by benzodiazepines [26,27] but also by sleep deprivation [25] or compounds that are not particularly anxiolytic, like H1-antagonists [50], α2-agonists [51,52], and anticholinergic agents [53]. All these drugs and circumstances cause reductions in VAS-alertness, saccadic peak velocity, latency and accuracy. In this respect, subjective alertness scores and saccadic eye movements can be considered as largely overlapping ven-diagrams, which both also show a considerable overlap with anxiolysis. Contrary to other compounds that fall into two or three of these categories, TPA023-effects seem to be restricted to SPV alone. The differences compared to lorazepam were quite apparent and could not be attributed to differences in test-sensitivity or statistical type II-errors. We have not been able to find other compounds that cause SPV-decreases without VAS- reductions or vice versa. This separation thus seems to be unique for TPA023. It is tempting to assign these divergent effects to the subtype-selectivity of TPA023, although the exact nature of the relationships between the pharmacological and functional effect profiles cannot be established from this study. A recent literature review showed clear relationships between anxiolytic doses of benzodiazepines and their SPV-effects [28]. For full benzodiazepines, anxiolytic effects are inseparable from the sedative effects. SPV-reduction is usually (although not always statistically significantly) accompanied by effects on latency and accuracy. But for a subtype-selective GABA agonist, SPV reduction without an effect on latency or any subjective indication for sedation could signify anxiolysis without impairment of alertness. If SPV-reduction is predictive of anxiolysis, TPA023 1.5 mg could be equally anxiolytic as lorazepam, but considerably less sedative. Clearly, this remains to be established in clinical trials.

TPA023 did not cause any effect on memory which was expected since TPA023 has antagonistic effects at the α5-subunit that is believed to be involved in memory and cognition [54,55]. Lorazepam is known to affect memory [19,56], which was also confirmed in this study. Based on lack of effects on memory testing and body sway, TPA023 could also have fewer effects on cognition and postural stability, perhaps leading to a decreased chance of memory impairment or falls.

In conclusion, this study showed a clear differentiation in pharmacodynamic effects for the selective $GABA_A$ agonist TPA023, which was not found for the non-selective benzodiazepine lorazepam. This differentiation seems to reflect the TPA023's selectivity for different $GABA_A$ receptor subtypes, although preclinical pharmacological profiles could not be immediately translated into predictions of clinical effects. TPA023 1.5 mg and lorazepam 2 mg showed equipotent reductions of saccadic peak velocity, which could point to comparable anxiolytic efficacy. Contrary to lorazepam, TPA023 did not have any effect on subjective alertness, memory or postural stability. It remains to be established whether the selectivity of TPA023 is reflected into an improved therapeutic window.

Table 1 Effects on Saccadic Eye Movements, Visual Analogue Scales and Body Sway

Variable	Overall treatment effect (p-value)	Placebo - TPA023 0.5mg	Placebo - TPA023 1.5mg	Lorazepam 2mg - TPA023 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

Treatment differences in pharmacodynamic measurements in AUC 0-6hr relative to baseline; ANOVA results are shown as contrasts (95% CI) and p-value

Figure 1 Average drug concentration profiles (mean + SD) of TPA023 0.5mg (squares), TPA023 1.5mg (circles) and lorazepam 2mg (triangles) after oral administration.

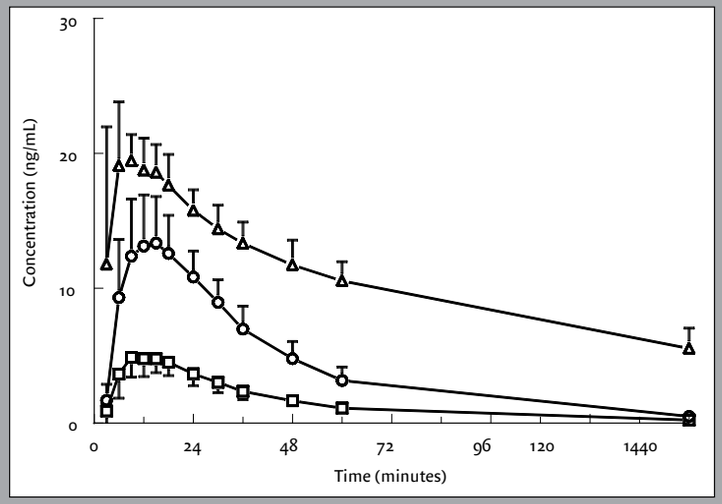


Figure 2 Average time profile (mean + SD) of Saccadic Peak Velocity (change from baseline) after oral administration of placebo (closed circles), TPA023 0.5mg (squares), TPA023 1.5mg (open circles) and lorazepam 2mg (triangles).

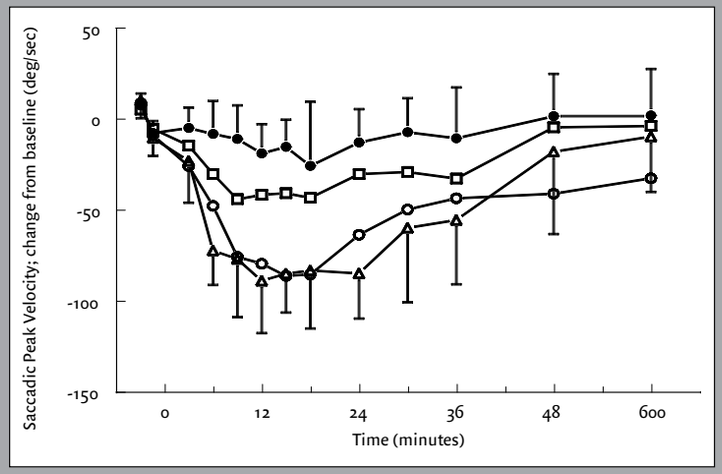


Figure 3 Average time profile (mean + SD) of VAS Alertness (change from baseline) after oral administration of placebo (closed circles), TPA023 0.5mg (squares), TPA023 1.5mg (open circles) and lorazepam 2mg (triangles).

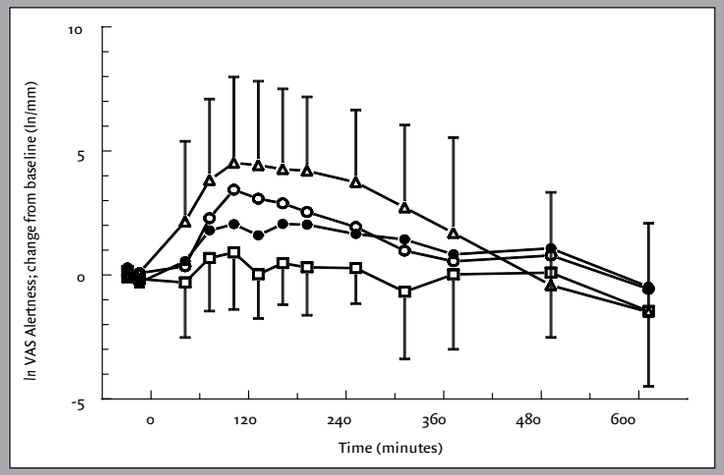


Figure 4 Average time profile (mean + SD) of LOG Body Sway Eyes Closed (change from baseline) after oral administration of placebo (closed circles), TPA023 0.5mg (squares), TPA023 1.5mg (open circles) and lorazepam 2mg (triangles).

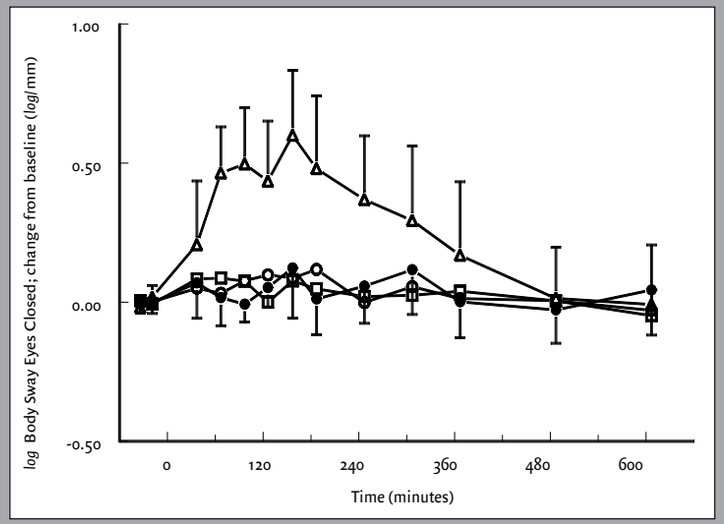
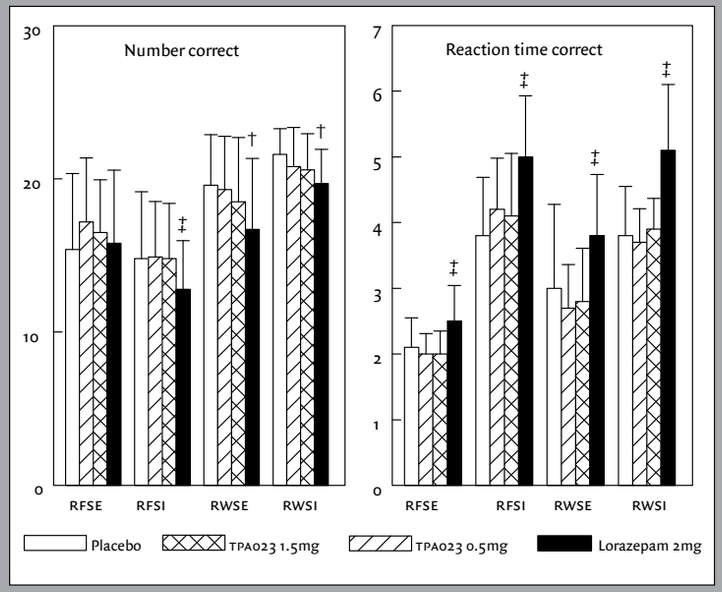


Figure 5 Effects on cognitive function tests (mean + SD).



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 TPA023 1.5mg.

REFERENCES

1 Gliatto MF. Generalized anxiety disorder. Am Fam Physician 2000; 62: 1591-600, 1602.
2 Clark RE, Xie H, Brunette MF. Benzodiazepine prescription practices and substance abuse in persons with severe mental illness. J Clin Psychiatry 2004; 65: 151-155.
3 Brunette MF, Noordsy DL, Xie H, Drake RE. Benzodiazepine use and abuse among patients with severe mental illness and co-occurring substance use disorders. Psychiatr Serv 2003; 54: 1395-1401.
4 Ray WA, Thapa PB, Gideon P. Benzodiazepines and the risk of falls in nursing home residents. J Am Geriatr Soc 2000; 48: 682-685.
5 Madhusoodanan S, Bogunovic OJ. Safety of benzodiazepines in the geriatric population. Expert Opin Drug Saf 2004; 3: 485-493.
6 Paterniti S, Dufouil C, Alperovitch A. Long-term benzodiazepine use and cognitive decline in the elderly: the Epidemiology of Vascular Aging Study. J Clin Psychopharmacol 2002; 22: 285-293.
7 Low K, Crestani F, Keist R, Benke D, Brunig I, Benson JA, Fritschy JM, Rulicke T, Bluethmann H, Mohler H, Rudolph U. Molecular and neuronal substrate for the selective attenuation of anxiety. Science 2000; 290: 131-134.
8 Rudolph U, Crestani F, Mohler H. GABA(A) receptor subtypes: dissecting their pharmacological functions. Trends Pharmacol Sci 2001; 22: 188-194.
9 Atack JR, Hutson PH, Collinson N, Marshall G, Bentley G, Moyes C, Cook SM, Collins I, Wafford K, McKernan RM, Dawson GR. Anxiogenic properties of an inverse agonist selective for alpha3 subunit-containing GABA A receptors. Br J Pharmacol 2005; 144: 357-366.
10 Atack JR. The benzodiazepine binding site of GABA(A) receptors as a target for the development of novel anxiolytics. Expert Opin Investig Drugs 2005; 14: 601-618.
11 Dias R, Sheppard WF, Fradley RL, Garrett EM, Stanley JL, Tye SJ, Goodacre S, Lincoln RJ, Cook SM, Conley R, Hallett D, Humphries AC, Thompson SA, Wafford KA, Street LJ, Castro JL, Whiting PJ, Rosahl TW, Atack JR, McKernan RM, Dawson GR, Reynolds DS. Evidence for a significant role of alpha3-containing $GABA_A$ receptors in mediating the anxiolytic effects of benzodiazepines. J Neurosci 2005; 25: 10682-10688.
12 Atack JR, Wafford KA, Tye SJ, Cook SM, Sohal B, Pike A, Sur C, Melillo D, Bristow L, Bromidge F, Ragan I, Kerby J, Street L, Carling R, Castro JL, Whiting P, Dawson GR, McKernan RM. TPA023 [7-(1,1-Dimethylethyl)-6-(2-ethyl-2H-1,2,4-triazol-3-ylmethoxy)-3-(2-fluor ophenyl)-1,2,4-triazolo[4,3-b]pyridazine], an Agonist Selective for {alpha}2- and {alpha}3-Containing $GABA_A$ Receptors, Is a Nonsedating Anxiolytic in Rodents and Primates. J Pharmacol Exp Ther 2006; 316: 410-422.
13 Carling RW, Madin A, Guiblin A, Russell MG, Moore KW, Mitchinson A, Sohal B, Pike A, Cook SM, Ragan IC, McKernan RM, Quirk K, Ferris P, Marshall G, Thompson SA, Wafford KA, Dawson GR, Atack JR, Harrison T, Castro JL, Street LJ. 7-(1,1-Dimethylethyl)-6-(2-ethyl-2H-1,2,4-triazol-3-ylmethoxy)-3-(2-fluoro phenyl)-1,2,4-triazolo[4,3-b]pyridazine: a functionally selective gamma-aminobutyric acid(A) (GABA(A)) alpha2/alpha3-subtype selective agonist that exhibits potent anxiolytic activity but is not sedating in animal models. J Med Chem 2005; 48: 7089-7092.
14 Griebel G, Perrault G, Simiand J, Cohen C, Granger P, Decobert M, Francon D, Avenet P, Depoortere H, Tan S, Oblin A, Schoemaker H, Evanno Y, Sevrin M, George P, Scatton B. SL651498: an anxioselective compound with functional selectivity for alpha2- and alpha3-containing gamma-aminobutyric acid(A) (GABA(A)) receptors. J Pharmacol Exp Ther 2001; 298: 753-768.
15 McKernan RM, Rosahl TW, Reynolds DS, Sur C, Wafford KA, Atack JR, Farrar S, Myers J, Cook G, Ferris P, Garrett L, Bristow L, Marshall G, Macaulay A, Brown N, Howell O, Moore KW, Carling RW, Street LJ, Castro JL, Ragan CI, Dawson GR, Whiting PJ. Sedative but not anxiolytic properties of benzodiazepines are mediated by the GABA(A) receptor alpha1 subtype. Nat Neurosci 2000; 3: 587-592.
16 Rudolph U, Crestani F, Benke D, Brunig I, Benson JA, Fritschy JM, Martin JR, Bluethmann H, Mohler H. Benzodiazepine actions mediated by specific gamma-aminobutyric acid(A) receptor subtypes. Nature 1999; 401: 796-800.
17 Tobler I, Kopp C, Deboer T, Rudolph U. Diazepam-induced changes in sleep: role of the alpha 1 GABA(A) receptor subtype. Proc Natl Acad Sci USA 2001; 98: 6464-6469.
18 Micallef J, Soubrouillard C, Guet F, Le Guern ME, Alquier C, Bruguerolle B, Blin O. A double blind parallel group placebo controlled comparison of sedative and amnesic effects of ETifoxine and lorazepam in healthy subjects. Fundam Clin Pharmacol 2001; 15: 209-216.
19 Green JF, McElholm A, King DJ. A comparison of the sedative and amnestic effects of chlorpromazine and lorazepam. Psychopharmacology (Berl) 1996; 128: 67-73.
20 Sigel E, Buhr A. The benzodiazepine binding site of $GABA_A$ receptors. Trends Pharmacol Sci 1997; 18: 425-429.
21 Smith GB, Olsen RW. Functional domains of $GABA_A$ receptors. Trends Pharmacol Sci 1995; 16: 162-168.
22 Ihmsen H, Albrecht S, Hering W, Schuttler J, Schwilden H. Modelling acute tolerance to the EEG effect of two benzodiazepines. Br J Clin Pharmacol 2004; 57: 153-161.
23 Carling RW, Moore KW, Street LJ, Wild D, Isted C, Leeson PD, Thomas S, O'Connor D, McKernan RM, Quirk K, Cook SM, Atack JR, Wafford KA, Thompson SA, Dawson GR, Ferris P, Castro JL. 3-phenyl-6-(2-pyridyl)methyloxy-1,2,4-triazolo[3,4-a]phthalazines and analogues: high-affinity gamma-aminobutyric acid-A benzodiazepine receptor ligands with alpha 2, alpha 3, and alpha 5-subtype binding selectivity over alpha 1. J Med Chem 2004; 47: 1807-1822.
24 van Steveninck AL, Cohen AF, Ward T. A microcomputer based system for recording and analysis of smooth pursuit and saccadic eye movements. Br J Clin Pharmacol 1989; 27: 712P-713P.
25 van Steveninck AL, van Berckel BN, Schoemaker RC, Breimer DD, van Gerven JM, Cohen AF. The sensitivity of pharmacodynamic tests for the central nervous system effects of drugs on the effects of sleep deprivation. J Psychopharmacol 1999; 13: 10-17.
26 van Steveninck AL, Verver S, Schoemaker HC, Pieters MS, Kroon R, Breimer DD, Cohen AF. Effects of temazepam on saccadic eye movements: concentration-effect relationships in individual volunteers. Clin Pharmacol Ther 1992; 52: 402-408.
27 van Steveninck AL, Schoemaker HC, Pieters MS, Kroon R, Breimer DD, Cohen AF. A comparison of the sensitivities of adaptive tracking, eye movement analysis and visual analog lines to the effects of incremental doses of temazepam in healthy volunteers. Clin Pharmacol Ther 1991; 50: 172-180.
28 de Visser SJ, van der Post JP, de Waal PP, Cornet F, Cohen AF, van Gerven JM. Biomarkers for the effects of benzodiazepines in healthy volunteers. Br J Clin Pharmacol 2003; 55: 39-50.
29 Norris H. The action of sedatives on brain stem oculomotor systems in man. Neuropharmacology 1971; 10: 181-191.
30 Bond A, Lader M. The use of analogue scales in rating subjective feelings. Br J Med Psychol 1974; 47: 211-218.
31 Wright BM. A simple mechanical ataxia-meter. J Physiol 1971; 218: 27P-28P.
32 Alpherts WCJ. Computers as a technique for neurphysiological assessment in epilepsy. In: Education and Epilepsy eds Aldenkamp AP, Alpherts WCJ, Meinardi H, Stores G, Lisse/ Berwyn: Swets & Zeitlinger, 1987: 101-109.
33 Aldenkamp A, Vermeulen J, Alpherts WCJ, Overweg J, van Parijs JAP, Verhoeff NPLG. Validity of computerized testing: patient dysfunction and complaints versus measured changes.In: Assessment of Cognitive Function ed W.E.Dodson and M.Kinsbourne, New York: Demos, 1992: 51-68.
34 Nelson RE, Dickson AL, Banos JH. An automated administration of Corsi's Block-tapping Test. Percept Mot Skills 2000; 91: 578-580.
35 Schoemaker RC, Cohen AF. Estimating impossible curves using NONMEM. Br J Clin Pharmacol 1996; 42: 283-290.
36 Mohler H, Crestani F, Rudolph U. GABA(A)-receptor subtypes: a new pharmacology. Curr Opin Pharmacol 2001; 1: 22-25.
37 van Steveninck AL, Gieschke R, Schoemaker HC, Pieters MS, Kroon JM, Breimer DD, Cohen AF. Pharmacodynamic interactions of diazepam and intravenous alcohol at pseudo steady state. Psychopharmacology (Berl) 1993; 110: 471-478.
38 van Steveninck AL, Gieschke R, Schoemaker RC, Roncari G, Tuk B, Pieters MS, Breimer DD, Cohen AF. Pharmacokinetic and pharmacodynamic interactions of bretazenil and diazepam with alcohol. Br J Clin Pharmacol 1996; 41: 565-573.
39 Griffiths AN, Jones DM, Richens A. Zopiclone produces effects on human performance similar to flurazepam, lormetazepam and triazolam. Br J Clin Pharmacol 1986; 21: 647-653.
40 van Steveninck AL, Wallnofer AE, Schoemaker RC, Pieters MS, Danhof M, van Gerven JM, Cohen AF. A study of the effects of long-term use on individual sensitivity to temazepam and lorazepam in a clinical population. Br J Clin Pharmacol 1997; 44: 267-275.
41 Green JF, King DJ, Trimble KM. Antisaccade and smooth pursuit eye movements in healthy subjects receiving sertraline and lorazepam. J Psychopharmacol 2000; 14: 30-36.
42 Basile AS, Lippa AS, Skolnick P. Anxioselective anxiolytics: can less be more? Eur J Pharmacol 2004; 500: 441-451.

43 Atack JR. Anxioselective compounds acting at the GABA(A) receptor benzodiazepine binding site. Curr Drug Targets CNS Neurol Disord 2003; 2: 213-232.
44 Puia G, Ducic I, Vicini S, Costa E. Molecular mechanisms of the partial allosteric modulatory effects of bretazenil at gamma-aminobutyric acid type A receptor. Proc Natl Acad Sci USA 1992; 89: 3620-3624.
45 Dingemanse J, Bury M, Bock J, Joubert P. Comparative pharmacodynamics of Ro 41-3696, a new hypnotic, and zolpidem after night-time administration to healthy subjects. Psychopharmacology (Berl) 1995; 122: 169-174.
46 Pollack MH, Worthington JJ, Manfro GG, Otto MW, Zucker BG. Abecarnil for the treatment of generalized anxiety disorder: a placebo-controlled comparison of two dosage ranges of abecarnil and buspirone. J Clin Psychiatry 1997; 58 Suppl 11: 19-23.
47 Aufdembrinke B. Abecarnil, a new beta-carboline, in the treatment of anxiety disorders. Br J Psychiatry Suppl 1998; 55-63.
48 Johnstone TB, Hogenkamp DJ, Coyne L, Su J, Halliwell RF, Tran MB, Yoshimura RF, Li WY, Wang J, Gee KW. Modifying quinolone antibiotics yields new anxiolytics. Nat Med 2004; 10: 31-32.
49 Griebel G, Perrault G, Simiand J, Cohen C, Granger P, Depoortere H, Francon D, Avenet P, Schoemaker H, Evanno Y, Sevrin M, George P, Scatton B. SL651498, a $GABA_A$ receptor agonist with subtype-selective efficacy, as a potential treatment for generalized anxiety disorder and muscle spasms. CNS Drug Rev 2003; 9: 3-20.
50 Cohen AF, Hamilton MJ, Peck AW. The effects of acrivastine (BW825C), diphenhydramine and terfenadine in combination with alcohol on human CNS performance. Eur J Clin Pharmacol 1987; 32: 279-288.
51 Glue P, White E, Wilson S, Ball DM, Nutt DJ. Pharmacology of saccadic eye movements in man. 2. Effects of the alpha 2-adrenoceptor ligands idazoxan and clonidine. Psychopharmacology (Berl) 1991; 105: 368-373.
52 de Visser SJ, van Gerven JM, Schoemaker RC, Cohen AF. Concentration-effect relationships of two infusion rates of the imidazoline antihypertensive agent rilmenidine for blood pressure and development of side-effects in healthy subjects. Br J Clin Pharmacol 2001; 51: 423-428.
53 Oliva GA, Bucci MP, Fioravanti R. Impairment of saccadic eye movements by scopolamine treatment. Percept Mot Skills 1993; 76: 159-167.
54 Dawson GR, Maubach KA, Collinson N, Cobain M, Everitt BJ, MacLeod AM, Choudhury HI, McDonald LM, Pillai G, Rycroft W, Smith AJ, Sternfeld F, Tattersall FD, Wafford KA, Reynolds DS, Seabrook GR, Atack JR. An inverse agonist selective for alpha5 subunit-containing $GABA_A$ receptors enhances cognition. J Pharmacol Exp Ther 2006; 316: 1335-1345.
55 Collinson N, Kuenzi FM, Jarolimek W, Maubach KA, Cothliff R, Sur C, Smith A, Otu FM, Howell O, Atack JR, McKernan RM, Seabrook GR, Dawson GR, Whiting PJ, Rosahl TW. Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the alpha 5 subunit of the $GABA_A$ receptor. J Neurosci 2002; 22: 5572-5580.
56 Soo-Ampon S, Wongwitdecha N, Plasen S, Hindmarch I, Boyle J. Effects of word frequency on recall memory following lorazepam, alcohol, and lorazepam alcohol interaction in healthy volunteers. Psychopharmacology (Berl) 2004; 176: 420-425.

CHAPTER 3

Pharmacodynamic and pharmacokinetic effects of MK-0343, a $GABA_A$ α2,3 subtype selective agonist, compared to lorazepam and placebo in healthy male volunteers

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ABSTRACT

The use of nonselective GABA enhancers, such as benzodiazepines in the treatment of anxiety disorders is still widespread but hampered by unfavourable side-effect s. Some of these may be associated with binding properties to certain subtypes of the $GABA_A$ receptor that are unnecessary for therapeutic effects. MK-0343 was designed to be a less sedating anxiolytic, based on reduced efficacy at the $\alpha 1$ subtype and significant efficacy at $\alpha 2$ and $\alpha 3$ subtypes of the $GABA_A$ receptor.

This study was a double-blind, 4-way cross-over (n=12) study to investigate the effects of MK-0343 (0.25 and 0.75 mg) in comparison to placebo and an anxiolytic dose (2mg) of the non-selective agonist lorazepam. Effects were measured by eye movements, body sway, Visual Analogue Scales and memory tests.

Lorazepam impaired Saccadic Peak Velocity (SPV), VAS alertness scores, postural stability and memory and increased saccadic latency and inaccuracy. MK-0343 0.75 mg was equipotent with lorazepam as indicated by SPV (-42.4 deg/sec), saccadic latency (0.02 sec) and VAS alertness scores (1.50 ln mm), while effects on memory and postural stability were smaller. MK-0343 0.25mg only affected postural stability to a similar extent as MK-0343 0.75mg.

The effect profile of MK-0343 0.75 mg is different from the full agonist lorazepam, which could reflect the selective actions of this compound. Although less effect on VAS alertness was expected, diminished effects on memory and postural stability were present. Clinical studies in anxiety patients should show whether this dose of MK-0343 is therapeutically effective with a different side-effect profile.

INTRODUCTION

Benzodiazepines are effective and widely used for the treatment of panic disorder and generalised anxiety disorder. Despite the effectiveness of these drugs, the clinical usefulness is limited due to side effects like amnesia, ataxia, sedation and impaired concentration and memory [1,2]. Tolerance and abuse are additional problems after long-term use [3]. This side-effect profile is caused by the non-selective properties of benzodiazepines for the different α-subtypes of the $GABA_A$ receptor [4]. The non-selective full agonist benzodiazepines led to the development of non-selective partial agonists with a lower maximum effect for some of the adverse properties. Although the preclinical profile improved, a translation to non-sedating anxiolytics failed [5-7]. Subsequently, rodent studies revealed the involvement of the different $GABA_A$ subtypes [4,8-12]. Knock-in and knock-out mice experiments clarified the association between the $GABA_A$ subtypes and their pharmacological response. This knowledge has stimulated the search for ligands for different $GABA_A$-receptor subtypes, with a purported higher therapeutic selectivity and an improved side effect profile.

Pre-clinical studies indicated that the $\alpha 2,3$ subunit of the $GABA_A$ receptor is responsible for anxiolysis and muscle relaxation [11,13-15], while the $\alpha 1$ subunit is involved in sedation [8,10,12,16]. MK-0343 is a compound with a low efficacy at the $\alpha 1$ and $\alpha 5$ subunit (both 18% relative to chlordiazepoxide) and a high efficacy at the $\alpha 2,3$ subunit of the $GABA_A$ receptor (23 and 45% respectively), as assessed by wholecell patch clamp recordings with human recombinant $GABA_A$ receptors (MSD, data on file). Based on its functional selectivity and the results in several animal models it was thought to have anxiolytic efficacy while being less sedating compared to benzodiazepines. The maximum tolerated dose (MTD) of MK-0343 in healthy volunteers was 1.0 mg, as moderate and severe drowsiness was reported by two of the six subjects after a single dose of 2 mg (MSD, data on file). The doses chosen for this current study of 0.25 and 0.75 mg, represented 1/4 and 3/4 of the MTD. A full pharmacodynamic evaluation of the higher dose of MK-0343 (0.75 mg) would provide an assessment of the greatest sedative, cognitive, and motor effects expected with this drug in subsequent clinical development. The lower dose of MK-0343 (~0.25 mg MTD) was also tested in order to establish the pharmacodynamic effects expected at a dose that, while substantially lower than the high dose, might still demonstrate anxiolytic efficacy. Pharmacodynamic (PD) measurements included eye movements, body sway measurements, Visual Analogue Scales and memory testing, which have all been shown to be highly sensitive to the effects of non-selective benzodiazepines [5,17-19]. MK-0343 was compared

to lorazepam 2 mg, which is known to be therapeutically relevant, mildly sedative [20,21] and known to affect these PD measurements [19]. The more selective character of MK-0343 was expected to lead to diminished subjective sedation, reduced postural instability and less memory impairment, compared to a benzodiazepine. In contrast, MK-0343 was expected to cause SPV reduction. This parameter has been shown to be associated with the anxiolytic effects of benzodiazepines [22], and a previous study with the selective $GABA_A$ $\alpha 2,3$ partial agonist TPA023 showed effects only on SPV. Similar results could therefore be expected in the current study.

METHODS

Design

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

Subjects

Twelve healthy non-smoking volunteers were recruited from the database of the Centre for Human drug Research (CHDR) and gave written informed consent before medical screening. 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 (juice) and St John's Wort at least 2 weeks prior to study start until completion of the study. The study was approved by the Medical Ethics Review Board of Leiden University medical Centre, and performed according to their standards.

Treatments

Each subject received a single oral dose MK-0343 0.25mg, MK-0343 0.75mg, lorazepam 2mg or placebo, administered with 250 ml of water in a fasted state at approximately 9 to 10 AM on each treatment day. To maintain blinding, subjects always received 3 tablets of MK-0343 or matching placebo plus 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), showing an average value for two sequential (duplicate) measurements at each time point. All safety measurements were made after sitting in a semi-recumbent position for at least 5 minutes.

Drug analyses

Blood samples (5ml) were drawn on each occasion day within 30 minutes predose 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 MK-0343 concentrations. Lorazepam concentrations were not determined. Plasma was separated from heparinized blood samples by centrifugation (2000 gs, 10 min, 4°C) to 3.6 cc Nunc cryotubes and stored at -20°C within 30 minutes after sampling. MK-0343 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.

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. Subjects underwent pharmacodynamic tests individually in a quiet room with ambient illumination. Each session consisted of the following sequence of tests: saccadic eye movements; body sway eyes open/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 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) [23]. 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 [17,18]. Saccadic peak velocity has also been shown to be closely related to the anxiolytic properties of benzodiazepines [22].

Visual Analogue Scale

Visual analogue scales as originally described by Norris [24] were previously used to quantify subjective effects of benzodiazepines [17]. From the set of sixteen scales three composite factors were derived as described by Bond and Lader [25], corresponding to alertness, mood and calmness. These factors were used to quantify subjective drug effects.

Body Sway

Body sway was measured with an apparatus similar to the Wright ataxiameter [26], 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. Body sway is a measure of postural stability that has previously been shown to be sensitive to benzodiazepines [5].

Cognitive function tests

Memory testing was performed between one to three hours postdose, using the validated FePsy program (The Iron Psyche), an automated system containing a battery of computerised tests for cognitive (neuro-psychological) functions [27,28]. Word and picture recognition and recall tests were performed after presentation of words and pictures serially and simultaneously. Reaction time and number of correct and incorrect answers were assessed. The Corsi block tapping test, constructed according to the principles of the original Corsi block tapping task [29], assessed the nonverbal memory span. Memory tests have been shown to be affected by benzodiazepines [19,30].

ANALYSIS

Pharmacokinetics

Pharmacokinetics of MK-0343 were determined using an one-compartment model with first order absorption and a lag-time. Parameters determined were absorption half-life, absorption lag-time, apparent clearance (clearance divided by bioavailability) and elimination half-life. A constant coefficient of variation error model was used. Estimation was performed using NONMEM software (NONMEM Version V, GloboMax LLC, Hanover, MD, USA) providing NONMEM population estimates using the first-order conditional estimation method with interaction.

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 two 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 changes 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 (using a p-value cut-off of 5%), the analysis was rerun without the carry-over factor. The four treatments were compared within the ANOVA model using the following contrasts: placebo - MK-0343 0.25mg, placebo - MK-0343 0.75mg, lorazepam 2mg - MK-0343 0.75mg 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 V8.2 (SAS Institute, Inc., Cary, NC, USA).

RESULTS

Subjects

Twelve male subjects underwent medical screening after giving written informed consent and completed the study. Subjects had a mean age of 25 years (range 18-30), weight of 78 kg (range 55-98 kg) and height of 183 cm (range 178-192 cm).

Clinical observations

No serious adverse reactions occurred following any treatment. The most frequently reported adverse event (AE) was fatigue after administration of lorazepam (seven subjects), and the high and low doses of MK-0343 (five and three subjects, respectively). Other reported adverse events were sleepiness after MK-0343 0.25mg and 0.75 mg administration (four subjects in each group) and lorazepam (two subjects), drowsiness after MK-0343 0.25 mg and 0.75mg administration (four and three subjects, respectively) and lorazepam (six subjects) and headache after MK-0343 0.25mg and 0.75 mg (2 subjects in each group) and lorazepam (three subjects). In the placebo group fatigue and sleepiness were each reported by one subject. All AEs were of mild intensity, except for 3 severe sedative-related AEs in the lorazepam group. The AEs in the lorazepam group were, on average, of longer duration than those in the MK-0343 groups.

Pharmacokinetics

The average plasma concentration-time curves for both doses of MK-0343 are shown in figure 1. Both doses of MK-0343 showed maximum concentrations after approximately 1 hour. The C_{MAX} (mean (SD)) was 9.23 (1.58) ng/mL for the higher dose and 3.25 (0.57) for the lower. NONMEM population PK parameters obtained (mean (coefficient of inter-individual variability)) were an absorption half-life of 3.09 min (82%), an absorption lag time of 24.8 min (0.4%), a Clearance/F of 0.391 L/min (22%), an elimination half-life of 126 min (15%), and a residual variability of 7.0%.

Pharmacodynamics

Saccadic Eye Movements

Saccadic Peak Velocity (SPV) was decreased by MK-0343 0.75 mg and lorazepam 2 mg (42.4 deg/sec and 51.6 deg/sec respectively) (table 1, figure 2). These treatments also increased saccadic latency, while only lorazepam affected saccadic inaccuracy (table 1).

Visual Analogue Scales

Lorazepam caused the largest effects on VAS alertness (figure 3), while the effects of the higher dose of MK-0343 were somewhat smaller but also different from placebo (table 1). No changes occurred after the low dose of MK-0343 compared to placebo (table 1). None of the treatments did affect the VAS contentedness and calmness scale.

Body Sway

Both doses of MK-0343 increased body sway (eyes closed) to a similar extent (0.26 logmm; 95%CI: 0.08/0.44 and 0.22; 95%CI: 0.05/0.40) (figure 4). The effects differed from placebo and from the larger effects of lorazepam (table 1). Body sway measurements with open eyes were only affected by lorazepam intake (-0.51 logmm; 95%CI: -0.673/-0.348).

Cognitive Function Tests and Corsi Block Tapping Task

No treatment effects were observed on the Corsi block tapping test for both doses of MK-0343 (0.75 mg: 0.33; 95%CI: -0.50 / 1.17) or lorazepam (0.42; 95%CI: -0.42 / 1.25). For the recognition tests, only lorazepam decreased the number of correct words in the test with simultaneous words compared to placebo and MK-0343 0.75 mg. Lorazepam increased the reaction times of the correct answers in all tests compared to placebo and MK-0343 0.75 mg, while MK-0343 0.75 mg itself only increased the reaction time in one test compared to placebo (Figure 5).

The lower dose of MK-0343 did not show any effect on the cognitive function test, nor the Corsi block tapping task.

DISCUSSION

This study was performed to determine the CNS effects of two doses of a new subtype selective $GABA_A$ agonist, MK-0343, and to compare them to those of placebo and the full agonist lorazepam.

Pre-clinical studies showed that MK-0343 acts as a partial agonist at α2,3 subtypes, with less efficacy at α1 and α5 subtypes (MSD, data on file). The selective efficacy profile of this compound seems to be reflected in the clinical effect profile in this study. As expected from previous studies with benzodiazepines [5,17-19], lorazepam impaired saccadic eye movements, VAS alertness scores, memory and postural stability. The higher dose of MK-0343 caused similar reductions in saccadic peak velocity (SPV) and VAS alertness scores compared to lorazepam, showing rough equipotency for these CNS-effects. In contrast, effects on postural stability and memory were present to a significantly lesser degree with the subtype specific agent than with the full benzodiazepine agonist. Although the therapeutic equipotency is not yet known due to lack of studies with MK-0343 in anxiety patients, both doses of MK-0343 are anxiolytic in pre-clinical studies. The current study suggests that MK-0343 will show less memory impairment and postural instability than lorazepam in patient studies.

Pre-clinical studies indicated that the α2,3 subunit of the $GABA_A$ receptor is responsible for anxiolysis and muscle relaxation [11,13-15], while the α1 subunit is involved in sedation [8,10,12,16]. These findings have stimulated the search for ligands for different $GABA_A$ receptor subtypes, with a higher therapeutic selectivity. Alpha-1 selective agents were developed for the indication of insomnia, while α2,3 selective compounds were developed as anxiolytics.

Pre-clinical studies showed that MK-0343 was anxiolytic in different rodent models and one primate model, similar to the effects of diazepam (MSD, data on file). Sedation models like the mouse rotarod test, the rat sensitivity test and an equivalent monkey test showed a clear separation between the doses required to produce sedation and anxiolysis. However, it is still unclear if the preclinical mechanisms seen in rodents and to a lesser extent in primates can be translated to humans. So far, the non-selective partial agonists bretazenil and abecarnil have failed as they did not have sufficient separation between anxiolytic and sedative effects [31]. In this respect, a partial agonist seems to behave much like a low dose of a full agonist, with a built in limit to its adverse as well as therapeutic efficacy. In theory, subtype selective compounds should not have this disadvantage, although it has proven difficult to translate this into practice. For a few compounds, like L-838417 [32], compound 4 [33] and nsx, only pre-clinical results have been published yet. For TPA023, pre-clinical and human, although not patient, data are available [19,34,35]. Development of other compounds has been stopped due a lack of anxiolytic efficacy [36,37] despite very promising pre-clinical data. Only ocinaplon [38] and ELB139, a $GABA_A$ α3 subtype-selective agonist [39], seem to be most advanced in their clinical development [36]. Unexpectedly, ocinaplon seems anxiolytic and non-sedating in patients despite a relatively α1 subtype selective efficacy profile [38]. This does not seem consistent with the other pre-clinical data and hypotheses that anxiolysis is mediated by $GABA_A$ α2 and α3, and sedation by α1 receptor subtypes [11,13,14]. These inconsistencies could reflect differences in preclinical predictivity of $GABA_A$ subtype-selectivity for the situation in humans. This may explain that such a small number of subtype-selective compounds have so far been shown to be therapeutically relevant. Comparison of adverse and clinical effect profiles, for different subtype-selective and non-selective $GABA_A$ agonists will improve the predictability of preclinical experiments with these compounds.

The differences in effects in the current study compared to lorazepam are very likely to reflect the selective efficacy profile of MK-0343, although they do not necessarily correspond with preclinical predictions. VAS alertness was decreased for both the higher dose of MK-0343 and lorazepam, which suggests that the sedative effects

for both lorazepam 2mg and MK-0343 0.75 mg are similar. This was not expected, based on the low efficacy at the α1 subtype of the compound. The lower dose of MK-0343 did not show significant effects on VAS alertness scores. But for this dose no significant SPV-reductions were present, and this dose was not equipotent to lorazepam for any effect that was measured.

The different results of this study are in some ways comparable to those of a previous study of our research group, in which another α2,3 selective partial $GABA_A$ agonist, TPA023, was studied [19]. However, in that study no effects on VAS alertness, postural stability and memory were present, while similar SPV-reductions were seen compared to lorazepam. The difference in efficacy profiles between the two compounds could be responsible for the differences in pharmacodynamic effects seen in human. Although both compounds share a selectivity for the α2,3 subtype, TPA023 is an antagonist at the α1 and α5 subtype while MK-0343 has shown low but at least some efficacy at these subtypes (relative efficacy 18% for both). It could be that the low efficacy at the α1 subtype is already enough to induce sedative effects, even similar to those of the full-agonist lorazepam, as seen in this study.

This low efficacy of MK-0343 at the α1 subtype could also be responsible for the effects on postural stability, although they were much smaller compared to the effects of lorazepam. This association is supported by the fact that zolpidem, selective for the α1 subtype, also increases body sway even more than diazepam [40] and that benzodiazepine induced ataxia was blocked in monkeys by an α1 $GABA_A$ receptor selective antagonist [41]. This may represent a significant therapeutic advantage of MK-0343, since several studies have shown that benzodiazepines increase body sway [42,43] and cause falls due to postural instability in elderly [44].

MK-0343 did not show any effects on memory. Lorazepam affected all memory reaction times, but only one of the four memory tests (serial word recognition). Consequently, the contrast with MK-0343 was small, and it cannot be fully excluded that the observed lack of memory effects of MK-0343 is due to an insensitivity of the memory tests in this study. However, a previous study with TPA023 showed no memory effects of the partial subtype selective $GABA_A$ agonist, as opposed to significant impairment on all tests with lorazepam [19]. This suggests that subtype-selective partial $GABA_A$ agonists with low α5-efficacy are memory sparing in humans.

The current study has shown that the subtype-selective $GABA_A$ agonist MK-0343 has a different effect profile compared to the benzodiazepine lorazepam. These differences may reflect the subtype-selectivity, although more subjective sedation was observed with the higher dose than would be expected from preclinical predictions.

Although this could translate into an improved safety profile, the clinical meaning of these differences is not yet fully known, because the anxiolytic dose of MK-0343 has not been established. Future studies in patients with anxiety disorders should reveal if anxiety can be suppressed using a dose of MK-0343 with relative few side effects.

Table 1 Differences in pharmacodynamic measurements

Variable	Overall treatment effect (p-value)	Placebo - MK-0343 0.25mg		Placebo - MK-0343 0.75mg		Lorazepam 2mg - MK-0343 0.75mg		Placebo - Lorazepam 2mg	
Saccadic Peak Velocity (deg/sec)	<.0001	11.54 (-4.63 / 27.71)	p = 0.155 F = 2.13	42.37 (26.2 / 58.54)	p < 0.001 F = 28.62	-9.26 (-25.43 / 6.91)	p = 0.251 F = 1.37	51.63 (35.46 / 67.80)	p < 0.001 F = 42.51
Saccadic Latency (sec)	<.0001	0.000 (-0.009 / 0.010)	p = 0.959 F = 0.00	-0.015 (-0.025 / -0.006)	p = 0.003 F = 10.96	0.014 (0.005 / 0.024)	p = 0.005 F = 9.24	-0.029 (-0.039 / -0.020)	p < 0.001 F = 40.32
Saccadic Inaccuracy (%)	0.0518	-1.02 (-3.14 / 1.10)	p = 0.335 F = 0.96	-1.54 (-3.66 / 0.57)	p = 0.147 F = 2.22	1.45 (-0.67 / 3.57)	p =0.173 F = 1.96	-2.99 (-5.11 / -0.87)	p = 0.007 F = 8.29
vas Alertness (ln mm)	0.0039	-0.88 (-1.86 / 0.10)	p = 0.078 F = 3.31	-1.50 (-2.49 /-0.52)	p = 0.004 F = 9.73	0.31 (-0.67 / 1.29)	p = 0.525 F = 0.41	-1.81 (-2.80 /-0.83)	p < 0.001 F = 14.14
vas Contentedness (ln mm)	0.3004	0.45 (-0.03 / 0.93)	p = 0.065 F = 3.65	0.30 (-0.18 / 0.78)	p = 0.208 F = 1.66	0.08 (-0.40 / 0.56)	p = 0.733 F = 0.12	0.22 (-0.26 / 0.70)	p = 0.354 F = 0.88
vas Calmness (ln mm)	0.5211	0.24 (-0.16 / 0.65)	p = 0.230 F = 1.51	0.11 (-0.30 / 0.51)	p = 0.589 F = 0.30	-0.15 (-0.56 / 0.25)	p = 0.446 F = 0.59	0.26 (-0.14 / 0.67)	p = 0.197 F = 1.74
Log Body Sway Eyes Closed (log mm)	0.0001	-0.262 (-0.441 /-0.083)	p = 0.005 F = 9.00	-0.224 (-0.403 /-0.046)	p = 0.016 F = 6.55	0.247 (0.068 / 0.425)	p = 0.008 F = 7.95	-0.471 (-0.650 /-0.292)	p < 0.001 F = 28.94
Log Body Sway Eyes Open (log mm)	<.0001	0.147 (-0.310 / 0.015)	p = 0.074 F = 3.42	-0.152 (-0.315 / 0.010)	p = 0.065 F = 3.69	0.358 (0.196 / 0.521)	p < 0.001 F = 20.25	-0.511 (-0.673 /-0.348)	p < 0.001 F = 41.22

Pharmacodynamic measurements in AUC0-6hr relative to baseline for Saccadic Eye Movements, Visual Analogue Scales and Body Sway; ANOVA results are shown as contrasts (95% CI) p-value and F-value (1, 30 df).

Figure 1 Average drug concentration profiles (mean + SD) of MK-0343 0.25mg (squares), MK-0343 0.75mg (circles) after oral administration.

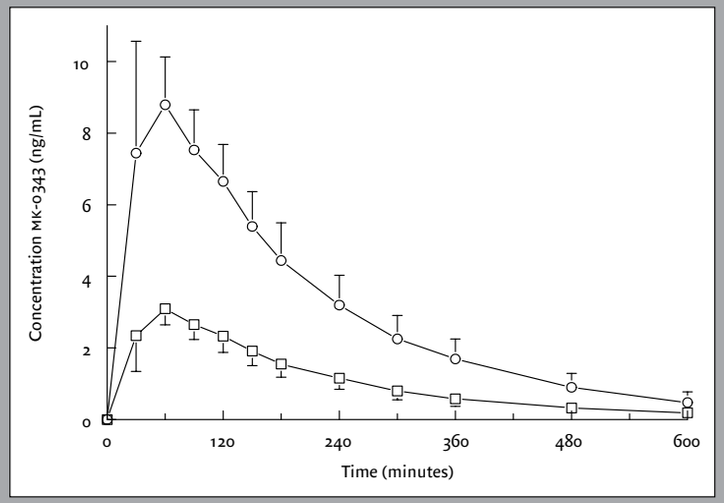


Figure 2 Average time profile (mean + SD) of Saccadic Peak Velocity (change from baseline) after oral administration of placebo (closed circles), MK-0343 0.25mg (squares), MK-0343 0.75mg (open circles) and lorazepam 2mg (triangles).

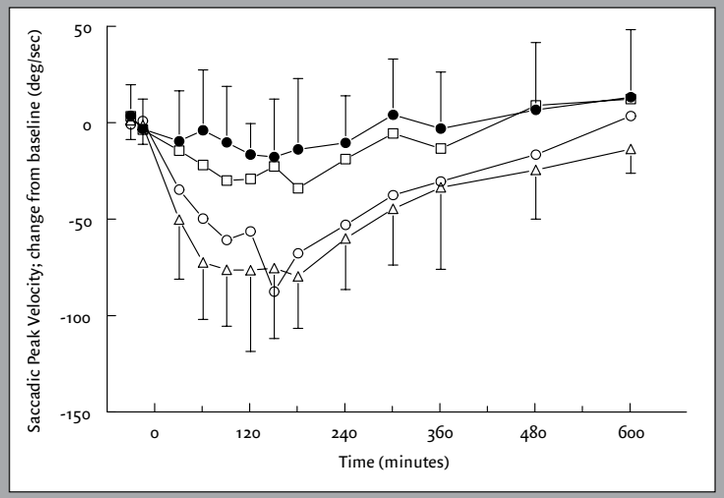


Figure 3 Average time profile (mean + SD) of VAS Alertness (change from baseline) after oral administration of placebo (closed circles), MK-0343 0.25mg (squares), MK-0343 0.75mg (open circles) and lorazepam 2mg (triangles).

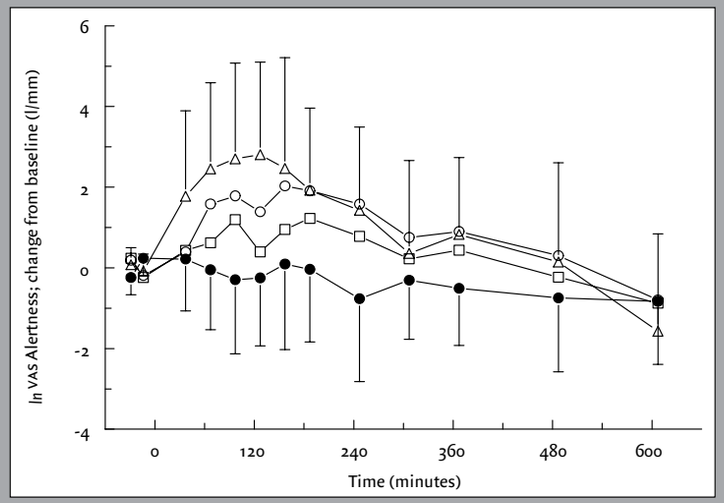


Figure 4 Average time profile (mean + SD) of LOG Body Sway Eyes Closed (change from baseline) after oral administration of placebo (closed circles), MK-0343 0.25mg (squares), MK-0343 0.75mg (open circles) and lorazepam 2mg (triangles).

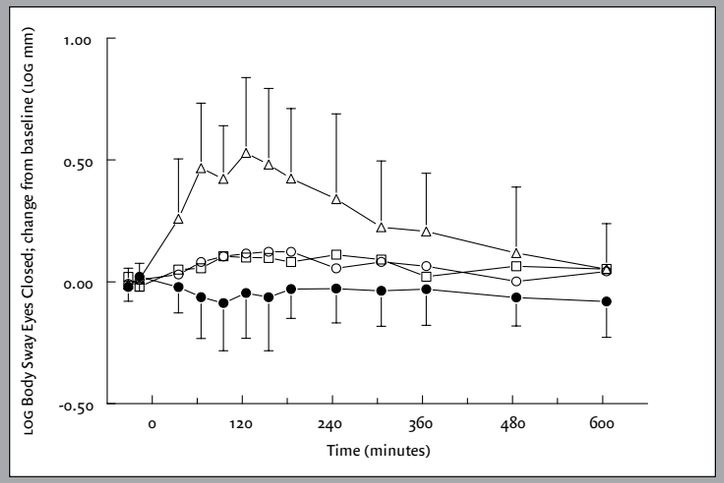
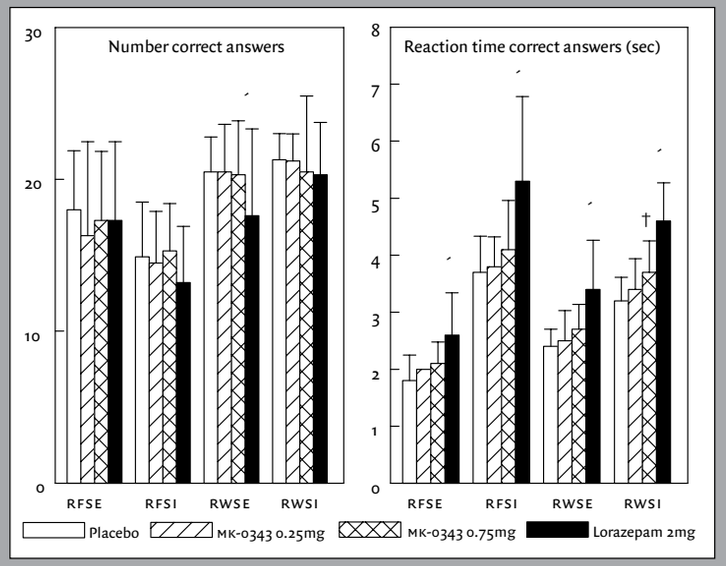


Figure 5 Effects on cognitive function tests (mean + SD).



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 MK-0343 0.75mg.

REFERENCES

1 Buffett-Jerrott SE, Stewart SH, Bird S, Teehan MD. An examination of differences in the time course of oxazepam's effects on implicit vs explicit memory. J Psychopharmacol 1998; 12: 338-347.

2 Barbee JG. Memory, benzodiazepines, and anxiety: integration of theoretical and clinical perspectives. J Clin Psychiatry 1993; 54 Suppl: 86-97.

3 O'brien CP. Benzodiazepine use, abuse, and dependence. J Clin Psychiatry 2005; 66 Suppl 2: 28-33.

4 Mohler H, Crestani F, Rudolph U. GABA(A)-receptor subtypes: a new pharmacology. Curr Opin Pharmacol 2001; 1: 22-25.

5 van Steveninck AL, Gieschke R, Schoemaker RC, Roncari G, Tuk B, Pieters MS, Breimer DD, Cohen AF. Pharmacokinetic and pharmacodynamic interactions of bretazenil and diazepam with alcohol. Br J Clin Pharmacol 1996; 41: 565-573.

6 Aufdembrinke B. Abecarnil, a new beta-carboline, in the treatment of anxiety disorders. Br J Psychiatry Suppl 1998; 55-63.

7 Evans SM, Foltin RW, Levin FR, Fischman MW. Behavioral and subjective effects of DN-2327 (pazinaclone) and alprazolam in normal volunteers. Behav Pharmacol 1995; 6: 176-186.

8 Griebel G, Perrault G, Simiand J, Cohen C, Granger P, Decobert M, Francon D, Avenet P, Depoortere H, Tan S, Oblin A, Schoemaker H, Evanno Y, Sevrin M, George P, Scatton B. SL651498: an anxioselective compound with functional selectivity for alpha2- and alpha3-containing gamma-aminobutyric acid(A) (GABA(A)) receptors. J Pharmacol Exp Ther 2001; 298: 753-768.

9 Griebel G, Perrault G, Simiand J, Cohen C, Granger P, Depoortere H, Francon D, Avenet P, Schoemaker H, Evanno Y, Sevrin M, George P, Scatton B. SL651498, a $GABA_A$ receptor agonist with subtype-selective efficacy, as a potential treatment for generalized anxiety disorder and muscle spasms. CNS Drug Rev 2003; 9: 3-20.

10 McKernan RM, Rosahl TW, Reynolds DS, Sur C, Wafford KA, Atack JR, Farrar S, Myers J, Cook G, Ferris P, Garrett L, Bristow L, Marshall G, Macaulay A, Brown N, Howell O, Moore KW, Carling RW, Street LJ, Castro JL, Ragan CI, Dawson GR, Whiting PJ. Sedative but not anxiolytic properties of benzodiazepines are mediated by the GABA(A) receptor alpha1 subtype. Nat Neurosci 2000; 3: 587-592.

11 Rudolph U, Crestani F, Mohler H. GABA(A) receptor subtypes: dissecting their pharmacological functions. Trends Pharmacol Sci 2001; 22: 188-194.

12 Rudolph U, Crestani F, Benke D, Brunig I, Benson JA, Fritschy JM, Martin JR, Bluethmann H, Mohler H. Benzodiazepine actions mediated by specific gamma-aminobutyric acid(A) receptor subtypes. Nature 1999; 401: 796-800.

13 Low K, Crestani F, Keist R, Benke D, Brunig I, Benson JA, Fritschy JM, Rulicke T, Bluethmann H, Mohler H, Rudolph U. Molecular and neuronal substrate for the selective attenuation of anxiety. Science 2000; 290: 131-134.

14 Atack JR, Hutson PH, Collinson N, Marshall G, Bentley G, Moyes C, Cook SM, Collins I, Wafford K, McKernan RM, Dawson GR. Anxiogenic properties of an inverse agonist selective for alpha3 subunit-containing GABA A receptors. Br J Pharmacol 2005; 144: 357-366.

15 Dias R, Sheppard WF, Fradley RL, Garrett EM, Stanley JL, Tye SJ, Goodacre S, Lincoln RJ, Cook SM, Conley R, Hallett D, Humphries AC, Thompson SA, Wafford KA, Street LJ, Castro JL, Whiting PJ, Rosahl TW, Atack JR, McKernan RM, Dawson GR, Reynolds DS. Evidence for a significant role of alpha3-containing $GABA_A$ receptors in mediating the anxiolytic effects of benzodiazepines. J Neurosci 2005; 25: 10682-10688.

16 Tobler I, Kopp C, Deboer T, Rudolph U. Diazepam-induced changes in sleep: role of the alpha 1 GABA(A) receptor subtype. Proc Natl Acad Sci USA 2001; 98: 6464-6469.

17 van Steveninck AL, Schoemaker HC, Pieters MS, Kroon R, Breimer DD, Cohen AF. A comparison of the sensitivities of adaptive tracking, eye movement analysis and visual analog lines to the effects of incremental doses of temazepam in healthy volunteers. Clin Pharmacol Ther 1991; 50: 172-180.

18 van Steveninck AL, Verver S, Schoemaker HC, Pieters MS, Kroon R, Breimer DD, Cohen AF. Effects of temazepam on saccadic eye movements: concentration-effect relationships in individual volunteers. Clin Pharmacol Ther 1992; 52: 402-408.

19 de Haas SL, de Visser SJ, van der Post JP, de smet M, Schoemaker RC, rijnbeek B, Cohen AF, Vega JM, Agrawal NG, Goel TV, Simpson RC, Pearson LK, Li S, Hesney M, Murphy MC, van Gerven MA. Pharmacodynamic and pharmacokinetic effects of TPA023, a $GABA_A$ {alpha}2,3 subtype-selective agonist, compared to lorazepam and placebo in healthy volunteers. J Psychopharmacol 2007; 21: 374-383.

20 Micallef J, Soubrouillard C, Guet F, Le Guern ME, Alquier C, Bruguerolle B, Blin O. A double blind parallel group placebo controlled comparison of sedative and amnesic effects of etifoxine and lorazepam in healthy subjects. Fundam Clin Pharmacol 2001; 15: 209-216.
21 Green JF, McElholm A, King DJ. A comparison of the sedative and amnestic effects of chlorpromazine and lorazepam. Psychopharmacology (Berl) 1996; 128: 67-73.
22 de Visser SJ, van der Post JP, de Waal PP, Cornet F, Cohen AF, van Gerven JM. Biomarkers for the effects of benzodiazepines in healthy volunteers. Br J Clin Pharmacol 2003; 55: 39-50.
23 van Steveninck AL, Cohen AF, Ward T. A microcomputer based system for recording and analysis of smooth pursuit and saccadic eye movements. Br J Clin Pharmacol 1989; 27: 712P-713P.
24 Norris H. The action of sedatives on brain stem oculomotor systems in man. Neuropharmacology 1971; 10: 181-191.
25 Bond A, Lader M. The use of analogue scales in rating subjective feelings. Br J Med Psychol 1974; 47: 211-218.
26 Wright BM. A simple mechanical ataxia-meter. J Physiol 1971; 218: 27P-28P.
27 Alpherts WCJ. Computers as a technique for neurphysiological assessment in epilepsy. In: Education and Epilepsy eds Aldenkamp AP, Alpherts WCJ, Meinardi H, Stores G, Lisse/ Berwyn: Swets & Zeitlinger, 1987: 101-109.
28 Aldenkamp A, Vermeulen J, Alpherts WCJ, Overweg J, van Parijs JAP, Verhoeff NPLG. Validity of computerized testing: patient dysfunction and complaints versus measured changes.In: Assessment of Cognitive Function ed W.E.Dodson and M.Kinsbourne, New York: Demos, 1992: 51-68.
29 Nelson RE, Dickson AL, Banos JH. An automated administration of Corsi's Block-tapping Test. Percept Mot Skills 2000; 91: 578-580.
30 Izaute M, Bacon E. Specific effects of an amnesic drug: effect of lorazepam on study time allocation and on judgment of learning. Neuropsychopharmacology 2005; 30: 196-204.
31 Atack JR. Anxioselective compounds acting at the GABA(A) receptor benzodiazepine binding site. Curr Drug Targets CNS Neurol Disord 2003; 2: 213-232.
32 Scott-Stevens P, Atack JR, Sohal B, Worboys P. Rodent pharmacokinetics and receptor occupancy of the $GABA_A$ receptor subtype selective benzodiazepine site ligand L-838417. Biopharm Drug Dispos 2005; 26: 13-20.
33 Johnstone TB, Hogenkamp DJ, Coyne L, Su J, Halliwell RF, Tran MB, Yoshimura RF, Li WY, Wang J, Gee KW. Modifying quinolone antibiotics yields new anxiolytics. Nat Med 2004; 10: 31-32.
34 Atack JR, Wafford KA, Tye SJ, Cook SM, Sohal B, Pike A, Sur C, Melillo D, Bristow L, Bromidge F, Ragan I, Kerby J, Street L, Carling R, Castro JL, Whiting P, Dawson GR, McKernan RM. TPA023 [7-(1,1-Dimethylethyl)-6-(2-ethyl-2H-1,2,4-triazol-3-ylmethoxy)-3-(2-fluor ophenyl)-1,2,4-triazolo[4,3-b]pyridazine], an Agonist Selective for {alpha}2- and {alpha}3-Containing $GABA_A$ Receptors, Is a Nonsedating Anxiolytic in Rodents and Primates. J Pharmacol Exp Ther 2006; 316: 410-422.
35 Carling RW, Madin A, Guiblin A, Russell MG, Moore KW, Mitchinson A, Sohal B, Pike A, Cook SM, Ragan IC, McKernan RM, Quirk K, Ferris P, Marshall G, Thompson SA, Wafford KA, Dawson GR, Atack JR, Harrison T, Castro JL, Street LJ. 7-(1,1-Dimethylethyl)-6-(2-ethyl-2H-1,2,4-triazol-3-ylmethoxy)-3-(2-fluoro phenyl)-1,2,4-triazolo[4,3-b]pyridazine: a functionally selective gamma-aminobutyric acid(A) (GABA(A)) alpha2/alpha3-subtype selective agonist that exhibits potent anxiolytic activity but is not sedating in animal models. J Med Chem 2005; 48: 7089-7092.
36 Atack JR. The benzodiazepine binding site of GABA(A) receptors as a target for the development of novel anxiolytics. Expert Opin Investig Drugs 2005; 14: 601-618.
37 Whiting PJ. GABA-A receptors: a viable target for novel anxiolytics? Curr Opin Pharmacol 2006; 6: 24-29.
38 Lippa A, Czobor P, Stark J, Beer B, Kostakis E, Gravielle M, Bandyopadhyay S, Russek SJ, Gibbs TT, Farb DH, Skolnick P. Selective anxiolysis produced by ocinaplon, a GABA(A) receptor modulator. Proc Natl Acad Sci USA 2005; 102: 7380-7385.
39 Langen B, Egerland U, Bernoster K, Dost R, Unverferth K, Rundfeldt C. Characterization in rats of the anxiolytic potential of ELB139 [1-(4-chlorophenyl)-4-piperidin-1-yl-1,5-dihydro-imidazol-2-on], a new agonist at the benzodiazepine binding site of the $GABA_A$ receptor. J Pharmacol Exp Ther 2005; 314: 717-724.
40 Mattila MJ, Vanakoski J, Kalska H, Seppala T. Effects of alcohol, zolpidem, and some other sedatives and hypnotics on human performance and memory. Pharmacol Biochem Behav 1998; 59: 917-923.
41 Licata SC, Platt DM, Cook JM, Sarma PV, Griebel G, Rowlett JK. Contribution of $GABA_A$ receptor subtypes to the anxiolytic-like, motor, and discriminative stimulus effects of benzodiazepines: studies with the functionally selective ligand SL651498 [6-fluoro-9-methyl-2-phenyl-4-(pyrrolidin-1-yl-carbonyl)-2,9-dihydro-1H-py ridol[3,4-b]indol-1-one]. J Pharmacol Exp Ther 2005; 313: 1118-1125.
42 Patat A, Foulhoux P. Effect on postural sway of various benzodiazepine tranquillizers. Br J Clin Pharmacol 1985; 20: 9-16.
43 van Steveninck AL, Gieschke R, Schoemaker HC, Pieters MS, Kroon JM, Breimer DD, Cohen AF. Pharmacodynamic interactions of diazepam and intravenous alcohol at pseudo steady state. Psychopharmacology (Berl) 1993; 110: 471-478.
44 Allain H, Bentue-Ferrer D, Polard E, Akwa Y, Patat A. Postural instability and consequent falls and hip fractures associated with use of hypnotics in the elderly: a comparative review. Drugs Aging 2005; 22: 749-765.

CHAPTER 4

The pharmacokinetic and pharmacodynamic effects of SL65.1498, a GABA$_A$ α2,3 selective agonist, in comparison with lorazepam in healthy volunteers

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ABSTRACT

Benzodiazepines are effective short-term treatments for anxiety disorders, but their use is limited by undesirable side-effects related to central nervous system (CNS) impairment and tolerance development. SL65.1498 is a new compound that acts in vitro as a full agonist at the $GABA_A$ $\alpha 2$ and $\alpha 3$ receptor and as a partial agonist at the $\alpha 1$ and $\alpha 5$ receptor-subtype. It is thought that the compound could be anxiolytic by its activation at the $\alpha 2$ and $\alpha 3$ receptor subtypes, without causing unfavourable side effects, which are believed to be mediated by the $\alpha 1$and $\alpha 5$ subtypes. This study was a double-blind, five way cross-over study to investigate the effects of three doses of SL65.1498 in comparison to placebo and lorazepam 2 mg in healthy volunteers. The objective was to select a dose level (expected to be therapeutically active), free of any significant deleterious effect. Psychomotor and cognitive effects were measured using a validated battery of measurements, including eye movements, body sway, memory tests, reaction time assessments and visual analogue scales (VAS).

The highest dose of SL65.1498 showed small effects on saccadic peak velocity and smooth pursuit performance, although to a much lesser extent than lorazepam. In contrast to lorazepam, none of the SL65.1498 doses affected body sway, VAS alertness, attention or memory tests.

This study showed that the three doses of SL65.1498 were well tolerated and induced no impairments on memory, sedation and psychomotor and cognitive functions.

INTRODUCTION

In the 1960s, benzodiazepines were considered the gold standard for treatment of anxiety and various phobias. Although they seemed the perfect drugs, based on their rapid onset of efficacy, they have become less favourable for prolonged therapy, due to their propensity for development of tolerance and dependency, and their adverse side-effect profile related to central nervous system (CNS) impairment. These side effects are caused by the non-selective binding profile of the full agonists to the different $GABA_A$ receptor subtypes. Several pre-clinical studies have shown that stimulation of receptors containing subunits are associated with anxiolysis [1,2]. Receptors with $\alpha 1$ subunits are thought to be responsible for sedation, and the $\alpha 5$ subtype for memory. Therefore, new compounds have been developed that are more selective agonists for the $GABA_A$ $\alpha 2,3$ subtype receptors, and are antagonists or partial agonists at $\alpha 1$ and $\alpha 5$ subtypes. This should result in an anxiolytic compound with less of the unwanted side effects that existing benzodiazepines possess.

SL65.1498 is a full agonist at receptors containing $\alpha 2$ and $\alpha 3$ subunits with an efficacy of 115 and 83% respectively, relative to a full-agonist. It is a partial agonist at those containing $\alpha 1$ and $\alpha 5$, showing a relative efficacy of 45 and 50%, respectively [3]. Behavioural studies in rodents demonstrated that SL65.1498 elicited similar anxiolytic-like activity to that of diazepam [3,4]. Other effects like muscle weakness, ataxia, and sedation were also induced but at much higher doses than those producing anxiolytic-like effects. In non-human primates, SL65.1498 also showed anxiolytic-like (anti-conflict) effects as assessed by a conditioned conflict test model, without showing sedation [5]. For the current study, three doses of SL65.1498 were selected that produced plasma concentrations in Phase I studies, which were predicted to be in the therapeutic range. At these plasma concentrations, animal studies showed potent anxiolytic-like activity similar to that of benzodiazepines, without any sedative effects [3,4].

To determine the psychopharmacological profile of these three doses, they were investigated using a validated battery of Central Nervous System (CNS) measurements in comparison to the effects of lorazepam and placebo. The measurements included saccadic eye movements, smooth pursuit, body sway, visual analogue scales and memory, cognition and attention tests. Previous studies have shown that benzodiazepines significantly decrease saccadic peak velocity, postural stability and memory [6-11]. The objective was to identify a dose level that was expected to be in the therapeutic range and that was free of any clinically significant deleterious effect compared to placebo.

METHODS

Design

This study was a placebo controlled, randomised, double-blind, five-way, cross-over, single-centre study in twenty healthy male volunteers, with a washout period between 7 and 14 days.

Subjects

Twenty healthy male and female volunteers were recruited from the CHDR database. All volunteers gave written informed consent and were medically screened before entry to the study. Subjects were not allowed to smoke more than five cigarettes per day and had to refrain from smoking during the study day. They were asked not to drink alcohol 48 hours prior to and 24 hours following a study day and to refrain from drinking xanthine- based and grapefruit-containing products from 24 hours before until the end of the study day. The use of medication or products containing St John's Wort was not allowed during the study period. The study was approved by the Medical Ethics Review Board of Leiden University medical Centre.

Treatments

On randomized treatment days, each subject received a single oral dose of SL65.1498-00 2.5 mg, 7.5 mg, 25 mg, lorazepam 2 mg (2*1 mg) or placebo administered with 250 ml of water in a fasted state in the morning. All treatments looked identical and consisted of 2 capsules. Lorazepam and placebo tablets were enclosed in capsules for blinding purposes. The treatment sequences were determined using 5x5 Williams design with two subjects per sequence.

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 10 minutes.

Pharmacokinetics

Blood samples (7 ml) were drawn on each treatment occasion within 1 hour predose and 0.5, 1, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 24 and 48 hours postdose to obtain plasma for assay of SL65.1498 and lorazepam concentrations.

Plasma was separated from heparinized blood samples by centrifugation (2000 g, 10 min, 4°C) to 3.6 ml Nunc cryotubes and stored at -20°C within 30 minutes after sampling. SL65.1498 analysis was accomplished using an Atmospheric Pressure Chemical Ionisation validated LC-ms/ms method. The quantitation limit of the assay was 0.5 ng/mL. Assays were performed in the Department of Clinical Metabolism and Pharmacokinetics at Sanofi-Aventis Research, Alnwick, Northumberland, UK.

A LC-ms/ms method using positive ion Turbo Ionspray with multiple monitoring (mrm) was validated for the quantification of lorazepam in human plasma. The calibration curves of lorazepam were linear between 0.500 and 50.0 ng/mL in human plasma and the limit of quantification (loq) was 0.500 ng/mL. Assays were performed by Ppd Development, Richmond, Virginia, USA.

Pharmacodynamics

Pharmacodynamic measurements were performed predose (within 30 minutes prior to dosing) and 0.5, 1, 2, 2.5, 3, 3.5, 4, 5, 6, 8 and 10 hours postdose. Pharmacodynamic tests were performed 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: body sway eyes closed; VAS; saccadic eye movements. Cognitive function tests were performed at fixed times within the 2-4 hours-postdose period between the other measurements. All subjects were thoroughly trained and familiarized with the psychometric tests within 7 days preceding study start to minimize learning effects before proceeding to the study.

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) [12]. Saccadic peak velocity has been validated as the most sensitive measure for the sedative effects of benzodiazepines [6-8].

Smooth Pursuit

The same system as used for saccadic eye movements was also used for measurement of smooth pursuit. For smooth pursuit eye movements, the target moves sinusoidally at frequencies ranging from 0.3 to 1.1 Hz, by steps of 0.1 Hz. The amplitude of target displacement corresponds to 20 degrees eyeball rotation to both sides. Four cycles were recorded for each stimulus frequency. The method has been validated at CHDR by Van Steveninck et al [12] based on the work of Bittencout et al [13] and the original description of Baloh [14]. The time in which the eyes were in smooth pursuit of the target was calculated for each frequency and expressed as a percentage of stimulus duration. The average percentage of smooth pursuit for all stimulus frequencies was the parameter used.

Visual Analogue Scale

Visual analogue scales as originally described by Norris [15] were previously used to quantify subjective effects of benzodiazepines [7]. From the set of sixteen scales three composite factors were derived as described by Bond and Lader [16], corresponding to alertness, contentedness and calmness. These factors were used to quantify subjective drug effects.

Body Sway

Body sway was measured with an apparatus similar to the Wright ataxiameter [17], which integrates the amplitude of unidirectional body movements transferred through a string attached to the subject's waist. Two-minute measurements were made in the antero-posterior direction with eyes closed, with subjects standing comfortably on a firm surface with their feet slightly apart. Body sway is a measure of postural stability that has previously been shown to be sensitive to benzodiazepines [18]

Cognitive Function Tests

Memory testing was performed using the validated Eprime program [19,20]. At 2 hours post-dose, subjects were presented 30 words in three consecutive word trials (word learning test). Each trial ended with a free recall of the presented words (Immediate Recall). Approximately thirty minutes after start of the first trial, the volunteers were asked to recall as many words as possible (Delayed Recall). Immediately thereafter, the volunteers underwent the delayed memory recognition test, which consisted of 15 presented words and 15 'distractors' (Delayed Recognition). At 3 hours post-dose, when the subjects were presented with 14 abstract visual patterns for 3 seconds. Hereafter, the same visual patterns were presented along side a 'distractor'. Subjects were then asked to indicate which visual pattern was previously presented. This testing was repeated 30 minutes later. Word and picture recognition and recall tests were performed to assess reaction time and number of correct and incorrect answers. The Corsi block tapping test, constructed according the principles of the original Corsi block tapping task [21], assessed the nonverbal memory span. The visual and auditory reaction times tests were performed using the validated FePsy program (The Iron Psyche) [22,23].

Memory tests have been shown to be affected by benzodiazepines [9,10].

Analysis

Pharmacokinetics

Pharmacokinetics of SL65.1498 were determined using a non-compartmental analysis model. Parameters determined were maximum plasma concentrations, time to maximum plasma concentration, AUC, apparent clearance (clearance divided by bioavailability) and elimination half-life. Estimation was performed using WinNonlin software (WinNonlin Network Version 3.1, Pharsight, Cary, NC, USA).

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.

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 - SL65.1498 2.5 mg, placebo - SL65.1498 7.5 mg, lorazepam 2 mg - SL65.1498 25 mg and placebo - lorazepam 2 mg. Overall p-value for the treatment effect was reported along with the specified contrasts with 95% confidence intervals and p-values.

With 20 subjects, there was 95% power to detect a SPV-reduction of at least 35 degree/sec/h after SL65.1498 treatment when compared with placebo in aueo-6h saccadic peak velocity, assuming the within-subject SD equal to 30 degree/sec/h.

RESULTS

Subjects

Eleven male and eleven female subjects were medically screened after giving written informed consent and ten of each group completed the study. Subjects were on average 25 years of age (range 19-38), had an average weight of 74 kg (range 58-98 kg) and average height of 175 cm (range 163-191 cm).

Clinical observations

No serious adverse reactions occurred following any of the treatments. The most frequently reported adverse event was sedation, which was reported in the lorazepam (fourteen subjects), SL65.1498 2.5 mg (six subjects), SL65.1498 7.5 mg (three subjects), SL65.1498 25 mg (eight subjects) and placebo group (seven subjects). Another reported adverse event was dizziness which was reported by nine subjects in the lorazepam group and one subject each in the placebo, SL65.1498 7.5 mg and 25 mg groups.

Pharmacokinetics

The mean (SD) plasma concentration-time curves for the three doses of SL65.1498 are shown in figure 1. All doses of SL65.1498 showed maximum concentrations between 3.01 and 3.75 hours.
The mean C_{MAX} x (SD) was dose-proportional at 375 (129) ng/mL for the highest dose, 126 (40.1) for the middle and 37.3 (12.7) for the lowest. Elimination half-life was 11.0 (2.9) h, 10.7(2.9) and 12.2 (3.4) h for SL65.1498 25, 7.5 mg and 2.5 mg respectively.

Lorazepam 2 mg showed maximum concentrations between 0.50 and 3.50 h with a mean (SD) C_{MAX} of 22.8 (5.1) ng/mL. Elimination half-life (SD) was 17.3 (4.3) h. These pharmacokinetic properties of lorazepam were in agreement with published data [24,25].

Pharmacodynamics

Saccadic Eye Movements

Lorazepam 2 mg and SL65.1498 25 mg decreased Saccadic Peak Velocity (SPV) compared with placebo (decreases in AUC 0-6hr 45.7 deg/sec and 15.0 deg/sec respectively (Table 1)). The lower doses of SL65.1498 doses did not affect the eye movements significantly (table 1, figure 2).

Smooth Pursuit

Lorazepam and to a lesser extent SL65.1498 25 mg decreased smooth pursuit performance compared with placebo, while the other two doses of SL65.1498 did not affect this parameter (table 1, figure 3).

Visual Analogue Scale

Only lorazepam caused effects on VAS alertness (Figure 4) and VAS contentedness compared with placebo, while none of the SL65.1498 doses caused changes in any VAS subscale (table 1).

Body Sway

Body sway was only affected by lorazepam compared with placebo and not by one of the three doses of SL65.1498 (table 1, figure 5).

Cognitive Function Tests and Corsi Block Tapping Task

During the learning phase of word recall test, the mean number of correct responses increased from the first to the third test in all treatment groups. Lorazepam decreased the number of correct responses in both the immediate and delayed word recall test, while responses after each dose of SL65.1498 were comparable with responses after placebo treatment (table 2). The number of correct responses for the delayed word recognition test was comparable between placebo and SL65.1498 treatment. Lorazepam decreased this number by 6.1 words compared with placebo (table 2).

The number of correct responses of the immediate/delayed picture recognition was similar between all study groups (results not shown).

The mean latency of correct responses after the simple auditory and visual reaction time test was increased after lorazepam administration (table 3). Results for the dominant and non-dominant hand (results not shown) were comparable.

For the binary choice reaction time test, there was no difference in mean number of correct responses between study treatments and placebo (table 3)

DISCUSSION

This study investigated the effects on CNS measurements of three doses of a new $GABA_A$ subtype selective agonist, SL65.1498, and compared these with the effects of a full $GABA_A$ agonist and placebo in healthy volunteers. The main aim was to determine whether SL65.1498 was free of deleterious effects at a dose level that was expected to be therapeutically active, based on animal models [3,4]. It was compared with lorazepam 2 mg, which is known to be a therapeutically relevant dose [26,27].

This study showed that the three doses of SL65.1498 induced no impairments on memory, subjective alertness and psychomotor and cognitive functions. Only the highest dose of SL65.1498 showed effects on saccadic peak velocity (SPV) and smooth pursuit performance, although much less than lorazepam. In this respect, the aim of the study was achieved as even the highest dose did not show clinically significant deleterious effects compared to placebo. The lack of effects on memory, body sway, attention and VAS alertness could mean a more favourable side effect profile compared to the commonly used benzodiazepines. The only significant effects of the highest dose of SL65.1498 were on eye movements. The reductions in SPV and smooth pursuit were only about one third of the effects of lorazepam. SPV reduction has been shown to be a quite sensitive biomarker for sedation caused by several different CNS-depressants, including GABAergic [7,8], histaminergic (H1) [28] and noradrenergic [29] or physiological conditions [6]. The limited SPV-decrease of 15 deg/sec with SL65.1498 25 mg is probably still compatible with the lack of subjective sedation [6].However, a recently published review indicated that a reduction in SPV is also quantitatively associated with the anxiolytic effects of benzodiazepines [11]. Since the effects on SPV are very low in comparison to those of lorazepam 2 mg or other anxiolytic benzodiazepines in the literature [11], this may imply that SL65.1498 25 mg not only has a lower sedative propensity, but also a lower anxiolytic efficacy. Recently, two other partial subtype-selective $GABA_A$ agonists showed SPV-reductions that were quite similar to lorazepam, hence much larger than for SL65.1498 [9,30]. These compounds had hardly any other CNS-effects, indicating that significant SPV-reductions can occur without reductions of alertness. It remains to be seen whether this translates into anxiolysis without sedation. So far, no clinical trials have been reported with partial subtype-selective $GABA_A$ agonists.

The lack of significant CNS effects does not seem to be related to low plasma concentrations. In our study, the plasma levels were comparable to those in rats receiving doses that produced anxiolytic-like effects in the punished drinking test and elevated plus-maze test [3,4]. Plasma levels were high compared to lorazepam, which is in keeping with the relatively low affinity of SL65.1498 for the $GABA_A$ receptor subtypes (Ki: 6.8-117 nM) compared to those of other $GABA_A$ receptor ligands [31]. Healthy humans showed small CNS-effects on some CNS-functions but not on others, at plasma concentrations of SL65.1498 that were anxiolytic but devoid of sedative effects in animal models [3,5]. This could be related to the selective pharmacological profile of SL65.1498. However, selectivity cannot be proven, since none of the three doses of SL65.1498 was equipotent to lorazepam for any effect that was measured. To date the compound has not been registered, and no results of clinical trials in anxiety or other conditions have been published. The putative wider therapeutic window that is suggested by preclinical experiments and supported by our results cannot therefore be confirmed at present.

The current study showed that SL65.1498 at doses of 2.5-25 mg is well tolerated and induces no impairments on memory, sedation and psychomotor and cognitive functions. It is unclear whether this is related to subtype selectivity or to relatively low doses.

Table 1 Pharmacodynamic differences in AUE 0-6hr relative to baseline for Saccadic Eye Movements, Smooth Pursuit Performance, Visual Analogue Scales and Body Sway; ANOVA results are shown as contrasts (95% CI) with p-value.

Variable	Overall treatment effect (p-value)	Placebo - SL65.1498 2.5 mg	Placebo - SL65.1498 7.5 mg	Placebo - SL65.1498 25 mg	Lorazepam 2mg - SL65.1498 25 mg	Placebo - Lorazepam 2 mg
Saccadic Peak Velocity (deg/sec)	0.0001	5.52 (4.41/15.44) p = 0.272	8.07 (1.86/17.99) p = 0.120	15.02 (5.09/24.94) p = 0.0035	30.67 (20.75/40.60) p =0.0001	45.69 (35.76/55.62) p = 0.0001
Smooth pursuit (%)	0.0001	0.62 (-2.96/4.19) p = 0.733	1.21 (-2.37/4.79) p = 0.502	5.27 (1.69/8.84) p = 0.005	-12.73 (-16.31/-9.15) p = 0.0001	18.00 (14.42/21.57) p = 0.0001
VAS Alertness (ln mm)	0.0001	1.42 (-24.68/27.52) p = 0.914	3.63 (-22.47/29.73) p = 0.7822	-13.01 (-39.11/13.09) p = 0.324	82.54 (56.44/108.63) p = 0.0001	-95.54 (-121.64/-69.45) p = 0.0001
VAS Contentedness (ln mm)	0.0356	-3.95 (-12.83/4.94) p = 0.3786	1.78 (-7.11/10.66) p = 0.6914	-2.15 (-11.03/6.74) p = 0.6316	9.53 (0.64/18.41) p = 0.036	-11.67 (-20.55/-2.79) p = 0.0107
VAS Calmness (ln mm)	0.9471	-1.19 (-6.84/4.47) p = 0.677	0.09 (-5.56/5.74) p = 0.975	1.20 (-4.45/6.85) p = 0.673	1.46 (-4.19/7.11) p = 0.608	-0.26 (-5.91/5.39) p = 0.927
Body Sway Eyes Closed (mm)	0.0001	6.0 (11.0/21.0) p =0.476	6.0 (11.0/21.0) p = 0.441	-2.0 (-14.0/21.0) p = 0.786	54.0 (62.0/46) p = 0.0001	-225.0 (-89/-168) p = 0.0001

Table 2 Mean number of correct responses (SEM) for immediate word recall (three consecutive trials) and delayed word recall and recognition.

	Immediate word recall						Delayed			
	1st trial		2nd trial		3rd trial		Word Recall		Word Recognition	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Placebo	11.1	0.7	15.0	0.9	18.1	1.1	16.2	1.4	25.5	1.3
SL65.1498 2.5 mg	**9.2***	0.7	15.2	0.9	17.9	1.5	15.2	1.4	25.4	1.5
SL65.1498 7.5 mg	10.3	0.7	15.0	0.9	17.4	1.1	15.2	1.2	26.4	0.6
SL65.1498 25 mg	10.0	0.8	14.5	1.1	18.2	1.1	14.8	1.4	25.7	0.6
Lorazepam	**5.6**	0.6	**8.6**	0.9	**11.1**	0.9	**7.4**	1.0	**19.4**	1.5

Bold values show significant effects compared to placebo ($p < 0.0001$, * $p < 0.05$).

Table 3 Mean latency times for correct response (SEM) for auditory and visual reaction time tests and mean number of correct responses (SEM) for binary choice reaction time test.

	Auditory reaction time test		Visual reaction time test		Binary Choice reaction time test	
Treatment	Mean latency for correct response (ms)	SEM	Mean latency for correct response (ms)	SEM	Mean number correct	SEM
Placebo	250.40	6.16	271.45	6.60	58.4	0.4
SL65.1498 2.5 mg	259.20	7.27	275.75	7.04	58.2	0.4
SL65.1498 7.5 mg	253.20	6.75	280.60	7.56	58.2	0.6
SL65.1498 25 mg	259.60	5.21	290.45	15.83	58.4	0.3
Lorazepam	**302.90**	13.49	**360.70**	31.38	58.0	0.7

Bold values show differences compared to placebo ($p < 0.0001$)

Figure 1 Average plasma drug concentration-time profiles (mean + SD)

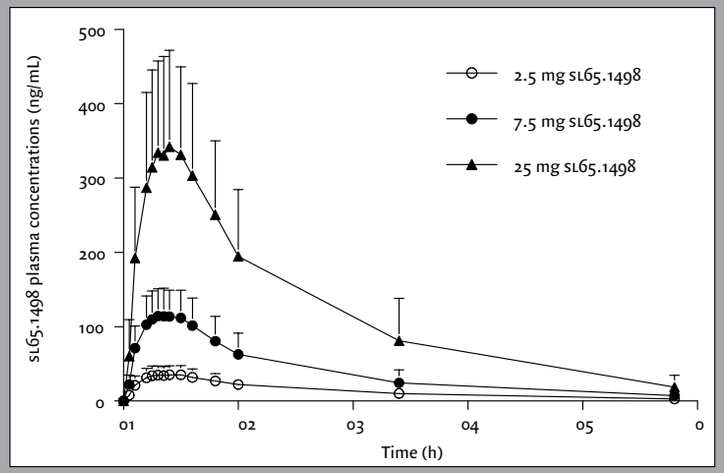


Figure 2 Average graph of Saccadic Peak Velocity (deg/sec) with SD error bars for Placebo (up) and Lorazepam 2 mg (down). Open circle: SL 2.5 mg; open square: SL65.1498 7.5 mg; open triangle: SL65.1498 25 mg; closed circle: Lorazepam 2 mg; closed square: Placebo.

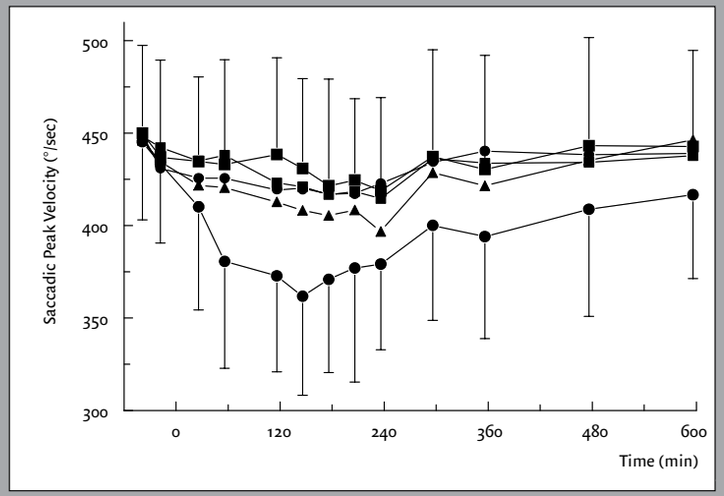


Figure 3 Average graph of Smooth Pursuit (%) with SD error bars for Placebo (up) and Lorazepam 2 mg (down). Open circle: SL 2.5 mg; open square: SL65.1498 7.5 mg; open triangle: SL65.1498 25 mg; closed circle: Lorazepam 2 mg; closed square: Placebo.

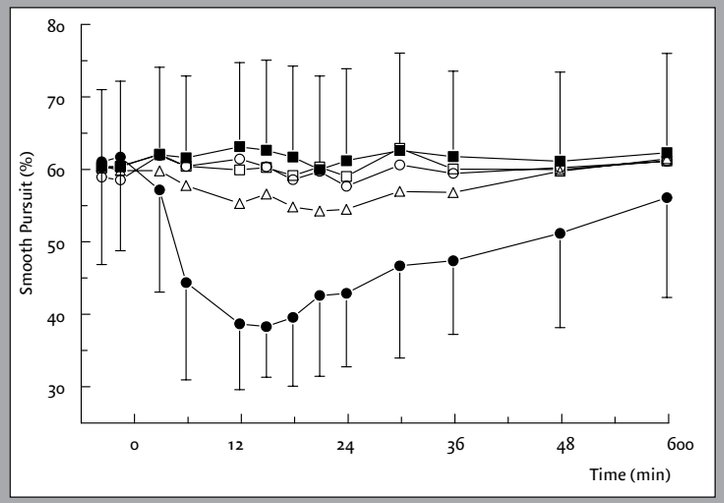


Figure 4 Average graph of VAS alertness (mm) with SD error bars for Lorazepam 2 mg (up) and Placebo (down). Open circle: SL 2.5 mg; open square: SL65.1498 7.5 mg; open triangle: SL65.1498 25 mg; closed circle: Lorazepam 2 mg; closed square: Placebo.

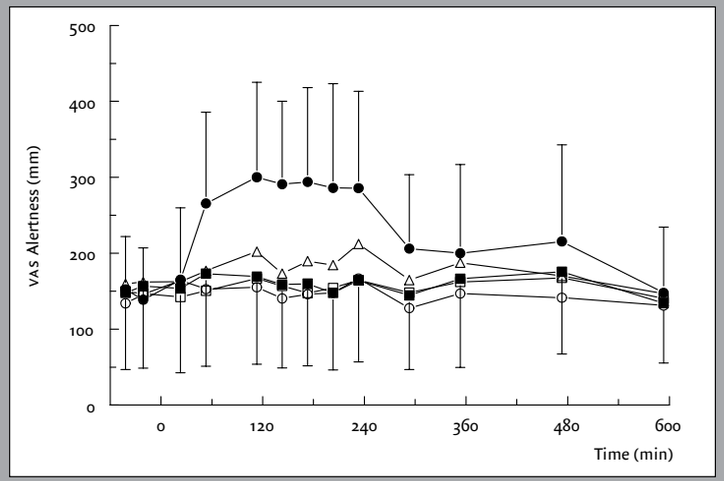
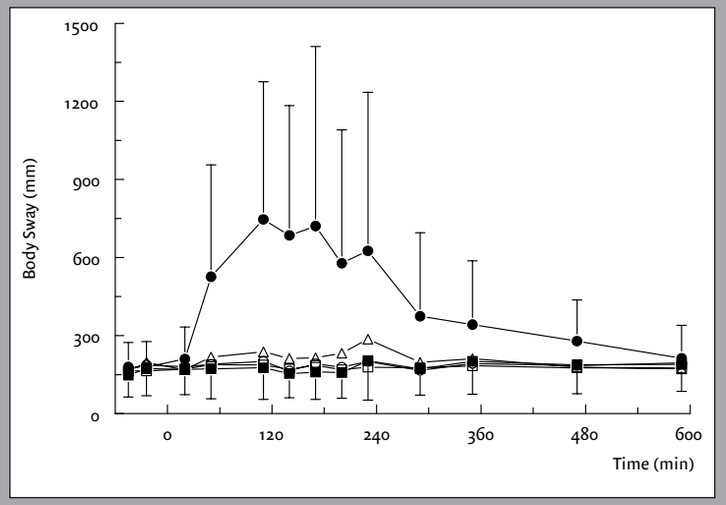


Figure 5 Average graph of Body Sway (mm) with SD error bars for Lorazepam 2 mg (up) and Placebo (down). Open circle: SL 2.5 mg; open square: SL65.1498 7.5 mg; open triangle: SL65.1498 25 mg; closed circle: Lorazepam 2 mg; closed square: Placebo.



REFERENCES

1 Low K, Crestani F, Keist R, Benke D, Brunig I, Benson JA, Fritschy JM, Rulicke T, Bluethmann H, Mohler H, Rudolph U. Molecular and neuronal substrate for the selective attenuation of anxiety. Science 2000; 290: 131-134.

2 Rudolph U, Crestani F, Mohler H. GABA(A) receptor subtypes: dissecting their pharmacological functions. Trends Pharmacol Sci 2001; 22: 188-194.

3 Griebel G, Perrault G, Simiand J, Cohen C, Granger P, Decobert M, Francon D, Avenet P, Depoortere H, Tan S, Oblin A, Schoemaker H, Evanno Y, Sevrin M, George P, Scatton B. SL651498: an anxioselective compound with functional selectivity for alpha2- and alpha3-containing gamma-aminobutyric acid(A) (GABA(A)) receptors. J Pharmacol Exp Ther 2001; 298: 753-768.

4 Griebel G, Perrault G, Simiand J, Cohen C, Granger P, Depoortere H, Francon D, Avenet P, Schoemaker H, Evanno Y, Sevrin M, George P, Scatton B. SL651498, a $GABA_A$ receptor agonist with subtype-selective efficacy, as a potential treatment for generalized anxiety disorder and muscle spasms. CNS Drug Rev 2003; 9: 3-20.

5 Licata SC, Platt DM, Cook JM, Sarma PV, Griebel G, Rowlett JK. Contribution of $GABA_A$ receptor subtypes to the anxiolytic-like, motor, and discriminative stimulus effects of benzodiazepines: studies with the functionally selective ligand SL651498 [6-fluoro-9-methyl-2-phenyl-4-(pyrrolidin-1-yl-carbonyl)-2,9-dihydro-1H-py ridol[3,4-b]indol-1-one]. J Pharmacol Exp Ther 2005; 313: 1118-1125.

6 van Steveninck AL, van Berckel BN, Schoemaker RC, Breimer DD, van Gerven JM, Cohen AF. The sensitivity of pharmacodynamic tests for the central nervous system effects of drugs on the effects of sleep deprivation. J Psychopharmacol 1999; 13: 10-17.

7 van Steveninck AL, Schoemaker HC, Pieters MS, Kroon R, Breimer DD, Cohen AF. A comparison of the sensitivities of adaptive tracking, eye movement analysis and visual analog lines to the effects of incremental doses of temazepam in healthy volunteers. Clin Pharmacol Ther 1991; 50: 172-180.

8 van Steveninck AL, Verver S, Schoemaker HC, Pieters MS, Kroon R, Breimer DD, Cohen AF. Effects of temazepam on saccadic eye movements: concentration-effect relationships in individual volunteers. Clin Pharmacol Ther 1992; 52: 402-408.

9 de Haas SL, de Visser SJ, van der Post JP, de smet M, Schoemaker RC, rijnbeek B, Cohen AF, Vega JM, Agrawal NG, Goel TV, Simpson RC, Pearson LK, Li S, Hesney M, Murphy MC, van Gerven MA. Pharmacodynamic and pharmacokinetic effects of TPA023, a $GABA_A$ {alpha}2,3 subtype-selective agonist, compared to lorazepam and placebo in healthy volunteers. J Psychopharmacol 2007; 21: 374-383.

10 Izaute M, Bacon E. Specific effects of an amnesic drug: effect of lorazepam on study time allocation and on judgment of learning. Neuropsychopharmacology 2005; 30: 196-204.

11 de Visser SJ, van der Post JP, de Waal PP, Cornet F, Cohen AF, van Gerven JM. Biomarkers for the effects of benzodiazepines in healthy volunteers. Br J Clin Pharmacol 2003; 55: 39-50.

12 van Steveninck AL, Cohen AF, Ward T. A microcomputer based system for recording and analysis of smooth pursuit and saccadic eye movements. Br J Clin Pharmacol 1989; 27: 712P-713P.

13 Bittencourt PR, Wade P, Smith AT, Richens A. The relationship between peak velocity of saccadic eye movements and serum benzodiazepine concentration. Br J Clin Pharmacol 1981; 12: 523-533.

14 Baloh RW, Sills AW, Kumley WE, Honrubia V. Quantitative measurement of saccade amplitude, duration, and velocity. Neurology 1975; 25: 1065-1070.

15 Norris H. The action of sedatives on brain stem oculomotor systems in man. Neuropharmacology 1971; 10: 181-191.

16 Bond A, Lader M. The use of analogue scales in rating subjective feelings. Br J Med Psychol 1974; 47: 211-218.

17 Wright BM. A simple mechanical ataxia-meter. J Physiol 1971; 218: 27P-28P.

18 van Steveninck AL, Gieschke R, Schoemaker RC, Roncari G, Tuk B, Pieters MS, Breimer DD, Cohen AF. Pharmacokinetic and pharmacodynamic interactions of bretazenil and diazepam with alcohol. Br J Clin Pharmacol 1996; 41: 565-573.

19 Schmitt JA, Jorissen BL, Sobczak S, van Boxtel MP, Hogervorst E, Deutz NE, Riedel WJ. Tryptophan depletion impairs memory consolidation but improves focussed attention in healthy young volunteers. J Psychopharmacol 2000; 14: 21-29.

20 Rubinsztein JS, Rogers RD, Riedel WJ, Mehta MA, Robbins TW, Sahakian BJ. Acute dietary tryptophan depletion impairs maintenance of 'affective set' and delayed visual recognition in healthy volunteers. Psychopharmacology (Berl) 2001; 154: 319-326.

21 Nelson RE, Dickson AL, Banos JH. An automated administration of Corsi's Block-tapping Test. Percept Mot Skills 2000; 91: 578-580.
22 Alpherts WCJ. Computers as a technique for neurphysiological assessment in epilepsy. In: Education and Epilepsy eds Aldenkamp AP, Alpherts WCJ, Meinardi H, Stores G, Lisse/ Berwyn: Swets & Zeitlinger, 1987: 101-109.
23 Aldenkamp A, Vermeulen J, Alpherts WCJ, Overweg J, van Parijs JAP, Verhoeff NPLG. Validity of computerized testing: patient dysfunction and complaints versus measured changes.In: Assessment of Cognitive Function ed W.E.Dodson and M.Kinsbourne, New York: Demos, 1992: 51-68.
24 Blin O, Jacquet A, Callamand S, Jouve E, Habib M, Gayraud D, Durand A, Bruguerolle B, Pisano P. Pharmacokinetic-pharmacodynamic analysis of mnesic effects of lorazepam in healthy volunteers. Br J Clin Pharmacol 1999; 48: 510-512.
25 Greenblatt DJ. Clinical pharmacokinetics of oxazepam and lorazepam. Clin Pharmacokinet 1981; 6: 89-105.
26 Green JF, McElholm A, King DJ. A comparison of the sedative and amnestic effects of chlorpromazine and lorazepam. Psychopharmacology (Berl) 1996; 128: 67-73.
27 Micallef J, Soubrouillard C, Guet F, Le Guern ME, Alquier C, Bruguerolle B, Blin O. A double blind parallel group placebo controlled comparison of sedative and amnesic effects of ETifoxine and lorazepam in healthy subjects. Fundam Clin Pharmacol 2001; 15: 209-216.
28 Cohen AF, Hamilton MJ, Peck AW. The effects of acrivastine (BW825C), diphenhydramine and terfenadine in combination with alcohol on human CNS performance. Eur J Clin Pharmacol 1987; 32: 279-288.
29 Glue P, White E, Wilson S, Ball DM, Nutt DJ. Pharmacology of saccadic eye movements in man. 2. Effects of the alpha 2-adrenoceptor ligands idazoxan and clonidine. Psychopharmacology (Berl) 1991; 105: 368-373.
30 de Haas SL, de Visser SJ, van der Post JP, Schoemaker RC, van Dyck K, Murphy MG, de smet M, Vessey LK, Ramakrishnan R, Xue L, Cohen AF, Gerven JMAv. Pharmacodynamic and pharmacokinetic effects of MK-0343, a $GABA_A$ α2,3 subtype selective agonist, compared to lorazepam and placebo in healthy male volunteers. J Psychopharmacol 2007; (In Press).
31 Sieghart W. Structure and pharmacology of gamma-aminobutyric acidA receptor subtypes. Pharmacol Rev 1995; 47: 181-234

CHAPTER 5

Pharmacokinetics, pharmacodynamics, and the pharmacokinetic/pharmaco-dynamic relationship of zolpidem in healthy subjects

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ABSTRACT

Zolpidem is one of the most frequently prescribed hypnotics, as it is a very short- acting compound with relatively few side effects. Zolpidem's short duration of action is partly related to its short elimination half-life, but the associations between plasma levels and pharmacodynamic effects are not precisely known. In the current study, the concentration-effect relationships for zolpidem were modelled. A dose of 10 mg zolpidem was administered in a double-blind randomised placebo-controlled trial to determine pharmacodynamics (PD) and pharmacokinetics (PK) in 14 healthy volunteers, as an active control for a novel sleep-promoting drug. A validated battery of central nervous system (CNS) tests was performed frequently. Zolpidem was absorbed and eliminated quickly, with a median t_{MAX} of 0.78 h (range: 0.33-2.50) and t1/2 of 2.2 h. Zolpidem reduced saccadic peak velocity (SPV), adaptive tracking performance, EEG alpha power and VAS alertness score, and increased body sway, EEG beta power and VAS 'feeling high'. Short- and long-term memory was not affected. CNS-effects normalised more rapidly than plasma concentrations decreased. For most effects, zolpidem's short duration of action could be adequately described by both a sigmoid E_{MAX} model and a transit tolerance model. For SPV and EEG alpha power, the tolerance model seemed less suitable. These PK/PD models have different implications for the mechanism underlying zolpidem's short duration of action. A sigmoid E_{MAX} -model would imply a threshold-value for the drug's effective concentrations. A transit tolerance model is compatible with a rapid reversible desensitisation of GABAergic subunits.

INTRODUCTION

Zolpidem is one of the most frequently prescribed hypnotic drugs in the United States and Europe [1]. It is selective for the $GABA_A$ $\alpha 1$ receptor due to its high affinity for this subtype. The $\alpha 1$-subtype is believed to be associated with sedation, while other subtypes are responsible for different effects of GABAergic ligands, such as muscle relaxation, anxiolysis and memory impairment [2,3]. The compound is absorbed quickly, which makes it very suitable to rapidly induce sleep. The short half-life of the compound may be responsible for fewer residual effects the next morning when taken at bedtime, since central nervous system (CNS)-depression has been shown to last less than 5 to 7 hours [4]. Several articles reported on the pharmacokinetics and pharmacodynamic effects of zolpidem [5-9]. However, the pharmacokinetic/pharmacodynamic (PK/PD) relationships have only been determined once in healthy volunteers [10]. In this earlier PK/PD study, an E_{MAX} model was used to describe the PK/PD relationship but this model was based on only three parameters and does not seem to explain the rapid increase and decrease in effects. Therefore, we performed a detailed PK/PD-analysis of a range of CNS-effects elicited by zolpidem 10 mg, given as an active control in a study in which pharmacodynamics and pharmacokinetics were determined frequently during day time. Several CNS measurements were performed including saccadic and smooth pursuit eye movements, visual analogue score (VAS) of alertness, body sway, pharmaco-EEG and adaptive tracking. These parameters have previously shown to be sensitive to the effects of non-selective benzodiazepines [11-14]. Zolpidem was used as a positive control in a study of a novel sleep-promoting compound, which has been reported in a previous publication [15]. The current article describes the PK, PD and PK/PD relationships of 10 mg zolpidem, in comparison to placebo.

METHODS

Subjects

A total of 70 male subjects were selected to participate in the trial with the novel drug, of which 14 subjects were planned to receive a single dose of zolpidem 10 mg as active control. After signing informed consent, subjects were medically screened within 3 weeks prior to study participation. Subjects were not allowed to smoke more than five cigarettes per day and had to refrain from smoking during the study period. They were asked to refrain from strong physical exercise and consumption of grapefruit (juice) from screening until the end of the

study period. The use of medication was not allowed during the study period. The study was approved by the Medical Ethics Review Board of Leiden University medical Centre.

Safety

Adverse events, ECG, body temperature, blood pressure and heart rate measurements were performed throughout the study after having been in a supine position for at least 5 minutes. 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). Vital signs consisted of both supine and corresponding standing measurements after 1 minute standing position.

Pharmacodynamics

Pharmacodynamic measurements were performed pre-dose (within 30 minutes prior to dosing) and 10, 20, 40, 60, 80 min, 2, 3, 4, 6, 8, 10 and 12 h post-dose. Average baseline values for each variable were obtained by calculation of the mean of two baseline assessments. Pharmacodynamic tests were performed in a quiet room with ambient illumination with only one subject in the same room per session. Tests were performed in the following order: saccadic eye movements (-5 min), smooth pursuit measurement (-4 min), pharmaco-EEG (-2 min), body sway (+1 min), adaptive tracking (+3 min), VAS Bond&Lader (+6 min), VAS Bowdle (+7 min). Cognitive function tests were performed at fixed times within the 2-6 hours post-dose period between the other measurements. All subjects were thoroughly trained and familiarised with the psychometric tests within 14 days preceding study start to minimise learning effects during the study.

Saccadic Eye Movements

Measurement of saccadic eye movements was recorded as previously described [16,17]. Average values of saccadic peak velocity (SPV), latency (= reaction time) and inaccuracy were calculated for all artefact-free saccades.

Smooth Pursuit

Smooth pursuit was measured as previously described [17]. The average percentage of smooth pursuit for all stimulus frequencies was used as response parameter.

Visual Analogue Scales

The Bond and Lader Visual Analogue Scale was performed to measure subjective alertness, mood and calmness [17]. The Bowdle VAS of psychedelic effects was also used in this study. It evaluates psychedelic effects that cluster into two distinct total sum scores: internal perception (reflects inner feelings that do not correspond with reality, including mistrustful feelings); and external perception (reflects a misperception of an external stimulus or a change in the awareness of the subject's surroundings) [18]. The Bowdle VAS was expanded with an additional subscore for 'feeling high'.

Body Sway

Two-minute body sway measurements were performed as previously described [17].

Adaptive Tracking

The adaptive tracking test was performed as originally described by Borland and Nicholson [19] using customised equipment and software (Hobbs, 2004, Hertfordshire, UK). The average performance and the standard deviation of scores over a 3.5-minute period were used for analysis, including a 0.5 minute run-in time, during which data were not recorded. Adaptive tracking is a pursuit-tracking task with a circle moving randomly on a computer screen. The subject must try to keep a dot inside the moving circle by operating a joystick. If this effort is successful, the speed of the moving circle increases. Conversely, the velocity is reduced if the test subject cannot maintain the dot inside the circle.

Pharmaco-EEG

EEG recordings were made using silver chloride electrodes with the same common ground electrode as for the eye movement registration (international 10/20 system). The electrode resistances were kept below 5 kOhm. EEG signals were obtained from leads Fz-Cz and Pz-Oz and a separate channel to record eye movements (for artefacts). The signals were amplified by use of a Grass 15LT series Amplifier Systems with a time constant of 0.3 seconds and a low pass filter at 100 Hz. Data collection and analysis were performed using customised CED and Spike2 for Windows software (Cambridge Electronics Design, Cambridge, UK). Per session eight consecutive blocks of eight seconds were recorded. The signal was AD-converted

using a CED 1401 Plus or Power (Cambridge Electronics Design, Cambridge, UK) and stored on hard disk for subsequent analysis. Data blocks containing artefacts were excluded from analysis. For each lead, fast Fourier transform analysis was performed to obtain the sum of amplitudes in the delta-, theta-, alpha- and beta-frequency ranges. The duration of EEG measurements was 64 seconds per session.

Cognitive function tests

Short-term and long-term memory was similarly tested as recently described in another publication [17]. The 30-word memory learning test was performed at two hours post-dose with 'Immediate Recall' immediately hereafter. Approximately four hours after start of the Immediate Recall test, 'Delayed Recall' was performed and followed by the 'Delayed Recognition'.

Pharmacokinetics

Blood samples (4 mL) were drawn within 1 hour pre-dose and 10, 20, 40, 60, 80 min, 2, 2.5, 3, 4, 6, 8, 10 and 12 h post-dose to obtain plasma for assay of zolpidem concentrations. Plasma was separated from heparinised blood samples by centrifugation (1500 g, 10 min, 4°C) to 3.6 cc Nunc cryotubes and stored at -20°C within 30 minutes after sampling. Zolpidem analysis was accomplished using HPLC with fluorescence detection with a quantification limit of 1.00 ng/mL. Assays were performed by Xendo Drug Development B.V. (Groningen, The Netherlands). Precision and accuracy of the quality control samples showed an accuracy (bias between 3.3% and 7.5%) and precision (CV% from 2.9% to 5.1%) and were in compliance with generally accepted requirements [20].

Statistical analysis

Pharmacodynamics

For all variables with repeated measurements, the area-under-the-effect-curve (AUE) 0-4 h was calculated. VAS Bowdle scores were log transformed. Inferential analyses to compare the different treatments were performed using mixed models for repeated measurements. The two-sample, two-sided t-test was performed to explore treatment differences with placebo for the variable measured only once during the course of the study. The results were compared with the standard nominal alpha value of 0.05.

PK and PK/PD analysis

The measured individual plasma concentrations of zolpidem were used directly to obtain C_{MAX} and T_{MAX}. AUC was calculated according to the linear trapezoidal rule using the measured concentration-time values above the limit of quantification (1.00 ng/mL) and was extrapolated to infinity using log-linear regression of the terminal part of the curve. For the calculation of other PK parameters (clearance, absorption half-life, volume of distribution) and PK/PD modelling, non-linear mixed effect modelling as implemented in NONMEM [21] was performed. Non-linear mixed effect modelling allows the description of a population of individuals using a common structural model while allowing the individuals to vary. Estimates of location and spread between individuals are estimated for the model parameters. Using the population values, individual-specific empirical Bayes estimates can be determined that allow description of individual time profiles. Because the average placebo profile for VAS alertness and adaptive tracking showed a small diurnal change, the average placebo profile was subtracted from these data at corresponding protocol time points and the result was subject to PK/PD analysis. Body sway and EEG data were log-transformed. Empirical Bayes pharmacokinetics estimates were generated and used to describe the concentration profile for investigation of the PK/PD relationship between zolpidem and PD variables. Different linear and non-linear concentration-effect models were estimated to find the best fitting model to describe these data. Nested competing models were compared using the log-likelihood for the models (or minimum value of the objective function: MVOF). First-order condition estimation (foce) was used throughout, with additive (on log-scale for concentrations) residual error models.

RESULTS

Clinical observations

Thirteen of the 14 subjects who received zolpidem experienced at least one adverse event. The most frequently reported adverse events were dizziness (n=6), somnolence (n=5), coordination disturbance (n=4) and diplopia (n=4). One subject in the zolpidem group reported vivid visual pseudo-hallucinations, which are separately described in a case-report [22]. This subject was included in all group analyses. In the placebo group, only single cases of pyrexia and increased lipase were reported. No changes were observed in vital signs, ECG or laboratory safety parameters.

Pharmacodynamics

Eye Movements (Saccadic and Smooth Pursuit)

Zolpidem decreased saccadic peak velocity by 22.9 deg/sec compared to placebo (95%CI: -38.8, -7.0). Reaction time and accuracy were also diminished after zolpidem treatment (Table 1). Smooth pursuit decreased by 8.3% compared to placebo (95%CI: -12.2, -4.4).

Visual Analogue Scale

For VAS Bond&Lader, the VAS alertness scale was affected by zolpidem treatment with a decrease of 12.0 mm compared to placebo (95%CI: -20.1, -4.0). No effects were seen on the mood and calmness subscales. For the Bowdle VAS, only an increase in VAS 'feeling high' was seen, while no changes were detected in internal and external perception (Table 1).

Body Sway

Body sway increased with 261 mm after zolpidem treatment compared to placebo (95%CI: 160, 362).

Adaptive Tracking

Zolpidem influenced adaptive tracking performance with a decrease of 4.6% performance compared to placebo (95%CI: -6.8, -2.3). Standard deviation of performance was also decreased with 0.5% (95%CI: -0.8, -0.2).

Pharmaco-EEG

The EEG power bands that were affected by zolpidem treatment were alpha Pz-Oz (-1.31; 95%CI: -2.15, -0.47) and beta Fz-Cz (0.36; 95%CI: 0.12, 0.60) compared to placebo.

Cognitive Function Tests

Zolpidem had hardly any effect on memory, compared to placebo. Only a decrease in double words during delayed word recall was seen ($p = .021$; data not shown)

Pharmacokinetics and pharmacokinetic / pharmacodynamic (PK/PD) relationships

Under fasting conditions, zolpidem was absorbed rapidly (median T_{MAX} = 0.78 h; range 0.33–2.50 h). The (geometric) mean of C_{MAX} was 132.0 (range 59.2-136.0) ng/mL. Plasma concentrations decreased rapidly with a terminal half-life of 2.2 (range 1.2-3.1) h. The AUC 0-∞ was 422 (range 208-808) ng h/mL. Zolpidem pharmacokinetics were described using a one-compartment model with first- order absorption. Inclusion of a lag-time markedly improved the data description. Figure 1 shows the average concentration-time profile with the average NONMEM predicted profile superimposed. Results are presented in Table 2. In order to derive a plausible PK/PD-model, initially a linear plasma concentration-effect relationship was estimated. The results for almost all PD responses indicated that the peak effect was underestimated and the decline after the peak was too slow. Two other options for further modelling were explored: using a non-linear concentration-effect relationship and implementing a model describing acute tolerance development.

For all parameters, a sigmoid E_{MAX} concentration effect relationship was estimated which resulted in clear improvement of the data description. Table 3 shows the population parameters for this model. This model generally predicts that lower doses (e.g., 5 mg instead of the applied 10 mg) will have much smaller effects, although it has been reported that 5 mg zolpidem still result in noticeable effects [6,23].

The second option, a tolerance model, proved difficult to implement. The classic approach to acute tolerance is implementation of an extra compartment, which is assumed to interfere with the plasma concentration-effect relationship [24,25]. This compartment reflects a hypothesised metabolite that antagonises the effect of zolpidem or a situation in which receptors are (temporarily) inactivated after binding to the drug resulting in a reduction of binding sites. This model provides an empirical description and is therefore hypothetical, as the mechanism responsible for the acute tolerance development is rarely known exactly. Such a classic tolerance compartment did not lead to a clear improvement of the data description: induction of tolerance takes longer than can be described using such a model, but when it occurs it is quite pronounced. Since lag-times cannot be implemented in the differential equations used to describe such compartmental models, a number of intermediate compartments were included in the model

that incurred such a delay (Figure 2). Such a transit compartmental approach has been advocated before [26], and has found applications in modelling drug absorption [27] and in oncology [28]. The number of intermediate compartments was iteratively tested for some of the PD responses. Implementation of this tolerance model with 15 transit compartments provided a satisfactory description of the data for all PD parameters examined (Table 3).

Concentration-effect relationships were assumed to be linear and the increasing tolerance was assumed to follow a simple E_{MAX} model with full suppression at E_{MAX}. Figure 3 shows the observed and predicted data for VAS alertness using the tolerance model. The predicted time profile of the zolpidem concentrations and those of the hypothetical compound are shown in Figure 4.

For the SPV and the EEG alpha (Pz-Oz) power, data were more difficult to fit. Both the sigmoid E_{MAX} and transit tolerance model were able to describe the SPV data with a small improvement of fit compared to a linear model. For EEG (Pz-Oz) alpha power, there seemed to be a better fit for both the linear and sigmoid E_{MAX} model compared with the transit tolerance model. The concentration of the hypothetical tolerance metabolite was predicted to be low when SPV and EEG alpha Pz-Oz were modelled by the tolerance model, which indicated less tolerance than for the other parameters.

In order to visually indicate the difference in expected profiles between a sigmoid E_{MAX} model and the final tolerance model, simulations were performed for the effect profiles after administration of two doses of 5 mg separated by one hour. The effect after the second dose is predicted to be larger for the sigmoid E_{MAX} model and smaller for the tolerance model (Figure 5). Ultimately, such an experiment is required to arrive at a final conclusion regarding the adequacy of either model.

DISCUSSION

In this study the pharmacodynamics (PD) and pharmacokinetics (PK) of zolpidem 10 mg were assessed and related to each other in a PK/PD analysis. The study showed that zolpidem was quickly absorbed and affected many central nervous system (CNS) variables. PK/PD analysis resulted in two models to describe zolpidem's concentration-effect relationships.

Zolpidem has been developed as a selective hypnotic with higher affinity for the the α1-subtype $GABA_A$ receptor, which is believed to be associated with sedation. The lower affinity for the other subtypes was expected to cause fewer effects on CNS functions than are normally seen with non-selective agents such as benzodiazepines. However, the behavioural effects of zolpidem are generally similar to those of benzodiazepines [29]. The current study also showed that zolpidem affected most CNS functions, although the effects of the 10 mg dose seemed less intense than generally observed with hypnotic doses of non-selective benzodiazepines [11,14,16]. At first sight, these results seem to contradict a selective pharmacological profile of this compound. However, most adverse events of benzodiazepines or zolpidem can be either directly or indirectly influenced by α1-activation. Muscle relaxation has been attributed to the α2,3-subtype [30,31], but functions related to muscle tone such as postural stability or saccadic peak velocity may have been indirectly affected by sedation and lack of concentration. Memory impairment is linked to the α5-receptor subtype [32-34] but is also affected by attention [5,7,35-37]. The lack of a memory effect in our study could be related to the late assessment, around six hours after dosing.

Zolpidem has been investigated in several studies but PK/PD analyses are rarely described. In a previous study, an E_{MAX} model was used to describe the data, but the fit was less than optimal immediately after dosing and after the peak [10]. A study in rats used a sigmoid E_{MAX} model to describe the concentration-EEG effect relationship [38]. Our analysis showed two models describing the PK/PD relationship for zolpidem 10 mg, which are both different from the model previously published for the relationship in man. All pharmacodynamic effects increased rapidly and disappeared while concentrations were still present, which suggested a sigmoid E_{MAX} model or a tolerance model. Both models show a comparable fit for the 10 mg dose, but have different implications for a wider dose-effect range. The sigmoid E_{MAX} model is widely used in PK/PD modelling, and previous studies have shown that such models yield a proper fit of the concentration-EEG effect relationships for benzodiazepines [25,39] as well as zolpidem [38]. A typical characteristic of this model is the 'lack' of effects at lower concentrations. Figure 5 shows that the sigmoid E_{MAX} -model would predict very small effects of zolpidem after a single low dose of 5 mg. However, studies with 5 mg of zolpidem showed effects on SPV [6] and on VAS subscales [23]. In another study zolpidem 7.5 mg showed effects on alertness scales, EEG β-activity, the digit-symbol substitution test (DSST) and tapping speed [40]. Three other studies showed no effects after 5 mg [41-43]. Unfortunately, the results of these studies could not be accurately compared with predictions from our two PK/PD-models, as measurements differed and not enough quantitative data were reported in these publications. Considering the effects with low doses in some studies, however, the sigmoid E_{MAX} model is probably less accurate, although it may suffice to describe the PK/PD-relations for some CNS-effects (notably SPV- and EEG alpha reductions). The

sigmoid E_{MAX} model also predicts that the effects of zolpidem will surge with a second 5 mg dose after one hour, and such a repeated low-dose experiment would be required to examine the validity of the sigmoid E_{MAX} -model.

A tolerance model was also investigated. With a single 'classic' tolerance compartment, the PK/PD-model could not capture the relatively slow onset of the tolerance that was actually observed. The shift in time needed to accurately describe the development of tolerance resulted in the implementation of 15 serial tolerance compartments, to mimic the apparent delay in tolerance development. To our knowledge, the development of acute tolerance in man has not previously been described for zolpidem. Theoretically, acute tolerance could be due to (local) formation of an antagonistic metabolite or to acute receptor-site adaptation. Zolpidem has no identified active metabolites. Downregulation of $GABA_A$ receptors is known to be involved in tolerance development, and may therefore also play a role for zolpidem.

The development of tolerance is very well known after chronic benzodiazepine use [44]. It may play a role in the rebound effects, the loss of effects and the dependence that generally limit the prolonged use of benzodiazepines [45]. The literature is less clear on acute tolerance development with benzodiazepines. It has been described for different short-acting benzodiazepines on a range of CNS-effects [25,46-48]. Other studies did not report any tolerance for these compounds [12,13], but this could have been masked by the assessment of performance while plasma benzodiazepine concentrations are declining [49]. In general, the short-acting benzodiazepines, such as midazolam, have been found to produce more tolerance, dependence, withdrawal symptoms [50,51] and abuse liability [52] than the longer-acting benzodiazepines. It has been suggested that acute tolerance development might be the first step in the development of chronic tolerance [53]. Despite zolpidem's rapid onset, short duration of action and pronounced tolerance development, most studies in animals and humans show few indications for long-term adaptive adverse events [29,54-57]. This suggests that acute tolerance development by zolpidem is related to a particular molecular-pharmacological characteristic of this compound. Different mechanisms have been discussed in the literature [58]. It has been suggested that $GABA_A$ receptor ligands affect sensitivity, synthesis and degradation of $GABA_A$ receptors, or elicit uncoupling of the allosteric linkage between GABA and benzodiazepine sites [58]. On the longer run, alterations in mRNA synthesis may cause changes in $GABA_A$ receptor subunits, thereby reducing the sensitivity of the GABA receptor to GABA [58]. Our PK/PD-model indicates that zolpidem's pd effects are attenuated from two hours post-dose, and this raises the question which of these tolerance mechanisms best fits this relatively slow process. One study in rats showed that the number of binding sites for zolpidem was diminished 40 minutes after zolpidem intake while affinity for the receptor was not changed [59]. It was hypothesised that this decrease in binding sites was due to a degradation of the α1-subtype $GABA_A$ receptor, or that zolpidem binding induces a substitution of the α1-subtype by one of the other subunits [59]. The time course of these events is compatible with our PK/PD-tolerance model, but unfortunately most in vivo studies did not provide evidence of acute tolerance in rodents [60,61]. If the acute tolerance is based on one of the outlined molecular changes, the lack of chronic tolerance would imply that these changes are rapidly reversible after clearance of the drug, without affecting the response upon long-term use.

Our findings indicate that tolerance development can differ among the range of $GABA_A$-sensitive CNS-functions. Some functions showed more acute tolerance than others, and the time-scales also differed, These phenomena could be described by different PK/PD-models, reflecting more or less distinct underlying pharmacological or functional tolerance mechanisms. This is in line with the different receptor subtypes for which development of acute tolerance has been reported [62-65]. Acute tolerance could also be caused by functional adaptations of different post-receptor systems or brain networks. PK/PD-models help to quantitatively describe different tolerance phenomena, but preclinical research is required to further elucidate the underlying mechanisms.

In conclusion, zolpidem causes a quick rise in plasma levels that is associated with a rapid onset of PD effects. This rapid onset seems to be responsible for the main clinical benefit of this sleep-inducing hypnotic. The rapid decrease in PD effects, which can be described by a sigmoid E_{MAX} model or a tolerance model, might contribute to zolpidem's short duration of action. It is unknown which molecular or functional characteristics underlie these concentration-effect relationships. It seems these may differ among the various CNS effects, since not all CNS effects can be described by the same model. In order to determine the exact PK/PD model to describe zolpidem's concentration-effect relationship, a study with repeated lower doses of zolpidem should be performed.

Table 1 Pharmacodynamic differences in AUE 0-4 h relative to placebo for Saccadic Eye Movements, Smooth Pursuit Performance, Visual Analogue Scales, Body Sway, Adaptive Tracking and Pharmaco-EEG; ANOVA results are shown as contrasts (SE), 95% CI and p-value.

Variable	Effect Treatment - Placebo (SE)	95%CI		P-value
Saccadic Peak Velocity (deg/sec)	-22.9 (8.1)	-38.8	-7.00	0.007
Saccadic Reaction Time (sec)	0.01 (0.0)	0.01	0.01	0.01
Saccadic Accuracy (%)	0.9 (0.4)	0.07	1.7	0.04
Smooth Pursuit (%)	-8.3 (2.0)	-12.2	-4.4	0.0001
VAS Alertness (mm)	-12.0 (4.1)	-20.1	-4.0	0.005
VAS Mood (mm)	-4.0 (2.9)	-9.6	1.6	0.2
VAS Calmness (mm)	-5.0 (3.5)	-11.8	1.8	0.2
VAS Internal Perception (logmm)	0.07 (0.05)	-0.03	0.2	0.2
VAS External Perception (logmm)	0.09 (0.06)	-0.03	0.2	0.1
VAS "feeling high" (logmm)	0.3 (0.09)	0.1	0.5	0.002
Body Sway Eyes Closed (mm)	261 (51.49)	160	362	0.0000
Adaptive Tracking Performance (%)	-4.6 (1.15)	-6.8	-2.3	0.0002
SD of Tracking Performance (%)	-0.5 (0.15)	-0.8	-0.2	0.001
EEG α Pz-Oz (μV)	-1.31 (0.43)	-2.15	-0.47	0.0009
EEG β Fz-Cz (μV)	0.36 (0.12)	0.12	0.60	0.004

Table 2 Population PK parameters of zolpidem. Mean: population average; SEM: Standard error population mean; IIVAR: Inter-individual variability.

	Mean	SEM	IIVar
Clearance (L/min)	0.405	0.0459	42.1%
Absorption half-life (min)	10.5*	2.86	90.3%
Distribution volume (L)	63.7	4.87	26.2%
Lag-time (min)	16.9*	1.54	35.1%
Residual error (SD/Mean)	0.18	0.0164	

* Correlation Lag-time/Absorption half-life: 0.716

Table 3 Population parameters of PK/PD analysis for the Sigmoid E_{MAX} and transit tolerance model

	Log Body Sway* Mean (IIVar)	VAS Alertness Mean (IIVar)	Tracking Performance Mean (IIVar)	Smooth Pursuit* Mean (IIVar)	Saccadic Peak Velocity* Mean (IIVar)	Log EEG beta Fz-Cz* Mean (IIVar)	Log EEG alpha Pz-Oz* Mean (IIVar)
Sigmoid E_{MAX}							
Intercept	2.46 (6%)	2.72 (10.9)	-0.829 (3.3)	51.8 (21%)	503 (14%)	0.278 (36.5%)	0.712 (31%)
E_{MAX}	0.693 (60%)	-74.7 (0%)	-100 (fixed)	-51.8	-70.1 (34%)	0.256 (0%)	-0.283 (0.1%)
EC50 (ng/mL)	113 (0%)	149 35%)	604 (36%)	206 (31%)	96.8 (43%)	150 (0%)	108 (0%)
Hill factor	2.81 (54%)	3.42 (56%)	1.58 (20%)	1.59 (34%)	2.58 (0%)	2.15 (170%)	2.42 (84%)
Residual error SD	0.136	8.37	2.74	5.03	28	0.043	0.0834
Tolerance with 15 transit compartments							
Intercept	2.49 (6%)	3.22 (10.5)	-0.761 (3.4)	51.5 (22%)	505 (14%)	0.27 (38%)	0.719 (31%)
Slope	0.0031 (58%)	-0.191 (50%)	-0.0638 (65%)	-0.132 (50%)	-0.36 (54%)	0.00148 (78%)	-0.00123 (14%)
IC50 (ng/mL)	8.52 (0%)	23.7 (95%)	66 (0%)	64.4 (0%)	0.01 (1068%)	65.8 (109%)	200 (0%)
Transit Time (min)	190 (0%)	161 (29%)	120 (0%)	146 (40%)	1390 (0%)	44.2 (13.6%)	658 (0%)
Residual error SD	0.125	8.93	2.59	4.82	26.4	0.0352	0.0866

* Placebo results not subtracted

Figure 1 Average observed (stars) and predicted zolpidem plasma concentrations with SD error bars (n=14). A one-compartment model with first-order absorption and lag time is shown by the solid black line.

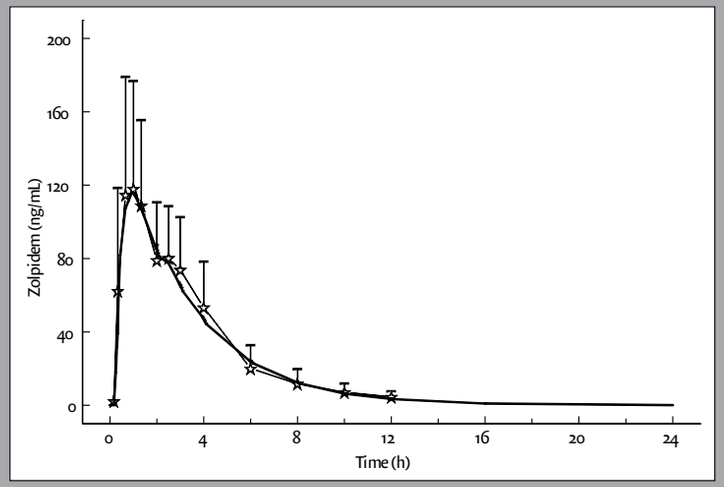


Figure 2 Pharmacokinetic/dynamic model with an interaction between the parent drug and a hypothetical drug in an additional tolerance compartment.

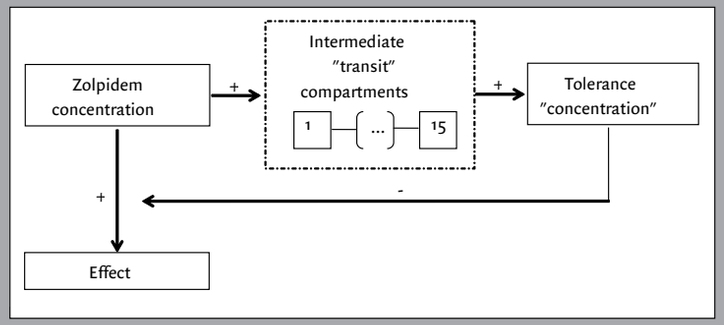


Figure 3 Average time profile (mean + SD) of VAS alertness (change from placebo) after oral administration of zolpidem 10 mg (stars) and the estimated effect-profile using a tolerance model with 15 intermediate transit compartments (solid black line).

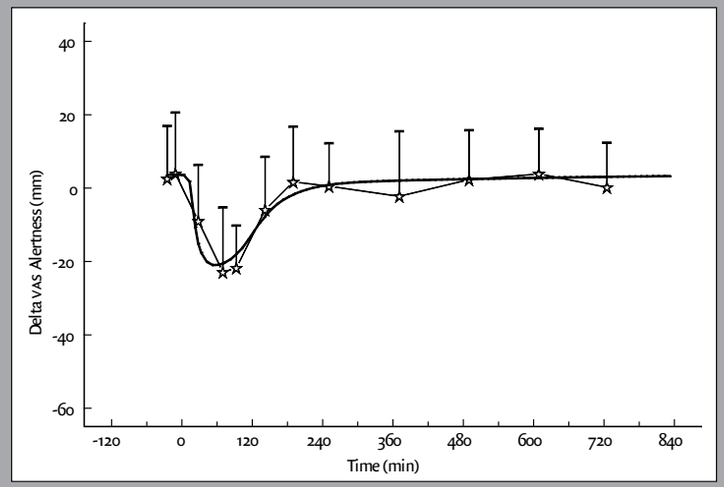


Figure 4 Average time profile (mean + SD) of predicted zolpidem plasma levels after oral administration of zolpidem 10 mg (stars) and the predicted concentration of the hypothetical antagonising metabolite in the transit tolerance model (solid black line) for the VAS alertness parameter.

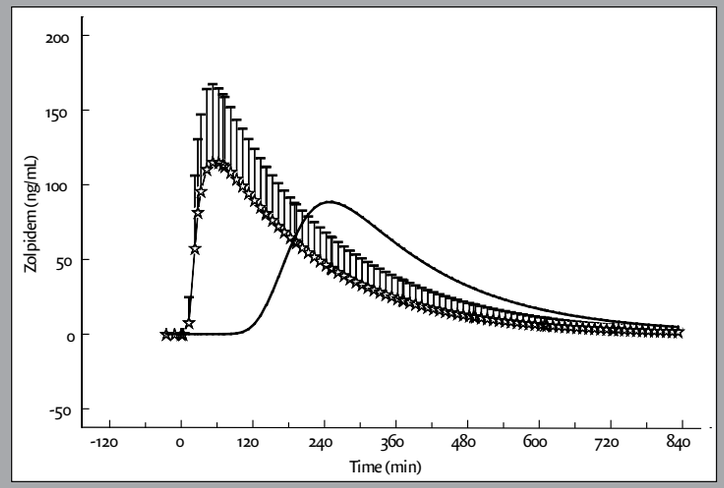
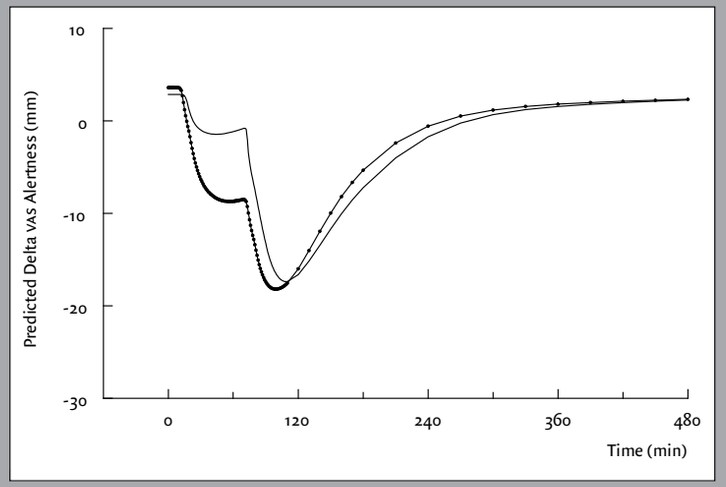


Figure 5 Average predicted VAS alertness values for two doses zolpidem 5 mg, separated by one hour, for a sigmoid E_{MAX} model (thin line) and a transit tolerance model (solid line).



REFERENCES

1 Morlock RJ, Tan M, Mitchell DY. Patient characteristics and patterns of drug use for sleep complaints in the United States: analysis of National Ambulatory Medical Survey data, 1997-2002. Clin Ther 2006; 28: 1044-1053.

2 Mohler H, Fritschy JM, Rudolph U. A new benzodiazepine pharmacology. J Pharmacol Exp Ther 2002; 300: 2-8.

3 Rowlett JK, Platt DM, Lelas S, Atack JR, Dawson GR. Different $GABA_A$ receptor subtypes mediate the anxiolytic, abuse-related, and motor effects of benzodiazepine-like drugs in primates. Proc Natl Acad Sci usa 2005; 102: 915-920.

4 Vermeeren A. Residual effects of hypnotics: epidemiology and clinical implications. CNS Drugs 2004; 18: 297-328.

5 Dingemanse J, Bury M, Bock J, Joubert P. Comparative pharmacodynamics of Ro 41-3696, a new hypnotic, and zolpidem after night-time administration to healthy subjects. Psychopharmacology (Berl) 1995; 122: 169-174.

6 Richens A, Mercer AJ, Jones DM, Griffiths A, Marshall RW. Effects of zolpidem on saccadic eye movements and psychomotor performance: a double-blind, placebo controlled study in healthy volunteers. Br J Clin Pharmacol 1993; 36: 61-65.

7 Mattila MJ, Vanakoski J, Kalska H, Seppala T. Effects of alcohol, zolpidem, and some other sedatives and hypnotics on human performance and memory. Pharmacol Biochem Behav 1998; 59: 917-923.

8 Patat A, Paty I, Hindmarch I. Pharmacodynamic profile of Zaleplon, a new non-benzodiazepine hypnotic agent. Hum Psychopharmacol 2001; 16: 369-392.

9 Drover DR. Comparative pharmacokinetics and pharmacodynamics of short-acting hypnosedatives : zaleplon, zolpidem and zopiclone. Clin Pharmacokinet 2004; 43: 227-238.

10 Drover D, Lemmens H, Naidu S, Cevallos W, Darwish M, Stanski D. Pharmacokinetics, pharmacodynamics, and relative pharmacokinetic/pharmacodynamic profiles of zaleplon and zolpidem. Clin Ther 2000; 22: 1443-1461.

11 van Steveninck AL, Schoemaker HC, Pieters MS, Kroon R, Breimer DD, Cohen AF. A comparison of the sensitivities of adaptive tracking, eye movement analysis and visual analog lines to the effects of incremental doses of temazepam in healthy volunteers. Clin Pharmacol Ther 1991; 50: 172-180.

12 van Steveninck AL, Verver S, Schoemaker HC, Pieters MS, Kroon R, Breimer DD, Cohen AF. Effects of temazepam on saccadic eye movements: concentration-effect relationships in individual volunteers. Clin Pharmacol Ther 1992; 52: 402-408.

13 van Steveninck AL, Gieschke R, Schoemaker RC, Roncari G, Tuk B, Pieters MS, Breimer DD, Cohen AF. Pharmacokinetic and pharmacodynamic interactions of bretazenil and diazepam with alcohol. Br J Clin Pharmacol 1996; 41: 565-573.

14 de Haas SL, de Visser SJ, van der Post JP, de smet M, Schoemaker RC, rijnbeek B, Cohen AF, Vega JM, Agrawal NG, Goel TV, Simpson RC, Pearson LK, Li S, Hesney M, Murphy MC, van Gerven MA. Pharmacodynamic and pharmacokinetic effects of TPA023, a $GABA_A$ {alpha}2,3 subtype-selective agonist, compared to lorazepam and placebo in healthy volunteers. J Psychopharmacol 2007; 21: 374-383.

15 Brisbare-Roch C, Dingemanse J, Koberstein R, Hoever P, Aissaoui H, Flores S, Mueller C, Nayler O, van Gerven J, de Haas SL, Hess P, Qiu C, Buchmann S, Scherz M, Weller T, Fischli W, Clozel M, Jenck F. Promotion of sleep by targeting the orexin system in rats, dogs and humans. Nat Med 2007; 13: 150-155.

16 de Haas SL, de Visser SJ, van der Post JP, Schoemaker RC, van Dyck K, Murphy MG, de smet M, Vessey LK, Ramakrishnan R, Xue L, Cohen AF, van Gerven JM. Pharmacodynamic and pharmacokinetic effects of MK-0343, a $GABA_A$ {alpha}2,3 subtype selective agonist, compared to lorazepam and placebo in healthy male volunteers. J Psychopharmacol 2008; 22: 24-32.

17 de Haas SL, Franson K, Schmitt JA, Cohen AF, Fau J-B, Dubruc C, van Gerven JMA. The pharmacokinetic and pharmacodynamic effects of SL65.1498, a GABA-A {alpha}2,3 selective agonist, in comparison with lorazepam in healthy volunteers. J Psychopharmacol 2008; (In Press).

18 Zuurman L, Roy C, Schoemaker RC, Hazekamp A, den Hartigh J, Bender J, Verpoorte R, Pinquier PL, Cohen AF, van Gerven JMA. Effect of intrapulmonary THC administration in humans. Submitted 2007.

19 Borland RG, Nicholson AN. Comparison of the residual effects of two benzodiazepines (nitrazepam and flurazepam hydrochloride) and pentobarbitone sodium on human performance. Br J Clin Pharmacol 1975; 2: 9-17.

20 Shah VP, Midha KK, Findlay JW, Hill HM, Hulse JD, McGilveray IJ, McKay G, Miller KJ, Patnaik RN, Powell ML, Tonelli A, Viswanathan CT, Yacobi A.

Bioanalytical method validation--a revisit with a decade of progress. Pharm Res 2000; 17: 1551-1557.
21 Version VI Users Guides (1989-2006). Beal, S. L., Sheiner, L. B., and Boeckmann, A. J. 1989. Ellicott City, Maryland, usa, Icon Development Solutions.
22 de Haas S, Dingemanse J, Hoever P, Cohen A, van Gerven J. Pseudohallucinations after zolpidem intake: a case report. J Clin Psychopharmacol 2007; 27: 728-730.
23 Rush CR, Madakasira S, Goldman NH, Woolverton WL, Rowlett JK. Discriminative stimulus effects of zolpidem in pentobarbital-trained subjects: II. Comparison with triazolam and caffeine in humans. J Pharmacol Exp Ther 1997; 280: 174-188.
24 Holford NH, Sheiner LB. Kinetics of pharmacologic response. Pharmacol Ther 1982; 16: 143-166.
25 Ihmsen H, Albrecht S, Hering W, Schuttler J, Schwilden H. Modelling acute tolerance to the EEG effect of two benzodiazepines. Br J Clin Pharmacol 2004; 57: 153-161.
26 Sun YN, Jusko WJ. Transit compartments versus gamma distribution function to model signal transduction processes in pharmacodynamics. J Pharm Sci 1998; 87: 732-737.
27 Yu LX, Amidon GL. A compartmental absorption and transit model for estimating oral drug absorption. Int J Pharm 1999; 186: 119-125.
28 Zandvliet AS, Siegel-Lakhai WS, Beijnen JH, Copalu W, Etienne-Grimaldi MC, Milano G, Schellens JH, Huitema AD. PK/PD Model of Indisulam and Capecitabine: Interaction Causes Excessive Myelosuppression. Clin Pharmacol Ther 2007.
29 Rush CR. Behavioral pharmacology of zolpidem relative to benzodiazepines: a review. Pharmacol Biochem Behav 1998; 61: 253-269.
30 Low K, Crestani F, Keist R, Benke D, Brunig I, Benson JA, Fritschy JM, Rulicke T, Bluethmann H, Mohler H, Rudolph U. Molecular and neuronal substrate for the selective attenuation of anxiety. Science 2000; 290: 131-134.
31 Rudolph U, Crestani F, Mohler H. GABA(A) receptor subtypes: dissecting their pharmacological functions. Trends Pharmacol Sci 2001; 22: 188-194.
32 Collinson N, Kuenzi FM, Jarolimek W, Maubach KA, Cothliff R, Sur C, Smith A, Otu FM, Howell O, Atack JR, McKernan RM, Seabrook GR, Dawson GR, Whiting PJ, Rosahl TW. Enhanced learning and memory and altered gabaergic synaptic transmission in mice lacking the alpha 5 subunit of the $GABA_A$ receptor. J Neurosci 2002; 22: 5572-5580.
33 Hopfenbeck JR, Cowley DS, Radant A, Greenblatt DJ, Roy-Byrne PP. Effects of diphenhydramine on human eye movements. Psychopharmacology (Berl) 1995; 118: 280-286.
34 Dawson GR, Maubach KA, Collinson N, Cobain M, Everitt BJ, MacLeod AM, Choudhury HI, McDonald LM, Pillai G, Rycroft W, Smith AJ, Sternfeld F, Tattersall FD, Wafford KA, Reynolds DS, Seabrook GR, Atack JR. An inverse agonist selective for alpha5 subunit-containing $GABA_A$ receptors enhances cognition. J Pharmacol Exp Ther 2006; 316: 1335-1345.
35 Lader MH. Limitations on the use of benzodiazepines in anxiety and insomnia: are they justified? Eur Neuropsychopharmacol 1999; 9 Suppl 6: S399-S405.
36 Hindmarch I, Patat A, Stanley N, Paty I, Rigney U. Residual effects of zaleplon and zolpidem following middle of the night administration five hours to one hour before awakening. Hum Psychopharmacol 2001; 16: 159-167.
37 Danjou P, Paty I, Fruncillo R, Worthington P, Unruh M, Cevallos W, Martin P. A comparison of the residual effects of zaleplon and zolpidem following administration 5 to 2 h before awakening. Br J Clin Pharmacol 1999; 48: 367-374.
38 Tuk B, van Gool T, Danhof M. Mechanism-based pharmacodynamic modeling of the interaction of midazolam, bretazenil, and zolpidem with ethanol. J Pharmacokinet Pharmacodyn 2002; 29: 235-250.
39 Danhof M, Mandema JW. Modelling of the pharmacodynamics and pharmacodynamic interactions of CNS active drugs. Int J Clin Pharmacol Ther Toxicol 1992; 30: 516-519.
40 Cysneiros RM, Farkas D, Harmatz JS, von Moltke LL, Greenblatt DJ. Pharmacokinetic and pharmacodynamic interactions between zolpidem and caffeine. Clin Pharmacol Ther 2007; 82: 54-62.
41 Rush CR, Griffiths RR. Zolpidem, triazolam, and temazepam: behavioral and subject-rated effects in normal volunteers. J Clin Psychopharmacol 1996; 16: 146-157.
42 Mintzer MZ, Frey JM, Yingling JE, Griffiths RR. Triazolam and zolpidem: a comparison of their psychomotor, cognitive, and subjective effects in healthy volunteers. Behav Pharmacol 1997; 8: 561-574.
43 Wesensten NJ, Balkin TJ, Belenky GL. Effects of daytime administration of zolpidem versus triazolam on memory. Eur J Clin Pharmacol 1995; 48: 115-122.
44 van Steveninck AL, Wallnofer AE, Schoemaker RC, Pieters MS, Danhof M, van Gerven JM, Cohen AF. A study of the effects of long-term use on individual sensitivity to temazepam and lorazepam in a clinical population. Br J Clin Pharmacol 1997; 44: 267-275.
45 Schweizer E, Rickels K. Benzodiazepine dependence and withdrawal: a review of the syndrome and its clinical management. Acta Psychiatr Scand Suppl 1998; 393: 95-101.
46 Coldwell SE, Kaufman E, Milgrom P, Kharasch ED, Chen P, Mautz D, Ramsay DS. Acute tolerance and reversal of the motor control effects of midazolam. Pharmacol Biochem Behav 1998; 59: 537-545.
47 Kroboth PD, Bertz RJ, Smith RB. Acute tolerance to triazolam during continuous and step infusions: estimation of the effect offset rate constant. J Pharmacol Exp Ther 1993; 264: 1047-1055.
48 Ellinwood EH, Jr., Nikaido AM, Heatherly DG, Bjornsson TD. Benzodiazepine pharmacodynamics: evidence for biophase rate limiting mechanisms. Psychopharmacology (Berl) 1987; 91: 168-174.
49 Laurijssens BE, Greenblatt DJ. Pharmacokinetic-pharmacodynamic relationships for benzodiazepines. Clin Pharmacokinet 1996; 30: 52-76.
50 Hallfors DD, Saxe L. The dependence potential of short half-life benzodiazepines: a meta-analysis. Am J Public Health 1993; 83: 1300-1304.
51 Rickels K, Schweizer E, Case WG, Greenblatt DJ. Long-term therapeutic use of benzodiazepines. I. Effects of abrupt discontinuation. Arch Gen Psychiatry 1990; 47: 899-907.
52 Victorri-Vigneau C, Dailly E, Veyrac G, Jolliet P. Evidence of zolpidem abuse and dependence: results of the French Centre for Evaluation and Information on Pharmacodependence (CEIP) network survey. Br J Clin Pharmacol 2007; 64: 198-209.
53 Kalant H, LeBlanc AE, Gibbins RJ. Tolerance to, and dependence on, some non-opiate psychotropic drugs. Pharmacol Rev 1971; 23: 135-191.
54 Griffiths RR, Sannerud CA, Ator NA, Brady JV. Zolpidem behavioral pharmacology in baboons: self-injection, discrimination, tolerance and withdrawal. J Pharmacol Exp Ther 1992; 260: 1199-1208.
55 Weerts EM, Griffiths RR. Zolpidem self-injection with concurrent physical dependence under conditions of long-term continuous availability in baboons. Behav Pharmacol 1998; 9: 285-297.
56 Soldatos CR, Dikeos DG, Whitehead A. Tolerance and rebound insomnia with rapidly eliminated hypnotics: a meta-analysis of sleep laboratory studies. Int Clin Psychopharmacol 1999; 14: 287-303.
57 Ware JC, Walsh JK, Scharf MB, Roehrs T, Roth T, Vogel GW. Minimal rebound insomnia after treatment with 10-mg zolpidem. Clin Neuropharmacol 1997; 20: 116-125.
58 Bateson AN. Basic pharmacologic mechanisms involved in benzodiazepine tolerance and withdrawal. Curr Pharm Des 2002; 8: 5-21.
59 Fahey JM, Grassi JM, Reddi JM, Greenblatt DJ. Acute zolpidem administration produces pharmacodynamic and receptor occupancy changes at similar doses. Pharmacol Biochem Behav 2006; 83: 21-27.
60 Elliot EE, White JM. Precipitated and spontaneous withdrawal following administration of lorazepam but not zolpidem. Pharmacol Biochem Behav 2000; 66: 361-369.
61 Perrault G, Morel E, Sanger DJ, Zivkovic B. Lack of tolerance and physical dependence upon repeated treatment with the novel hypnotic zolpidem. J Pharmacol Exp Ther 1992; 263: 298-303.
62 Holt RA, Bateson AN, Martin IL. Chronic treatment with diazepam or abecarnil differently affects the expression of $GABA_A$ receptor subunit mRNAs in the rat cortex. Neuropharmacology 1996; 35: 1457-1463.
63 van Rijnsoever C, Tauber M, Choulli MK, Keist R, Rudolph U, Mohler H, Fritschy JM, Crestani F. Requirement of alpha5-$GABA_A$ receptors for the development of tolerance to the sedative action of diazepam in mice. J Neurosci 2004; 24: 6785-6790.
64 Pericic D, Strac DS, Jembrek MJ, Vlainic J. Allosteric uncoupling and up-regulation of benzodiazepine and GABA recognition sites following chronic diazepam treatment of HEK 293 cells stably transfected with alpha1beta2gamma2S subunits of GABA (A) receptors. Naunyn Schmiedebergs Arch Pharmacol 2007; 375: 177-187.
65 Birzniece V, Turkmen S, Lindblad C, Zhu D, Johansson IM, Backstrom T, Wahlstrom G. GABA(A) receptor changes in acute allopregnanolone tolerance. Eur J Pharmacol 2006; 535: 125-134.

CHAPTER 6

Pseudo-hallucinations after zolpidem intake: A case report

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