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Biomarkers in early phase development of central nervous system drugs: a conceptual framework

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Biomarkers in early phase development of central nervous system drugs: *a conceptual framework*

**BIOMARKERS IN EARLY PHASE
DEVELOPMENT OF CENTRAL
NERVOUS SYSTEM DRUGS:
*A CONCEPTUAL FRAMEWORK***

PROEFSCHRIFT ter verkrijging van de graad van Doctor
aan de Universiteit Leiden, op gezag van de Rector
Magnificus Dr.D.D.Breimer, hoogleraar in de faculteit
der Wiskunde en Natuurwetenschappen en die der
Geneeskunde, volgens besluit van het College voor
Promoties te verdedigen op dinsdag 26 september 2006
klokke 15.00 uur door **J.-P. VAN DER POST**
geboren te Den Haag in 1967

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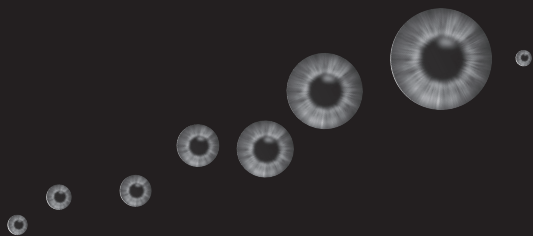
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OUTLINE OF THIS THESIS



Aim

The main objective of this thesis is to provide a conceptual framework for the use of central nervous system (CNS) biomarkers in early phase clinical drug development.

Outline

In the introduction the current use of biomarkers in early CNS drug development is discussed. A conceptual framework for the classification of biomarkers is suggested, based on general questions that these markers should provide information on. The body of this thesis (chapters 1-7) exemplifies the use of these markers within this conceptual framework.

Chapter 1 describes a study of the first administration in man of a potential drug with a novel mechanism of action (NAALADASE inhibition) and a relative uncertain therapeutic indication (e.g. neuropathic pain). Sensitive markers for functional domains were included in the test-battery to establish a general CNS-profile. In chapter 2, this approach was used to compare the effects of two drugs with a similar mechanism of action (sumatriptan and rizatriptan) relative to an active comparator of a different drug class (temazepam). Chapters 3 and 4 investigate the time course of central nervous system effects of centrally active anti-hypertensive agents (rilmenidine and moxonidine) in patients following prolonged treatment as estimated by sensitive markers for sedation.

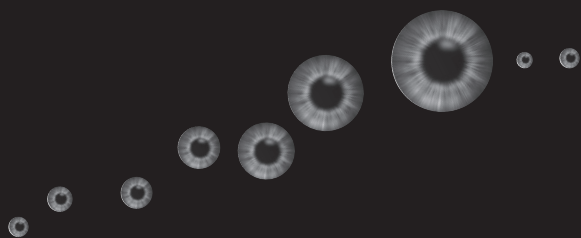
In the studies presented in chapters 1-4, each biomarker was studied separately, as a measure of the effects of a drug on a certain functional CNS-domain. It is possible that certain combinations of functionale CNS-effects characterize specific pharmacological actions. This possibility is explored in chapter 5, which investigates whether drug class specificity of sensitive markers can be enhanced using a composite of markers. A combination of effect scores on the some of the most commonly and reproducible CNS-biomarkers (saccadic peak velocity, visual analogue scales for alertness and electroencephalography) was determined from historical data from the Centre for Human Drug Research (CHDR) to examine the specificity of various composites of combined scores, for different drug classes.

This approach may lead to the identification of biomarker combinations that provide a “pharmacological footprint” for drugs or even a drug class. However, this use of composite biomarkers seems to have little face validity. In chapter 6, a potential model for cognitive impairment is explored, by exposing healthy volunteers to moderate levels of hypoxia. Hypoxia-induced cognitive impairment could be a useful model to show beneficial effects of (certain types of) cognitive enhancing agents, in the early phases of drug development.

Chapter 7 exemplifies the type of work needed for selection and validation of biomarkers for specific pharmacological (receptor-specific) activity. In this chapter, an attempt is made to validate a potential functional marker (eye-blinks) for in vivo central dopamine receptor activity. For this purpose, the effects of both a dopamine agonist and antagonist are investigated using eye blink rate and plasma prolactin levels as potential biomarkers for specific dopaminergic activity.

In the conclusions and discussion a critical evaluation of the presented conceptual biomarker framework is given and directions for future biomarker research are offered.

INTRODUCTION **BIOMARKERS IN EARLY PHASE DEVELOPMENT
OF CENTRAL NERVOUS SYSTEM DRUGS**



Background

The use of biomarkers in early phase development of central nervous system drugs seems challenged by the fact that a vast number of often poorly validated tests is being used as ill-defined biomarkers. Especially in the area of neuropsychological tests, the majority of tests are used sporadically or do not show a consistent treatment response, thus lacking basic test requirements such as reproducibility and sensitivity.

To date in early CNS drug development, the justification of a particular selection of tests still seems to lie mainly in its ability to show adverse effects. For older drugs with a small therapeutic range, this approach is useful since the maximum tolerated dose in healthy volunteers is often close to the dose that is both tolerable and effective in patients. For such drugs, the main focus of early phase drug development lies on evaluating safety and tolerability.

This situation is changing, or has been changing for a number of years. For novel CNS active agents the drug concentrations that are associated with pharmacological activity, therapeutic efficacy and adverse events are further apart. Due to the increased selectivity of novel CNS active drugs for receptors, neurotransmitters or even brain regions, the therapeutic range is broadened. The maximum tolerated dose in healthy volunteers can no longer be used as a reliable indicator for pharmacological activity and therapeutic efficacy.

This necessitates a new approach to CNS biomarkers in the early phases of drug development. There is an increasing need to include markers for the pharmacological activity of the drug and/or activity of the drug on disease related (patho)physiology, and wherever possible also markers for potential therapeutic effects. Indeed, it seems likely that biomarkers will play an increasingly important role in all phases of drug development, including regulatory review. It is unlikely, however, for many of these biomarkers to become established well enough to be used in regulatory decision making as surrogate endpoints, thereby substituting for traditional clinical endpoints.

It has been suggested that ideally the attributes of a biomarker should include the following*: clinical relevance, sensitivity and specificity to treatment effects, reliability, practicality and simplicity. Although based on these recommendations biomarkers can now be ordered according to their linkage to the disease or their clinical relevance, this does not form a conceptual framework for the selection of tests in early drug development. Many biomarkers may be closely linked to a particular disease in patients (e.g. memory testing related

Ref. 1

to dementia), but their relevance in early drug development in healthy volunteers may be entirely unknown (e.g. memory testing for cognitive enhancers). This apparent contradiction has contributed to the questionable reputation of CNS-biomarkers in early drug development – which is also fuelled by their vast number. Consequently, there is a need for a novel approach to biomarker selection and classification.

Objectives of biomarkers in early drug development

The main objective of CNS biomarkers in early drug development is that they provide reliable information that is of consequence to the development program, at the earliest possible time. In early CNS drug development, there are basically four general questions on which biomarkers can provide information:

1. Proof of CNS-penetration and functional CNS effects, which can be sub-divided into:
 - A. Biomarkers of CNS pharmacokinetics
 - B. Biomarkers of adverse effects
2. Proof of pharmacodynamic principle (biomarkers of mechanism of action)
3. Proof of pathophysiological principle (biomarkers of effects on relevant pathophysiological process)
4. Proof of therapeutic principle (biomarkers of therapeutic effects)

A single biomarker may provide information on more than one area.

Biomarkers of CNS penetration and functional CNS-effects

Currently, an overwhelming number of neuropsychological different tests (or tests variants) are being used by different research groups, often sporadically and with poor sensitivity. The majority of these tests can be classified as measures of ‘CNS-activity’. They are aimed at showing CNS penetration, or showing indications of adverse effects. Quite often, their interpretation is highly dependent on the experience of the investigator, and how the tests have previously been affected by other drugs. This poses problems for new drugs with innovative action mechanisms. The large number of CNS-tests also makes it impossible to identify ‘the best method’ with the most favourable test-characteristics, simply because suitable methods differ widely for each objective (CNS-penetration,

adverse event profiling, confirmation of action mechanism). It is better, therefore, to define the information that is required, and select the most accurate test that can provide this information. Information about 'CNS activity' can be divided into several primarily neurophysiological or neuropsychological domains.

Ref.2,3,4

This is illustrated by three recent literature reviews that evaluated biomarkers for neuroleptics, SSRI antidepressants and benzodiazepine anxiolytics.* In these reviews, the use of biomarkers in healthy volunteer studies was evaluated: 65 studies with neuroleptics, 56 with SSRI's and 56 with benzodiazepines, which reported the use of 101, 63 and 64 different neuropsychological tests, respectively. Only 15%, 20% and 32% of these tests were used five times or more. The percentages of tests that never showed any significant treatment effects were 48%, 54% and 29%, respectively. Despite the large number of tests, only a small number of primary cognitive domains were addressed (table 1).

Table 1 – p. 19

The domains which were most frequently affected by these psychotropic agents were 'attention', memory, visuomotor and motor functions. Only a few tests within these domains showed any drug effects. The majority did not provide meaningful information about the drug. A CNS-test-battery to quantitate 'CNS-activity' should at least cover these four domains. Within each domain, the method(s) with the best test characteristics should be used. These properties include not only basic methodological features like sensitivity and reproducibility, but also properties that are needed in early drug development, like repeatability (to show relationships to changing plasma concentrations) and responsiveness to therapeutic drug levels. These ideal methods have not yet been identified, although the reviews provide some indications, showing that a rational choice can start with gathering of available experience from the literature and among psychopharmacological research groups. This should lead to more uniform test batteries, which will be of great benefit to the development of novel psychoactive drugs.

Biomarkers for pharmacological effects

These biomarkers are expected to show a response to a specific pharmacodynamic effect of a CNS active drug. In early drug development there are only a very small number of validated biomarkers that provide information on pharmacodynamic CNS effects of drugs. Potentially these biomarkers could be valuable at the early stages of drug development, especially if its pharmacodynamic mechanisms of action in vivo are

not elucidated. These biomarkers then provide a pharmacological proof of principle for novel CNS drugs. This aim requires the marker to be as specific as possible to a certain pharmacodynamic pathway, as well as sensitive.

In the area of neuropsychological tests, no test can be mentioned that demonstrates a specific pharmacological effect. Most tests can be affected by a number of drugs with very different mechanisms of action (SSRI's, benzodiazepines and neuroleptics all similarly affect attention tasks). As mentioned, these tests at best show that a drug affects a certain neuropsychological domain, regardless of the pharmacological pathway of the drug. This is largely due to the fact that several functional neurological (neurotransmitter) systems must be orchestrated in order to perform on a neuropsychological test.

There are a few neuro-endocrine biomarkers that are able to indicate, with reasonable sensitivity and specificity, a pharmacodynamic mechanism of action. Plasma prolactin for example has been used frequently in drug research to demonstrate a D₂-receptor antagonist effect of drugs. This aided considerably in the development of many neuroleptic drugs, for which D₂-receptor antagonistic activity was an important pharmacodynamic effect and a predictor of therapeutic efficacy. Another example is the plasma cortisol response to serotonergic drugs. Although the specificity of the cortisol response to 5HT-receptor subtype agonism is not completely elucidated, it does demonstrate a 5HT-receptor agonistic drug effect.

An important limitation of these biomarkers is that the physiological activity of some neuro-endocrine systems is so low that a pharmacological effect is indiscernible. Furthermore, more subtle pharmacodynamic effects like those of modern neuro-modulatory drugs will only become apparent in neuro-endocrine systems with an abnormal activity. A way of enhancing the sensitivity of neuro-endocrine tests is by challenging the involved neurophysiological system, creating an elevated activity. These challenges can be pharmacological (MCPD or 5HT as a serotonin challenge) or physical (heat stress for cortisol).

Ref. 5

A relatively novel approach is the use of functional MRI (fMRI).* The blood oxygen level-dependent (BOLD) signal measured by fMRI is determined by local changes in the ratio of oxygenated to deoxygenated haemoglobin, resulting from increased blood flow in response to neural activity that is not matched proportionately by locally increased oxygen consumption. The pharmacological MRI (phMRI) involves measuring drug related changes in the BOLD signal. In humans, fMRI measurements are usually made while the subject is performing a cognitive task.

Therefore, the results of pHMRI potentially provide information about the pharmacodynamics of the drug ('neurocognitive profiling') and the transmitter mechanisms that normally underpin the coherent and adaptive activation of large-scale neurocognitive systems activated by task performance. However, this approach has important limitations. Drug effects on the BOLD signal could be mediated by effects on cerebral vasoactivity rather than direct effects on neurons. Often, changes in the BOLD response occur in the absence of behavioural changes, which raises questions about the significance of these findings. It seems that pHMRI can provide useful pharmacological information, but only when combined with neurophysiologic, neuroendocrine or neurocognitive tests.

Biomarkers for (patho)physiological principle

Many CNS-diseases can be viewed as derangements of normal physiological systems. Effects of a new drug on relevant system in healthy volunteers may also provide evidence that drug may correct pathophysiology in patients. For example, augmentation of serotonergic neurotransmission is associated with increased memory consolidation, which may be relevant to its therapeutic and cognitive actions in acutely depressed patients, which often show memory impairment.* In another study,* short-term administration of two different antidepressant types had similar effects on emotion-related tasks in healthy volunteers, reducing the processing of negative relative to positive emotional material. Such effects of antidepressants may ameliorate the negative biases in information processing that characterize mood and anxiety disorders. They also suggest a mechanism of action potentially compatible with cognitive theories of anxiety and depression.

Ref. 6

Ref. 7

As for most CNS (especially psychiatric) diseases no clear pathophysiological concept is known, the use of this type of biomarker is severely hindered.

Biomarkers for therapeutic principle

To state the obvious, diseases are not present in healthy volunteers. However, the use of 'subsyndromal' patient populations in early drug development may aid in the development of CNS drugs. This seems particularly relevant for psychiatric diseases, where no clear dichotomy

between healthy and disease states seems present. The clinical definition of psychiatric diseases such as affective and anxiety disorders are largely phenomenological and clear anatomical or functional distinguishing characteristics are rarely present. Although the use of ‘subsyndromal’ patient populations is not often used in phase one drug development, some studies in patients with ‘minor depression’ show a favourable treatment response to SSRI’s.*

Ref. 8

More often, biomarkers for therapeutic activity are evaluated in a ‘disease model’, in which a disease-like state is induced in healthy volunteers. In the development of most CNS drugs animal disease models are used to obtain an indication of therapeutic activity. The predictive quality of these models for therapeutic efficacy in humans is often very limited. Only a few disease models have been used in human pharmacology. In the development of cognitive enhancers two models have been used frequently: induced cerebral hypoxia* and scopolamine induced amnesia.* For modelling affective disorders, tryptophan depletion produces clear mood lowering effects.* For many CNS diseases no model is available in humans. Again, in the use of disease models, an important limitation is the lack of understanding of pathophysiological pathways in many of the CNS diseases.

Ref. 9, 10

Ref. 11, 12, 13, 14, 15, 16

Ref. 17

Conclusions

With the above mentioned limitations, is there any benefit in using biomarkers in early central nervous system drug development? Despite these limitations, different biomarker classes can contribute to early proof of different development principles. A necessary first step is to realise what a specific biomarker actually provides information on. The presented division into classes of biomarkers should present a conceptual framework to achieve this. Within each biomarker class, the most useful biomarkers should be identified. This is particularly relevant for the biomarkers for functional CNS-effects, where an overwhelming number of tests are currently used. Here, there is a great need for more uniform practical, sensitive and specific test batteries. Improved understanding of pathogenesis of CNS-diseases will hopefully lead to more useful biomarkers for pathophysiological and therapeutic activity. It will probably also lead to a mutual substitution of pharmacological, pathophysiological and therapeutic biomarkers, reducing the number of tests and increasing their value in early central nervous system drug development.

REFERENCES

- 1 Lesko LJ, Atkinson AJ, Jr. Use Of Biomarkers And Surrogate Endpoints In Drug Development And Regulatory Decision Making: Criteria, Validation, Strategies. *Annu.rev.Pharmacol.Toxicol.* 2001; 41: 347-366.
- 2 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.
- 3 De Visser SJ, Van Der PJ, Pieters MS, Cohen AF, Van Gerven JM. Biomarkers For The Effects Of Antipsychotic Drugs In Healthy Volunteers. *Br.J.Clin. Pharmacol.* 2001; 51:119-132.
- 4 Rijnbeek B, De Visser SJ, Franson KL, Cohen AF, Van Gerven JM. REM Sleep Effects As A Biomarker For The Effects Of Antidepressants In Healthy Volunteers. *J.Psychopharmacol.* 2003; 17:196-203.
- 5 Honey G, Bullmore E. Human Pharmacological MRI. *Trends Pharmacol.Sci.* 2004; 25:366-374.
- 6 Harmer CJ, Bhagwagar Z, Cowen PJ, Goodwin GM. Acute Administration Of Citalopram Facilitates Memory Consolidation In Healthy Volunteers. *Psychopharmacology (Berl)* 2002; 163:106-110.
- 7 Harmer CJ, Shelley NC, Cowen PJ, Goodwin GM. Increased Positive Versus Negative Affective Perception And Memory In Healthy Volunteers Following Selective Serotonin And Norepinephrine Reuptake Inhibition. *Am.J.Psychiatry* 2004; 161: 1256-1263.
- 8 Van Steveninck AL, Mandema JW, Tuk B *et al.* 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.
- 9 Saletu B, Grünberger J, Anderer P, Linzmayer L, König P. On The Cerebro-protective Effects Of Caroverine, A Calcium-channel Blocker And Antiglutaminergic Drug: Double-blind, Placebo-controlled, EEG-mapping And Psychometric Studies Under Hypoxia. *Brit.J.Clin. Pharmacol.* 1996; 41:89-99.
- 10 Van Der Post JP, Noordzij LA, De Kam ML, Blauw GJ, Cohen AF, Van Gerven JM. Evaluation Of Tests Of Central Nervous System Performance After Hypoxemia For A Model For Cognitive Impairment. *J.Psychopharmacol.* 2002; 16:337-343.
- 11 Broks P, Preston GC, Traub M, Poppleton P, Ward C, Stahl SM. Modelling Dementia: Effects Of Scopolamine On Memory And Attention. *Neuropsychologia* 1988; 26:685-700.
- 12 Broocks A, Little JT, Martin A *et al.* The Influence Of Ondansetron And M-chlorophenylpiperazine On Scopolamine-induced Cognitive, Behavioral, And Physiological Responses In Young Healthy Controls. *Biol.Psychiatry* 1998; 43:408-416.
- 13 Gilles C, De Buyl O, Genevrois C, Salama M, Mendlewicz J. Specificity Of Visual Evoked Potentials Alterations In Alzheimer's Disease: Comparison With Normal Aging, Depression And Scopolamine Administration In Young Healthy Volunteers. *Acta Neurol.Belg.* 1989; 89:226-227.
- 14 Mauri M, Sinforiani E, Reverberi F, Merlo P, Bono G. Pramiracetam Effects On Scopolamine-induced Amnesia In Healthy Volunteers. *Arch.Gerontol.Geriatr.* 1994; 18:133-139.
- 15 Preda L, Alberoni M, Bressi S *et al.* Effects Of Acute Doses Of Oxiracetam In The Scopolamine Model Of Human Amnesia. *Psychopharmacology (Berl)* 1993; 110:421-426.
- 16 Riedel W, Hogervorst E, Lebox R, Verhey F, Van Praag H, Jolles J. Caffeine Attenuates Scopolamine-induced Memory Impairment In Humans. *Psychopharmacology (Berl)* 1995; 122:158-168.
- 17 Van Der Does AJ. The Mood-lowering Effect Of Tryptophan Depletion: Possible Explanation For Discrepant Findings. *Arch.Gen.Psychiatry* 2001; 58:200-202.

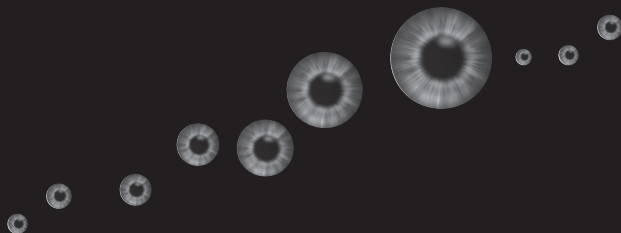
TABLE 1 **Number of tests within individual domains**

Neuropsychological domains	Neuroleptics	Selective Serotonin Re-uptake Inhibitors	Benzodiazepines
Achievement	4	0	1
Executive	15	6	11
Attention	24	14	15
Memory	21	9	20
Language	4	0	0
Visual, visuomotor and auditory	25	28	23
Motor	8	6	4

CHAPTER 1

**THE CENTRAL NERVOUS SYSTEM EFFECTS,
PHARMACOKINETICS AND SAFETY OF THE
NAALADASE-INHIBITOR GPI 5693**

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Abstract

AIM The aim was to assess the central nervous system (CNS) effects, pharmacokinetics and safety of GPI 5693, an inhibitor of a novel CNS-drug target, NAALADase which is being evaluated for the treatment of neuropathic pain.

METHODS This was a double-blind, placebo-controlled, exploratory study in healthy subjects receiving oral GPI 5693 single ascending doses of 100, 300, 750, 1125 mg with a placebo treatment randomly interspersed. An open-label, parallel extension examined the effects of food and sex on the pharmacokinetics of 750, 1125 and 1500 mg doses. Blood samples were collected for pharmacokinetic and biochemical/haematological safety analysis, vital signs, ECG and adverse event checks were performed regularly up to 48 h postdose. Postdose CNS effects were assessed using eye movements, adaptive tracking, electroencephalography (EEG), body sway and Visual Analogue Scales (VAS).

RESULTS CNS effects were mainly observed after the 1125 mg dose, showing a significant decrease of adaptive tracking performance, VAS alertness and VAS mood, and an increase of EEG occipital alpha and theta power. Gastro-intestinal (GI) adverse effects were frequent at higher doses. No clinically significant changes in vital signs or ECG were noted during any of the treatments. The therapeutically relevant concentration range (950–11 100 ng ml⁻¹) as determined from animal experiments was already reached after the 300 mg dose. C_{MAX} after the 300 mg and 750 mg dose was 2868 and 9266 ng ml⁻¹ with a t_{1/2} of 2.54 and 4.78 h, respectively. Concomitant food intake (with the 750 mg and 1125 mg doses) reduced C_{MAX} by approximately 66% and AUC by approximately 40%. With concomitant food intake, the dose-normalized C_{MAX} also decreased significantly by -5.6 (CI: -2.6 to -8.7) ng ml⁻¹ mg⁻¹. The pharmacokinetic variability was largest after the 300 mg and 750 mg dose, resulting in a SD of approximately 50% of the C_{MAX}.

CONCLUSION NAALADase inhibition with GPI 5693 was safe and tolerable in healthy subjects. Plasma concentrations that were effective in the reversal of hyperalgesia in the chronic constrictive injury animal model of neuropathic pain were obtained at doses of 300, 750 and 1125 mg in the fasted state. Concomitant food intake reduced C_{MAX} and AUC. CNS effects and GI AEs increased in incidence over placebo only at the 1125 mg dose.

Introduction

NAALADase (also termed glutamate carboxypeptidase II) is a membrane bound metalloprotease that hydrolyses the neuropeptide N-acetyl-aspartyl glutamate (NAAG) to N-acetyl-aspartate and glutamate. NAAG acts as an agonist at group II metabotropic glutamate receptors, and is also a mixed agonist/antagonist at the NMDA glutamate receptor. The functional relevance of NAALADase is unknown, but it is thought both to terminate the neurotransmitter activity of NAAG and to liberate glutamate from NAAG, which subsequently acts at the various glutamate receptor subtypes. NAALADase inhibition is thus primarily expected to cause indirect antiglutamatergic effects.*

Ref 1

GPI 5693 ((R,S)-2-(3-mercaptopropyl) pentanedioic acid) is an orally bioavailable, selective and potent NAALADase inhibitor.* Rat models of peripheral neuropathy suggested that daily oral administration of NAALADase inhibitors can reduce neuropathic pain signs and simultaneously reduce the rate of decline in nerve conduction velocity.* Pathology results indicate that NAALADase inhibition also protects against diabetic-induced axonal atrophy and Wallerian degeneration. These data suggest a potential use for GPI 5693 as a treatment for neuropathic pain as well as retarding the progression of diabetic neuropathy in patients. The indirect antiglutamatergic effects of NAALADase inhibition in the central nervous system (CNS) also suggest a role for NAALADase inhibition in a host of diseases where glutamate may be involved,* but this has not yet been studied extensively.

Ref 2

Ref 3

Ref 4, 5

Since the physiological role of NAALADASE is unknown, potential CNS effects can only be predicted indirectly, e.g. from the various known effects of inhibitors of excitatory amino acids on the CNS. Currently, no behavioural toxicity has actually been observed in animal experiments. Since it may be possible to detect subtle CNS effects in humans that might not be observed in animals, it is important to carefully study the potential CNS effects of NAALADASE-inhibition. Also, observed CNS effects may provide indications of the functional relevance of NAALADase inhibition, and provide methods to quantify these effects in future studies.

Ref 6, 7

Drugs affecting glutamatergic neurotransmission can cause a general depression of CNS activity, including sedation.* It is essential to know if significant CNS depression occurs, since this may limit applicability of the compound during prolonged treatment of neuropathy. There are many different methods to quantify CNS depression, and saccadic peak velocity is one of the most sensitive parameters.* Effects on motor performance could also occur with inhibitors of excitatory neurotransmitters. Different

Ref 8, 9

Ref 10 motor effects can be quantified using smooth pursuit eye movements, adaptive tracking and body sway.*

Some antiglutamatergic drugs have psychotomimetic properties. The illicit drug PCP ('angel's dust') and the related anaesthetic agent ketamine are direct NMDA receptor antagonists with psychotropic effects. It is unknown whether these effects are related to glutamatergic inhibition, but the subjective effects of subanaesthetic doses of ketamine can be quantified and related to central and peripheral drug concentrations, using specific questionnaires and visual analogue scales.* Although GPI 5693 does not possess direct NMDA-antagonist activity, potential psychotomimetic effects were evaluated for safety.

Ref 11, 12 In theory, the clinical effects of glutamatergic modulation could be measured most sensitively in the realms of attention, motor function and subjective changes. In addition, the pharmaco-electro-encephalogram (EEG) provides a sensitive general pharmacodynamic parameter of CNS penetration and activity.*

Ref 13 Glutamatergic agents have caused concentration-related changes in EEG-activity in healthy subjects.*

Ref 14

This study represents the first administration of a NAALADase inhibitor in humans. It aimed to describe the pharmacokinetics and safety of GPI 5693, and to determine the central nervous system effects of NAALADase inhibition concentrations that showed efficacy in neuropathic animal models.

Methods

After approval of the protocol by the Ethical Review Board of the Leiden University Medical Centre, 25 healthy male and female subjects (age 18–45 years) were asked to participate in this exploratory study. After giving written informed consent, subjects received a full medical examination before entering the study, including a pregnancy test for female participants of childbearing potential. Alcohol and xanthine use was restricted to two units per day, from two days prior to the study day. Furthermore, subjects refrained from alcohol, caffeine and xanthine containing foods and beverages 12 h prior to the study days and during the study days. Female subjects were instructed to use barrier contraceptives. Concomitant medication other than paracetamol was not permitted during the study period.

This was a double-blinded, partially randomized, placebo-controlled, ascending dose study of GPI 5693, with minimum washout periods of 48 h. The study aimed to reach a maximum concentration (C_{MAX}) and

area under the concentration curve (AUC) at the concentrations showing reversal of hyperalgesia in experimental animal models.

The study was performed in panels, with interim analyses of pharmacokinetics and safety to adapt the design of consecutive panels. Four male subjects participating in panel 1 received fasting oral GPI 5693 doses of 100 mg, 300 mg and 750 mg, with a placebo occasion randomly inserted. A fifth open-label study day was added to this panel to investigate the effects of food on the 750 mg dose. After an interim analysis, the selected doses for panel 2 (which also consisted of four male subjects) were 300 mg, 750 mg, 1125 mg and placebo. Interim analyses of the first two panels provided indications for a food effect on GPI 5693 pharmacokinetics. The safety and tolerability of the drug in men allowed inclusion of women in a third open-label panel, investigating the tolerability and pharmacokinetics after single oral doses of 1125 and 1500 mg in fed state for both sexes. No CNS effects were measured during panel 3 which consisted of six female and six male subjects.

The eye-movement recording, adaptive tracking, body sway and Visual Analogue Scales (VAS) were practised before the first study day to reduce learning effects during the study. GPI 5693 was administered as 100 mg and 375 mg capsules after overnight fasting. During all occasions of the first two panels without food interaction, EEG, eye movements, adaptive tracking, body sway and VAS were measured at baseline and at 30 min intervals for the first 2 h, and with increasing intervals until 8 h after drug administration. ECG, vital signs and adverse events were checked regularly and continuous two-lead ECG monitoring was performed for 4 h after drug administration. In the 24–48-h period after dosing, a fecal sample was taken to check for occult blood. Blood sampling for haematological and biochemical parameters was done at baseline and 24 h after dosing. Pharmacokinetic sampling was performed every 10 min for the first hour and with increasing intervals until 48 h after drug administration. GPI 5693 was measured by means of LC/MS/MS. Plasma GPI 5693 concentrations were linear from 1 to 2000 ng ml⁻¹ and urine GPI 5693 concentrations were linear from 10 to 2000 ng ml⁻¹. The average within assay CV was 2.5% and the average between assay CV was 1.6%.

Subjects remained at the study site until 24 h after each dosing. A poststudy check-up took place 48 h after their last study day including final follow-up of any adverse effects.

Electroencephalograms were recorded and analysed using CED software (Cambridge Electronics Design, Cambridge, UK), as described previously.* EEG recordings were made using silver-silver chloride electrodes, fixed with collodion at Fz, Cz, Pz and Oz, with the same common

Ref 10

ground electrode as for the eye movement registration (international 10/20 system). The electrode resistances were kept below 5 kOhm. All recordings were done with the subjects' eyes closed. EEG signals were obtained from leads Fz-Cz and Pz-Oz. The signals were amplified by use of a Nihon Kohden AB-621G bioelectric amplifier (Nihon Kohden Corporation, Tokyo, Japan) with a time constant of 0.3 s and a low pass filter at 100 Hz. Per session eight consecutive blocks of 8 s were recorded over a 2-min period. The sampling rate was 1024 Hz. Datablocks containing artefacts were identified by visual inspection and these were excluded from analysis. Fast Fourier transform analysis was performed to obtain the sum of amplitudes in the delta- (0.5–3.5 Hz), theta- (3.5–7.5 Hz), alpha- (7.5–11.5 Hz) and beta (11.5–30 Hz) frequency ranges. The total recording bandwidth was 0–50 Hz.

Ref 15 Recording and analysis of saccadic and smooth pursuit eye movements were done with a microcomputerbased system.* The equipment used for stimulus display, signal collection and amplification was from Nihon Kohden (Nihon Kohden Corporation, Tokyo, Japan). Saccadic eye movements were recorded for stimulus amplitudes of 15 degrees to either side. Interstimulus intervals varied randomly between 3 and 6 s, and 15 saccades were recorded. The average values of saccadic peak velocity, latency (reaction time) and inaccuracy were used as parameters. For smooth pursuit eye movements the target moved sinusoidal at frequencies range from 0.3 to 1.1 Hz, increasing by steps of 0.1 Hz. The amplitude of target displacement corresponds to 20 degrees eyeball rotations to both sides. Four cycles were recorded for each stimulus frequency.

Ref 16 The adaptive tracking test was performed as originally described by Borland and Nicholson,* using customized equipment and software (Hobbs, 2000, Hertfordshire, UK). The average performance and the standard deviation of scores over a 10-min period were used for analysis. Adaptive tracking is a pursuit tracking task. A circle moves randomly about a screen. The subject is instructed to 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. Performance was scored after a fixed 10-min period. The adaptive tracking test is more sensitive to impairment of eye–hand coordination by drugs than compensatory pursuit tasks or other pursuit tracking tasks, such as the pursuit rotor. The adaptive tracking test has proven to be useful for measurement of CNS effects of alcohol,* various psychoactive drugs and sleep deprivation.*

Ref 9

Ref 10

Ref 17

The body sway meter allows measurement of body movements in a single plane, providing a measure of postural stability. Body sway was measured with an apparatus similar to the Wright ataxiometer.* With a string attached to the waist, all body movements over a period of time were integrated and expressed as mm sway on a digital display. The contribution of vision to postural control was eliminated by asking subjects to close their eyes. Subjects were instructed to wear the same pair of comfortable, low-heeled shoes on each session. Before starting a measurement, subjects were asked to stand still and comfortable, with their feet approximately 10 cm apart and their hands in a relaxed position alongside the body. Subjects were not allowed to talk during the measurement. The total period of body-sway measurement was 2 min.

Ref 18

Visual analogue scales as originally described by Norris* were used to quantify subjective effects. From these measurements, three factors were derived as described by Bond and Lader,* corresponding to alertness, mood and calmness. Psychedelic effects were monitored by visual analogue scales, translated from scales described by Bowdle *et al.** since no validated version was available for the Dutch language and population.

Ref 19

Ref 20

All repeatedly measured dynamic variables were characterized using maximum change from pretreatment value (E_{MAX}) and using areas under the effect curve (AUECs). These AUECs were calculated using the linear trapezoidal rule and were divided by the corresponding time span resulting in a weighted average outcome. Nominal times were used. AUECs were calculated over the entire sampling period (12 h) and over the first 6 hrs, because most of the response is expected to occur during this period.

Descriptive statistics were obtained for each pharmacodynamic parameter. Time course as well as derived parameters were summarized (using means, standard deviation (SD), min, median, max, n). Derived pharmacodynamic parameters were analysed untransformed. EEG parameters were analysed as percentage change from prevalue. These parameters were analysed, from the fasted occasions only, using analysis of variance (ANOVA) accounting for subject and treatment.

Dose-independent parameters (clearance, $C_{MAX}/dose$, $AUC_{0-inf}/dose$ and T_{MAX}) were compared between the fasting and fed occasions using analysis of covariance with food and subject as factors, dose as covariate and including the dose–food interaction. This resulted in a predicted linear relationship between dose and the pharmacokinetic (PK) parameter for fed and fasted separately. This line was depicted in graphs of the individual PK parameters (clearance, $C_{MAX}/dose$, $AUC_{0-inf}/dose$

and T_{MAX}) plotted against dose, to visualize both dose dependency and potential differences between fasted and fed occasions. The line was calculated using the reported least square means (at the average administered dose: 907.5 mg) along with the slopes on dose for the two groups (fed/fasted). In absence of a significant dose by food interaction, the ANOVA model was recalculated assuming the interaction to be absent and in that case the resulting (parallel) lines are shown in the graphs.

Statistical calculations were performed using SPSS for Windows (SPSS, Inc., Chicago, IL) and SAS for Windows v8.1 (SAS Institute, Inc., Cary, NC, USA).

The pharmacokinetic parameters were calculated by standard noncompartmental analysis using the software package WinNonlin V3.1 (Pharsight, Inc., Mountainview, CA, USA).

Results

A total of 25 subjects were screened for this study. Twenty-one subjects received at least one dose of study medication: four males in panel 1, five males in panel 2 and 12 (six males, six females) in panel 3. One subject dropped out after the first dose of study medication in panel 2 for reasons unrelated to the study. All subjects who received at least one dose of study medication were included in the safety analysis. Subjects who participated in panel 1 (occasion 1–4) and panel 2 were included in the pharmacodynamic (CNS effect) and pharmacokinetic analysis if they had received at least one dose of study medication. The average (SD) weight of the participating subjects was 71 (10) kg, the height 178 (9) cm and age 23 (3) years.

The incidence of all adverse experiences judged as ‘treatment related’ are summarized in table 1. Most of the treatment-related adverse events occurred in the WHO body system ‘gastro-intestinal disorders’. After 53 administrations of active drug, 20 (38%) treatment related gastro-intestinal adverse events were reported. These consisted mainly of mild nausea, dyspepsia and reflux. Incidence of these adverse events was similar between placebo, 100 mg, 300 mg, and 750 mg. Incidence of these adverse events after the 1125 and 1500 mg dose was greater than placebo regardless of dosing in the fed or fasted state (approximately 50% of the subjects vs. incidence after placebo in 13% of the subjects). After concomitant food intake with the 750 mg dose, no gastro-intestinal adverse effects were reported. At the higher dose of 1125 mg the incidence was identical at the fed and fasted state.

Table 1 – p. 35

Other frequently reported adverse events were somnolence and headache, which were mostly judged as not treatment related (91% and 86%, respectively). No clinically significant changes in vital signs or ECG were noted during any of the treatments. Three positive fecal occult blood tests were reported; twice after the 300 mg dose, once after the 1500 mg dose (fed state) and once after placebo.

Fig. 1 – p. 33

Compared with placebo, small but consistent statistically significant CNS effects were observed with the highest dose of 1125 mg. As indicated by the average time profile (figure 1), EEG occipital alpha power AUC_{0-6 h} increased (43.9 (95% confidence interval (CI): 4.8–83.1)%). Occipital theta power AUC_{0-6 h} also increased after 1125 mg (17.1 (CI: 0.2–34.1)%).

Fig. 2 – p. 33

The effect of GPI 5693 on adaptive tracking performance is shown figure 2. Adaptive tracking performance AUC_{0-6 h} decreased after 1125 mg (-8.7 (CI: -14.0 to -3.5)%).

Fig. 3, 4 – p. 34

The average time profiles for the Bond and Lader visual analogue scales are presented in figures 3 and 4.

GPI 5693 1125 mg caused a reduction of the AUC_{0-6 h} for VAS alertness and the VAS mood (-9.9 (CI: -19.2 to -0.6) mm and -8.6 (CI: -16.7 to -0.5) mm, respectively).

At lower dose levels, inconsistent VAS effects were observed which did not occur at higher doses and thus did not show a dose relationship. The AUC_{0-6 h} for body sway after the 300 mg dose was decreased compared with placebo (-40.3 (CI: -66.6 to -14.0) mm min⁻²). The AUC_{0-6 h} of the VAS scores for meaning ('I had the idea that events, objects, or other people had particular meaning that was specific for me') and anxious ('I felt anxious') were decreased after the 300 mg dose (-2.8 (CI: -4.8 to -0.8) mm and -1.3 (CI: -2.6 to -0) mm, respectively). 750 mg showed a decrease of the VAS score for meaning (-2.2 (CI: -4.2 to -0.2) (mm)).

Table 2 – p. 36

No effects were seen on saccadic or smooth pursuit eye movements after any of the doses.

Fig. 5 – p. 35

The pharmacokinetic parameters are presented in table 2. The average concentration–time profiles of plasma GPI 5693 are presented in figure 5. The therapeutically relevant concentration range (950–11 100 ng ml⁻¹) as determined from animal experiments was already reached in the first panel after the 300 mg dose. The pharmacokinetic variability was largest after the 300 mg and 750 mg dose, resulting in a SD of approximately 50% of the C_{MAX}. Although no formal testing was performed, and pharmacokinetics in females were only studied in the fed state, there was no indication of sex differences in pharmacokinetics (third panel).

With concomitant food intake, the C_{MAX} decreased from 9266

to 3057 and from 15 742 to 5309 (ng ml⁻¹) after the 750 mg and 1125 mg, respectively. Food intake also reduced the AUC_{inf} from 8855 to 5144 and from 17 332 to 10 523 (h ng⁻¹ ml⁻¹) for the 750 mg and 1125 mg doses, respectively. These food effects were evaluated for these doses, where food and dose were used as ANOVA-covariates. The dose-normalized C_{MAX} decreased significantly by -5.6 (CI: -2.6 to -8.7) ng ml⁻¹ mg⁻¹. Food caused a significant reduction of the slope of the relationship between dose and AUC_{0-inf}. Dose-normalized T_{MAX} showed no significant food effects.

The effects of food on pharmacokinetic variability were unclear. Although no formal testing was performed, food seemed to reduce the variability following the 750 mg dose, from a CV of 50% to a CV of 34% for C_{MAX} and a CV of 28% to a CV of 24% for AUC_{0-inf}. Following the 1125 mg dose however, food caused a slight increase in the CV for C_{MAX} (from 25 to 39%) and for AUC_{0-inf} (from 26 to 31%).

Discussion

The main aim of this exploratory study was to assess the single-dose pharmacokinetics and to evaluate safety and tolerability, including CNS effects of a newly developed NAALADase-inhibitor. In all, five dose levels were administered, of which three were also dosed with concomitant food intake.

The pharmacokinetics showed that therapeutically relevant plasma concentrations were reached in this study. Although there was some pharmacokinetic variability, this will presumably not cause major problems in the development of the drug, since the therapeutic range was broad and it appears that the upper end of this range can be achieved without dose-limiting adverse effects. There were clear effects of food on pharmacokinetics, mainly resulting in a lower C_{MAX} and AUC, which should be taken into account when designing a dose schedule for further studies.

The main safety issues after single ascending dose administration of GPI 5693 were the relatively high incidence of gastro-intestinal side-effects, such as nausea and dyspepsia (generally mild and short lasting) which appeared to occur more often at higher dose levels. Three subjects presented with a positive feces occult blood test, but these were not necessarily related to GPI 5693. Firstly, there was no relation to dose or to repeated exposure. Secondly, subjects were not required to adhere to a strict diet and result could therefore have been false positive.

Also, one subject had a positive feces occult blood test following placebo. NAALADase inhibition per se is not expected to cause gastrointestinal effects. The gastrointestinal effects of GPI 5693 could be due to the acidic nature of the compound and could be a limiting factor during prolonged treatment of neuropathic pain patients with this agent. Firstly, gastrointestinal effects may be enhanced during repeated exposure with multiple dosing. Secondly, the associated autonomic neuropathy in diabetic patients often leads to changes in gastro-intestinal motility. A prolonged exposure of the gastric or duodenal epithelium to GPI 5693 may also increase the incidence of gastrointestinal adverse events. Furthermore, if the gut epithelium is affected by GPI 5693, pharmacokinetic properties of the drug may be altered.

Central nervous system aes were also reported although the incidence was not different following placebo or active treatment. A relatively high number of somnolence and headache AEs were reported after both GPI 5693 and placebo dosing. These effects may be partly due to caffeine abstinence and disruption of normal daily routines. At the highest tested dose (1125 mg) at which central nervous system tolerability was evaluated, some CNS effects were observed. The performance on adaptive tracking declined and the scores of the VAS alertness were significantly affected, even in the small group of four subjects. These effects could be associated with clinically relevant sedation by GPI 5693. The findings of these CNS effects warrant a more extensive CNS profiling of the drug with therapeutic dosages, focusing on sedation and on the possible development of tolerance to these effects after prolonged exposure in a multiple dose study. However, these effects also demonstrate CNS penetration of the drug. This may be important if the expansion of the area of development of this drug includes central neurodegenerative diseases. Pharmacokinetic-dynamic modeling was not performed because the effects were small and only consistently found at the highest dose level.

In summary, GPI 5693 was safe and generally well tolerated at plasma exposures that were effective in animal model of neuropathic pain. A clear food effect was observed on the pharmacokinetics, reducing C_{MAX} and AUC. Furthermore a relatively high, dose-dependant incidence of gastro-intestinal adverse events was observed. Although no significant drug related CNS adverse events were reported, mild CNS effects were observed following the highest dose level (at which these were assessed). These findings warrant a multiple dose study in healthy subjects in a fed state, to further evaluate the pharmacokinetic properties and adverse events profile of GPI 5693, prior to proceeding to patient studies.

REFERENCES

- 1 Jackson PF, Slusher BS. Design Of NAALADase Inhibitors: A Novel Neuroprotective Strategy. *Curr Med* 2001; 8: 949–57.
- 2 Jackson PF, Tays KL, Maclin KM, Ko YS, Li W, Vitharana D, Tsukamoto T, Stoermer D, Lu XC, Wazniak K, Slusher BS. Design And Pharmacological Activity Of Phosphinic Acid Based NAALADase Inhibitors. *J Med Chem* 2001; 44: 4170–5.
- 3 Zhang W, Slusher B, Murakawa Y, Wazniak K, Tsukamoto T, Jackson PF, Sima AA. GCPII (NAALADase) Inhibition Prevents Long-term Diabetic Neuropathy In Type 1 Diabetic Bb/wor Rats. *J Neurol Sci* 2002; 194: 21–8.
- 4 Slusher BS, Thomas A, Paul M, Schad CA, Ashby CR Jr. Expression And Acquisition Of The Conditioned Place Preference Response To Cocaine In Rats Is Blocked By Selective Inhibitors Of The Enzyme N-acetylated-alpha-linked-acidic Dipeptidase (NAALADase). *Synapse* 2001; 41: 22–8.
- 5 Slusher BS, Vornov JJ, Thomas AG, Hurn PD, Hacubuni I, Bhaedwaj A, Traystman RJ, Robinson MD, Britton P, Lu XC, Tortella FC, Wazniak KM, Yudhoff M, Potter BM, Jackson PF. Selective Inhibition Of NAALADase, Which Converts NAAG To Glutamate, Reduces Ischemic Brain Injury. *Nat Med* 1999; 5: 1396–402.
- 6 Wauquier A. EEG And Neuropharmacology. In: *Electroencephalography – Basic Principles, Clinical Applications, And Related Fields*, 3rd Edition., Ed. Niedermeyer E, Lopez Da Silva F, Baltimore: Williams & Wilkins 1993, 619–29.
- 7 Saletu B, Grünberger J, Anderer P, Linzmayer L, König P. On The Cerebro-protective Effects Of Caroverine, A Calcium-channel Blocker And Antiglutaminergic Drug: Double-blind, Placebocontrolled, EEG-mapping And Psychometric Studies Under Hypoxia. *Br J Clin Pharmacol* 1996; 41: 89–99.
- 8 Van Steveninck AL. *Methods Of Assessment Of Central Nervous System Effects Of Drugs In Man*. Thesis, State University Leiden 1993.
- 9 Van Steveninck AL, Schoemaker RC, Pieters MSM, Kroon JM, Breimer DD, Cohen AF. A Study Comparing The Sensitivities Of Adaptive Tracking, Eye Movement Analysis And Visual Analogue Lines To The Effects Of Incremental Doses Of Temazepam In Healthy Volunteers. *Clin Pharmacol Ther* 1991; 50: 172–80.
- 10 Van Steveninck AL, Van Berckel BNM, Schoemaker RC, Breimer DD, Van Gerven JMA, 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: 10–7.
- 11 Hartvig P, Valtysson J, Lindner K-J, Knstensen J, Karlsten R, Bustafsson LL, Persson J, Svensson JO, Oye I, Antoni G. Central Nervous System Effects Of Subdissociative Doses Of (S)-ketamine Are Related To Plasma And Brain Concentrations Measured With Positron Emission Tomography In Healthy Volunteers. *Clin Pharmacol Ther* 1995; 58: 165–73.
- 12 Harborne GC, Watson FL, Healy DT, Groves L. The Effects Of Sub-anaesthetic Doses Of Ketamine On Memory, Cognitive Performance And Subjective Experience In Healthy Volunteers. *J Psychopharmacol* 1996; 10: 134–40.
- 13 Cohen AF, Ashby L, Crowley D, Land G, Peck AW. Lamotrigine (Bw430c), A Potential Anticonvulsant. Effects On The Central Nervous System In Comparison With Phenytoin And Diazepam. *Br J Clin Pharmacol* 1985; 20: 619–29.
- 14 Muir Kw, Grosset DG, Gamzu E, Lees KR. Pharmacological Effects Of The Non-competitive NMDA Antagonist CNS 1102 In Normal Volunteers. *Br J Clin Pharmacol* 1994; 38: 33–8.
- 15 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: 712–3.
- 16 Borland RG, Nicholson AN. Visual Motor Coordination And Dynamic Visual Acuity. *Br J Clin Pharmacol* 1984; 18: 695–725.
- 17 Wright BM. A Simple Mechanical Ataxia-meter. *J Physiol* 1971; 218: 27p–28p.
- 18 Norris H. The Action Of Sedatives On Brain Stem Oculomotor Systems In Man. *Neuropharmacology* 1971; 10: 181–91.
- 19 Bond A, Lader M. Self-concepts In Anxiety States. *Br J Med Psychol* 1976; 49: 275–9.
- 20 Bowdle TA, Radant AD, Cowley DS, Kharasch ED, Strassman RJ, Roy-byrne PP. Psychedelic Effects Of Ketamine In Healthy Volunteers: Relationship To Steady-state Plasma Concentrations. *Anesthesiology* 1998; 88: 82–8.

FIGURE 1 Average EEG alpha power. Mean \pm SD time-effect profiles of EEG alpha power for placebo (●), GPI 5693 100 mg (○), 300 mg (■), 750 mg (□) and 1125 mg (▼).

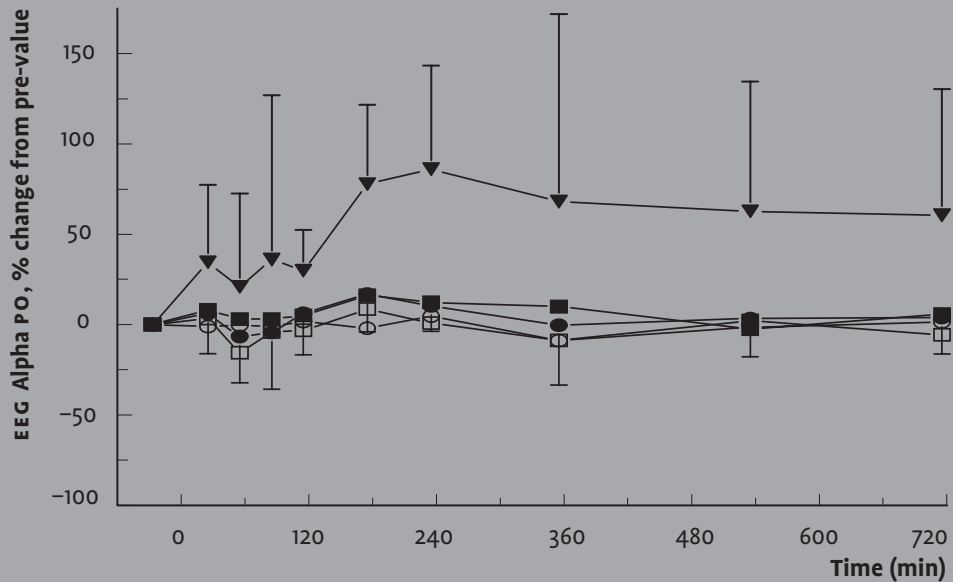


FIGURE 2 Average adaptive tracking performance. Mean \pm SD time-effect profiles of adaptive tracking performance for placebo (●), GPI 5693 100 mg (○), 300 mg (■), 750 mg (□) and 1125 mg (▼).

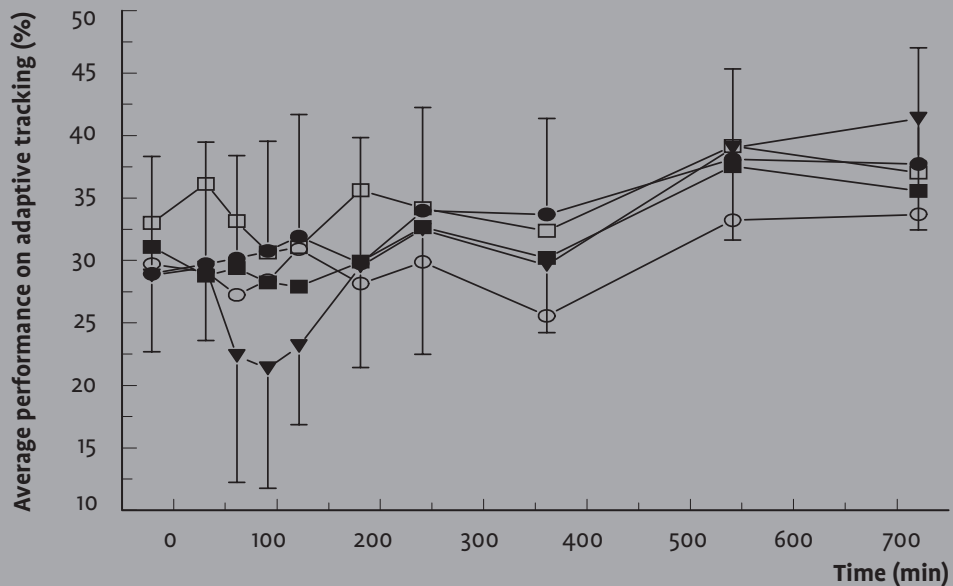


FIGURE 3 Average visual analogue scales alertness. Mean \pm SD time-effect profiles of visual analogue scales for alertness for placebo (●), GPI 5693 100 mg (○), 300 mg (■), 750 mg (□) and 1125 mg (▼).

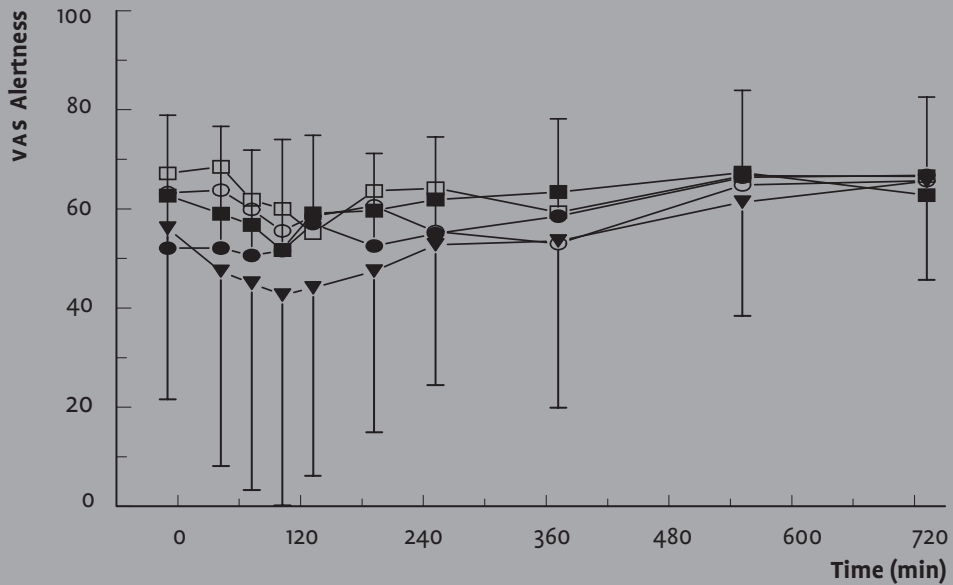


FIGURE 4 Average visual analogue scales mood. Mean \pm SD time-effect profiles of visual analogue scales for mood for placebo (●), GPI 5693 100 mg (○), 300 mg (■), 750 mg (□) and 1125 mg (▼).

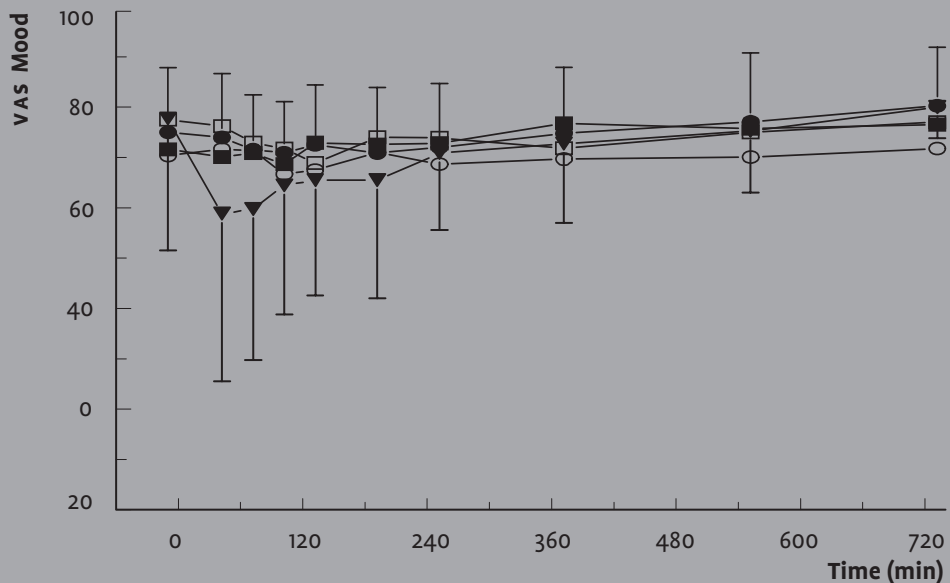


FIGURE 5 Average plasma concentrations of GPI 5693. Mean \pm SD time-concentration profiles after GPI 5693 100 mg (+), 300 mg (∇), 750 mg (\circ), 750 mg (fed) (\square), 1125 mg (\bullet), 1125 mg (fed) (\blacksquare), 1500 mg (fed) (\blacklozenge).

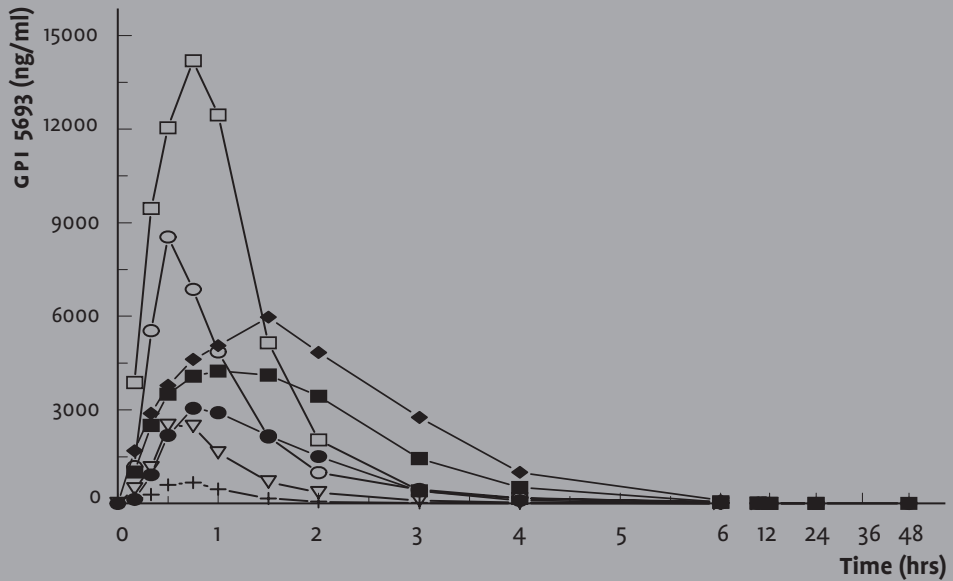


TABLE 1 Summary of treatment related adverse events

Body systems (preferred term)	Doses in mg (number of subjects)							
	100 (4)	300(9)	750(8)	750 ^f (4)	1125 (4)	1125 ^f (12)	1500 ^f (12)	Placebo (8)
Body as a whole – General disorders			1	1				4
Fatigue					1			3
Temperature changed sensation			1					1
Central & peripheral nervous system disorders				2		1	1	1
Dizziness						1	1	
Headache				2				1
Gastro-intestinal disorders	1	1	2		3	6	7	1
Abdominal pain					1			
Diarrhoea							1	
Dyspepsia	1	1	1		2	2	2	
Eructation							1	
Gastro-esophageal reflux						2	1	
Nausea			1			2	2	1
Psychiatric disorders				1			1	3
Insomnia				1				
Somnolence							1	3
Skin and appendages disorders				1			1	1
Pruritis				1				
Skin reaction localized							1	1
Vision disorders						1		
Vision abnormal						1		

* Dose administered with standardised meal

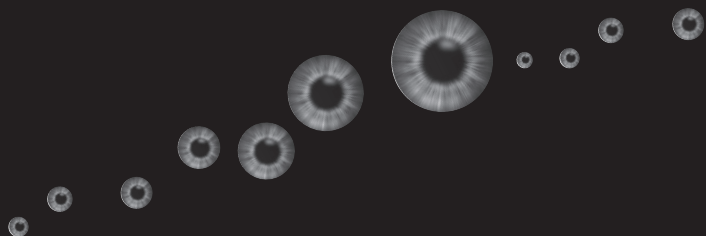
TABLE 2 GPI 5693 summary pharmacokinetic parameters

Dose GPI5693	N	C _{MAX} (ng/ml)	SD	T _{MAX} (min)	SD	AUC _{INF} (hr*ng/ml)	SD	T _{1/2} (hr)	SD
100 mg	4	723	201	38	9	684	205	0.87	0.02
300 mg	9	2868	1384	42	10	2733	779	2.54	0.71
750 mg	8	9266	4650	51	53	8855	2498	4.78	2.21
1125 mg	4	15742	3958	45	12	17332	4542	6.05	0.70
750 mg fed	4	3057	1046	45	0	5144	1239	8.83	7.03
1125 mg fed	12	5309	2086	74	33	10523	3292	6.88	6.71
1500 mg fed	12	6822	2229	93	37	15218	3708	8.62	5.48

CHAPTER 2

**CNS EFFECTS OF SUMATRIPTAN AND RIZATRIPTAN
IN HEALTHY FEMALE VOLUNTEERS**

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Abstract

This study investigates the CNS effects of sumatriptan and rizatriptan, with temazepam as an active comparator, in healthy female volunteers. Sixteen volunteers completed a randomized, double-blind, crossover study and on four separate occasions received either 100 mg sumatriptan, 20 mg rizatriptan or 20 mg temazepam. The main parameters were eye movements, EEG, body sway, visual analogue scales and a cognitive test battery. Rizatriptan and sumatriptan decreased saccadic peak velocity by 18.3 (95% CI: 5.7, 30.8) and 15.0 (2.2, 27.9) °/sec, respectively, about half the decrease induced by temazepam (35.0 (22.1, 47.8) °/sec). Body sway increased (30% for rizatriptan (16%, 45%) and 14% for sumatriptan (1%, 27%), respectively). Temazepam caused larger, similar effects. In contrast to temazepam, sumatriptan and rizatriptan decreased reaction times of recognition tasks and increased EEG alpha power (significant for sumatriptan, 0.477 (0.02, 0.935)). Therapeutic doses of sumatriptan and rizatriptan caused CNS effects indicative of mild sedation. For EEG and recognition reaction times the effects were opposite to temazepam, indicating central stimulation.

Introduction

Ref. 1 5HT_{1b/d} agonists are becoming increasingly popular in the treatment of migraine attacks. This type of drug is able to activate the 5HT₁-like receptors on the main cranial vessels, resulting in vasoconstriction and thus providing relief of symptoms.* The popularity of the 5HT agonists in the acute treatment of migraine is mainly due to the high efficacy and the relatively low rate of side-effects compared with other treatments.

Ref. 2 The first registered 5HT_{1b/d} agonist was sumatriptan, at a recommended oral dose of 50 mg followed by another 50 mg 2 h later in case of recurrence of symptoms. Following oral administration sumatriptan has a bioavailability of 14%,* which is controlled by dosing to effective concentrations. Sumatriptan is metabolized by monoamineoxidase (MAO) to an inactive N-desmethyl metabolite; the elimination half-life is around 2 h.* Other triptans quickly followed sumatriptan. Although these triptans demonstrate increased bioavailability over sumatriptan they appear to offer few clinical advantages.

Ref. 4 Rizatriptan has been demonstrated to be absorbed more rapidly compared with sumatriptan* and has a bioavailability of 40–45% after oral administration,* with an elimination half-life of around 2 h.* It is metabolized by MAO into an active N-desmethyl metabolite and may have a longer effect.

Ref. 5 / Ref. 3

Ref. 6-8

It has been claimed that rizatriptan has superior efficacy compared with sumatriptan, whereas at therapeutically relevant doses there are indications that rizatriptan may cause more adverse events, such as dizziness or drowsiness, compared with sumatriptan.* The mechanisms involved in these adverse events, and the best methods to quantify them, are unknown. It has long been assumed that triptans were not able to penetrate the central nervous system, which might have led to a relatively low number of studies and assessments of these types of adverse effects.

Ref. 9-11

The current study investigated the effects of clinical doses of sumatriptan and rizatriptan on the central nervous system, compared with placebo and temazepam as an active comparator for dizziness and drowsiness. An array of neuropsychometric parameters was used that has been shown to be sensitive to sedative agents.* Additionally, the relationship between the plasma concentrations and pharmacodynamics of sumatriptan and rizatriptan was evaluated. The study was performed in female subjects only, because of the higher prevalence of migraine in females.

Methods

Subjects

After approval of the protocol by the Ethical Review Board of the Leiden University Medical Centre, 16 healthy female volunteers were recruited, plus one replacement subject. Written informed consent was obtained before entering the study. After this the subjects received a full medical examination. Drugs were administered to the participants only after a negative pregnancy test (Pacific Biotech Inc., San Diego, USA) and urinary drug-screen (ONTRAK Rapid Assays for Drug Abuse; Roche (diagnostic systems), Mijdrecht, the Netherlands) had been obtained on the morning of each study day. Volunteers refrained from nicotine, alcohol and caffeine from 48 h prior to each study day until 24 h after dosing. Strenuous exercise was to be avoided for 48 h before each study safety screen and 12 before and after each dose. Concomitant medication was not permitted during each study period.

Design and procedure

The study was performed as a double-blind, randomized, placebo-controlled four period cross-over comparative trial with a minimum

washout period of 3 days and a maximum washout period of 1 week. The study medication contained sumatriptan 50 mg tablets, rizatriptan 10 mg encapsulated tablets, temazepam 20 mg capsules and placebo tablets and capsules. Rizatriptan and temazepam were encapsulated into identical hard gelatine capsules. On each study day subjects received either: a sumatriptan 50 mg tablet and a placebo capsule followed by the same treatment after 2 h; rizatriptan 10 mg capsule and a placebo tablet followed by the same treatment after 2 h; a placebo tablet and capsule followed by temazepam 20 mg capsule and a placebo tablet after 2 h; or a placebo tablet and capsule, followed by the same treatment after 2 h. The triptans were administered according to clinical practice for repeated dosing. Temazepam was administered at 2 h to have a comparable T_{MAX} for temazepam and the triptans (3–5 h after initial dose).

All tests (except the cognitive test battery) and blood sampling were performed before the intake of medication and at the following times after the first intake: 20, 40 min, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10 and 12 h. The cognitive test battery was executed in the 3–5-h period after the first administration of the study drug. Adverse events were checked regularly with openended questions ('How do you feel?'). Before the intake of medication (around 09.15) A light standard breakfast was served. Standard lunch and dinner were served 5 and 8 h post-dose.

Pharmacodynamics

EYE MOVEMENT ANALYSIS Recording and analysis of saccadic and smooth-pursuit eye movements was performed with a microcomputer-based system, as described previously.* The equipment used for stimulus display, signal collection and amplification was from Nihon Kohden (Nihon Kohden Corporation, Tokyo, Japan). Saccadic eye movements were recorded for stimulus amplitudes of 15 degrees to either side. Interstimulus intervals varied randomly between 3 and 6 s, 15 saccades were recorded. The average values of saccadic peak velocity, latency (reaction time) and inaccuracy were used as parameters. For smooth-pursuit eye movements the target moved sinusoidally at frequencies ranging from 0.3 to 1.1 Hz, increasing by steps of 0.1 Hz. The amplitude of target displacement corresponds to 20 degrees eyeball rotations to both sides. Four cycles were recorded for each stimulus frequency.

ELECTROENCEPHALOGRAMS Electroencephalograms were recorded and analysed using CED software (Cambridge Electronics Design,

Ref. 12

Ref. 13 Cambridge, UK), as described previously.* EEG recordings were made using silver-silver chloride electrodes, fixed with collodion at Fz, Cz, Pz and Oz, with the same common ground electrode as for the eye movement registration international 10/20 system). The electrode resistances were kept below 5 kOhm. All recordings were done with the subjects' eyes closed. EEG signals were obtained from leads Fz-Cz and Pz-Oz. The signals were amplified by use of a Nihon Kohden AB-621G bioelectric amplifier (Nihon Kohden Corporation) with a time constant of 0.3 s and a low pass filter at 100 Hz. Eight consecutive blocks of 8 s were recorded per session over a 2-min period. The sampling rate was 1024 Hz. Datablocks containing artefacts were identified by visual inspection and these were excluded from analysis. Fast Fourier transform analysis was performed to obtain the sum of amplitudes in the delta (0.5–3.5 Hz), theta (3.5–7.5 Hz), alpha (7.5–11.5 Hz) and beta (11.5–30 Hz) frequency ranges. The total recording bandwidth was 0–50 Hz.

Ref. 11 **BODY SWAY** Body sway was measured using an apparatus similar to the Wright ataxiometer,* which adds up the amplitude of body movement transferred through a string that is attached to the waist (apparatus: TNO/NIPG, Leiden, the Netherlands). Measurements of sagittal body sway (forwards/backwards) were made for 2 min with the eyes closed while standing on an unstable surface consisting of a 10-cm foam pad covered with a rigid plateau. The subjects were standing comfortably with their feet slightly apart and arms along their body.

Ref. 14 **VISUAL ANALOGUE SCALES** Visual analogue scales as originally described by Norris* were used to quantify subjective effects. Three factors corresponding to alertness, mood and calmness were derived from these measurements.*

Ref. 15

Cognitive test battery

Ref. 16 Cognitive performance was tested using the 'FePsy' software package (Instituut voor Epilepsiebestrijding, Heemstede, the Netherlands)*. This system contains a battery of computerized tests of different complexity measuring diverse cognitive skills. The battery has been extensively validated in epilepsy patients.*

Ref. 17, 18

- Simple auditory and visual reaction time, measuring attention.
- The binary choice task, a more complex reaction time task measuring information processing and response inhibition.

- The visual searching task, which evaluates the visual (complex) information processing and perceptual–mental strategies. The main parameter of the visual searching task was the number of errors, the secondary endpoint was the mean searching time of correct response.*
Ref. 19, 20
- The card sorting task, which assesses abstraction ability, conceptualization, failure to maintain set and perseverance.*
Ref 21
- Serial recognition of words and figures and simultaneous recognition of words and figures, which are recognition and short-term memory tasks.
- The corsi block tapping test,* a direct recall memory test.
Ref 22

Pharmacokinetics

Blood samples were drawn from an intravenous cannula inserted into a forearm vein. The blood samples were immediately centrifuged after collection at 4°C at 1000 g and the plasma was separated. The plasma samples were frozen at -20°C and stored until analysis. The plasma samples were analysed by the department of international bioanalysis (GaxoSmithKline, Ware, UK) using solid phase extraction in combination with liquid chromatography tandem mass spectrometry (LC-MS-MS) for sumatriptan and rizatriptan. For temazepam, the plasma samples were analysed by Simbec Research TLD (Merthyr Tydfil, Wales) using a gas chromatography method.

Sample times were related to the time of (first) dose (for study drugs administered twice). Pharmacokinetics for all drugs were assessed using non-compartmental methodology as implemented in WinNonlin V2.1 (Pharsight Corporation, Mountainview, California, USA). For each subject and each treatment the following pharmacokinetic parameters were determined: maximum plasma concentration (C_{MAX}), the time at which the maximum concentration was reached, relative to dosing (T_{MAX}), the terminal half-life ($t_{1/2}$) and the area under the plasma concentration-time curve up to the last quantifiable concentration (AUC_{last}) and extrapolated to infinity (AUC_{inf}). Descriptive statistics were reported for each pharmacokinetic parameter. The results for sumatriptan and rizatriptan were compared with the literature and with data on file to verify that the bioavailability was not altered by the reformulation.

Statistics

All repeatedly measured dynamic variables were characterized using changes from pre-treatment value expressed as areas under the effect curve (AUECs). These AUECs were calculated using the linear trapezoidal rule and were divided by the corresponding timespan, resulting in a time-weighted average outcome. AUECs were calculated over the entire sampling period (12 h) and over the first 6 h because most of the response was expected to occur during this period. Results are only reported relative to baseline over the 0–6-h period. The cognitive test results were compared among treatments using standard analysis of variance methods. The primary contrasts were sumatriptan vs. rizatriptan, sumatriptan vs. placebo and rizatriptan vs. placebo, calculated within the ANOVA model. The contrast with temazepam was used in an exploratory fashion, providing a frame of reference for the CNS effects of sumatriptan and rizatriptan. The results are presented as average differences with 95% confidence intervals (95% CI) unless indicated otherwise.

The relationship between concentrations and effects was assessed by plotting the effect vs. the interpolated concentration. The interpolated concentration was used because of a time difference between blood samples and dynamic parameters. Placebo correction was implemented by subtracting the response during the placebo occasion from the response during active treatment at corresponding nominal times on an individual basis. The relationships between interpolated concentrations and placebo corrected SPV and log body sway were assessed by calculating linear concentration-effect relationships using nonlinear mixed effect modelling (NONMEM version V; NONMEM Project Group, UCSF, California, USA). This procedure analyses all curves collectively while preserving differences between individuals. Different slope and intercept estimates for the different drugs were implemented. Additive intra- and interindividual error models were used and parameters were estimated using first order conditional estimation (FOCE) with the ‘interaction’ option. The significance of slope parameters was assessed by calculating 95% confidence intervals using the reported standard errors.

Results

Sixteen subjects were initially recruited. One subject repeated a dosing period, due to a non-treatment-related adverse event that led to discontinuation of the third study day. One subject was replaced

because of a non-serious adverse event during the third study day. The mean age of the 17 participants was 21.4 years (range 18–28). Body mass index (weight (kg)/length (m)²) was below 30 for all participants. The pharmacodynamic results are based upon 16 subjects who completed all treatment periods and one subject who completed two dosing periods. The pharmacokinetic results are based upon plasma concentrations of 17 subjects for rizatriptan and 16 subjects for sumatriptan and temazepam. The safety analysis was performed on all 17 subjects that received one or more treatments.

Adverse events

In all treatment groups sedation (40%), headache (19%) and dizziness (9%) were recorded most. Drug-related adverse events, as judged by the investigator, were also mainly sedation (75%), headache (13%) and dizziness (11%). No large differences were seen between the different treatments, but temazepam appeared to induce slightly more sedation of moderate intensity than the other treatments (table 1). Events were generally mild and did not require treatment. Two subjects experienced moderate adverse events (flu-like symptoms) that led to the discontinuation of a trial day. During those events paracetamol was used for symptomatic treatment.

Table 1 – p. 53

Pharmacodynamics

EYE MOVEMENTS Both rizatriptan and sumatriptan caused a small but statistically significant reduction in saccadic peak velocity of -18.3 (-30.8, -5.7) °/s (mean difference, 95% confidence interval) and -15.0 (-27.9, -2.2) °/s, respectively, compared with placebo. This was about half the effect of temazepam, which caused a reduction of -35.0 (-47.8, -22.1) °/s (fig. 1). The difference of 3.3 (-9.6, 16.1) °/s between the two triptans was not statistically significant. The triptans did not have significant effects on saccadic latency or inaccuracy. Temazepam showed a clear but non-significant effect on the smooth-pursuit eye movements. Both triptans did not alter smooth-pursuit eye movements compared with placebo.

Fig. 1 – p. 53

ELECTROENCEPHALOGRAMS Sumatriptan caused a statistically significant increase in alpha power compared with placebo for the

Fig.2A - p. 54

frontal leads (0.477 (0.020, 0.935) $\mu\text{v}/\text{min}$). Rizatriptan showed a non-significant increase in alpha power for the frontal leads (0.366 (-0.081, 0.812) $\mu\text{v}/\text{min}$). The average time-effect profiles of temazepam suggested a decrease of frontal and occipital alpha power, but this did not reach statistical significance relative to placebo (-0.399 (-0.856, 0.58), -0.439 (-1.171, 0.292) $\mu\text{v}/\text{min}$, frontal and occipital leads, respectively) (fig. 2A). The mean difference and confidence intervals for frontal leads alpha power are displayed graphically in fig. 2B. Temazepam showed a non-significant elevation of frontal beta power (0.142 (-0.025, 0.309) $\mu\text{v}/\text{min}$). No effect of temazepam or the triptans was seen on the occipital beta amplitude. There were no significant changes in delta power for any of the treatments compared with placebo. A non-significant decrease of frontal (-0.319 (-0.654, 0.017) $\mu\text{v}/\text{min}$) and occipital (-0.271 (-0.577, 0.035) $\mu\text{v}/\text{min}$) theta power was observed for temazepam compared with placebo.

Fig.2B - p. 54

Fig.3 - p. 55

BODY SWAY The blinded review of body sway data indicated that body sway needed to be analysed on a log-scale. Both triptans caused small statistically significant increases in body sway compared with placebo (fig. 3). Rizatriptan caused an increase of 30 (16, 45)% and sumatriptan had a smaller effect of 14 (1, 27)%. By comparison, temazepam caused an increase in body sway of 31 (17, 47)%. There was a statistically significant difference between the two triptans of 15 (2, 29)% ($p=0.022$).

VISUAL ANALOGUE SCALES Temazepam gave a clear decrease of the VAS alertness score of 4.35 (0.03, 8.68) cm compared with placebo. The triptans did not have any effect on VAS for alertness. No differences were seen in mood and calmness comparing the different treatments with placebo and the triptans with each other.

Cognitive test battery

SIMPLE AUDITORY AND VISUAL REACTION TIME The reaction time tests were performed with the dominant and the non-dominant hand. Temazepam caused an increase in mean auditive and visual reaction times for the dominant (51.8 (31.4, 72.1) and 35.9 (15.5, 56.4) ms) and the non-dominant hand (47.8 (32.3, 63.3) and 69.7 (24.3, 115.1) ms, respectively) compared with placebo. Rizatriptan and sumatriptan both caused an increase of mean auditive and visual reaction times. This was not significant for the dominant hand, but rizatriptan caused a significant

increase in the mean auditory reaction time for the non-dominant hand (20.9 (5.7, 36.1) ms) compared with placebo. A significantly longer reaction time for rizatriptan compared with sumatriptan was found for the mean auditory reaction times of the non-dominant hand (18.2 (3.0, 33.4) ms).

BINARY CHOICE TASK The mean reaction times for the binary choice task were significantly affected following temazepam. There was an increase compared with placebo of 99.5 (46.2, 152.8) ms. Rizatriptan and sumatriptan caused much smaller, non-significant increases in the mean reaction times.

COMPUTERIZED VISUAL SEARCHING TASK Temazepam caused a significant increase in the number of errors (0.85 (0.17, 1.53) items), the main parameter of the visual searching task, and prolonged the mean searching time for the correct responses (by 1.44 (0.37, 2.51) s). Sumatriptan and rizatriptan had no significant effects compared with placebo or each other.

SIMULTANEOUS AND SERIAL RECOGNITION OF WORDS AND FIGURES For simultaneous recognition of words, a significant increase in reaction times of correct responses of 0.34 (0.08, 0.60) s during temazepam treatment was observed. Also, for this task a significant reduction in the number of correct responses was seen for temazepam compared with placebo (2.02 (0.55, 3.49) items). Compared with temazepam, the average number of correct items during sumatriptan and rizatriptan treatments decreased slightly but not significantly. However, for simultaneous recognition of words the reaction times for the correct responses decreased significantly for both sumatriptan (0.31 (0.04, 0.58) (s) and rizatriptan (0.26 (0.00, 0.52) (s) compared to placebo. The reaction times for correct responses during temazepam treatment increased significantly compared with placebo for both simultaneous recognition of figures (0.67 (0.23, 1.11) (s) and the serial recognition of words (0.45 (0.23, 0.66) (s). No effect of temazepam was found on the number of correct responses of both tests. Both triptans showed no effect on the reaction times or numbers of correct responses. No significant effect of temazepam, rizatriptan or sumatriptan was observed on the serial recognition of figures.

CORSI BLOCK TAPPING TASK AND CARD SORTING TEST

No significant effect of temazepam, rizatriptan or sumatriptan was observed on the corsi block tapping task or card sorting test.

Pharmacokinetics

Table 2 - p. 56

Ref 23

Ref 23

Pharmacokinetic parameters are presented in table 2. A recent pharmacokinetic study reported C_{MAX} after rizatriptan 10 mg orally as 28.6 ± 13.5 ng/ml.* Thus, C_{MAX} was found to be approximately 14% lower after the first dose in the current study, with encapsulated rizatriptan. In the same study T_{MAX} was reported as 42 ± 12 min,* which is 56 min earlier than in the current study. Thus, encapsulation of rizatriptan caused a delay in absorption compared with the tablet formulation as presented in recent literature.

Ref 24-26

The pharmacokinetic properties of sumatriptan and temazepam were consistent with those presented in the literature. Dynamic measurements were performed after the second dose and around C_{MAX} for the second dose of all treatments.

Pharmacokinetic–pharmacodynamic modelling

Pharmacokinetic–pharmacodynamic relationships were investigated for all repeated measurements that showed a significant difference between both triptans and placebo, i.e. saccadic peak velocity (SPV) and log body sway.

For saccadic peak velocity corrected for placebo, the average slopes ($(^\circ/s)/(ng/ml)$) and their 95% confidence intervals (CI) were as follows: sumatriptan -0.168 ($-0.37, 0.04$), rizatriptan -0.494 ($-0.79, -0.20$) and temazepam -0.164 ($-0.21, -0.12$). Thus, no significant concentration-effect relationship was shown for sumatriptan, in contrast to rizatriptan and temazepam.

For log body sway corrected for placebo, the average slopes ($(\log mm/2 min)/(ng/ml)$) and their 95% confidence intervals (CI) were as follows: sumatriptan 0.76 ($-0.39, 1.91$), rizatriptan 2.98 ($1.40, 4.56$) and temazepam 0.495 ($0.34, 0.65$). As for saccadic peak velocity, a significant concentration-effect relationship was shown only for rizatriptan and temazepam.

Discussion

Until recently, it has been assumed that triptans do not penetrate the central nervous system. However, the current study showed that sumatriptan and rizatriptan had effects on several central nervous

system measurements. Both triptans reduced saccadic peak velocity, which is indicative of a mild sedative drug effect. The increase in body sway and the increases in simple auditive and visual reaction times during sumatriptan and rizatriptan treatment support this finding. The effects of these triptans were generally smaller and less consistent than those of temazepam. For instance, the decrease in saccadic peak velocity caused by sumatriptan and rizatriptan was only half that caused by a moderately sedative dose of temazepam. Sedation caused by a 5HT_{1B/D} agonist is rarely reported in human studies and could be of clinical importance. Sedative drug effects of sumatriptan have been demonstrated during safety studies in cynomolgus monkeys after administration of very high doses of 20 and 40 mg/kg.* In a recent meta-analysis of oral triptans, the incidence of CNS effects (consisting among others of agitation, confusion, dizziness and somnolence) was reported around 4% after sumatriptan 50 mg and around 9% for rizatriptan 10 mg.*

Ref 27

Ref 28

The apparent difference in effect size between sumatriptan and rizatriptan was small. Rizatriptan appeared to have a greater effect on the measurements than sumatriptan. This difference between these triptans only became significant for body sway and some of the cognitive tests. Rizatriptan CNS responses in this study may have been underestimated as rizatriptan encapsulation appears to have caused a decrease in expected C_{MAX} of around 14%, and a delay in T_{MAX} of about 56 min, compared with pharmacokinetic data available for rizatriptan.*

Ref 23

Cognitive testing showed clear neurocognitive effects of temazepam. The drug primarily caused an increase in reaction times for the different tests, an effect that is well known for benzodiazepines and other sedative agents.* There are indications that benzodiazepines interfere primarily with procural and/or storage of information.* This is supported by our findings. Temazepam decreased the number of correct responses on the simultaneous recognition of words and increased the number of errors on the visual searching task. Triptan effects on cognition and EEG were partly different from those of temazepam, suggesting slightly enhanced impulsivity. First, sumatriptan (and rizatriptan nonsignificantly) both caused an elevation of frontal-central alpha power. Such an increase in alpha power is compatible with a state of relaxed wakefulness.* As expected, temazepam caused a decrease in alpha power, which was nonsignificant in this case but has been described repeatedly.* A similar observation, an increase with the triptans and decrease with temazepam, was made in occipital-central theta power. This may be explained by subharmonic alpha waves that present as theta waves, a phenomenon

Ref 29

Ref 29

Ref 30-33

Ref 34-36

which is often seen in young people's EEGs. However, it must be noted that the relationship between states of alertness and frontal alpha power is complex.

Secondly, both sumatriptan and rizatriptan caused a small but significant decrease in reaction times for the simultaneous recognition of words, while the number of correct responses declined (though not significantly). These effects are opposite to temazepam, which caused a significant increase of reaction times, consistent with moderate sedation. These findings may be caused by a potentially class-specific effect of sumatriptan and rizatriptan on information or decision processes. A change in strategy under influence of these triptans may be responsible for the decrease in reaction times: instead of choosing for accuracy subjects may have favoured speed. The same changes in decision processes were not observed for other reaction time tasks (e.g. the visual searching task and the card sorting task). In contrast to the word recognition task, these tasks give feedback on the accuracy (but not the velocity) of the responses, which may have stimulated accuracy.

The enhanced speed and reduced accuracy suggests that sumatriptan and rizatriptan stimulate impulsivity, although the effects were very small. This may explain why they were seen in only one test of the cognitive test battery: simultaneous recognition of words is regarded as one of the most difficult memory tests in the battery, and could therefore be more sensitive to a slight impairment. On the other hand, this isolated finding may be spurious.

Ref 37-41

Previous reports of other 5HT₁ agonists, like buspirone and umespirone, have shown mixtures of sedative and stimulant effects.* Buspirone showed a subjective sedative effect on analogue rating scales, impaired memory and increased reaction times for cognitive tests. Umespirone also increased reaction times. Stimulant effects such as an increase in subjective alertness and a decrease in reaction times were observed for buspirone* and improvements in word recognition and mood were observed for umespirone* Our observations on the 5HT_{1b/d} agonist-triptans are partly compatible with these findings.

Ref 39

Ref 37

Recently, there is increasing evidence that triptan penetration into the central nervous system is not only responsible for some of the adverse effects, but also contributes to the relief of migraine symptoms. The mechanisms of action could be the inhibition of activity of the trigeminal nucleus caudalis activity* or the decrease of cortical spreading depression as occurs in the early onset of a migraine attack.* Sumatriptan was also shown to normalize EEG changes associated with

Ref 42

Ref 43

migraine, such as an increase in the theta and delta range and a decrease in the alpha and beta range.*

In summary, these results show that sumatriptan and rizatriptan cause small but clear effects on the central nervous system. These effects may differ from those caused by the benzodiazepine temazepam. They may be due to subtle changes in information and decision processing, compatible with enhanced impulsivity. The effects of rizatriptan appear to be slightly larger than for sumatriptan, although in most cases this did not reach statistical significance. The difference may have been decreased by an alteration of rizatriptan pharmacokinetics as a result of encapsulation. In any case, the CNS-effects of both sumatriptan and rizatriptan were small in comparison to those of 20 mg temazepam, and do not seem to compromise clinical tolerability to any appreciable extent. Although this study was performed in a small number of subjects with many parameters and does not univocally establish CNS effects of sumatriptan and rizatriptan, these results could be regarded as a guide for further studies.

REFERENCES

- 1 Tfelt-Hansen P. Sumatriptan For The Treatment Of Migraine Attacks—a Review Of Controlled Clinical Trials. *Cephalalgia* 1993; 13 (4):238–44.
- 2 Lacey LF, Hussey EK, Fowler PA. Single Dose Pharmacokinetics Of Sumatriptan In Healthy Volunteers. *Eur J Clin Pharmacol* 1995; 47 (6):543–8.
- 3 Duquesnoy C, Mamet JP, Sumner D, Fuseau E. Comparative Clinical Pharmacokinetics Of Single Doses Of Sumatriptan Following Subcutaneous, Oral, Rectal And Intranasal Administration. *Eur J Pharm Sci* 1998; 6 (2):99–104.
- 4 Cheng H, Polvino WJ, Sciberras D, Yogendran L, Cerchio KA, Christie K *et al.* Pharmacokinetics And Food Interaction Of Mk-462 In Healthy Males. *Biopharm Drug Dispos* 1996; 17 (1):17–24.
- 5 Sciberras DG, Polvino WJ, Gertz BJ, Cheng H, Stepanavage M, Wittreich J *et al.* Initial Human Experience With Mk-462 (Rizatriptan): A Novel 5-HT_{1D} Agonist [Published Erratum Appears In *Br J Clin Pharmacol* 1997; 43 (4) : 450]. *Br J Clin Pharmacol* 1997; 43 (1) : 49–54.
- 6 Cutler NR, Claghorn J, Sramek JJ, Block G, Panebianco D, Cheng H *et al.* Pilot Study Of Mk-462 In Migraine. *Cephalalgia* 1996; 16 (2):113–6.
- 7 Teall J, Tuchman M, Cutler N, Gross M, Willoughby E, Smith B *et al.* Rizatriptan (Maxalt) For The Acute Treatment Of Migraine And Migraine Recurrence. A Placebo-controlled, Outpatient Study. *Rizatriptan 022 Study Group. Headache* 1998; 38 (4):281–7.
- 8 Gijsman H, Kramer MS, Sargent J, Tuchman M, Matzura-Wolfe D, Polis A *et al.* Double-blind, Placebo-controlled, Dose-finding Study Of Rizatriptan (MK-462) In The Acute Treatment Of Migraine. *Cephalalgia* 1997; 17 (6):647–51.
- 9 Harron DW, Hasson B, Regan M, McClelland RJ, King DJ. Effects Of Rilmenidine And Clonidine On The Electroencephalogram, Saccadic Eye Movements, And Psychomotor Function. *J Cardiovasc Pharmacol* 1995; 26 (Suppl.):s48–54.
- 10 Van Steveninck AL, Wallnofer AE, Schoemaker RC, Pieters MS, Danhof M, Van Gerven JM *et al.* 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 (3):267–75.
- 11 Wright BM. A Simple Mechanical Ataxia-meter. *J Physiol* 1971; 218 (1):27p–8.
- 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 (5):712–3.
- 13 Steveninck A, Lv Berckel BN, Mv Schoemaker HC, Breimer DD, Gerven JM, Av Cohen AF. The Sensitivity Of Pharmacodynamic Tests For The Central Nervous System Effects Of Drugs On The Effects Of Sleep Deprivation. *J Psychopharm* 1999; 13 (1):10–7.
- 14 Norris H. The Action Of Sedatives On Brain Stem Oculomotor Systems In Man. *Neuropharmacology* 1971; 10 (21):181–91.
- 15 Bond A, Lader M. Self-concepts In Anxiety States. *Br J Med Psychol* 1976; 49 (3):275–9.
- 16 Moerland M, Aldenkamp AP, Alpherts WCJ. A Neuropsychological Test Battery For The Apple li-e. *Intern J Man-machine Studies* 1986; 25:453–67.
- 17 Alpherts WC, Aldenkamp AP. Computerized Neuropsychological Assessment Of Cognitive Functioning In Children With Epilepsy. *Epilepsia* 1990; 31 (Suppl. 4):s35–40.
- 18 Aldenkamp AP, Arends J, Overweg-plandsoen TC, Van Bronswijk KC, Schyns-soeterboek A, Linden I *et al.* Acute Cognitive Effects Of Nonconvulsive Difficult-to-detect Epileptic Seizures And Epileptiform Electroencephalographic Discharges. *J Child Neurol* 2001; 16 (2):119–23.
- 19 Goldstein G, Welch RB, Rennick PM, Shelly CH. The Validity Of A Visual Searching Task As An Indicator Of Brain Damage. *J Consult Clin Psychol* 1973; 41 (3):434–7.
- 20 Demita MA, Johnson JH. The Validity Of A Computerized Visual Searching Task As An Indicator Of Brain Damage. *Behav Res Meth Instrumentation* 1981; 13:592–4.
- 21 Anderson SW, Damasio H, Jones Rd, Tranel D. Wisconsin Card Sorting Test Performance As A Measure Of Frontal Lobe Damage. *J Clin Exp Neuropsychol* 1991; 13 (6):909–22.
- 22 Nelson RE, Dickson AL, Banos JH. An Automated Administration Of Corsi's Block-tapping Test. *Percept Mot Skills* 2000; 91 (2):578–80.
- 23 Lee Y, Conroy JA, Stepanavage ME, Mendel CM, Somers G, McLoughlin DA *et al.* Pharmacokinetics And Tolerability Of Oral Rizatriptan In Healthy Male And Female Volunteers. *Br J Clin Pharmacol* 1999; 47 (4):373–8.
- 24 Jhee SS, Shiovitz T, Crawford AW, Cutler NR. Pharmacokinetics And Pharmacodynamics Of The Triptan Antimigraine Agents: A Comparative Review. *Clin Pharmacokinetics* 2001; 40 (3):189–205.
- 25 Fowler PA, Lacey LF, Thomas M, Keene ON, Tanner RJ, Baber NS. The Clinical Pharmacology, Pharmacokinetics And Metabolism Of Sumatriptan. *Eur Neurol* 1991; 31 (5):291–4.
- 26 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 (2):172–80.

- 27 Humphrey PP, Feniuk W, Marriott AS, Tanner RJ, Jackson MR, Tucker ML. Preclinical Studies On The Anti-migraine Drug, Sumatriptan. *Eur Neurol* 1991; 31 (5): 282–90.
- 28 Ferrari MD, Roon KI, Lipton RB, Goadsby PJ. Oral Triptans (Serotonin 5-HT (1B/1D) Agonists) In Acute Migraine Treatment: A Meta-analysis Of 53 Trials. *Lancet* 2001; 358 (9294): 1668–75.
- 29 Kleidienst-vanderbeke O. *Psychopharmacology And Reaction Time*. Chichester: John Wiley, 1998.
- 30 Ray WJ, Cole HW. EEG Activity During Cognitive Processing: Influence Of Attentional Factors. *Int J Psychophysiol* 1985; 3 (1):43–8.
- 31 Cole HW, Ray WJ. EEG Correlates Of Emotional Tasks Related To Attentional Demands. *Int J Psychophysiol* 1985; 3 (1):33–41.
- 32 Valentino DA, Dufresne RL. Attention Tasks And EEG Power Spectra. *Int J Psychophysiol* 1991; 11 (3):299–301.
- 33 Lindsey DB. Attention, Consciousness, Sleep & Wakefulness. In: Field J, Magoun HW, Hall E, Eds. *Handbook Of Physiology*. Washington, DC: American Physiological Society, 1960:1553–93.
- 34 Van Steveninck AL, Schoemaker HC, Den Hartigh J, Pieters MS, Breimer DD, Cohen AF. Effects Of Intravenous Temazepam. ii. A Study Of The Long-term Reproducibility Of Pharmacokinetics, Pharmacodynamics, And Concentrationeffect Parameters. *Clin Pharmacol Ther* 1994; 55 (5):546–55.
- 35 Van Steveninck AL, Schoemaker HC, Den Hartigh J, Rijnkels J, Pieters MS, Breimer DD et al. Effects Of Intravenous Temazepam. I. Saccadic Eye Movements And Electroencephalogram After Fast And Slow Infusion To Pseudo Steady State. *Clin Pharmacol Ther* 1994; 55 (5):535–45.
- 36 Van Steveninck AL, Mandema JW, Tuk B, Van Dijk JG, Schoemaker HC, Danhof M et al. A Comparison Of The Concentration-effect Relationships Of Midazolam For EEGderived Parameters And Saccadic Peak Velocity. *Br J Clin Pharmacol* 1993; 36 (2):109–15.
- 37 Holland RL, Wesnes K, Dietrich B. Single Dose Human Pharmacology Of Umespirone. *Eur J Clin Pharmacol* 1994; 46 (5):461–8.
- 38 Lucki I, Rickels K, Giesecke MA, Geller A. Differential Effects Of The Anxiolytic Drugs, Diazepam And Buspirone, On Memory Function. *Br J Clin Pharmacol* 1987; 23 (2):207–11.
- 39 Alford C, Bhatti JZ, Curran S, McKay G, Hindmarch I. Pharmacodynamic Effects Of Buspirone And Clobazam. *Br J Clin Pharmacol* 1991; 32 (1):91–7.
- 40 Goa KL, Ward A. Buspirone. A Preliminary Review Of Its Pharmacological Properties And Therapeutic Efficacy As An Anxiolytic. *Drugs* 1986; 32 (2):114–29.
- 41 Bourin M, Auget JL, Colomel MC, Larousse C. Effects Of Single Oral Doses Of Bromazepam, Buspirone And Clobazam On Performance Tasks And Memory. *Neuropsychobiology* 1989; 22 (3):141–5.
- 42 Proietti-Cecchini A, Afra J, Schoenen J. Intensity Dependence Of The Cortical Auditory Evoked Potentials As A Surrogate Marker Of Central Nervous System Serotonin Transmission In Man: Demonstration Of A Central Effect For The 5HT_{1b/1d} Agonist Zolmitriptan (311C90, Zomig). *Cephalalgia* 1997; 17 (8):849–54.
- 43 Read SJ, Parsons AA. Sumatriptan Modifies Cortical Free Radical Release During Cortical Spreading Depression. A Novel Antimigraine Action For Sumatriptan? *Brain Res* 2000; 870 (1–2):44–53.
- 44 Thomaidis T, Tagaris G, Karageorgiou C. EEG And Topographic Frequency Analysis In Migraine Attack Before And After Sumatriptan Infusion. *Headache* 1996; 36 (2):111–40.

FIGURE 1 Average time-effect curve of the used drugs and saccadic eye movements peak velocity.

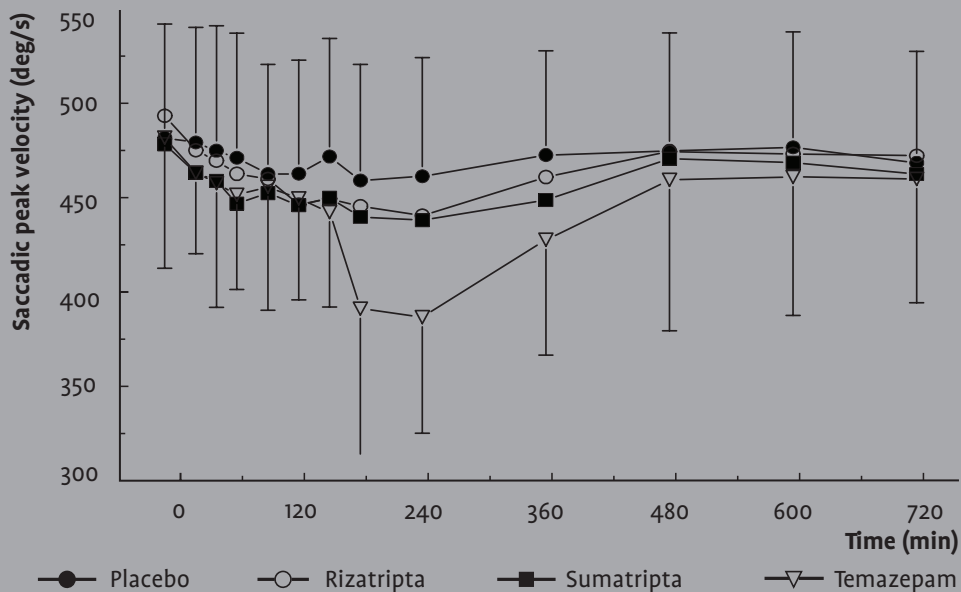


TABLE 1 Summary of main adverse events (number reported).

Main adverse events	Placebo oral (n = 17)	Sumatriptan (n = 17)	Rizatriptan (n = 17)	Temazepam (n = 16)
Any event	30	29	27	25
Any drug related event	13	13	13	17
Neurological event	19	16	20	23
Drug related neurological event	13	11	13	17
Sedation	8	9	11	14
Headache	3	1	2	1
Dizziness	3	1	0	2

FIGURE 2A Average time-effect curve for EEG alpha power (FC and PO leads).

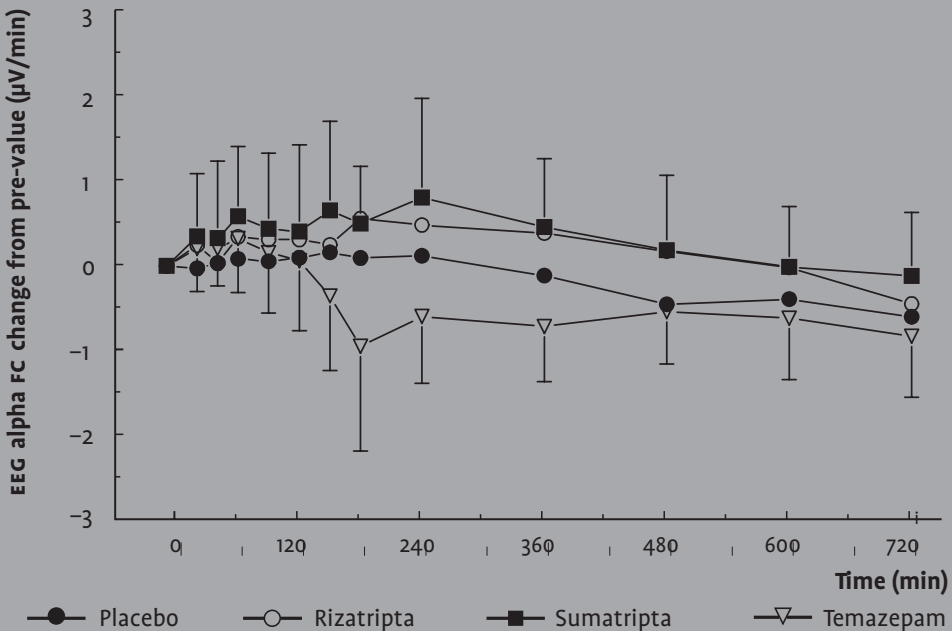
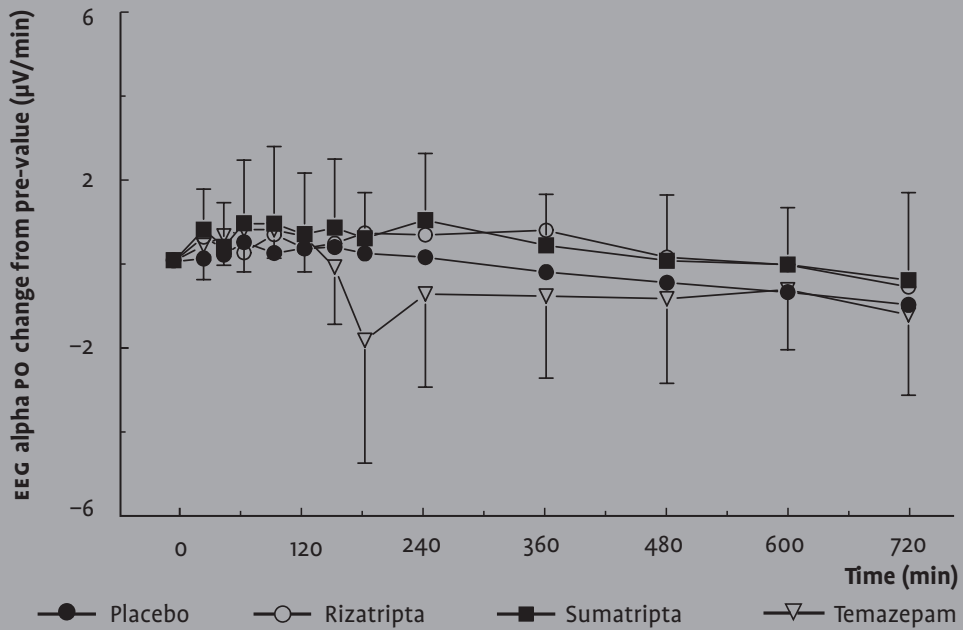


FIGURE 2B Mean difference from placebo and 95% confidence intervals of EEG alpha power.

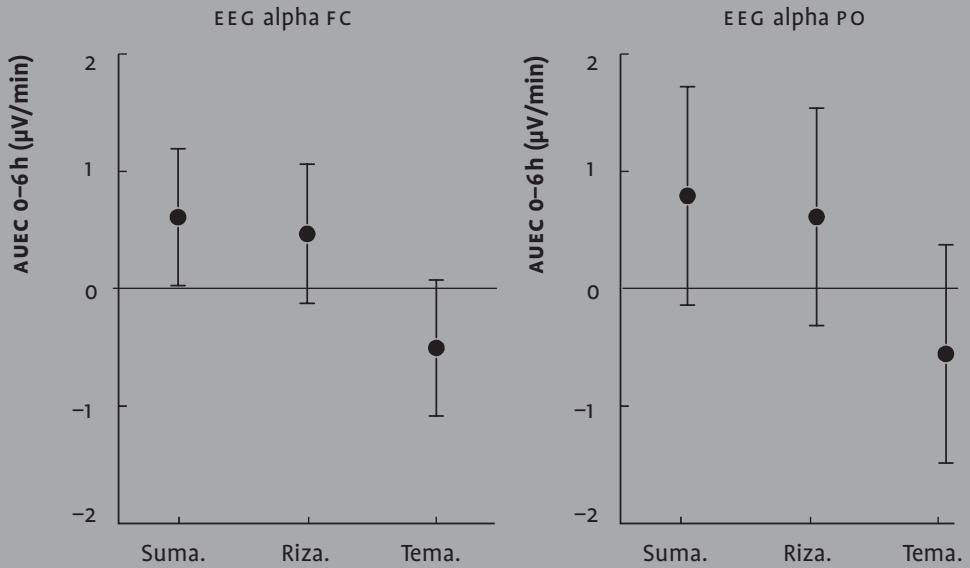


FIGURE 3 Average time-effect curve for log-transformed body sway.

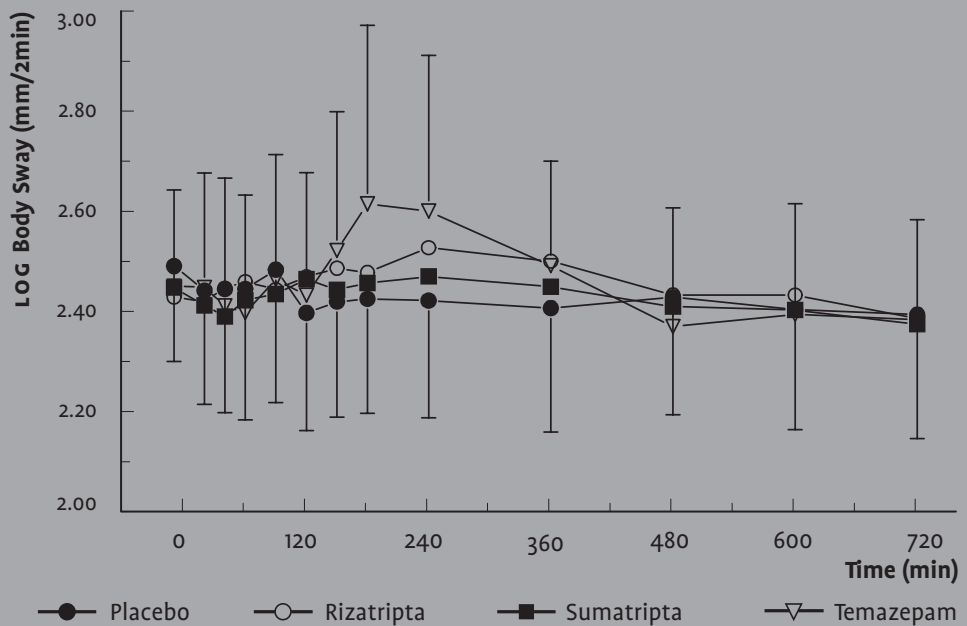


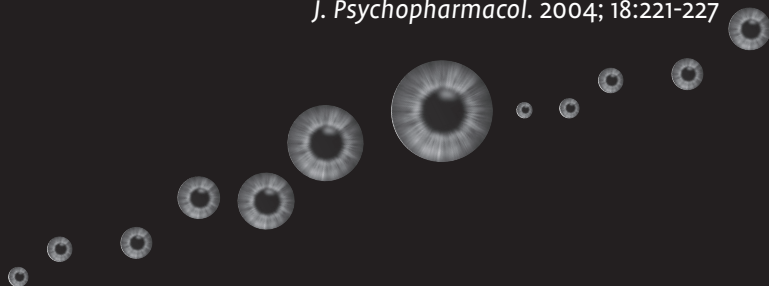
TABLE 2 Pharmacokinetic parameters.

Parameters		Sumatriptan	Rizatriptan	Temazepam
Geometric mean (95% confidence intervals)				
C_{MAX} (ng/ml)	1 st Dose	28.5 (19.9-40.7)	22.7 (18.2-28.3)	NA
	2 nd Dose	40.6 (34.1-48.3)	36.8 (31.6-42.8)	423 (364-493)
AUC_{0-1NF} (ng/ml*hr)		238 (200-284)	197 (173-224)	2600 (2100-3210)
$T_{1/2}$ (min)		111 (101-121)	105 (95-116)	350 (285-430)
Mean \pm SD (range)				
T_{MAX} (min)	1 st Dose	94 \pm 24 (60-120)	98 \pm 26 (40-120)	NA
	2 nd Dose	148 \pm 79 (50-230)	89 \pm 47 (50-230)	86 \pm 50 (20-230)

CHAPTER 3

**PHARMACOKINETIC/PHARMACODYNAMIC ASSESSMENT
OF TOLERANCE TO CENTRAL NERVOUS SYSTEM EFFECTS
OF A 3 MG SUSTAINED RELEASE TABLET OF RILMENIDINE
IN HYPERTENSIVE PATIENTS**

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Abstract

Previous single-dose studies have shown clear blood pressure-lowering effects of a potential sustained release (SR) profile of rilmenidine, with concentration-dependent effects on the central nervous system. The aim of this study was to evaluate potential changes in concentration-effect-relationships for these central nervous system effects during a 4-week treatment period with an experimental SR formulation of rilmenidine 3 mg once daily in 15 mild-to-moderate hypertensive patients. The central nervous system effects of the treatment were evaluated using saccadic eye movements for sedative effects and visual analogue scales for subjective effects on alertness, mood and calmness. Measurements for pharmacokinetic and pharmacodynamic evaluations were performed on the first day of the treatment period and repeated after 1 week and 4 weeks of treatment. Drug concentrations increased during the study, whereas treatment related reductions in saccadic peak velocity (SPV) remained similar on all three study days. The slopes of the concentration-effect-curves for SPV remained unchanged throughout the study, while the intercepts tended to increase as a result of increased pre-dose values. Similar effects were observed for visual analogue scales for alertness: pre-dose values increased significantly during the study, while the size of the treatment responses (slopes) remained unaltered. The reasons for these adaptations cannot be determined but may include drug tolerance and habituations to study procedures. Blood pressure control remained stable and adequate throughout the study.

Introduction

Rilmenidine [2-(dicyclopropylmethyl)-amino-2-oxazoline] is a centrally acting anti-hypertensive with binding selectivity to the I₁ imidazoline receptors over α ₂-adrenoceptors (Beau *et al.*, 1988; Verbeuren *et al.*, 1990; Institut de Recherches Internationales Servier (IRIS), 1993; Yu *et al.*, 1996). It has dose (concentration)-dependent blood pressure lowering effects above 0.5 subjects. Rilmenidine is registered in several European countries at a recommended dose of one tablet (1 mg) once or twice daily. Clinical experience indicates that with 1 mg dosing blood pressure control might not be maintained for 24 h per day in all patients. In a study of 146 patients with hypertension (diastolic blood pressure between 95 and 115 mmHg), trough level blood pressure control was considered inadequate in 56% of subjects after 4 weeks of treatment (De Cort *et al.*, 1993). An unspecified number of these patients became adequately

controlled after increasing the dosing frequency to 1 mg twice daily. This dosage regimen is less acceptable during chronic treatment. On the other hand, elevating the dose of once-daily administration may increase the incidence of peak concentration-related side-effects, such as sedation and dry mouth (xerostomia) (Dollery *et al.*, 1988).

A sustained release formulation of the drug could maintain plasma levels in between a minimum effective (anti-hypertensive) concentration and a maximum non-sedative peak level. In addition to the plasma concentrations, the rate of increase of concentration may also influence the effect. The classic example is provided by Kleinbloesem *et al.* (1987) who demonstrated that a high rate of increase of nifedipine concentrations did not lead to a blood pressure reduction in healthy volunteers, in contrast to a low rate of increase of nifedipine concentrations. However, a previously performed study showed no influence of the rate of infusion of rilmenidine on both blood pressure and central nervous system effects (visual analogue scales and saccadic eye movements) (de Visser *et al.*, 2001a). The current study was performed after several studies had investigated the design of an optimal slow release profile (de Visser *et al.*, 2001a,b, 2002). From these studies, it was concluded that a 3 mg sustained release formulation would have the optimal profile for adequate blood pressure control with an improved side-effect profile. Because many centrally active drugs show some tolerance development to side-effects during prolonged treatment, the current study aimed to investigate the effects of a 4-week treatment with a 3 mg sustained release formulation on the pharmacokinetic/pharmacodynamic (PK/PD) relationships between rilmenidine plasma concentrations and central nervous system effects (saccadic eye movements and visual analogue scales). Tolerance to these effects would be indicated by a reduced slope of the PK/PD relationship with saccadic eye movements and/or visual analogue scales. Increased intercepts of these relationships might also be an indication of tolerance, but this is more difficult to interpret because of the possible contributions of habituation to study procedures and other factors.

Methods

Design

This was a phase II, single-centre, open, non-controlled study without direct individual benefit for patients. Screening assessment took place

within 17 days before rilmenidine treatment. Patients were acquainted with the experimental methods and conditions in a short training session taking place within 1 week before rilmenidine treatment. Eligible patients were then withdrawn from their own anti-hypertensive treatment and switched directly into a 4-week 3 mg O.D. rilmenidine sustained release (SR) treatment. The withdrawal was gradual for β -blockers, and immediate for other anti-hypertensive agents but, in all cases, as short as possible to prevent loss of blood pressure control. Measurements for PK/PD evaluation were performed on the first day (d1–d2) of the rilmenidine treatment period and repeated after 1 week (d8) and 4 weeks (d29) of treatment. At the end of the 4-week rilmenidine treatment period, patients were reallocated to their own anti-hypertensive treatment.

Subjects

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

Treatments

Rilmenidine SR was presented as white round-shaped film coated tablets containing 3 mg of active medication. Patients were requested to take one tablet of rilmenidine every morning under fasting conditions, with approximately 150 ml of water, 30 min before breakfast. Patients were instructed to take their study medication regularly (between 07.00 h and 09.00 h in the morning). Patients were instructed to maintain a diary, where intake of study medication was to be recorded.

Blood pressure

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

Visual analogue scales

Visual analogue scales as originally described by Norris (1971) have been used previously to quantify subjective effects of benzodiazepines (van Steveninck *et al.*, 1991). From these scales, three factors can be derived, as described by Bond *et al.*, 1974, corresponding to alertness, mood and calmness. Increased scores on these scales indicate enhanced subjective feelings of alertness, mood (in general) and calmness. These visual analogue scales were practised at a training session (three times), and measured pre-dose and every 1 h for 12 h after dosing, on each of the three study days.

Saccadic eye movements

Saccadic eye movements have been used previously to quantify drug effects of rilmenidine (de Visser *et al.*, 2001A) and clonidine (Harron *et al.*, 1995). Saccadic eye movements were practised at a training session (three times), and measured pre-dose and every 1 h for 12 h after dosing, on each of the three study days, with an additional measurement after 24 h for the first dosing. Recording of eye movements was performed in a quiet room with ambient illumination. There was only one patient per session in the same room. Recording and analysis of saccadic eye movements was conducted with a microcomputer-based system for sampling and analysis of eye movements (Van Steveninck *et al.*, 1999). The equipment used for stimulus display, signal collection and amplification was from Nihon Kohden (Nihon Kohden Corporation, Tokyo, Japan). Disposable silver-silver chloride electrodes (Medicotest N-00-s, Olstykke, Denmark) were applied on the forehead and beside the lateral canthi of both eyes of the patient for registration of the electro-oculographic signals. Skin resistance was reduced to less than 5 k Ω before application of the electrodes. Head movements were restrained using a fixed head support. The target consisted of an array of light emitting diodes on a bar, fixed at 50 cm in front of the head support. Saccadic eye movements were recorded for stimulus amplitudes of $\pm 15^\circ$ to either side. Fifteen saccades were recorded for each stimulus amplitude with interstimulus intervals varying randomly between 3 and 6 s. Average values of latency (i.e. reaction time), saccadic peak velocity and inaccuracy of all artifact-free saccades were used as parameters. Saccadic inaccuracy was calculated as the absolute value of the difference between the stimulus angle and the corresponding saccade, expressed as a percentage of the stimulus angle.

Blood sampling

Patients were randomly allocated to one of the following investigation schedules for PK/PD evaluation.

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

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

Blood samples for rilmenidine assay (9 ml) were obtained in lithium heparin-containing polypropylene tubes. Blood samples were drawn from an i.v. cannula (inserted into the arm opposite to the one where blood pressure was measured), which was kept patent using a heparin-NaCl solution. Blood samples were taken after discarding the contents of the cannula. At the 24-h time point on day d2, blood was collected using a vacuette with a venapuncture.

Analyses

PHARMACOKINETICS Rilmenidine plasma levels were measured by using a gas chromatographic/mass spectrometric method (Ung *et al.*, 1987). Rilmenidine PK was modelled using a one-compartment model with first order absorption and a lag-time using NONMEM version V software (NONMEM Project Group, UCSF, San Francisco, CA, USA) using the first order conditional estimation method with interaction. Residual error was modelled as a combination of a constant coefficient of variation component and an additive component. Individual empirical Bayes estimates for absorption half-life, elimination half-life, clearance and lag-time were determined for all occasions separately, and predicted individual rilmenidine concentration profiles were obtained using these estimates.

PHARMACODYNAMICS Areas under the curve were calculated for saccadic eye movement data and visual analogue scale scores using the linear trapezoidal rule on (expected) protocol times (AUECS). These AUECS were subsequently divided by the corresponding time span resulting in a weighted average response. Additionally, the minimum measurement was determined with the associated actual time point for parameters with a clear response. In the case of multiple minima, the first occurrence

was taken. No corrections for baseline response were implemented for either AUECs or E_{MIN} , because the baseline results could be subject to changes during treatment.

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

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

Data management and additional calculations were performed using SAS for Windows V 8.2 (SAS institute, INC., Cary, NC, USA).

Results

Subjects

Fifteen (seven male, eight female) hypertensive patients were randomized to one of the blood sampling schedules. Their ages were in the range 41–65 years with a (mean \pm SD, 51.3 ± 7.2 years). All patients had mild-to-moderate essential hypertension, and received one or two anti-hypertensive agents (angiotensin-converting enzyme inhibitors 33.3%, β -blockers 26.7%, diuretics 26.7%, angiotensin II inhibitors 26.7%, calcium antagonists 6.7%, combined form (β -blocker and diuretic) 6.7% of the study population). Two patients suffered from arthrosis and these patients used allowed concomitant medication (Ibuprofen 400 mg prn). Baseline systolic/diastolic blood pressures on medication at screening (before switching to rilmenidine) were in the range 128/68–165/98 mmHg.

Safety assessments

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

Pharmacokinetics

Fig. 1, 2 – p. 69

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

Blood pressure

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

Saccadic eye movements

Table 1 - p. 71

Fig. 2 – p. 69

The effects of prolonged treatment on the AUECs of saccadic peak velocity (SPV), reaction time (RT) and inaccuracy are shown in table 1. The average curves for saccadic peak velocity (SPV) on days 1, 8 and 29 are presented in fig. 2. The primary endpoint of saccadic peak velocity (SPV AUEC) showed no significant changes during 4 weeks of treatment with rilmenidine SR 3.0 mg OD. The minimum SPV values during days 1, 8 and 29 of treatment were comparable, with mean \pm SD values of 413.2 ± 48.2 , 415.4 ± 53.7 and 401.5 ± 63.0 degrees/s, respectively. Hence, these data provide no indications for tolerance development. There are clear indications for a treatment effect that is comparable among the three treatment days. The average minimum values (E_{MIN}) on the three treatment days all represent decreases in excess of 15%, which is

well over the level of clinical significance of 10% below baseline (van Steveninck *et al.*, 1999) Associated with a decrease in SPV observed after the loss of one night of sleep. The other two parameters (reaction time and inaccuracy) did not show any significant effects, except for a decrease in AUEC inaccuracy for days 8 and 29 compared to day 1.

Visual analogue scales

Table 1 - p. 71

Fig. 3 - p. 70

The AUECs of VAS alertness, mood and calmness are represented in table 1. The average curves for visual analogue scale alertness on days 1, 8 and 29 are shown in fig. 3. Significant increases in subjective alertness, mood and calmness were observed from day 1 to day 8 and from day 1 to day 29. No significant changes were observed from day 8 to day 29. The VAS baseline values all increased from day 1 to day 8 and from day 1 to day 29.

PK/PD

Table 2 - p. 72

Fig. 4, 5 - p. 70, 71

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

Discussion

This study was part of a series of investigations, designed for the development of an optimal controlled release formulation of the centrally active antihypertensive agent rilmenidine. Previous studies showed clear concentration-dependent effects on blood pressure and the central nervous system of a potential sustained release profile of rilmenidine (de Visser *et al.*, 2001a,b, 2002). Furthermore, these studies suggested that the optimal therapeutic window would be 4–6 ng/ml.

Although effective, these concentrations have been shown to produce some changes in saccadic eye movements and visual analogue scales, which could be consistent with the clinical phenomenon of sedation (van Steveninck *et al.*, 1991). These effects could become less pronounced during prolonged treatment due to tolerance development (van Steveninck *et al.*, 1997). The aim of the current study was to evaluate potential changes in PK/PD-relationships for these central nervous system effects during a 4-week treatment period with rilmenidine SR 3 mg OD.

The design of the study was based on two assumptions. First, a rapid switch from prestudy antihypertensives to rilmenidine was considered unlikely to affect the central nervous system effects. A rapid switch could affect blood pressure control that, soon after the switch, would still be partly affected by the interrupted prestudy treatment and would not be individually optimized. However, adequate long-term blood pressure control has already been established with rilmenidine (Ostermann *et al.*, 1988), and this was not the aim of our study. The second assumption was that PK/PD analyses reduce the need for a placebo-control. Any major placebo response would dilute the relationship between the drug concentration and the pharmacodynamic parameter. Hence, a clear concentration-effect-relationship was considered a strong argument for drug-dependency of the parameter. PK/PD analyses were essential for the aims of the study because they can be used to quantify changes in sensitivity to the drug and the development of tolerance. For linear concentration-effect-relationships, changes can occur in the slope and/or the intercept of the concentration-effect curve. A decreased slope signifies that the same concentration range produces a less pronounced response. For example, in this case, the effect at the highest observed concentration is decreased due to desensitization or dynamic counter-regulation. An increase in the intercept signifies that the entire concentration-effect curve is right-shifted. Elevated pre-dose values will usually lead to an increased intercept of the concentration-effect curve. Rilmenidine concentrations accumulated significantly during the 4-week period, whereas central nervous system-effects did not increase. This is an indication of tolerance, which is also demonstrated in the concentration-effect-relationships for both SPV and VAS alertness scores. Figures 4 and 5 clearly show a parallel rightward shift of the concentration-effect curves (i.e. an increase in intercepts without any change in slopes). This increase could be largely attributed to an increase in pre-dose alertness. The treatment responses remained unchanged with rising rilmenidine concentrations. The mechanisms behind these

Fig. 4. 5 - p. 70, 71

changes are unclear. Pharmacological adaptations can cause an increased pre-dose effect (e.g. rebound after drug withdrawal), but this has not been reported for rilmenidine, and is particularly unlikely considering the accumulation (rather than withdrawal) of the drug with this SR profile. Pharmacological tolerance to the central nervous system effects of rilmenidine would primarily (or at least additionally) be expected to cause a reduction in the slopes of the concentration–effect relationships, which was not observed in this study. Therefore, explanations for the observed alterations do not appear to be purely pharmacological. Methodological causes can be considered, related to learning effects or habituation to the study procedures. If causes for the adaptations were methodological, this would also be expected in a placebo group. However, a recent 4-week placebo-controlled trial with the imidazoline antihypertensive moxonidine did not reveal any changes in the placebo treated group (Kempe *et al.*, 2003). The concentration–effect–relationships in the moxonidine-group displayed clear changes in intercepts but not in slopes that are comparable to the findings of the current study. This suggests that the gradual increase in predose alertness is drug-class specific. These changes are reminiscent of long-term adaptations observed with monoaminergic antidepressant treatments. Imidazoline agents show an increase in extracellular noradrenaline in the prefrontal cortex (Nutt *et al.*, 1997), and this could explain the observed increase in attention (Middelton, 1996). The neuropharmacological effects of imidazoline agents and their therapeutic potentials for psychiatric disorders warrant further study (Nutt *et al.*, 1997).

In conclusion, this study has shown an improvement in subjective pre-dose alertness (and mood and calmness) during prolonged rilmenidine treatment, whereas adequate blood pressure control was maintained throughout the 4-week treatment period.

REFERENCES

- Beau B, Mahieux F, Paraire M, Laurin S, Brisgand B, Vitou P (1988) Efficacy And Safety Of Rilmenidine For Arterial Hypertension. *Am J Cardiol* 61: 95d-95d
- Bond A J, Lader M H (1974) The Use Of Analogue Scales In Rating Subjective Feelings. *Br J Med Psychol* 47: 211-218
- De Cort P, Smilde J G, Arora A (1993) An Assessment Of The Antihypertensive Activity Of Rilmenidine (Ril) Before The First Daily Intake, In 146 Patients With Mild To Moderate Hypertension. Institut De Recherches Internationales Servier (IRIS), 18 March 1993, Paris, France
- De Visser S J, Gerven J M A, Schoemaker H C, Cohen A F (2001a) 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* 51: 423-428
- De Visser S J, Vis P W, Gerven J M A, Schoemaker H C, Cohen A F (2001b) Comparison Of Oral And Oral Sustained-release Rilmenidine In Healthy Volunteers And Correlation With In Vitro Sustained-release Properties. *Clin Drug Invest* 21: 579-586
- De Visser S J, Van Der Post J P, Nanhekhani L, Schoemaker R C, Cohen A F, Van Gerven J M (2002) Concentration-effect Relationships Of Two Rilmenidine Single-dose Infusion Rates In Hypertensive Patients. *Clin Pharmacol Ther* 72: 419-428
- Dollery C T, Davies D S, Duchier J, Pannier B, Safar M E (1988) Dose And Concentration-effect Relations For Rilmenidine. *Am J Cardiol* 61: 60d-60d
- Harron D W, Hasson B, Regan M, McClelland R J, King D J (1995) Effects Of Rilmenidine And Clonidine On The Electroencephalogram, Saccadic Eye Movements, And Psychomotor Function. *J Cardiovasc Pharmacol* 26 (Suppl 2): S48-S54
- Institut De Recherches Internationales Servier (IRIS) (1993) Rilmenidine (S 3341) Investigators Brochure, Version 3. IRIS, Paris, France
- Kemme M J, Van Der Post J P, Schoemaker R C, Straub M, Cohen A F, Van Gerven J M (2003) Central Nervous System Effects Of Moxonidine Experimental Sustained Release Formulation In Patients With Mild To Moderate Essential Hypertension. *Br J Clin Pharmacol* 55(6): 518-525
- Kleinbloesem C H, Van Brummelen P, Danhof M, Faber H, Urquhart J, Breimer D D (1987) Rate Of Increase In The Plasma Concentration Of Nifedipine As A Major Determinant Of Its Hemodynamic Effects In Humans. *Clin Pharmacol Ther* 41: 26-30
- Middelton H C (1996) Behavioural Pharmacology Of ∞ 2-adrenoceptors. *J Psychopharmacol* 10 (Suppl) 3: 19-25
- Norris H (1971) The Action Of Sedatives On Brain Stem Oculomotor Systems In Man. *Neuropharmacology* 10: 181-191
- Nutt D J, Lalies M D, Lione L A, Hudson A L (1997) Noradrenergic Mechanisms In The Prefrontal Cortex. *J Psychopharmacol* 11: 163-168
- Ostermann G, Brisgand B, Schmitt J, Fillastre J P (1988) Efficacy And Acceptability Of Rilmenidine For Mild To Moderate Systemic Hypertension. *Am J Cardiol* 61: 76d-80d
- Ung H L, Girault J, Lefebvre M A, Mignot A, Fourtillan J B (1987) Quantitative Analysis Of S3341 In Human Plasma And Urine By Combined Gas Chromatography-negative Ion Chemical Ionization Mass Spectrometry: 15 Month Inter-day Precision And Accuracy Validation. *Biomed Environ Mass Spectrom* 14: 289-293
- Van Steveninck A L, Schoemaker H C, Pieters M S, Kroon R, Breimer D D, Cohen A F (1991) A Comparison Of The Sensitivities Of Adaptive Tracking, Eye Movement Analysis And Visual Analog Lines To The Effects Of Incremental Doses Of Long-term Use On Healthy Volunteers. *Clin Pharmacol Ther* 50: 172-180
- Van Steveninck A L, Wallnofer A E, Schoemaker R C, Pieters M S, Danhof M, Van Gerven J M, Cohen A F (1997) A Study Of The Effects Of Long-term Use On Individual Sensitivity To Temazepam And Lorazepam In A Clinical Population. *Br J Clin Pharmacol* 44: 267-275
- Van Steveninck A L, Van Berckel B N, Schoemaker R C, Breimer D D, Van Gerven J M, Cohen A F (1999) The Sensitivity Of Pharmacodynamic Tests For The Central Nervous System Effects Of Drugs On The Effects Of Sleep Deprivation. *J Psychopharmacol* 13: 10-17
- Verbeuren T J, Dinh Xuan A T, Koenig-berard E, Vitou P (1990) Rilmenidine. *Cardiovasc Drug Rev* 8: 56-70
- Yu A, Frishman W H (1996) Imidazoline Receptor Agonist Drugs: A New Approach To The Treatment Of Systemic Hypertension. *J Clin Pharmacol* 36: 98-111

FIGURE 1 Average rilmenidine concentration-time profiles at days 1, 8 and 29 (mean \pm SD).

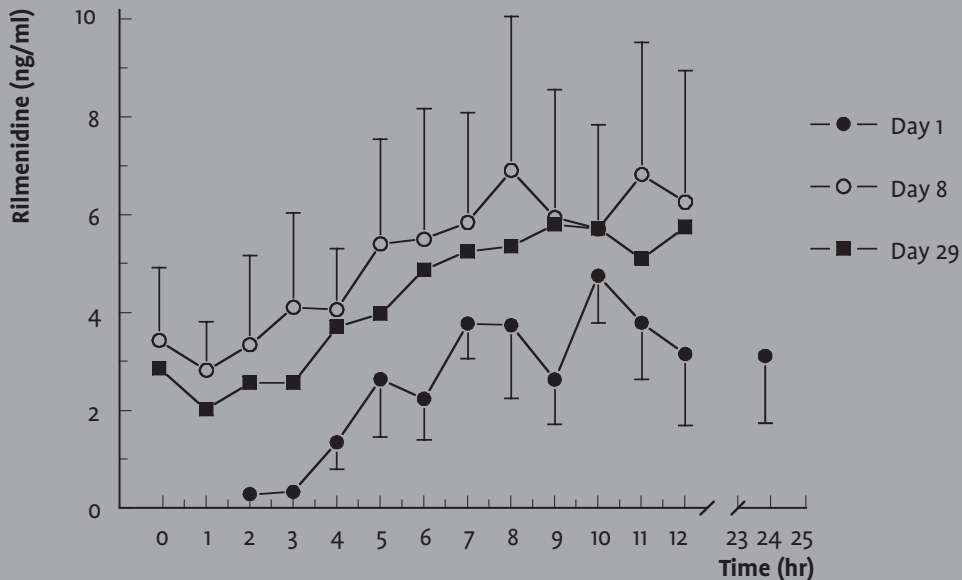


FIGURE 2 Average saccadic peak velocity-time profiles at days 1, 8 and 29.

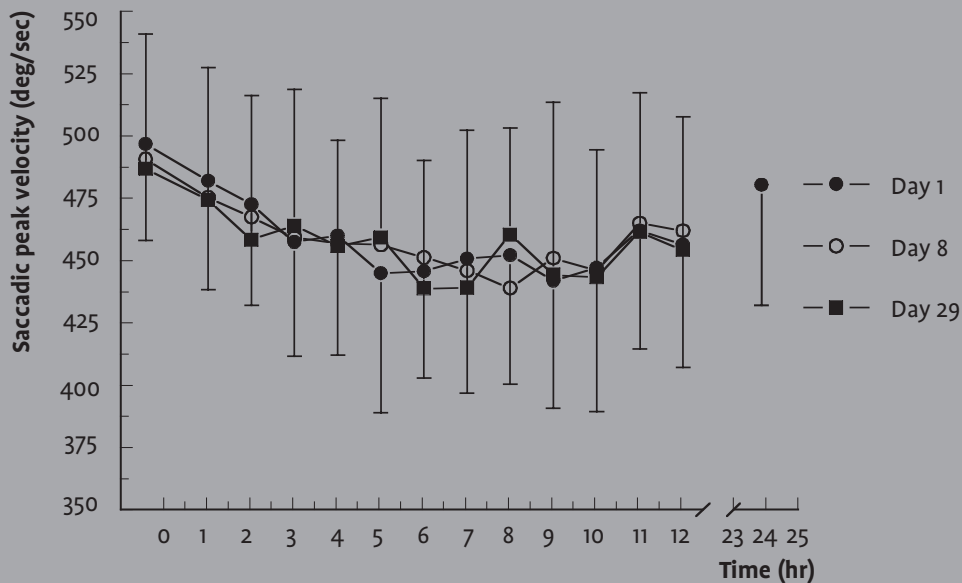


FIGURE 3 Average visual analogue scale alertness-time profiles at days 1, 8 and 29.

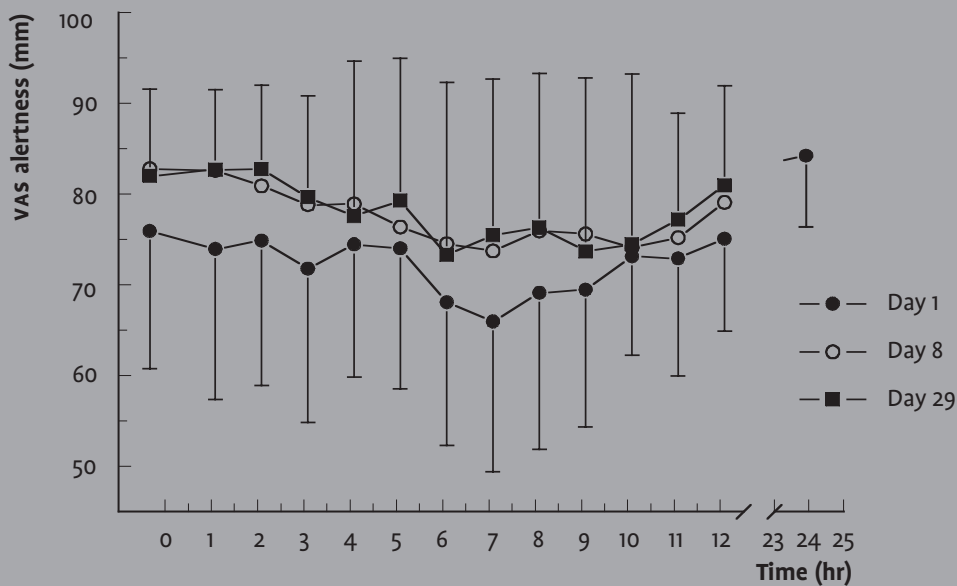


FIGURE 4 Average PK/PD relationship between predicted rilmenidine concentrations and saccadic peak velocity.

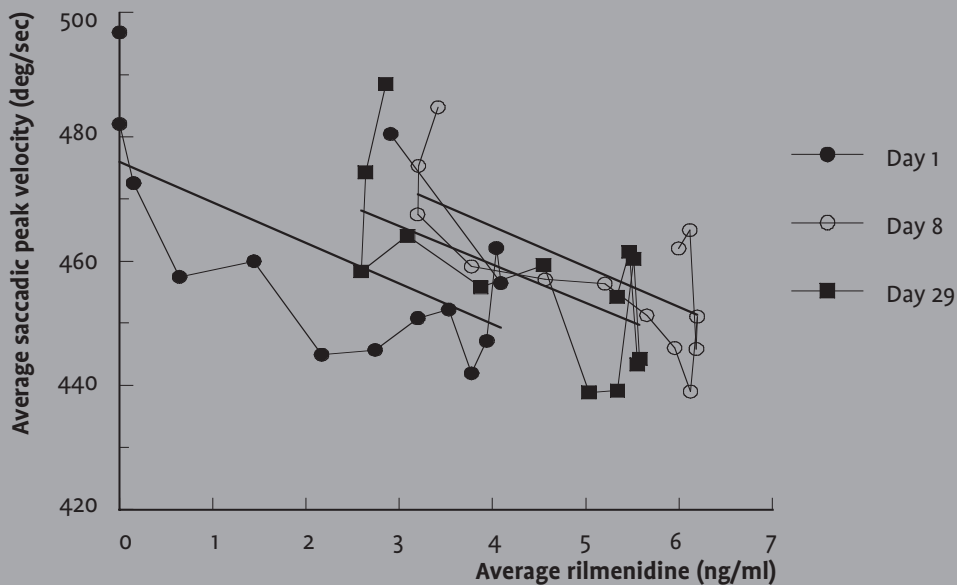


FIGURE 5 Average PK/PD relationship between predicted rilmenidine concentrations and VAS alertness.

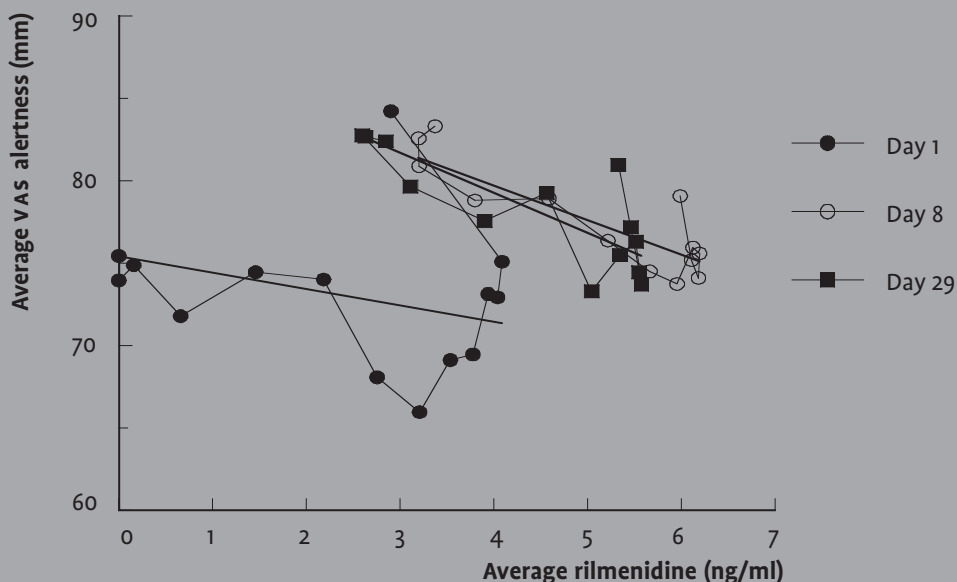


TABLE 1 Time-corrected AUEC's (0–12 h) for all pharmacodynamic parameters.

Data are shown as mean (SD) and contrasts with 95% confidence intervals (95% CI) between treatment days (*p < 0.05).

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

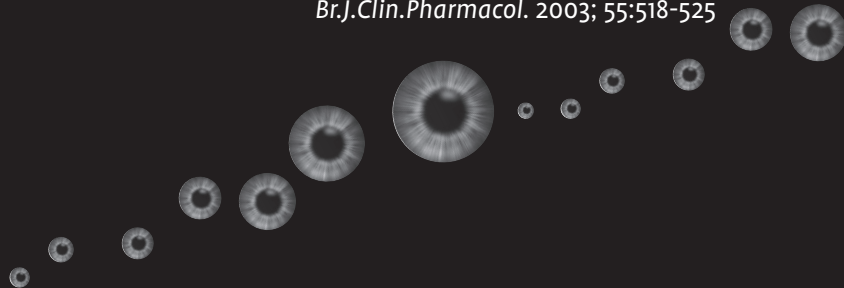
TABLE 2 PK/PD parameters using empirical Bayes estimates for saccadic peak velocity and VAS alertness. Data are population average, SE of the population average (mean), 95% CI and inter-individual variability as standard deviation (IISD).

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

CHAPTER 4

**CENTRAL NERVOUS SYSTEM EFFECTS OF MOXONIDINE
EXPERIMENTAL SUSTAINED RELEASE FORMULATION
IN PATIENTS WITH MILD TO MODERATE ESSENTIAL
HYPERTENSION**

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Abstract

OBJECTIVES The primary aim was to demonstrate that moxonidine, given in an experimental sustained release (SR) formulation, had no clinically relevant central nervous system (CNS) effects after 4 weeks of treatment. A clinically relevant CNS effect was predefined as more than $45^{\circ} \text{ s}^{-1}$ reduction in saccadic peak velocity (SPV), corresponding to the effects of one night's sleep deprivation.

METHODS In a randomized, double-blind fashion, 35 patients with mild to moderate essential hypertension received placebo run-in medication for 2 weeks, followed by 4 weeks' moxonidine sustained release (1.5 mg O.D.) or placebo. On the first day and 1 and 4 weeks following the start of treatment, blood pressure was measured and CNS effects were assessed using SPV, visual analogue scales and EEG.

RESULTS On day 1 there was a significant, but not clinically relevant, reduction in the time-corrected area under the effect curve (AUEC) for SPV in the moxonidine group compared with placebo [difference of $38^{\circ} \text{ s}^{-1}$; 95% confidence interval (CI) 23, 52]. This difference was no longer significant after one (9° s^{-1} ; 95% CI -17, 35) and 4 weeks ($6.9^{\circ} \text{ s}^{-1}$; 95% CI -16, 30). Visual analogue scales for alertness showed similar results. A decrease in EEG α - and β -power and an increase in delta-power were only found on day 1 of moxonidine treatment. The AUEC for systolic/diastolic blood pressure relative to placebo was 23 (95% CI 17, 29)/13 (9, 16) mmHg lower on day 1 and remained reduced by 20 (11, 30)/12 (6, 17) and 15 (6, 25)/9 (3,15) mmHg after 1 and 4 weeks' moxonidine treatment.

CONCLUSIONS Four weeks' treatment with an experimental SR formulation resulted in tolerance to CNS effects (equivalence to placebo) while blood pressure-lowering effects remained adequate. The tolerance to CNS effects was already observed after 1 week of treatment.

Introduction

Ref. 1

Moxonidine is a centrally acting antihypertensive agent, which binds selectively and with high affinity to the imidazoline- I_1 receptor in the ventrolateral medulla of the brainstem and the kidney.* This receptor is thought to play an important role in central blood pressure regulation.

Ref. 2

Ref. 2

Ref. 2

Ref. 2,3

Ref. 4-6

Ref. 7, 8

Ref. 9

Ref. 8

Ref. 10

Stimulation of I receptors induces sympatho-inhibition in the periphery, reflected by a lowering of blood pressure, heart rate, and plasma noradrenaline levels.* Moxonidine also binds to the β_2 -receptor in the medulla oblongata, albeit to a lesser extent than to the imidazoline- I_1 receptor.* Stimulation of the α_2 -receptor in the nucleus coeruleus appears to induce the side-effects of centrally acting antihypertensive drugs, such as sedation and dry mouth.* The agonistic activity of moxonidine on α_2 -receptors is low in comparison with other first-generation centrally acting antihypertensive agents like clonidine and methyl dopa.* The improved tolerability of moxonidine compared with these drugs appears to be related to this difference in receptor activity.*

The sedative properties of many drugs like benzodiazepines* or partial α_2 agonists like clonidine and rilmenidine* have been evaluated previously with measurements of saccadic eye movements and Visual Analogue Scales (VAS). Sedation is typically accompanied by a drop in saccadic peak velocity (SPV), as well as a decrease in subjective alertness as measured with VAS scores. During prolonged treatment with drugs like benzodiazepines, development of tolerance to the sedative effects has been shown.* So far, tolerance development to central nervous system (CNS) drug effects has not been studied for imidazoline I_1 agonists. This tolerance development may improve the clinical acceptability during prolonged treatment with centrally acting antihypertensive drugs.

In this study, the CNS effects of moxonidine were investigated using an experimental sustained release (SR) formulation during prolonged treatment of patients with mild to moderate hypertension. The aim was to demonstrate that moxonidine had no clinically relevant effect on the SPV after 4 weeks of treatment, compared with placebo. A clinically relevant effect on the SPV was predefined as an effect which is found after 24 h of sleep deprivation.* Secondary objectives were to assess the subjective effects (visual analogue scales of Bond and Lader), effects on two-lead pharmaco-EEG (fast Fourier analysis), blood pressure and heart rate. In addition, the relationship between moxonidine plasma concentration and its effects on SPV, visual analogue scales for alertness and blood pressure were modelled.

Methods

The study was conducted according to the principles of the Declaration of Helsinki and in accordance with the guideline for Good Clinical Practice. The study protocol was approved by the Committee on Medical

Ethics of Leiden University Medical Centre. All patients were included after giving written informed consent. This was a randomized, parallel study in which patients received placebo run-in medication for 2 weeks in a single-blind fashion, followed by a double-blind 4-week treatment with moxonidine using an experimental SR formulation of 1.5 mg O.D. or placebo. After screening and approval by their own physician, eligible patients entered a variable washout period of up to 8 weeks, in which their own antihypertensive treatment was withdrawn. As soon as a diastolic blood pressure of 90–104 mmHg had been reached and the patient was off antihypertensive medication for at least 2 weeks, the patient entered the placebo run-in period, during which the patients were acquainted with the experimental methods and conditions during a short training session. Patients with a diastolic blood pressure of 90–104 mmHg without previous antihypertensive treatment could directly enter the single-blind placebo run-in period. Patients whose blood pressure increased above 120 mmHg after withdrawal of their antihypertensive medication were excluded from further participation. At the end of the run-in period eligible patients were randomized to one of the study treatments: moxonidine or placebo. Patients were studied on the first day of allocated randomized treatment, and at 1 and 4 weeks following the start of treatment. After the final study day, medication was tapered off to 0.5 mg O.D. for 3 days. The patients were instructed to take their study medication regularly at breakfast with liquids. Compliance was checked by tablet counting. Sufficient compliance was assumed when the study drug intake was within 80–120% of the planned intake. The moxonidine SR dose was based on the maximum dose intended to be developed, to be able to investigate the course of effects of the highest dose likely to be used in clinical practice.

On the study days, the patients arrived at the facility in the morning in a fasted state. Inclusion/exclusion criteria were checked, if applicable a pregnancy test (CARDS O.S.®H.C.G.-Urine test kits; Pacific Biotech, INC., San Diego, CA, USA) was performed and the urine was checked for use of illicit or sedative drugs (amphetamines, cocaine, morphine, tetrahydrocannabinol, barbiturates and benzodiazepines using Abuscreen ONTRAK®Rapid Assays for Drug Abuse; Roche Diagnostic Systems, Mijdrecht, the Netherlands). In addition, an alcohol breath test was performed with a Lion Alcol Meter (TAXA meter, Amsterdam, the Netherlands). After cannulation of a forearm vein and attachment of scalp, periorbital and ECG electrodes, patients received a light standardized breakfast, and were allowed a 30-min adaptation period in quiet surroundings. Randomized trial medication was administered

($t=0$ h) after a baseline blood sample (drug-assay) had been drawn, and after two ($t=-0.5$ and 0 h) baseline measurements of heart rate, blood pressure, saccadic eye movements, pharmaco-EEG and VAS. These measurements were repeated every half hour for the first hours, and every hour from 3 to 10 h.

Measurements

The blood pressure measurements were made using an automated oscillometric blood pressure monitor (MPV1072; Nihon Kohden, Tokyo, Japan), which displays an average value for two sequential (duplicate) measurements at each time point. All measurements were made after the patient had been in a semirecumbent position for at least 5 min.

Recording of eye movements was performed in a quiet room with ambient illumination. Recording and analysis of saccadic eye movements was conducted with a microcomputer-based system for sampling and analysis of eye movements (Customized CED Software; Cambridge Electronic Design, Cambridge, UK) as described previously.*

Ref. 7, 10

The equipment used for stimulus display, signal collection and amplification was from Nihon Kohden. Disposable silver-silver chloride electrodes (Medicotest N-00-S, Olstykke, Denmark) were applied on the forehead and beside the lateral canthi of both eyes of the patient for registration of the electrooculographic signals. Skin resistance was reduced to <5 k Ω before application of the electrodes. Head movements were restrained using a fixed head support. The target consisted of an array of light-emitting diodes on a bar, fixed at 50 cm in front of the head support. Saccadic eye movements were recorded for stimulus amplitudes of $\pm 15^\circ$ to either side. Fifteen saccades were recorded for each stimulus amplitude with interstimulus intervals varying randomly between 3 and 6 s. Average values of latency (= reaction time), SPV and inaccuracy of all artefact-free saccades were used as parameters. Saccadic inaccuracy was calculated as the absolute value of the difference between the stimulus angle and the corresponding saccade, expressed as a percentage of the stimulus angle.

Ref. 7

EEG registrations were made as described previously*, using silver-silver chloride electrodes, fixed with collodion at median frontal (Fz), median central (Cz), median parietal (Pz) and median occipital (Oz), with the same common ground electrode as for the eye movement registration (international 10/20 system). The electrode resistances were kept below 5 k Ω . EEG signals were obtained from leads Fz-Cz and Pz-Oz.

The signals were amplified by use of a Nihon Kohden AB-621G bioelectric amplifier with a time constant of 0.3 s and a low pass filter at 100 Hz. For the fast Fourier analysis, data collection and analysis were performed using customized CED software (Cambridge Electronics Design). Per session, eight consecutive blocks of 8 s were recorded. The signal was AD-converted using a CED 1401 laboratory interface (Cambridge Electronics Design) and electronically stored for subsequent analysis. Data blocks containing artefacts were identified by visual inspection and these were excluded from analysis. Fast Fourier analysis was performed to obtain the sum of amplitudes in the delta (0.5–3.5 Hz), theta (3.5–7.5 Hz), alpha (7.5–11.5 Hz) and beta (11.5–30 Hz) frequency ranges.

Ref. 11

Ref. 12

Visual analogue scales were used as described by Bond and Lader* and Norris.* From the lines three factors were derived, corresponding to alertness, mood and calmness.

Blood samples for drug assay were collected using lithium heparin-containing polystyrene tubes (Vacuette; Greiner, Alphen a/d Rijn, the Netherlands) and centrifuged immediately after sampling for 10 min at 4°C and 1500 g. Plasma was stored at -20°C until shipment and drug analysis. Drug concentrations were measured using HPLC/MS with atmospheric pressure chemical ionization at oneida research services (Whitesboro, NY, USA). Validation range was 0.050–8.000 ng ml⁻¹, accuracy and precision were < 14% and < 4%.

Calculations and statistical analysis

Data from all subjects were used in the analysis, including the dropouts during treatment with randomized trial medication. The primary statistical analysis was aimed at differences between the two study treatments at day 29 (after 4 weeks' treatment) in overall effects on SPV. The overall effect was characterized by calculating the area under the effect curves (AUEC) over 10 h and dividing this area by the corresponding time span. An analysis of covariance was performed with the value prior to initial drug administration (on day 1) as covariate. A one-sided t-test using the mean square error from the ANCOVA was used for testing one-sided equivalence of the SPV response at day 29. Clinical equivalence was predefined to be proven, if the upper value of the 90% confidence interval of the difference was below 45° s⁻¹. This difference is found after one night of sleep deprivation and can be considered to be a maximally acceptable sedative effect.* All secondary parameters were analysed using an analysis of covariance. For the estimates of the treatment

Ref. 10

differences, 95% confidence intervals (CIs) were calculated. All parameters are given as time-corrected AUECs as a representation of the average effect over the day.

Ref. 13

SPV, VAS alertness and diastolic blood pressure concentration–effect relationships were investigated using nonlinear, mixed effect modelling as implemented in NONMEM (Version 5; NONMEM Project Group, UCSF, CA, USA). This methodology analyses all occasions simultaneously: all subjects are described using the same structural model, and mean and interindividual variability estimates are generated for the entire population.* Significance of parameters was assessed by comparing models with and without the specific parameter(s) using a likelihood ratio test, where a conservative p-value of 0.001 was used to compensate for the large sample normal approximation. In order to correct for the placebo response, the average placebo vs. time profile was calculated over all placebo subjects and subtracted from the individual moxonidine responses at corresponding protocol times. The relationship was described using a simple linear model. In order to relate concentrations to effects, linear interpolation was used. This was implemented in NONMEM by adding, for each data point, the corresponding slope and intercept of the interpolation line for that particular segment. This makes it possible to assess and estimate the need for an effect compartment to model the possible delay between concentrations and effects. Concentration–effect parameters were estimated assuming a parameter value for day 1 and a within-subject deviation (with a fixed and random component) for days 8 and 29. Fixed and/or random components were subsequently eliminated using the likelihood ratio test. An effect compartment was implemented in all cases.

EEG data were analysed after log transformation because of clear indications of skewness and only log EEG results were reported. All other variables were analysed untransformed.

Results

Subjects

Two patients dropped out during the placebo run-in period, one because of an adverse event (depression) and one because of frequent benzodiazepine use. Thirty-five patients were randomized into the doubleblind treatment period: 16 to moxonidine SR and 19 to placebo. Demographics and baseline characteristics of the subjects who started randomized trial medication are summarized in table 1. Two patients

Table 1 – p. 87

in the moxonidine SR group dropped out: one patient after 1 week's treatment because of adverse event (dizziness), and one patient after day 1 because of a serious adverse event (aorta dissection leading to hospitalization and death). This serious adverse event was regarded as unrelated to the study drug. In the placebo group, also two patients dropped out: one patient because of severe hypertension and one patient because of depression, both after day 1.

Adverse events

A total of 30 patients (86%) reported treatment-emergent adverse events. The most frequently reported adverse events were somnolence and dry mouth. On the first treatment day, somnolence was reported by all 16 subjects on moxonidine SR (100%) and by 10/19 patients (58%) in the placebo group, and dry mouth by 10 patients (63%) in the moxonidine group, vs. two patients (11%) on placebo. After 4 weeks of treatment, the number of moxonidine-treated patients reporting sedation had diminished to 9/16 (56%), compared with 7/19 (37%) on placebo. At that time, dry mouth was noted by 7/16 patients on moxonidine SR (44%) and 2/19 on placebo (11%).

Compliance

The median percentage of tablets taken during treatment was 100.0% (range 96.0–105.6%) in the moxonidine treatment group, and 100.0% (95.5–114.3%) in the placebo group.

Saccadic peak velocity

The sPV profile showed a clear treatment-related curve for all moxonidine SR treatment study days (figure 1). On day 1 the time-corrected AUEC for peak velocity was significantly lower in the moxonidine SR group (38°s^{-1} ; 95% CI 23, 52). However, as predose moxonidine baseline values increased, the moxonidine treatment profile shifted upwards, and the difference with placebo was no longer present on day 8 (difference 9°s^{-1} ; 95% CI -17, 35) and day 29 (difference 7°s^{-1} ; 95% CI -16, 30). The upper value of the 90% CI (-12, 26) was well below 45°s^{-1} , the predefined level of clinical significance*. The two treatments were therefore considered

Fig. 1 – p. 87

Ref. 10

equivalent with respect to the AUEC curve for SPV after 4 weeks of treatment. No significant differences were observed for accuracy and reaction times (data not shown).

Visual analogue scales

Fig. 2 – p. 88

Analogously to the SPV profile, the alertness VAS showed a treatment-related curve for all moxonidine SR treatment study days (figure 2). The time-corrected alertness AUEC was significantly lower on day 1 in the moxonidine SR group (6.7 mm; 95% CI 0.2, 13.2). As predose baseline VAS alertness increased and the profile shifted upwards, this difference disappeared on day 8 (difference of 5.4 mm; 95% CI -2.6, 13.5) and day 29 (difference of 0.9 mm; 95% CI -8.3, 10.1). No significant differences between the moxonidine SR and placebo groups were observed with respect to the mood and calmness AUECs (data not shown).

Electroencephalogram

No clear treatment profiles were observed for EEG parameters. Significant differences between treatment groups were shown for all mid-parietal–occipital (PzOz) leads. In the moxonidine SR group on day 1, log transformed AUEC of total α - and β -power was significantly lower (difference 0.08, 95% CI 0.005, 0.16; and 0.12, 95% CI 0.01, 0.23, respectively), while total delta-power was significantly higher (difference -0.10; 95% CI -0.20, -0.01). These differences were not observed on day 8 and day 29, nor were there significant differences between study days (data not shown). The log transformed AUEC of total theta-power was higher in the moxonidine SR group on day 8 and day 29 (difference -0.16, 95% CI -0.33, -0.001; and -0.21, 95% CI -0.40, -0.03, respectively). No significant differences between treatment groups were observed for any of the mid-frontal–central (FzCz) lead EEG parameters. The log transformed AUEC of total theta-power (FzCz lead) on day 1, however, came close to a significant increase for the moxonidine SR group compared with placebo (difference -0.15; 95% CI -0.31, 0.003).

Blood pressure and heart rate

Fig. 3.4 – p. 88, 89

Systolic and diastolic blood pressure profiles showed clear treatment profiles on all study days (figures 3 and 4). Mean (95% CI) systolic AUECs were significantly lower in the moxonidine SR group on all study days: 23 (17, 29), 20 (11, 30) and 15 (6, 25) mmHg difference for days 1, 8 and 29. Also the mean (95% CI) diastolic blood pressure AUECs were significantly lower in the moxonidine SR group on all study days: 13 (9, 16), 12 (6, 17) and 9 (3, 15) mmHg difference for days 1, 8 and 29. Only on day 8 was predose (trough) diastolic blood pressure significantly lower in the moxonidine SR group compared with placebo (difference 5.3 mmHg; 95% CI 0.2, 10.5). Average heart rate did not show a treatment-related response (not shown).

Pharmacokinetics

Fig. 5 – p. 89

The moxonidine time profiles were clearly the result of an extended release oral formulation. Average moxonidine concentration profiles shifted upwards on day 8 and day 29 as baseline values increased (figure 5). The area under the curve (AUC) increased 18% (95% CI 2, 37) from day 1 to day 8 and the AUC increased 14% (95% CI -1, 33) from day 1 to day 29. The maximum concentration increased 19% (95% CI 6, 34) from day 1 (2.6 ± 0.7 ng ml⁻¹) to day 8 (3.1 ± 1.1 ng ml⁻¹) and 20% (95% CI 6, 35%) from day 1 to day 29 (3.1 ± 0.8 ng ml⁻¹). The time to maximum concentration (4 h 28 min on day 1 and 3 h 26 min on day 29) did not change significantly between study days.

Concentration–effect modelling

Concentration–effect modelling was performed by linearly interpolating the concentration profile and by linking these concentrations to the effects. In the concentration–effect graphs the average effect (moxonidine minus placebo effect) is plotted against the corresponding average interpolated moxonidine concentration. All effects tended to lag behind relative to the plasma concentrations. This hysteresis was dealt with by assuming a hypothetical effect compartment, characterized by an equilibration half-life that describes the speed with which plasma concentrations would be equilibrated with effect compartment concentrations if the drug was to be infused at a constant rate.

Saccadic peak velocity

Fig. 6 – p. 90

For SPV the best fit was given by a model including an effect compartment where the equilibration half-life (58 min; 95% CI 39, 77) and slope ($-22.9^{\circ} \text{ s}^{-1} \text{ ng}^{-1} \text{ ml}$; 95% CI -4, -42) were similar between study days (figure 6). From day 1 to 8 and 29 the profile shifted upwards, leading to an increase in intercept of $36^{\circ} \text{ s}^{-1}$ (95% CI 30, 41) from day 1 to day 8 and an increase of $41^{\circ} \text{ s}^{-1}$ (95% CI 26, 55) from day 1 to day 29.

VAS alertness

Fig. 7 – p. 90

For VAS alertness, the best model was analogous to SPV. The slope ($-3.9 \text{ mm ng}^{-1} \text{ ml}$; 95% CI -1.6, -6.2) and equilibration half-life (43 min; 95% CI 10, 76) did not differ between occasions (figure 7). Only the intercept was different between occasions: a nonsignificant increase of 3.0 mm (95% CI -1.3, 7.3) from day 1 to day 8 and a significant increase of 6.6 mm (95% CI 3.6, 9.6) from day 1 to day 29.

Diastolic blood pressure

Fig. 8 – p. 91

For diastolic blood pressure, no shift of the concentration–delta diastolic blood pressure curve was observed, but the profile levelled off on day 8 and day 29 (figure 8). The best fit was given by a model in which only the slope was significantly different between study days. Estimated equilibration half-life was 86 min (95% CI 52, 119) and intercept was -0.15 mmHg (95% CI -4.5, 4.2). The slope decreased $4.4 \text{ mmHg ng}^{-1} \text{ ml}$ (95% CI 2.3, 6.6) from day 1 to day 8 and $3.2 \text{ mmHg ng}^{-1} \text{ ml}$ (95% CI 0.3, 6.1) from day 1 to day 29.

Discussion

This study showed that after 4 weeks of treatment, moxonidine 1.5 mg O.D. given in an experimental SR formulation was equivalent to placebo for the overall effects on SPV, an objective measure of alertness. On each study day, a treatment-related decrease in SPV relative to predose values at each study day was observed, but only on the first day of moxonidine SR treatment was the SPV AUEC significantly lower compared with placebo. As a result of an upward shift of the curve, the average effects

Ref. 10

of moxonidine SR on SPV were at placebo level after 8 and 29 days of treatment. Although its face validity is not very high, SPV is one of the most reproducible and sensitive measures of sedation.* No method applicable in drug research is available that shows direct relationships to clinical consequences of sedation, like reduced working performance or increased accident rates. The SPV results were corroborated by similar findings with visual analogue scales for alertness, which have a higher face validity. The subjective VAS score for alertness showed treatment-related curves, which shifted upwards during treatment. The EEG results further support these observations, as only on day 1 were significant effects consistent with CNS depression observed.* All these results point to the development of tolerance to the CNS effects of moxonidine 1.5 mg. It should be noted that the recommended starting dose for moxonidine is considerably lower than the dosage used in this study. The observed CNS effects for the first treatment day are therefore likely to be smaller during normal clinical use. Both the average subjective and objective CNS effects on day 1 returned to placebo levels within the first week of treatment, although treatment-related decreases in SPV and VAS were still present after 1 and 4 weeks. These observations are further clarified by concentration–effect modelling. The slope of the modelled effect remained unchanged, indicating that the response to moxonidine was similar on all study days. However, the concentration–effect curves shifted upwards during treatment, resulting in net CNS effects comparable to placebo.

Ref. 14

Ref. 2

The increase in baseline alertness as shown by two independent measures of alertness (SPV and VAS) indicates that after 1 and 4 weeks of treatment the patients in the Moxonidine SR group became more alert in the morning before receiving their daily dose. This phenomenon has not been observed previously during treatment with moxonidine, and its causes are unclear. The CNS effects of moxonidine are thought to be mainly due to weak α_2 receptor agonism, which decreases central noradrenergic activity.* An increase in alertness during treatment with α_2 agonists could be the result of a hyperresponsiveness of central noradrenergic pathways during prolonged treatment.* However, such a putative central noradrenergic increase did not seem to affect blood pressure.

Ref. 15

Systolic and diastolic blood pressure showed clear treatment effects in the moxonidine SR group, with a lowering of blood pressure to nonhypertensive levels. Despite this clear treatment effect, predose blood pressure did not differ between the treatment groups, except for a lower diastolic blood pressure in the moxonidine SR group on day 8. Thus, 24-h effective control of systolic and diastolic blood pressure was

not fully maintained with a single dose of this experimental moxonidine SR formulation. However, it has to be considered that the study was not powered to detect clinically meaningful blood pressure effects at trough. Further improvement of the extended release profile could increase the trough levels of 0.7 ng ml⁻¹ observed on day 29, and also reduce the mean maximum concentrations of 3.1 ng ml⁻¹ that were found in this study. Moxonidine plasma concentrations increased by 14–18% compared with day 1, but the average blood pressure remained unchanged throughout the 4-week treatment period. Modelling showed a small decrease in slopes of the concentration–effect curves, which may be interpreted as an indication of tolerance. Tolerance is often observed among agonist-stimulated, G-protein-coupled receptors, such as the I₁-receptor. On the other hand, no rebound hypertension has been reported after the discontinuation of moxonidine treatment,* arguing against such a phenomenon. Systemic counter-regulation could be a more plausible explanation, considering the strong antihypertensive effects of this moxonidine dose, as exemplified by an average maximum systolic/diastolic blood pressure reduction of 36/21 mmHg on day 1 (figures 3 and 4). In view of the sustained antihypertensive effects, the lack of further blood pressure reduction despite increasing moxonidine concentrations may be regarded as clinically nonsignificant.

Ref. 16

Fig. 3, 4, p. 88, 89

In conclusion, this study showed that after 4 weeks of treatment, moxonidine SR 1.5 mg O.D. is equivalent to placebo for average CNS effects. Treatment–effect curves over the day and modelling of the pharmacodynamic and pharmacokinetic data provided indications of an increased alertness at predose (trough level) after 1 and 4 weeks treatment.

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REFERENCES

- 1 Ernsberger P, Damon TH, Graff LM, Schafer SG, Christen MO. Moxonidine, A Centrally Acting Antihypertensive Agent, Is A Selective Ligand For 11-imidazoline Sites. *J Pharmacol Exp Ther* 1993; 264: 172–182.
- 2 Van Zwieten PA. The Renaissance Of Centrally Acting Antihypertensive Drugs. *J Hypertens* 1999; 17 (Suppl 3): S15–S21.
- 3 Prichard BN, Graham BR, Owens CW. Moxonidine: A New Antiadrenergic Antihypertensive Agent. *J Hypertens* 1999; 17 (Suppl 3): S41–S54.
- 4 Macphee GJ, Howie CA, Elliott HL, Reid JL. A Comparison Of The Haemodynamic And Behavioural Effects Of Moxonidine And Clonidine In Normotensive Subjects. *Br J Clin Pharmacol* 1992; 33: 261–267.
- 5 Planitz V. Crossover Comparison Of Moxonidine And Clonidine In Mild To Moderate Hypertension. *Eur J Clin Pharmacol* 1984; 27: 147–152.
- 6 Schachter M. Moxonidine: A Review Of Safety And Tolerability After Seven Years Of Clinical Experience. *J Hypertens* 1999; 17 (Suppl 3): S37–S39.
- 7 Van Steveninck AL, Mandema JW, Tuk B *et al.* 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.
- 8 Van Steveninck AL, Wallnofer AE, Schoemaker RC *et al.* 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.
- 9 Harron DW, Hasson B, Regan M, McClelland RJ, King DJ. Effects Of Rilmenidine And Clonidine On The Electroencephalogram, Saccadic Eye Movements, And Psychomotor Function. *J Cardiovasc Pharmacol* 1995; 26 (Suppl 2): S48–S54.
- 10 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.
- 11 Bond AJ, Lader MH. The Use Of Analogue Scales In Rating Subjective Feelings. *Br J Med Psychol* 1974; 47: 211–218.
- 12 Norris H. The Action Of Sedatives On Brain Stem Oculomotor Systems In Man. *Neuropharmacology* 1971; 10: 181–191.
- 13 Schoemaker HC, Cohen AF. Estimating Impossible Curves Using NONMEM. *Br J Clin Pharmacol* 1996; 42: 283–290.
- 14 Santamaria J, Chiappa KH. The EEG Of Drowsiness In Normal Adults. *J Clin Neurophysiol* 1995; 4: 327–382.
- 15 Geyskes GG, Boer P, Dorhout Mees EJ. Clonidine Withdrawal. Mechanism And Frequency Of Rebound Hypertension. *Br J Clin Pharmacol* 1979; 7: 55–62.
- 16 Webster J, Koch HF. Aspects Of Tolerability Of Centrally Acting Antihypertensive Drugs. *J Cardiovasc Pharmacol* 1996; 27 (Suppl 3): S49–S54.

TABLE 1 Demographic characteristics and baseline variables

		Moxonidine SR (n=16)	Placebo (n=19)
Age (years) ^a		55 (34-71)	56 (35-78)
Numbers / %	<i>per age group:</i>		
	< 50	6 / 38	3 / 16
	50 – 59	3 / 19	9 / 47
	≥ 60	7 / 44	7 / 37
Sex (n, Male / female)		7/9	10/9
Weight (kg) ^a		78 (59-102)	81 (61-105)
Height (cm) ^a		172 (160-190)	172 (158-186)
Body Mass Index (kg/m ²) ^a		26 (22-37)	28 (21-38)
Systolic blood pressure ^b		157 (135-189)	161 (146-178)
Diastolic blood pressure ^b		91 (72-106)	91 (77-100)
Heart rate ^b		71 (60-86)	72 (56-91)
Ethnic origin (n/%)	Caucasian	15 / 94	19 / 100
	Negroid	1 / 6	0 / 0

a given as means (range)

b pre-dose value at first study-day

FIGURE 1 Mean \pm SD saccadic peak velocity profiles for the moxonidine sustained release (SR) (●) and placebo (○) treatment groups during study day 1, day 8 and day 29. Each profile represents a different study day; measurements were performed from predose (first two data points) until 10 h postdose.

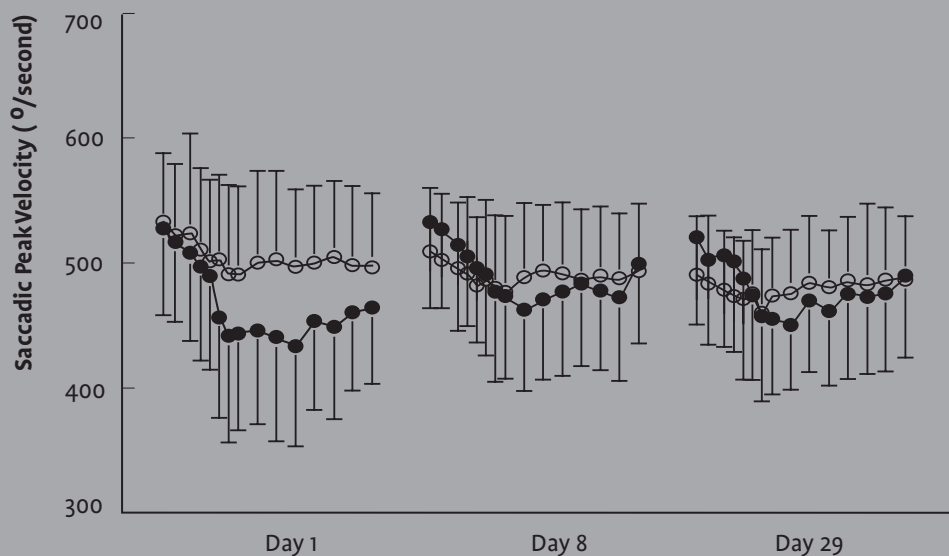


FIGURE 2 Mean \pm SD visual analogue scale (VAS) alertness profiles for the moxonidine sustained release (SR) (●) and placebo (○) treatment groups during study day 8 and day 29. Each profile represents a different study day; measurements were performed from predose (first two data points) until 10 h postdose.

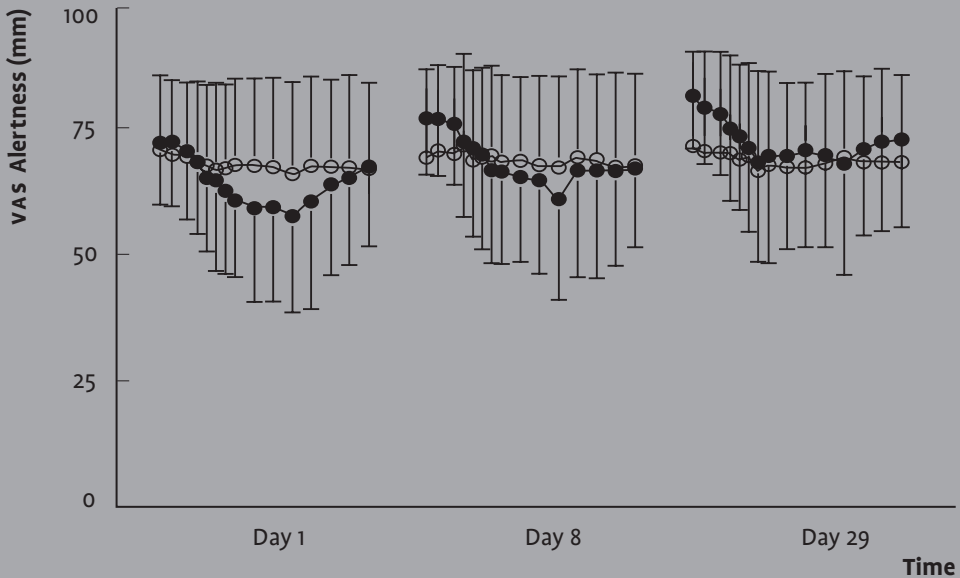


FIGURE 3 Mean \pm SD systolic blood pressure profiles for the moxonidine sustained release (SR) (●) and placebo (○) treatment groups during study day 1, day 8 and day 29. Each profile represents a different study day; measurements were performed from predose (first two data points) until 10 h postdose.

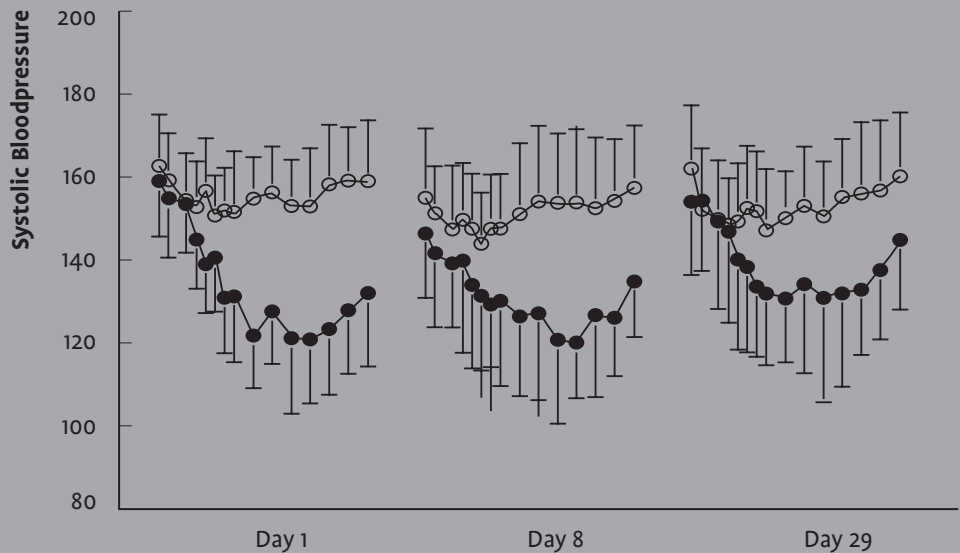


FIGURE 4 Mean \pm SD diastolic blood pressure profiles for the moxonidine sustained release (SR) (●) and placebo (○) treatment groups during study day 1, day 8 and day 29. Each profile represents a different study day; measurements were performed from predose (first two data points) until 10 h postdose.

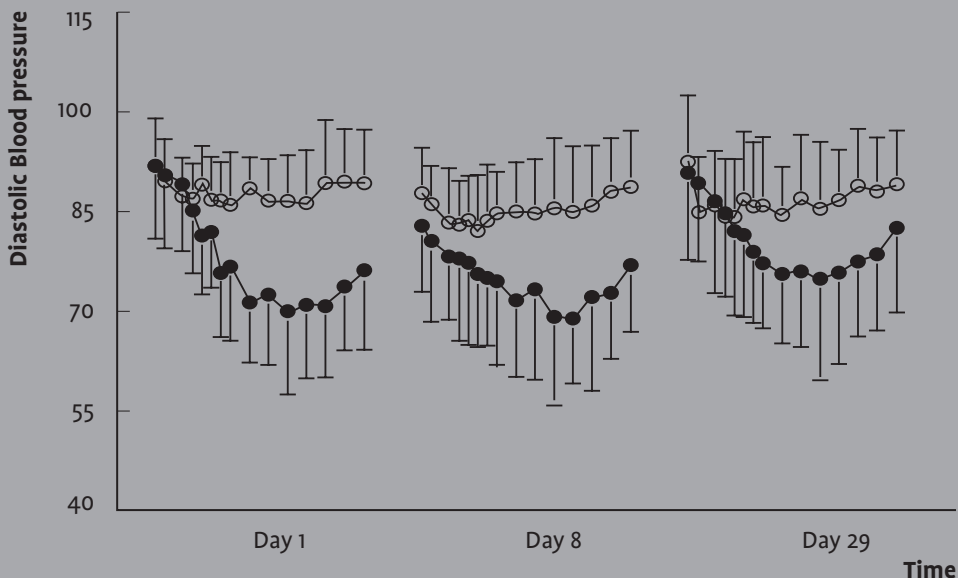


FIGURE 5 Mean \pm SD moxonidine plasma concentration profiles during study day 1, day 8 and day 29. Each profile represents a different study day; measurements were performed from predose (first two data points) until 10 h postdose.

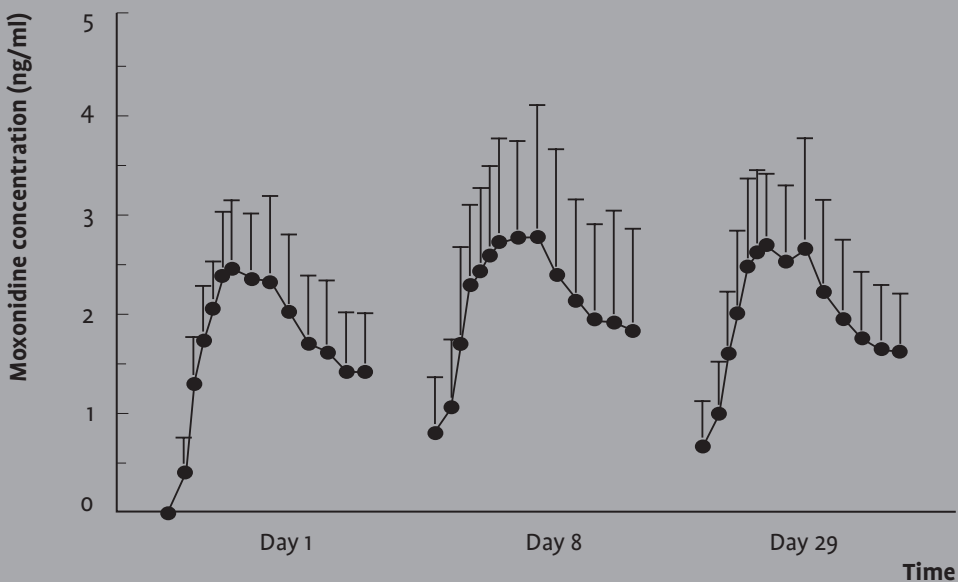


FIGURE 6 Delta (placebo minus moxonidine response) saccadic peak velocity profiles vs. interpolated moxonidine concentration. The broken line represents the modelled profile for day 1 (with average original data, ○), the solid lines represent the modelled profile for days 8 and 29.

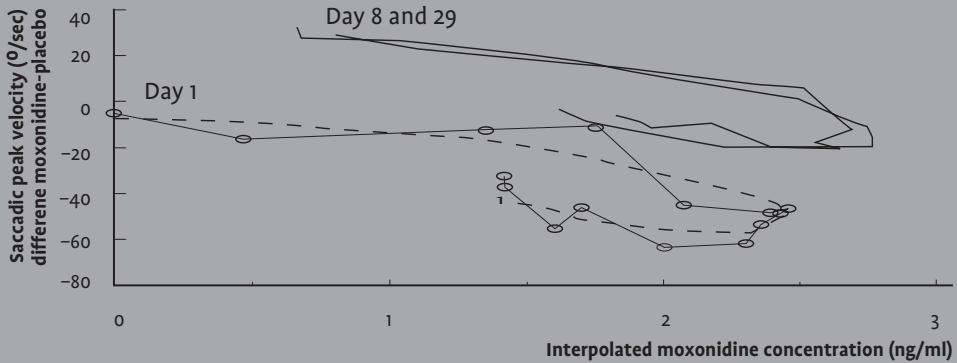


FIGURE 7 Delta (placebo minus moxonidine response) visual analogue scale (VAS) alertness vs. interpolated moxonidine concentration. The broken line represents the modelled profile for day 1 (with average original data, ○), the solid lines represent the modelled profile for days 8 and 29.

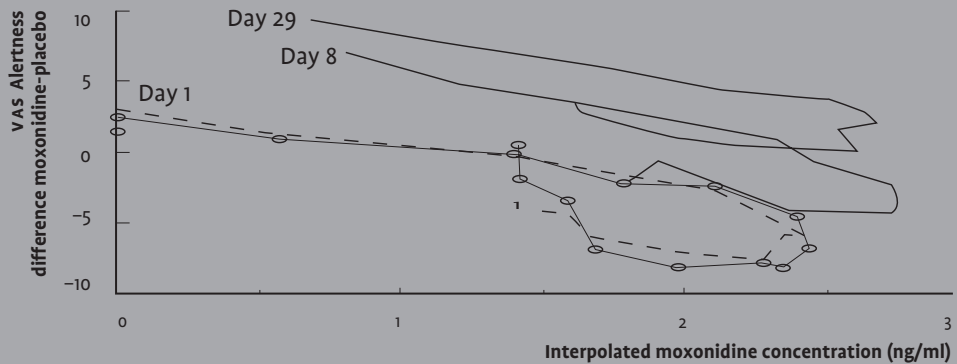
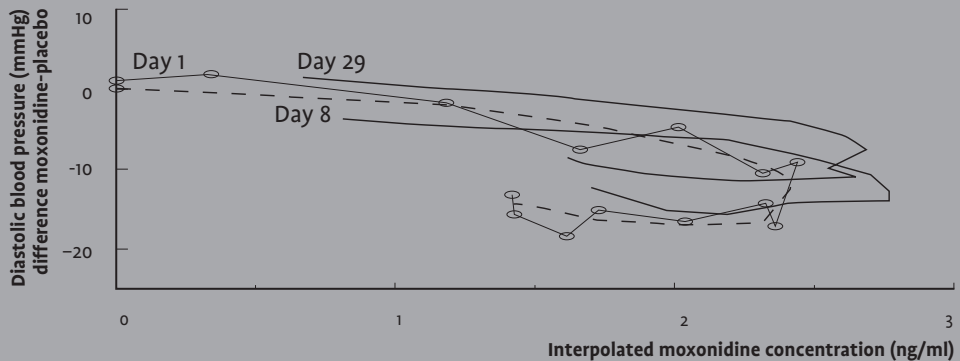
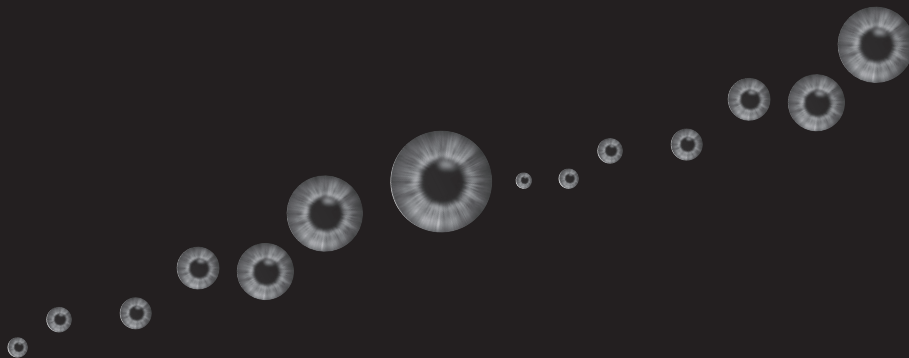


FIGURE 8 Delta (placebo minus moxonidine response) diastolic blood pressure vs. interpolated moxonidine concentration. The broken line represents the modelled profile for day 1 (with average original data, ○), the solid lines represent the modelled profile for days 8 and 29.



CHAPTER 5

**DRUG CLASS SPECIFIC COMPOSITE EFFECTS ON SACCADIC
PEAK VELOCITY, VISUAL ANALOGUE SCALES AND
ELECTROENCEPHALOGRAPHY**



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Abstract

AIMS This study aims to explore whether drug (class) specific relationships are present between saccadic peak velocity (SPV), Visual Analogue Scales (VAS) and electro-encephalography (EEG).

METHODS From studies that were performed at centre for human drug reasearch (CHDR) data was included that had showed a statistically significant treatment effect on SPV, and included either VAS or EEG measurements or both in the evaluation of a range of CNS active drugs. All data were analysed simultaneously, resulting in a population average estimate for slope and intercept and estimates of the inter-individual variability in parameters. Analysis was performed using linear mixed effect modelling. Two models (estimation of a a common slope and intercept for all drugs, estimation of a different slope for each drug with a reference slope for placebo) were tested. These two models were statistically compared using a likelihood ratio. Differences in slope from placebo with approximate 95% confidence intervals (CI) were calculated, allowing an assessment of difference in slopes between the different drugs.

RESULTS Delta SPV-delta VAS relationship showed a mean slope 0.125 (95% CI of 0.110/0.140). The difference in minimum value of the objective function between the two models (with and without separate slopes for the different drugs) was 56.5 ($p < 0.001$, likelihood ratio test with 10 degrees of freedom (DF)). The most clear discrimination between drugs could be observed for SPV relationships with beta Fc, alpha Po and theta Fc. The difference in minimum value of the objective function between the two models was 144.55 for beta Fc ($p < 0.001$, 10 DF), 79.55 for alpha Po ($p < 0.001$, 8 DF) and 55.79 ($P < 0.001$, 8 DF).

CONCLUSIONS Combining the diferent slopes of the SPV-VAS and SPV-EEG relationships, a drug class specific picture emerges. With decreasing SPV, benzodiazepines/propofol show a strong subjective response on VAS, increase beta Fc power and decrease alpha and theta power. The serotonergic drugs show only a small VAS response and increase alpha and theta power. The imidazoline modulators show a VAS response above serotonergic drugs but under benzodiazepines, and decrease alpha power and increase theta power.

Introduction

There is a high level of interest in biomarkers in the pharmaceutical industry, which is faced with the ever-increasing cost of research and development, and with growing pressure to accelerate the rate of bringing new drugs to the marketplace. Appropriate use of suitable biomarkers can facilitate this process. A biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. In general, biomarkers can be used for the following applications: as a diagnostic tool (for the identification of those patients with a disease or abnormal condition), as a tool for staging a disease or classification of the extent of disease, as an indicator of disease prognosis or for prediction and monitoring of clinical response to an intervention.

Ref. 1

Biomarkers are perhaps most useful in the early phases of drug development, when measurement of clinical endpoints may be too time-consuming or cumbersome to provide timely proof of concept or dose-ranging information. Ideally, the attributes of a biomarker should include the following*: clinical relevance, sensitivity and specificity to treatment effects, reliability, practicality and simplicity.

This investigation focusses on biomarkers for pharmacological activity of central nervous system (CNS) active drugs in phase 1 development. The identification of biomarkers in this area poses specific problems. The overwhelming amount of different tests (or tests variants) used by different research groups makes it difficult to identify even basic test characteristics such as sensitivity, and severely limits the reproducibility of findings. This compromises the ideal biomarker attributes of sensitivity and reliability. In addition, the specificity of these biomarkers seems to be low. The clinical relevance of tests results is also often difficult to assess: most CNS biomarkers are used during a broad evaluation of adverse CNS effects, and seldomly focuss on the intended pharmacological effect.

Showing CNS activity of drugs can be informative on another level than assessing adverse reactions. Potentially, a marker can be sensitive to a certain receptor modulating effect (such as plasma prolactin for D₂-antagonistic activity) or effects on a specific neurotransmitter (such as plasma cortisol for serotonergic action, or memory impairment for achetylcholine inhibition). These markers are not necessarily pathophysiologically close to a clinical endpoint, but can demonstrate a certain mechanism of action. An important shortcoming of these,

although sensitive, ‘single biomarkers’ (one test, one measurement) is their frequent lack of specificity. Frequently drugs will show similar results on a marker, although their pharmacological properties vary widely.

A potentially more fruitful approach could be to identify a combination of sensitive markers that show a specific co-dependence to a drug (or preferably a drug class) action. If such a ‘composite biomarker’ can be identified, it could allow establishing ‘biomarker templates’ of drug classes with specific receptor binding profiles or mechanisms of action. It is important to realize that these composite markers will initially not allow a strong link to the pathophysiology of a disease or intended clinical effect. However, it will allow compounds with a new or unknown mechanism of action to be placed in a relation to existing drugs, based on their effects on this composite marker. This could guide further evaluation of the CNS effects of such a drug, based on the cumulative knowledge of existing drugs.

Ref. 2

Ref 3

Table 1, 2 – p. 106, 107

Recent structured literature evaluation of tests used in neuroleptic* and anxiolytic* drug development as well as studies on drugs from other drug classes (studies presented in tables 1&2) showed that saccadic peak velocity (SPV), Visual Analogue Scales (VAS) and electroencephalography (EEG) are frequently used and are sensitive to a wide range of drugs. This study aims to explore whether drug (class) specific relationships are present between SPV, VAS and EEG.

Methods

Study selection

The studies all included SPV and either VAS or EEG measurements or both. The data used was selected from studies that were performed at CHDR during the past 20 years. The drugs under investigation and the number of study occasions for studies that had both SPV and VAS measurements are presented in table 1. The same data is presented in table 2 for drugs that had both SPV and EEG measurements.

Table 1 – p. 106

Table 2 – p. 107

Saccadic eye movements

Saccadic peak velocity as used in our experiments is the maximum velocity that the eye ball reaches while following a light that jumps

Ref. 4

on a horizontal bar. The head of the subject is fixated in a headrest. Recording and analysis of saccadic eye movements was done with a microcomputer-based system.* The equipment used for stimulus display, signal collection and amplification was from Nihon Kohden (Nihon Kohden Corporation, Tokyo, Japan). Saccadic eye movements were recorded for stimulus amplitudes of 15 degrees to either side. Interstimulus intervals varied randomly between 3 and 6 seconds, and fifteen saccades were recorded. The average values of saccadic peak velocity were used as parameters.

Visual analogue scales

Ref. 5

Visual analogue scales as originally described by Norris* were used to quantify subjective effects. The factors corresponding to alertness were derived from these measurements.* These consisted of 7 of the total of 16 scales. The direction corrected mean score in mm of these scales was used.

Ref. 6

Electroencephalography

Ref. 7

Electroencephalograms were recorded and analysed using CED software (Cambridge Electronics Design, Cambridge, UK), as described previously.* EEG recordings were made using silver-silver chloride electrodes, fixed with collodion at Fz, Cz, Pz and Oz, with the same common ground electrode as for the eye movement registration (international 10/20 system). The electrode resistances were kept below 5 kOhm. All recordings were done with the subjects' eyes closed. EEG signals were obtained from leads Fz-Cz and Pz-Oz. The signals were amplified by use of a Nihon Kohden ab-621g bioelectric amplifier (Nihon Kohden Corporation, Tokyo, Japan) with a time constant of 0.3 seconds and a low pass filter at 100 Hz. Per session eight consecutive blocks of eight seconds were recorded over a 2 minute period. The sampling rate was 1024 Hz. Datablocks containing artefacts were identified by visual inspection and these were excluded from analysis. Fast Fourier transform analysis was performed to obtain the sum of amplitudes in the delta- (0.5-3.5 Hz), theta- (3.5-7.5 Hz), alpha- (7.5-11.5 Hz) and beta (11.5-30 Hz) frequency ranges. The total recording bandwidth was 0-50 Hz.

Statistical analysis

Ref. 2, 3

Studies were included in the analysis that showed a statistically significant treatment effect on SPV, and included either VAS or EEG measurements or both. VAS and EEG were related to SPV because SPV has shown to be the most sensitive test for a wide range of CNS active drugs.* Relating VAS and EEG test results to SPV, thus normalising results to SPV, allows comparison of SPV-VAS and SPV-EEG relationships for different drugs and dosages.

The SPV profiles were linearly interpolated to obtain interpolated SPV measurements for corresponding VAS measurements. The value just prior to drug administration was used as pre-value and pre-values were subtracted for both SPV and VAS measurements, resulting in changes from baseline (Δ SPV and Δ VAS). The SPV measurements were always close to the EEG measurements and were therefore shifted slightly to the corresponding EEG measurements. The value just prior to drug administration was used as pre-value and pre-values were subtracted for both SPV and EEG measurements, resulting in changes from baseline (Δ SPV and Δ EEG). Data analysis was performed using SPSS for Windows V10.1.4 (SPSS, INC., Chicago, IL).

SPV-VAS, SPV-EEG RELATIONSHIPS Each individual's relationship between SPV and VAS or EEG was described using a straight line. More complex relationships did not result in improvement of fit. Instead of estimating all individual linear regressions, all data were analysed simultaneously, resulting in a population average estimate for slope and intercept and estimates of the inter-individual variability in parameters. The advantage of simultaneous estimation is that this allows a single test to determine if the relationship is the same for all the different drugs. This analysis was performed using linear mixed effect modelling with NONMEM Version V software (NONMEM Project Group, UCSF, CA), where 'first order conditional estimation' was employed. For each set of parameters, two models were estimated. The first model estimated a common slope and intercept for all drugs and the second estimated a different slope for each drug (a reference slope for placebo and changes in slope (from placebo) for the different drugs were determined). These two models were statistically compared using the likelihood ratio test that compares the difference in minimum value of the objective function between the two models with a chi-square distribution. Differences in slope from placebo with approximate 95% confidence intervals (using $2 \times \text{SEM}$) were calculated, allowing an assessment of difference in slopes between the different drugs.

Results

SPV-VAS relationships

Linear mixed effect modelling used to estimate slope and intercept of the delta SPV-delta VAS relationship showed a mean slope of 0.125 with a 95% confidence interval of 0.110/0.140. The 95% confidence interval for the slope indicates a highly significant relationship between the two measures. Subsequently, relationships were simultaneously estimated for the different drugs and placebo. The difference in minimum value of the objective function between these two models (with and without separate slopes for the different drugs) was 56.5 which is significant at $p < 0.001$ using the likelihood ratio test with 10 degrees of freedom. This proves that the different drugs have different slopes for the delta SPV-delta VAS relationship. Regression estimates for the different drugs are presented in figure 1.

Fig. 1 – p. 104

SPV-EEG relationships

The most clear discrimination between drugs could be observed for SPV relationships with beta Fc, alpha Po and theta Fc. Using the likelihood ratio test, the difference in minimum value of the objective function between these two models (with and without separate slopes for the different drugs) was 144.55 for beta Fc ($p < 0.001$, 10 degrees of freedom), 79.55 for alpha Po ($p < 0.001$, 8 degrees of freedom (DF)) and 55.79 ($p < 0.001$, 8 DF). This proves that the different drugs have different slopes for the SPV-EEG relationships. Regression estimates of these SPV-EEG relationships for the different drugs are presented in figures 2-4.

Fig 2, 3, 4 – p. 104, 105

SPV-EEG-VAS relationships

Drugs were grouped into three classes; benzodiazepines, serotonergic drugs and imidazoline receptor modulators. The mean slope ranges for the drug classes are presented in table 3. The composition of these drug classes differs for each column, since not all measurements were available for all the different drugs. Only three studies included all measurements (SPV, VAS, EEG), which did not allow for discriminant analysis.

Table 3 – p. 108

Discussion

Fig. 1 / Table 3 –
p. 104 / 108

The delta SPV-delta VAS relationships showed a strong correlation between the two measures for all drugs that had a decreasing effect on SPV. However, the drugs appear to have a clear differential effect. With a comparable effect size on SPV, the slopes for benzodiazepines (lorazepam, temazepam) seemed to be largest (figure 1, table 3). The slopes for the imidazoline receptor modulators (rilmenidine, moxonidine) were lower than those of the benzodiazepines, followed by the slopes for serotonergic drugs (suma- and rizatriptan). This indicates that SPV changes can lead to a different subjective notion of decreased alertness.

The interpretation of these results does have a number of pitfalls. First of all, the study information that the participating subjects received, may have led to the difference in subjective alertness scores. The information will usually state what the treatment indication for a drug is, and will inform subjects on the expected side effects. For a number of studies, the study medication consisted of benzodiazepine and placebo treatment in a cross-over design. The subjective interpretation of decreased alertness by subjects in such a study may lead to a stronger VAS response if they know one of the drugs is a hypnotic (temazepam) or anxiolytic (lorazepam). Furthermore, although most studies used healthy volunteers (mostly between 18-40 years), the populations used in the studies may have been heterogenous, which could influence subjective scores.

The relatively small slope of the benzodiazepine diazepam combined with the large effect size on SPV, indicated a non-linear and potentially sigmoidal relationship between SPV and VAS (no increase of VAS with increasing SPV change). However, examination of individual SPV-VAS relationships for diazepam showed no evidence of non-linearity.

In conclusion, the consistency in methodology (identical for all studies, all studies were performed in one centre) and the highly significant difference in slopes for the drugs, warrants the presumption of a unique SPV-VAS relationship for different drugs or perhaps drug classes.

The relationship between SPV and EEG showed clearly different slopes for benzodiazepines/propofol, serotonergic drugs (suma- and rizatriptan, M-CPP) and the imidazoline receptor modulators (rilmenidine, moxonidine). SPV-beta Fc relationships showed a large positive slope for temazepam, midazolam and propofol, with comparable small slopes for the other drugs (figure 2, table 3). The specific effect of benzodiazepines and propofol on beta Fc is well documented (9-14), and

Fig. 2 / Table 3 –
p. 104 / 108
Ref. 9, 10, 11, 12, 13, 14

Fig. 3 / Table 3 – p. 106

confirmed in this study. For the SPV-alpha Po relationships a positive slope is observed with the serotonergic drugs, contrasting with the negative slope for the other drugs (figure 3, table 3). M-CPP actually increases SPV and decreases alpha power, leading to a positive slope for the SPV-alpha Po relationship. The SPV-theta Fc relationship showed a contrast between the benzodiazepines/propofol and the other drugs, showing a negative slope for the first and a positive slope for the latter (figure 4, table 3).

Fig. 4 / Table 3 – p. 106

Combining the different slopes of the SPV-VAS and SPV-EEG relationships, a drug class specific picture emerges (table 3). With decreasing SPV, benzodiazepines/propofol show a strong subjective response on VAS, increase beta Fc power and decrease alpha and theta power. The serotonergic drugs show only a small VAS response and increase alpha and theta power. The imidazoline modulators show a VAS response above serotonergic drugs but under benzodiazepines, and decrease alpha power and increase theta power.

Unfortunately only three studies included both SPV, VAS and EEG. A larger number of overlapping studies would allow a discriminant analysis that could quantify the discriminating power of the combination of these measures. Although this is an exploratory study, the presented results should be viewed as a stimulant to include SPV, VAS and EEG in the evaluation of CNS active drugs in phase 1 research. Ideally, more data on composite effects, also of other relatively receptor specific drugs, will be generated. If the observed composite effects on these measures is thus prospectively validated, they may allow drugs with (partly) unknown mechanisms of action to be compared to the ‘composite biomarker templates’ of receptor specific drugs.

REFERENCES

- 1 Lesko LJ, Atkinson AJ, Jr. Use Of Biomarkers And Surrogate Endpoints In Drug Development And Regulatory Decision Making: Criteria, Validation, Strategies. *Annu Rev Pharmacol Toxicol* 2001; 41:347-366.
- 2 Visser SJ, Post V.d. J, Pieters MSM, Cohen AF, Gerven JMA. Biomarkers For The Effects Of Antipsychotic Drugs In Healthy Volunteers. *Brit J Clin Pharmacol* 2001; 51:119-132.
3. Visser SJ, Post V.d. J, Waal P, Pieters MSM, Cohen AF, Gerven JMA. Biomarkers For The Effects Of Anxiolytic Drugs In Healthy Volunteers. *Br J Clin Pharmacol* 2002; Submitted For Publication.
- 4 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(5):712-713.
- 5 Norris H. The Action Of Sedatives On Brain Stem Oculomotor Systems In Man. *Neuropharmacology* 1971; 10(21):181-191.
- 6 Bond A, Lader M. Self-concepts In Anxiety States. *Br J Med Psychol* 1976; 49(3):275-279.
- 7 Steveninck AL v, Berckel BNM v, Schoemaker HC, Breimer DD, Gerven JMA, Cohen AF. The Sensitivity Of Pharmacodynamic Tests For The Central Nervous System Effects Of Drugs On The Effects Of Sleep Deprivation. *J Psychopharm* 1999; 13(1):10-17.
- 8 Visser SJ, Gerven JMA, Schoemaker HC, 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. *Brit J Clin Pharmacol* 2001; 51:423-428.
- 9 Steveninck AL v, Schoemaker HC, Hartigh JD, Rijnkels JM, Pieters MSM, Breimer DD *et al.* Effects Of Intravenous Temazepam. I. Saccadic Eye Movements And Electroencephalogram After Fast And Slow Infusion To Pseudo Steady State. *Clin Pharmacol Ther* 1994; 55:535-545.
- 10 Dingemans J, Van Gerven JM, Schoemaker RC, Roncari G, Obery J, Van Oostenbruggen MF *et al.* Integrated Pharmacokinetics And Pharmacodynamics Of Ro 48-6791, A New Benzodiazepine, In Comparison With Midazolam During First Administration To Healthy Male Subjects. *Br J Clin Pharmacol* 1997; 44(5):477-486.
- 11 Van Gerven JM, Roncari G, Schoemaker RC, Massarella J, Keesmaat P, Kooyman H *et al.* Integrated Pharmacokinetics And Pharmacodynamics Of Ro 48-6884, A New Benzodiazepine, In Comparison With Midazolam During First Administration To Healthy Male Subjects. *Br J Clin Pharmacol* 1997; 44(5):487-493.
- 12 Greenblatt DJ, Harmatz JS, Gouthro TA, Locke J, Shader RI. Distinguishing A Benzodiazepine Agonist (Triazolam) From A Nonagonist Anxiolytic (Buspirone) By Electroencephalography: Kinetic-dynamic Studies. *Clin Pharmacol Ther* 1994; 56(1):100-111.
- 13 Kaplan GB, Greenblatt DJ, Ehrenberg BL, Goddard Je, Harmatz JS, Shader RI. Single-dose Pharmacokinetics And Pharmacodynamics Of Alprazolam In Elderly And Young Subjects. *J Clin Pharmacol* 1998; 38(1):14-21.
- 14 Billard V, Gambus PI, Chamoun N, Stanski Dr, Shafer Sl. A Comparison Of Spectral Edge, Delta Power, And Bispectral Index As EEG Measures Of Alfentanil, Propofol, And Midazolam Drug Effect. *Clin Pharmacol Ther* 1997; 61(1):45-58.
- 15 Van Steveninck AL, Gieschke R, Schoemaker RC, Roncari G, Tuk B, Pieters MS *et al.* Pharmacokinetic And Pharmacodynamic Interactions Of Brexazenil And Diazepam With Alcohol. *Br J Clin Pharmacol* 1996; 41(6):565-573.
- 16 Aldenkamp AP, Baker G, Pieters MS, Schoemaker HC, Cohen AF, Schwabe S. The Neurotoxicity Scale: The Validity Of A Patient-based Scale, Assessing Neurotoxicity. *Epilepsy Res* 1995; 20(3):229-239.
- 17 Verkes RJ, Gijssman HJ, Pieters MSM, Schoemaker HC, Visser SJ, Kuijpers M *et al.* Cognitive Performance And Serotonergic Function In Users Of Ecstasy. *Psychopharmacology* 2001; 153:196-202.
- 18 Kemme MJB, Post V.d. J, Schoemaker HC, Gerven V. JMA, Cohen AF. Central Nervous System Effects Of Moxonidine Sustained Release Formulation In Patients With Mild To Moderate Essential Hypertension. *Br J Clin Pharmacol* Submitted For Publication. 2002.
- 19 Post V.d. J, Schram MT, Schoemaker RC, Pieters MSM, Fuseau E, Pereira A *et al.* CNS Effects Of Sumatriptan And Rizatriptan In Healthy Female Volunteers. *Cephalalgia* 2002; Accepted For Publication.
- 20 Van Steveninck AL, Wallnofer AE, Schoemaker RC, Pieters MS, Danhof M, Van Gerven JM *et al.* 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(3):267-275.
- 21 Steveninck AL v, Schoemaker HC, Hartigh JD, Pieters MSM, Breimer DD, Cohen AF. Effects Of Intravenous Temazepam. II. A Study Of The Long-term Reproducibility Of Pharmacokinetics, Pharmacodynamics, And Concentration-effect Parameters. *Clin Pharmacol Ther* 1994; 55:546-555.
- 22 Gijssman HJ, Gerven JMA, Tieleman NJ, Schoemaker HC, Pieters MSM, Ferrari MD *et al.* Pharmacokinetic And Pharmacodynamic Profile Of Oral And Intravenous Meta-chlorophenylpiperazine In Healthy Volunteers. *J Clin Psychopharm* 1998; 18(4):289-295.

- 23 Tuk B, Obery JJ, Pieters MS, Schoemaker RC, Kemp B, Van Gerven J *et al.* Pharmacodynamics Of Temazepam In Primary Insomnia: Assessment Of The Value Of Quantitative Electroencephalography And Saccadic Eye Movements In Predicting Improvement Of Sleep. *Clin Pharmacol Ther* 1997; 62(4):444-452.
- 24 Karlsson MO, Schoemaker RC, Kemp B, Cohen AF, Van Gerven JM, Tuk B *et al.* A Pharmacodynamic Markov Mixed-effects Model For The Effect Of Temazepam On Sleep. *Clin Pharmacol Ther* 2000; 68(2):175-188.
25. Gijsman HJ, Van Gerven JM, Tieleman MC, Schoemaker RC, Pieters MS, Ferrari Md *et al.* Pharmacokinetic And Pharmacodynamic Profile Of Oral And Intravenous Meta-chlorophenylpiperazine In Healthy Volunteers. *J Clin Psychopharmacol* 1998; 18(4):289-295.

FIGURE 1 Regression estimates for the different drugs; endpoints (on the x-axis) correspond to 95% of the deltaSPV values for that drug.

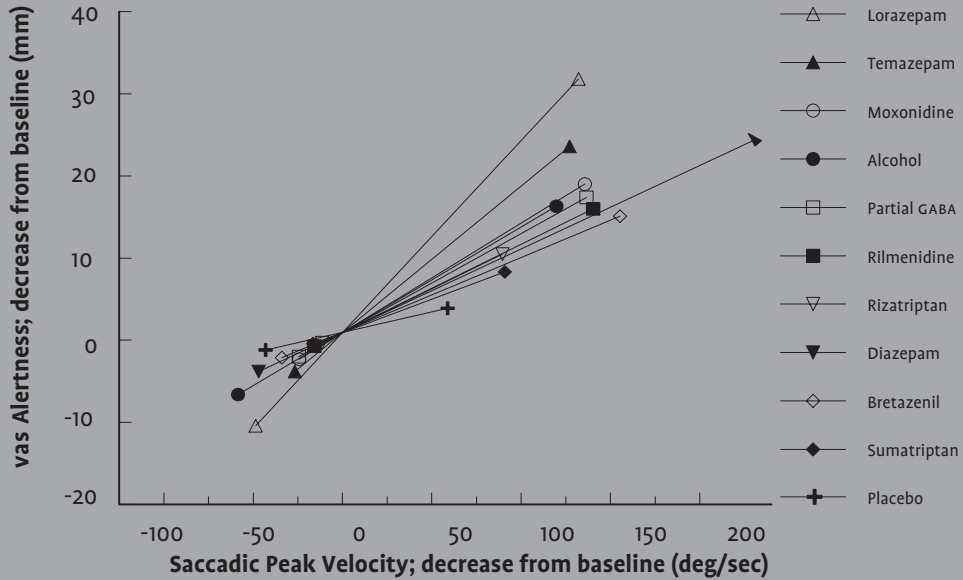


FIGURE 2 Regression estimates for the different drugs; endpoints (on the x-axis) correspond to 95% of the delta SPV values for that drug.

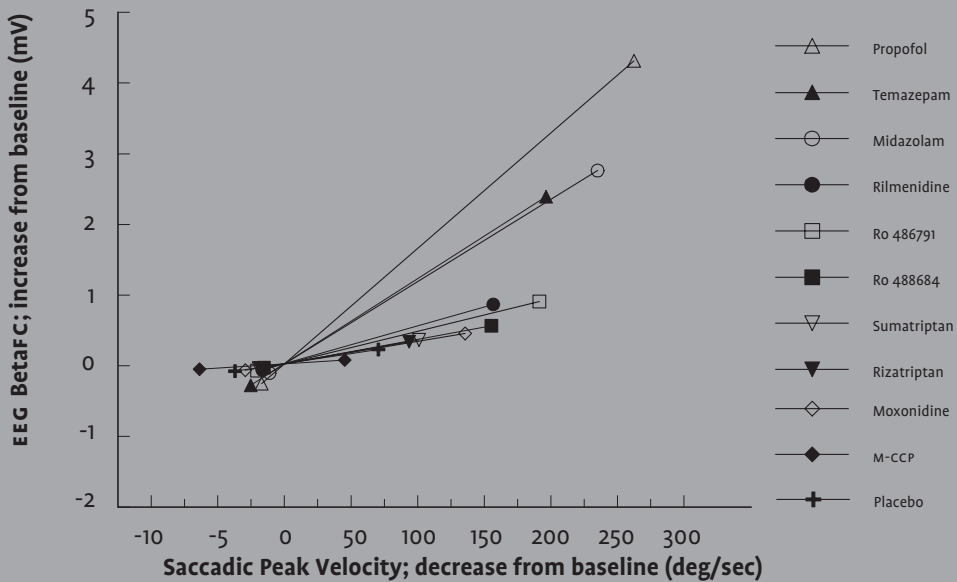


FIGURE 3 Regression estimates for the different drugs; endpoints (on the x-axis) correspond to 95% of the delta SPV values for that drug.

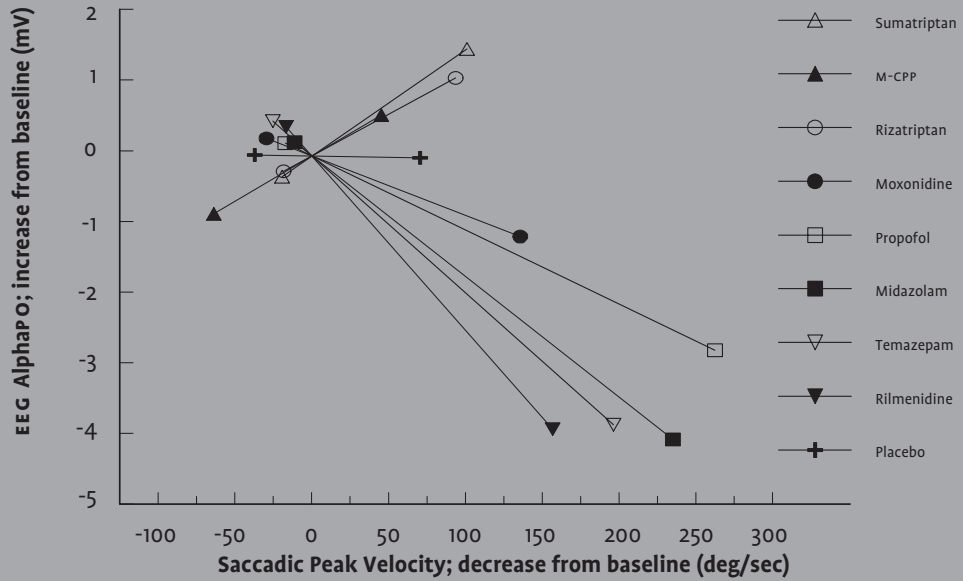


FIGURE 4 Regression estimates for the different drugs; endpoints (on the x-axis) correspond to 95% of the delta SPV values for that drug.

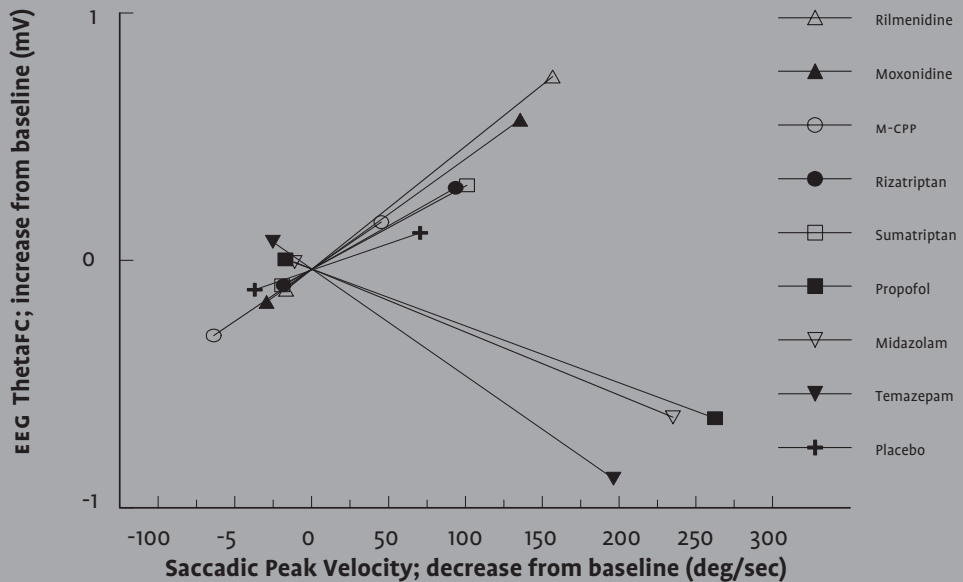


TABLE 1 Studies (refs. or drug) and number of occasions used for delta SPV-delta VAS relationships.

Treatments	(study reference) {number of study occasion}
Placebo	(15) {13}, (16) {10}, (8) {9}, (17) {63}, (18) {53}, (19) {17} (partial GABA agonist) {13}
Sumatriptan	(19) {16}
Rizatriptan	(19) {16}
Rilmenidine	(8) {18}, (rilmenidine) {43}
Partial GABA-agonist	(partial GABA agonist) {12}
Alcohol	(15) {12}
Lorazepam	(20) {28}, (partial GABA agonist) {14}
Temazepam	(20) {48}, (16) {19}, (19) {15}
Moxonidine	(18) {44}
Diazepam	(15) {13}
Bretazenil	(15) {13}

TABLE 2 Studies (refs. or drug) and number of occasions used for delta SPV-delta EEG relationships.

Treatments	(study reference) {number of study occasion}
Placebo	(9;21) {9}, (22) {11}, (10;11) {19}, (propofol) {10} (8) {9}, (18) {53}, (19) {14}
Sumatriptan	(19) {16}
Rizatriptan	(19) {16}
Rilmenidine	(8) {18}
Ro 486791	(10) {30}
Ro 488684	(11) {30}
Temazepam	(9;21) {35}, (23;24) {21}, (19) {15}
Moxonidine	(18) {44}
Midazolam	(10;11) {20}, (propofol) {22}
Propofol	(propofol) {23}
M-CPP	(25) {27}

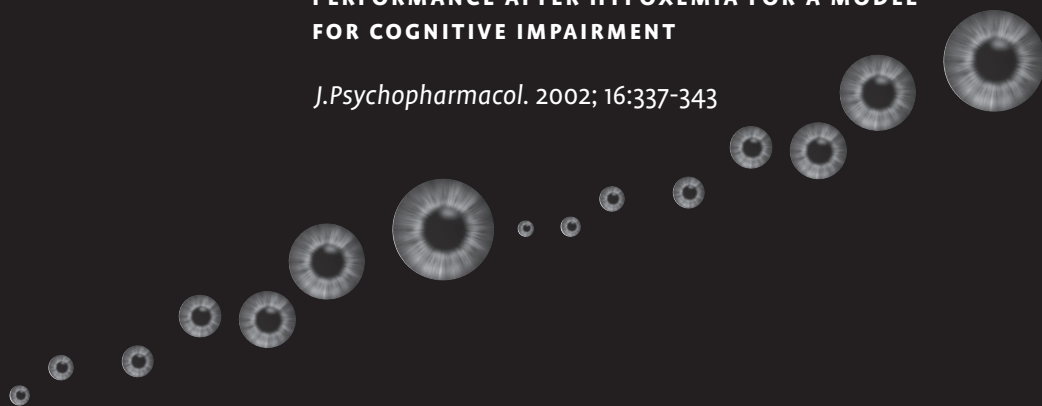
TABLE 3 Slope ranges of the different drug classes for SPV, EEG beta Fc, theta Fc and alpha Po.

SLOPE RANGES				
drug class	SPV	EEG beta Fc	EEG theta Fc	EEG alpha Po
<i>benzodiazepines</i>	0.0912 / 0.2338	0.0035 / 0.0164	-0.0043 / -0.0023	-0.0105 / -0.0194
<i>imidazoline modulators</i>	0.1077 / 0.1331	0.0032 / 0.0054	0.0045 / 0.0050	-0.0247 / -0.0084
<i>serotonergic drugs</i>	0.0805 / 0.1072	0.0012 / 0.0034	0.0034 / 0.0035	0.0118 / 0.0150

CHAPTER 6

**EVALUATION OF TESTS OF CENTRAL NERVOUS SYSTEM
PERFORMANCE AFTER HYPOXEMIA FOR A MODEL
FOR COGNITIVE IMPAIRMENT**

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Abstract

The sensitivity of several neurophysiological and cognitive tests to different levels of hypoxia was investigated. Cerebral hypoxia in healthy volunteers may be a disease model for dementia or other forms of brain dysfunction. Twelve healthy subjects were included in a randomized, single-blind, placebocontrolled, three-period cross-over trial. They received three air/N₂ gas mixtures via mask breathing [aimed at peripheral oxygen saturation (SPO₂) values of > 97% (placebo), 90% and 80%, with normal end-tidal CO₂. Central nervous system effects were tested regularly for 130 min by saccadic and smooth pursuit eye movements, electro-encephalogram, visual analogue scales and cognitive tests. Treatments were well tolerated. Compared to SPO₂ 90%, SPO₂ 80% reduced saccadic peak velocity by 16.4 °/s [confidence interval (CI) -26.3, -6.4], increased occipital delta power by 14.3% (CI 3.6, 25.1), and significantly increased most cognitive reaction times. SPO₂ 80% also decreased correct responses for the binary choice task and serial word recognition [-1.3 (-2.2, -0.3) and -3.5 (-6.2, -0.8), respectively] compared to SPO₂ 90%. Cognitive performance was decreased by SPO₂ 80% and increased by SPO₂ 90% compared to placebo. Sensitive effect measurements can be identified for these interventions. The applicability as a model for cognitive impairment should be investigated further.

Introduction

Many investigators have found cerebral hypoxia to have an effect on cognitive performance as well as on several neurophysiological measures. Much of this research has been conducted in the fields of mountaineering and aviation physiology (Denison *et al.*, 1966; Clark *et al.*, 1983; Jason *et al.*, 1989; Regard *et al.*, 1989; Kida and Imai, 1993; Bonnon *et al.*, 1995). More recently, it has been recognized that hypoxemia-induced cognitive impairment and neurophysiological changes share similarities with the findings in dementia of both the vascular and degenerative subtypes (Saletu *et al.*, 1991A,b; Saletu *et al.*, 1992). This has led to the suggestion that hypoxic conditions in healthy volunteers may be used as a model for cognitive impairment (Saletu and Grunberger, 1984).

To date, no widely applicable model for cognitive impairment is available. Although other pharmacological models have been suggested (Patel and Tariot, 1991), currently the most used method for inducing cognitive impairment is a challenge with scopolamine, a muscarinic

receptor antagonist. Scopolamine has been shown to increase delta and theta power and decrease alpha and beta frequencies in the electroencephalogram (EEG) of healthy young volunteers (Ebert and Kirch, 1998). The drug also increases reaction times and generally impairs tasks with minimal memory requirements, without showing effects on word recognition and delayed word recall (Ebert and Kirch, 1998). Furthermore, the administration of scopolamine induces sedative effects, accommodation disturbances and dryness of the mouth. These characteristics limit the use of the scopolamine model in several ways.

First, induced symptoms do not overlap completely with those of cognitive impairment resulting from Alzheimers' disease. This may be partly due to the fact that other neurotransmitter systems, apart from acetylcholine, are also involved in cognition and memory (Ellis and Nathan, 2001). Moreover, scopolamine-induced cognitive impairment may in part be caused by sedation. In addition, the scopolamine model has proven useful during the development of cholinergic drugs, although its applicability for drugs with other mechanisms of action is doubtful.

The hypoxemia model would potentially allow the testing of treatments for cognitive impairment, without the disadvantages of drug interaction. In contrast to the scopolamine model, the induced cognitive deficit will possibly be more widespread and less specific to one neurotransmitter system. Also, sedation does not appear to be an important symptom, which possibly clarifies the interpretation of the results.

Most studies have shown that cerebral hypoxia induces EEG changes, such as an increase in delta, theta and fast-beta waves, and a decrease in alpha activity (Kraaier *et al.*, 1988; Saletu *et al.*, 1990; Van der Worp *et al.*, 1991). Similar changes are found in dementia both of the vascular and degenerative subtypes. Furthermore, a deterioration of cognitive performance, such as a decline in recognition and recall reminiscent of Alzheimer's dementia, has also been reported under cerebral hypoxia (Crow and Kelma, 1971; Berry *et al.*, 1989; Noble *et al.*, 1993; Bonnon *et al.*, 1995). It is still unclear which tests are the most sensitive to evaluate the effects of cerebral hypoxia on cognitive and psychomotor functions. The relationship between the levels of hypoxemia and the impairment of central nervous system (CNS) function is also unknown.

The aim of this study was to investigate the potential of hypoxemia as a model for cognitive impairment, by evaluating the effect of different levels of hypoxemia on a selection of cognitive and neurophysiological tests.

Methods

Subjects

After approval of the protocol by the Ethical Review Board of the Leiden University Medical Center, 12 healthy volunteers were asked to participate in the study. After providing their written informed consent, subjects received a full medical examination before entering the study. A training session for mask breathing was performed as part of their eligibility assessment. The CNS test battery was practised to reduce learning effects during the study. Subjects refrained from alcohol, caffeine and xantine containing foods and beverages 12 h prior to the study day and during the study days. Concomitant medication other than paracetamol or oral contraceptives was not permitted during the study period. A light standard breakfast was served before the start of the experiment. After the experiment, subjects were allowed to go home if all vital signs were back to normal and stable and significant adverse events had subsided.

Design and procedure

The study was designed as a blinded, randomized, placebo-controlled three-period cross-over trial with a minimum washout period of 2 days. Starting at approximately the same time each study day (approximately 09.30 h) subjects breathed an air/N₂ gas mixture through a well-fitted facemask (VBM Medizintechnik GmbH, Sulz a.N., Germany), which maintained their peripheral SPO₂ at either > 97% (placebo, normal air breathing) or decreased their SPO₂ percentages to 90% or 80% for a duration of 2 h 10 min. The air/N₂ gas mixtures were adapted continuously to the peripheral SPO₂ percentages (Peripheral Saturation meter, Life scope EC BSM-1101K, Nihon Kohden, Tokyo, Japan) by a single investigator, who was not involved in other study procedures. All other investigators and the study subject remained blinded to the administered gas mixture and the oxygen saturation levels. Eye movements, EEG and Visual Analogue Scale (VAS) scores were performed at baseline and at the following time points after reaching the hypoxemic plateau phase: 0 h 00 min, 0 h 30 min, 1 h 00 min, 1 h 30 min, 2 h 00 min, 2 h 10 min and 2 h 20 min (after the subjects SPO₂ was back and stable at baseline levels). The cognitive test battery was conducted intermittently between 10 min and 1 h and 50 min during breathing of the gas mixture. One-lead electro-cardiogram, heart rate, peripheral SPO₂, and end-tidal CO₂ were monitored continuously and recorded at intervals.

CNS function tests

COGNITIVE TEST BATTERY Cognitive performance was tested using the 'FePsy' software package (Instituut voor Epilepsiebestrijding, Heemstede, the Netherlands) (Alphers and Aldenkamp, 1994). This system contains a battery of computerized tests of different complexity measuring diverse cognitive skills.

MEMORY TASKS To evaluate memory function, serial and simultaneous recognition of words and figures, which are recognition and short memory tasks, were evaluated. For serial recognition, six words/four figures are presented in serial order, at 1 s per word with a 250-ms interval. After a delay of 2 s, one of these words/figures is presented for recognition, and the subject is instructed to indicate the presentation order in the study phase. Recall of the order of stimuli was required for recognition. For simultaneous word/figure recognition, six words/four figures are presented simultaneously for 6 s. After a delay of 2 s, six words/four figures are presented. The subjects are asked to indicate which of these words/figures was presented earlier.

The Corsi Block Tapping task was used to assess direct recall. This test is constructed according the principles of the original Corsi block tapping task (Nelson *et al.*, 2000), and assesses nonverbal memory span. In this test, nine buttons appear on a screen. Three buttons flash in serial order in a tempo of one per second. Subjects are instructed to indicate the order in which the buttons appear. After each correct response, one extra button is presented until two consecutive trials fail. The 'span' is the maximum correct number of buttons.

To address visual (complex) information processing and perceptual-strategies, the visual searching task was performed. It consists of finding a grid pattern out of 24 that matches the one in the centre of the screen. The target pattern is marked by an arrow on the right side and is selected by typing the correct number from the keyboard. After 12 presentations, the surrounding grids change. The subject is asked to react as fast as possible. The main parameter of the visual searching task was the number of errors, the secondary endpoint was the mean searching time of correct response (Goldstein *et al.*, 1973; Demita and Johnson, 1981).

The wisconsin card sorting task was an adaptation of the original wisconsin card sorting task, assessing abstraction ability, conceptualization, failure to maintain set and perseveration (Anderson *et al.*, 1991). The four stimuli cards are shown on the top row of the

screen. The deck of 128 response cards is shown on the bottom row. The subject is instructed to sort the cards. The categories (colour, number or form) are not communicated. The subject has to type the number of the stimulus card he thinks the response card belongs to. The response card is then placed beneath the stimulus card. Hereafter, the subject receives feedback from the computer by showing 'correct' or 'incorrect' on the screen and an accompanying high or low beep. After identification of the correct category, the sorting strategy is changed. The test proceeds until six shifts of categories have taken place or until the deck of response cards is exhausted. Main parameters are: total number of errors and number of persisting errors.

REACTION TIMES To measure attention, simple auditory and visual reaction times were performed. Stimulus exposure endures until a response (space bar) is given. The subject is asked to react as quickly as possible. The inter-stimulus interval is randomly varied from 2.5–4 s. For both the dominant and the non-dominant hand, 30 stimuli are presented. The main parameters are average response latency and the standard deviation of all scores.

The binary choice task, which is a more complex reaction time task, was added to measure information processing and response inhibition. Attention and speed of information processing are evaluated. Either a red or a green block is displayed in random order in either the left or the right half of the screen. The subject reacts by pushing a button on either side of the keyboard, corresponding to the position of the coloured block on the screen. The test is self-paced and continuous, which implies that a response is immediately followed by substitution of another block in the same or the opposite position. Sixty stimuli are presented during 1 min. The main parameters are the number of correct responses, mean reaction time of correct response and the SD of correct responses.

Neurophysiological tests

EYE MOVEMENTS Saccadic eye movements have been shown to be very sensitive to sedation, specifically saccadic peak velocity, in studies on sleep deprivation and sedative compounds (van Steveninck *et al.*, 1991; Harron *et al.*, 1995; Steveninck *et al.*, 1999). Smooth pursuit eye movements are altered in a number of neuropsychiatric diseases (e.g. schizophrenia and stroke), and are also affected by sedative agents such

as barbiturates and alcohol (van Steveninck, 1993; van Steveninck *et al.*, 1997). The sensitivity to cerebral hypoxia remains to be assessed.

Recording and analysis of saccadic and smooth pursuit eye movements was performed with a microcomputer-based system (van Steveninck *et al.*, 1989). The equipment used for stimulus display, signal collection and amplification was obtained from Nihon Kohden. Saccadic eye movements were recorded for stimulus amplitudes of 15° to either side. Interstimulus intervals varied randomly from 3–6 s, and 15 saccades were recorded. The average values of saccadic peak velocity, latency (reaction time) and inaccuracy were used as parameters. For smooth pursuit eye movements the target moved sinusoidal at frequencies ranging from 0.3–1.1 Hz, increasing by steps of 0.1 Hz. The amplitude of target displacement corresponds to 20° eyeball rotations to both sides. Four cycles were recorded for each stimulus frequency.

ELECTRO-ENCEPHALOGRAMS Electro-encephalograms were recorded and analysed using CED software (Cambridge Electronics Design, Cambridge, UK), as described previously (Steveninck *et al.*, 1999). EEG recordings were made using silver-silver chloride electrodes, fixed with collodion at Fz, Cz, Pz and Oz, with the same common ground electrode as for the eye movement registration (international 10/20 system). The electrode resistances were maintained below 5 kOhm. All recordings were carried out with the subjects' eyes closed. EEG signals were obtained from leads Fz–Cz and Pz–Oz. The signals were amplified by use of a Nihon Kohden AB-621G bio-electric amplifier with a time constant of 0.3 s and a low pass filter at 100 Hz. Per session, eight consecutive blocks of 8 s were recorded over a 2-min period. The sampling rate was 1024 Hz. Datablocks containing artefacts were identified by visual inspection and these were excluded from analysis. Fast Fourier transform analysis was performed to obtain the sum of amplitudes in the delta- (0.5–3.5 Hz), theta- (3.5–7.5 Hz), alpha- (7.5–11.5 Hz) and beta (11.5–30 Hz) frequency ranges. The total recording bandwidth was 0–50 Hz.

Subjective assessments

VISUAL ANALOGUE SCALES Visual analogue scales, as originally described by Norris (Norris, 1971), were used to quantify subjective effects. Three factors corresponding to alertness, mood and calmness were derived from these measurements (Bond and Lader, 1976).

Statistical analysis

All data were analysed by factorial one-way analysis of variance (ANOVA), with subject, treatment and study day as factors. Contrasts between treatments and between study days were calculated within the ANOVA model and are presented with 95% confidence intervals. For saccadic peak velocity, saccadic accuracy, saccadic latency, VAS alertness, VAS mood and VAS calmness, area under the effect curves (AUEC) were calculated using the linear trapezoidal rule on the protocol times from prevalue to the final value. For the EEG variables, the AUEC of the percentage change from baseline was calculated. AUECs were divided by the corresponding time span, resulting in weighted averages. Calculations were performed using the SPSS statistical software package (Version 9.0.1, SPSS INC., Chicago, IL, USA).

Results

Fifteen subjects, nine males and six females (aged 19–27 years, with normal weight to height ratio), were enrolled in the study. Twelve subjects completed all three treatment periods, and were included in the dynamic analysis. Two female and one male subject dropped out of the study due to adverse events, and were replaced. All subjects who had one or more treatments were included in the safety analysis.

Adverse events

The adverse events seen in this study were mainly headache and mild drowsiness (table 1). Most of the adverse events (52%) and treatment related adverse events (70%) were seen during SPO₂ 80%. Three subjects dropped out of the study due to non-serious adverse events: one female subject experienced tachy-dysrhythmia and anxiety during SPO₂ 90%, one male subject experienced a sinus tachy dysrhythmia and blackouts during SPO₂ 80% and one female subject did not tolerate the mask breathing during the Placebo occasion. All adverse events abated immediately after interruption of mask-breathing.

Table 1 – p. 126

SPO₂ and end-tidal CO₂

The peripheral SPO₂ of > 97%, 90% and 80%, aimed for on individual study days was reached accurately and remained stable throughout the study period (98.7 ± 0.8%, 90 ± 0.9%, 80.3 ± 1.2%, respectively). Return to normal SPO₂ was reached almost instantaneously after the oxygen concentration in the gas mixture was returned to 21%. End-tidal CO₂ remained stable at baseline levels throughout the study period (table 2).

Table 2 – p. 126

Blood pressure and heart rate

No difference in systolic or diastolic blood pressure was observed between any of the treatments. An increase in heart rate occurred during the SPO₂ 90% and 80%, which appeared to mirror the level of hypoxemia (fig. 1).

Fig. 1 – p. 124

Cognitive effects

Generally, the observed cognitive effects were small and showed an improvement under SPO₂ 90% and a deterioration under SPO₂ 80%.

RECOGNITION OF WORDS AND FIGURES A significant reduction in the number of correct responses for serial recognition of words was observed during SPO₂ 80% compared to SPO₂ 90% [mean difference -3.5, confidence interval (CI) -6.2, -0.8]. No noteworthy effects were seen on serial recognition of figures, simultaneous recognition tasks or any of their reaction times.

BINARY CHOICE TASK A significantly smaller number of correct responses was observed during SPO₂ 80% compared to SPO₂ 90% (mean difference -1.25, CI -2.17, -0.33). The mean difference of incorrect responses was larger during SPO₂ 80% compared to SPO₂ 90% (mean difference 101.3, CI 19.5, 183.0). During SPO₂ 80%, the reaction time for incorrect responses was significantly longer (mean difference 99.9, CI 18.5, 181.3) (ms). The SD of incorrect responses was larger (mean difference 13.3, CI 0.6, 25.9), compared to SPO₂ 90%.

SIMPLE AUDITORY AND VISUAL REACTION TIMES Compared to placebo, SPO₂ 90% caused a decrease in mean difference auditory reaction times for the dominant hand (mean difference -13.2, CI -1.5, -24.9) (ms). Compared to SPO₂ 90%, SPO₂ 80% increased mean auditory reaction time for the dominant hand (mean 31.8, CI 0.8, 62.9) (ms) and caused a longer mean difference auditory reaction time for the non-dominant hand (mean difference 18.4, CI 2.9, 33.9) (ms). A longer (mean difference 40.1, CI 12.6, 67.5) (ms) mean difference visual reaction time for the non-dominant hand was also observed for SPO₂ 80% compared to SPO₂ 90%.

OTHER COGNITIVE TESTS No significant effects were seen on the visual searching task, the card-sorting task or the corsi block tapping task.

Neurophysiological effects

EYE MOVEMENTS SPO₂ 80% caused a small reduction in peak velocity (mean difference -7.6, CI -17.5, 2.4) (°/s) compared to placebo. SPO₂ 90% caused a non-significant increase in saccadic peak velocity (mean difference 8.8, CI -1.2, 18.8) (°/s) compared to placebo. The difference in saccadic peak velocity of 16.4 (CI 26.3, 6.4) (°/s) between SPO₂ 90% and 80% was statistically significant (fig. 2). No effects on saccadic accuracy, latency and smooth pursuit eye movements were seen after both SPO₂ 80% and 90%.

Fig. 2 – p. 124

ELECTRO-ENCEPHALOGRAMS 80% SPO₂ caused an increase in occipital delta power compared to placebo and 90% SPO₂ [mean difference 10.7, CI 0.3, 21.1 and mean difference 14.3, CI 3.6, 25.1 (%) respectively] (fig. 3A). Also, an increase in occipital theta power occurred, compared to 90% SPO₂ (mean difference 9.9, CI -5.4, 25.3) (%) was found. Small decreases in frontal alpha power, compared to placebo and 90% SPO₂ [mean difference -8.4, CI -19.7, 3.0 and mean difference -9.2, CI -20.6, 2.2 (%), respectively] were seen (fig. 3B).

Fig. 3A – p. 125

Fig. 3B – p. 125

SPO₂ 90% caused a decrease in occipital delta and theta power compared to placebo (mean difference -3.6, CI -14.4, 7.2 and mean difference -7.2, CI -22.5, 8.1 (%), respectively). No effects of SPO₂ 80% and 90% were seen on occipital alpha power, frontal and occipital beta power, frontal delta power and frontal theta power.

Subjective effects

VISUAL ANALOGUE SCALE No significant differences were seen in alertness, mood and calmness on comparing the different SPO_2 levels.

Discussion

This study aimed to explore the effects of two levels of hypoxemia on CNS function in healthy human volunteers, and to identify sensitive tests to measure these effects. Although small, a number of effects of hypoxemia on CNS function have been observed.

Because this was an exploratory study with a small number of subjects, and a large number of tests, these effects may be spurious. However, although in general these effects were small, a consistent picture emerged from these results. The main contrasts were seen between SPO_2 90% and SPO_2 80%.

SPO_2 80% seemed to decrease cognitive performance, in contrast to the increase observed under SPO_2 90%, with placebo in-between. A decrease in the number of correct responses was found for the serial recognition of words and for the binary choice task when SPO_2 80% was compared to SPO_2 90%. Generally, reaction times increased under SPO_2 80% and decreased under SPO_2 90%.

SPO_2 80% caused cognitive effects that differed from those observed during sedation, either by pharmacological intervention or sleep deprivation (Saletu *et al.*, 1991A; Visser *et al.*, 2001). In mild sedation, an increase in reaction times is expected, while the number of correct responses for cognitive tasks will remain unchanged. In contrast, the current study showed a combination of an increase in reaction time and a decrease in correct responses, which was also reported previously in a hypoxemia study in human volunteers (Noble *et al.*, 1993). This seems to indicate that hypoxia does not simply cause a 'slowing' of the brain, but affects memory and decision making processes.

This study showed an improvement in cognitive performance above normal values under mild hypoxemia (SPO_2 90%) similar observations have been made previously. Acute exposure to mild hypoxia, a fractional inspiratory oxygen concentration of 14.5% (comparable to SPO_2 90%) has improved a simple measure of cognitive performance above normal values (Schlaepfer *et al.*, 1992).

Neurophysiological tests showed similar results. SPO_2 90% appears to have a general stimulant effect on CNS function as opposed to

SPO₂ 80%, which seems to be slightly impairing. This is indicated by a reduction in saccadic peak velocity under SPO₂ 80% compared to placebo. This can be regarded as a very mild sedative effect, less than would be observed after one night of sleep deprivation (Steveninck *et al.*, 1999). However, the absence of significant effects on subjective rating scales for alertness would argue against a clear sedative effect (Saletu *et al.*, 1991A; Saletu *et al.*, 1992). This lack of subjective effects is in accordance with the finding that, even with severely hypoxic aircraft pilots, feelings of somnolence are rarely reported prior to loss of consciousness (Brown, 1994). The slight elevation in saccadic peak velocity compared to placebo that was observed with SPO₂ 90%, suggests an improvement of alertness (Steveninck *et al.*, 1999).

The study showed an increase in EEG occipital delta power (endrenyi and Zha, 1994; Sheiner, 1994) and a decrease of alpha power (levy, 1994), which is comparable to findings in Alzheimers' disease. SPO₂ 90%, however, caused an increase in alpha power compared to placebo, suggesting an improvement in alert wakefulness. An increase in delta and theta power and a decrease in alpha power can also indicate hyperventilation (Van der Worp *et al.*, 1991). This is unlikely to have caused the EEG changes in our study, since end-tidal CO₂ values were not decreased by the hypoxemic conditions. These results are in agreement with previous reports, in which increases in delta EEG activity and decreases in alpha activity were observed in healthy volunteers during hypobaric hypoxia (Kraaier *et al.*, 1988; Saletu *et al.*, 1990; Van der Worp *et al.*, 1991).

The observed activation and increased performance during SPO₂ 90% could be the result of a functional adaptation to the effects of mild hypoxia; if cognitive function is only slightly affected, the impairment could be noticed, and consequently overcompensated by the subjects. Similar effects have been reported with benzodiazepines (van Steveninck *et al.*, 1991) and after sleep deprivation (Steveninck *et al.*, 1999). Alternatively, this might also arise from CNS dysinhibition under mild hypoxia. Furthermore, the sensitivity of different neurotransmitter systems in the brain could differ for various levels of hypoxia, resulting in the observed bidirectional effect of hypoxemia.

Hypoxemia down to 80% SPO₂ over an extended period of time appears to be generally well tolerated by healthy young volunteers. Most of the adverse events, comprising mainly headache and drowsiness, were mild and ceased usually within minutes of ending the mask breathing. The three subjects that dropped out due to nonserious adverse events appeared to have intolerance to the test procedures, resulting in tachy-

dysrhythmia and anxiety. A 25% dropout rate could seriously affect the efficient use of this model. However, more careful training of subjects on the ability to breathe through a mask could potentially avoid these events. The increase in heart rate was the only observed effect on vital signs, and remained within normal limits (60–100 b.p.m.) for most subjects. Hypocapnia was excluded as a contributor to either the adverse or the CNS effects of the procedure.

In conclusion, it appears that a number of cognitive (auditory and visual reaction times, binary choice task and serial recognition of words) and neurophysiological (saccadic eye movements, EEG) are sensitive to the effects of hypoxemia. The effects seem to be most outspoken on simple and complex reaction times. In our study, hypoxemia showed only limited cognitive effects, and the main contrasts were found between SpO_2 80% and 90%. This could set a limit to the usefulness of hypoxemia as a model for cognitive impairment. An elderly (target) population may show a different more unidirectional response to hypoxemia. A repetition of this study in this population could also increase the effect size, due to a diminished capacity for compensation.

REFERENCES

- Alpers W C J, Aldenkamp A P (1994) FePsy, The Iron Psyche. Manual 5.0. Publisher, Publisher's Location
- Anderson S W, Damasio H, Jones R D, Tranel D (1991) Wisconsin Card Sorting Test Performance As A Measure Of Frontal Lobe Damage. *J Clin Exp Neuropsychol* 13: 909–922
- Berry D T, McConnell J W, Phillips B A, Carswell C M, Lamb D G, Prine B C (1989) Isocapnic Hypoxemia And Neuropsychological Functioning. *J Clin Exp Neuropsychol* 11: 241–251
- Bond A, Lader M (1976) Self-concepts In Anxiety States. *Br J Med Psychol* 49: 275–279
- Bonnnon M, Noel-jorand M C, Therme P (1995) Psychological Changes During Altitude Hypoxia. *Aviat Space Environ Med* 66: 330–335
- Brown P (1994) Pupil Test For Alheimer's. *New Sci* 5
- Clark C F, Heaton R K, Wiens A N (1983) Neuropsychological Functioning After Prolonged High Altitude Exposure In Mountaineering. *Aviat Space Environ Med* 54: 202–207
- Crow T J, Kelman G R (1971) Effect Of Mild Acute Hypoxia On Human Short-term Memory. *Br J Anaesth* 43: 548–552
- Demita M A, Johnson J H (1981) The Validity Of A Computerized Visual Searching Task As An Indicator Of Brain Damage. *Behav Res Meth Instrument* 13: 592–594
- Denison D M, Ledwith F, Poulton E C (1966) Complex Reaction Times At Simulated Cabin Altitudes Of 5000 Feet And 8000 Feet. *AErosp Med* 37: 1010–1013
- Ebert U, Kirch W (1998) Scopolamine Model Of Dementia: Electroencephalogram Findings And Cognitive Performance. *Eur J Clin Invest* 28: 944–949
- Ellis K A, Nathan P J (2001) The Pharmacology Of Human Working Memory *Int J Neuropsychopharmacol* 4: 299–313
- Endrenyi L, Zha J (1994) Comparative Efficiencies Of Randomized Concentration- And Dose-controlled Clinical Trials *Clin Pharmacol Ther* 56: 331–338
- Goldstein G, Welch RB, Rennick PM, Shelly Ch (1973) The Validity Of A Visual Searching Task As An Indicator Of Brain Damage. *J Consult Clin Psychol* 41: 434–437
- Harron D W, Hasson B, Regan M, McClelland R J, King D J (1995) Effects Of Rilmenidine And Clonidine On The Electroencephalogram, Saccadic Eye Movements, And Psychomotor Function. *J Cardiovasc Pharmacol* 26 (Suppl.): 548–554
- Jason G W, Pajurkova E M, Lee R G (1989) High-altitude Mountaineering And Brain Function: Neuropsychological Testing Of Members Of A Mount Everest Expedition. *Aviat Space Environ Med* 60: 170–173
- Kida M, Imai A (1993) Cognitive Performance And Event-related Brain Potentials Under Simulated High Altitudes. *J Appl Physiol* 74: 1735–1741
- Kraaier V, Van Huffelen A C, Wieneke G H (1988) Quantitative EEG Changes Due To Hypobaric Hypoxia In Normal Subjects. *Electroencephalogr Clin Neurophysiol* 69: 303–312
- Levy G (1994) Pharmacologic Target-mediated Drug Disposition. *Clin.pharmacol.ther* 56: 248–252
- Nelson R E, Dickson A L, Banos J H (2000) An Automated Administration Of Corsi's Block-tapping Test. *Percept Motor Skills* 91: 578–580
- Noble J, Jones J G, Davis E J (1993) Cognitive Function During Moderate Hypoxaemia. *Anaesth Intens Care* 21: 180–184
- Norris H (1971) The Action Of Sedatives On Brain Stem Oculomotor Systems In Man. *Neuropharmacology* 10: 181–191
- Patel S, Tariot P N (1991) Pharmacologic Models Of Alzheimer's Disease. *Psychiatr Clin North Am* 14: 287–308
- Regard M, Oelz O, Brugger P, Landis T (1989) Persistent Cognitive Impairment In Climbers After Repeated Exposure To Extreme Altitude. *Neurology* 39: 210–213
- Saletu B, Grunberger J (1984) The Hypoxia Model In Human Psychopharmacology: Neurophysiological And Psychometric Studies With Aniracetam i.v. *Hum Neurobiol* 3: 171–181
- Saletu B, Grunberger J, Anderer R (1990) On Brain Protection Of Codergocrine Mesylate (Hydergine) Against Hypoxic Hypoxidosis Of Different Severity: Double-blind Placebo-controlled Quantitative EEG And Psychometric Studies. *Int J Clin Pharmacol Ther Toxicol* 28: 510–524
- Saletu B, Anderer P, Paulus E (1991a) EEG Brain Mapping In Diagnostic And Therapeutic Assessment Of Dementia. *Alzheimer Dis Assoc Disord* 5: 57–75
- Saletu B, Paulus E, Linzmayer L (1991b) Nicergoline In Senile Dementia Of Alzheimer Type And Multi-infarct Dementia: A Double-blind, Placebo-controlled, Clinical And EEG Erp Mapping Study. *Psychopharmacology* 117: 385–395
- Saletu B, Anderer P, Fischhof PK, Lorenz H, Barousch R, Bohmer F (1992) EEG Mapping And Psychopharmacological Studies With Denbutylline In Sdat And Mid. *Biol Psychiatry* 32: 668–681
- Schlaepfer T E, Bartsch P, Fisch H U (1992) Paradoxical Effects Of Mild Hypoxia And Moderate Altitude On Human Visual Perception. *Clin Sci (Colch)* 83: 633–636
- Sheiner L B (1994) A New Approach To The Analysis Of Analgesic Drug Trials, Illustrated With BroMFenac Data *Clin Pharmacol Ther* 56: 309–321
- Steveninck A L, Berckel B N M, Schoemaker H C, Breimer D D, Gerven J M A, Cohen A F (1999) The Sensitivity Of Pharmacodynamic Tests For The Central Nervous System Effects Of Drugs On The Effects Of Sleep Deprivation. *J Psychopharmacol* 13: 10–17

- Van Der Worp H B, Kraaier V, Wieneke G H, Van Huffelen A C (1991) Quantitative EEG During Progressive Hypocarbica And Hypoxia. Hyperventilation-induced EEG Changes Reconsidered. *Electroencephalogr Clin Neurophysiol* 79: 335-341
- Van Steveninck A L (1993) *Methods Of Assessment Of Central Nervous System Effects Of Drugs In Man* Thesis. State University, Leiden
- Van Steveninck A L, Cohen A F, Ward T (1989) A Microcomputer Based System For Recording And Analysis Of Smooth Pursuit And Saccadic Eye Movements *Br J Clin Pharmacol* 27: 712-713
- Van Steveninck A L, Schoemaker H C, Pieters M S, Kroon R, Breimer D D, Cohen A F (1991) 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* 50: 172-180
- Van Steveninck A L, Wallnofer A E, Schoemaker R C, Pieters M S, Danhof M, Van Gerven J M, Cohen A F (1997) A Study Of The Effects Of Long-term Use On Individual Sensitivity To Temazepam And Lorazepam In A Clinical Population. *Br J Clin Pharmacol* 44: 267-275
- Visser S J D, Post V D J, Pieters M s M, Cohen A F, Gerven J M A (2001) Biomarkers For The Effects Of Antipsychotic Drugs In Healthy Volunteers. *Br J Clin Pharmacol* 51: 119-132

FIGURE 1 Average heart rates. Mean \pm SD time-effect profiles of heartrate for SPO₂ 80% (●), 90% (○) and 100% (■).

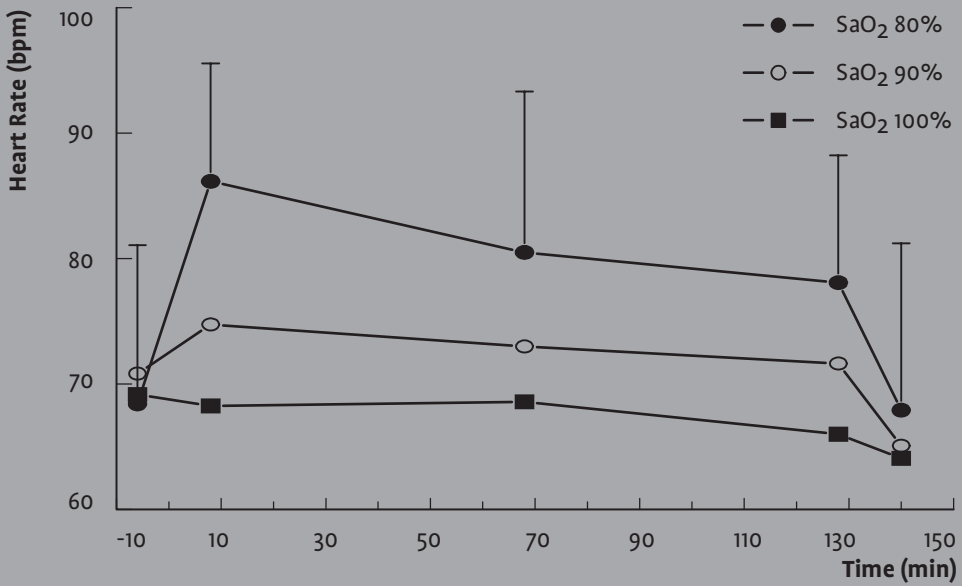


FIGURE 2 Average saccadic peak velocity. Mean \pm SD time-effect profiles of saccadic peak velocity for SPO₂ 80% (●), 90% (○) and 100% (■).

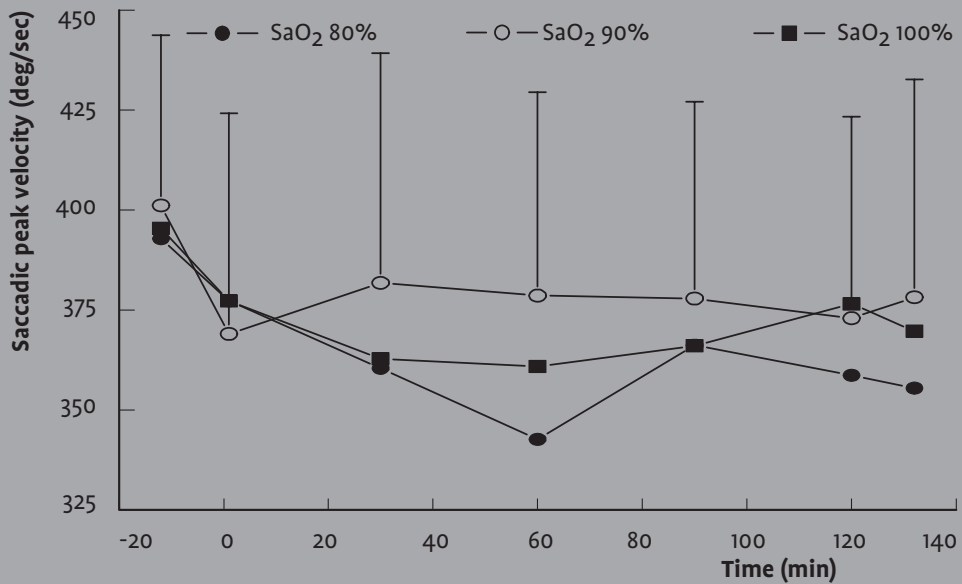


FIGURE 3A Average occipital delta power. Mean \pm SD time-effect profiles of occipital delta power for SPO₂ 80% (●), 90% (○) and 100% (■). (B)

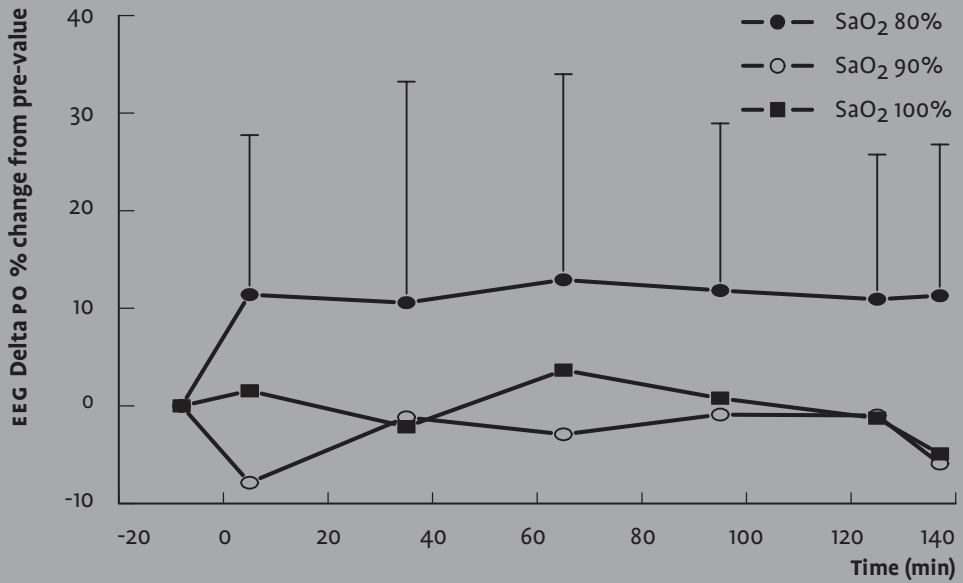


FIGURE 3B Frontal alpha power. Mean \pm SD time-effect profiles of frontal alpha power for SPO₂ 80% (●), 90% (○) and 100% (■).

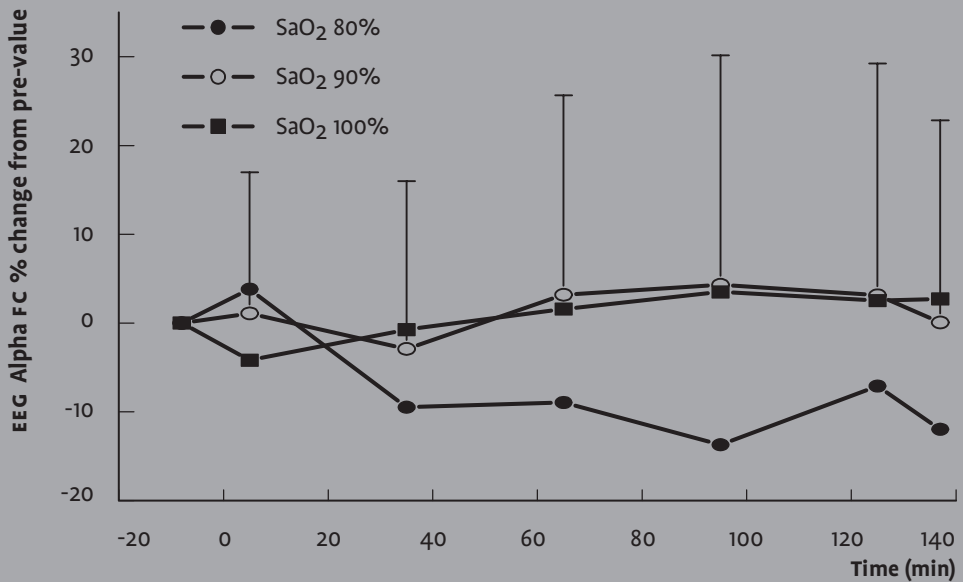


TABLE 1 Adverse events for different SpO_2 .

Adverse events	100% n = 15	90% n = 14	80% n = 13
Any event	8	8	17
Any treatment related event	1	5	14
Headache	3	1	5
Drowsiness	3	3	5

TABLE 2 End-tidal CO_2 percentages.

Treatment	pre-treatment <i>mean ± SD</i>	during treatment <i>mean ± SD</i>	post-treatment <i>mean ± SD</i>
HbSaO ₂ 80%	5.4 (0.3)	5.1 (0.3)	4.9 (0.3)
HbSaO ₂ 90%	5.3 (0.2)	5.1 (0.3)	5.0 (0.3)
HbSaO ₂ 100%	5.3 (0.2)	5.2 (0.3)	5.1 (0.3)

CHAPTER 7

**NO EVIDENCE OF THE USEFULNESS OF EYE BLINKING
AS A MARKER FOR CENTRAL DOPAMINERGIC ACTIVITY**

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Abstract

This study aimed to evaluate eye blinking as a marker for central dopaminergic activity by investigating the effects of sulpiride (D₂-antagonist) and lisuride (D₂-agonist) on spontaneous eye blinks. Twelve healthy subjects were included in a randomized, double-blind, placebo-controlled, three-period crossover trial. They received sulpiride 400 mg, lisuride 0.2 mg and placebo on different occasions. Eye blinks, prolactin, finger tapping, eye movements and visual analogue scales were measured at baseline and regularly for 12 h after administration. No effect of sulpiride or lisuride was observed on the number of eye blinks. Sulpiride caused an increase in prolactin (643 U/ml) [confidence interval (CI) 549–737]. Lisuride caused a decrease in smooth pursuit eye movements (–4.1%) (CI –7.3 to –0.9) and visual analogue scales for mood (–2.1 mm) (CI –3.7 to –0.4). Spontaneous eye blink rate was not affected by sulpiride and lisuride, which makes eye blinking not suitable as a marker for central D₂ activity.

Introduction

Spontaneous eye blinking occurs in humans approximately 15 000 times per day, 15–20 times per minute in a quiet relaxed state. The biological function of eye blinking is to keep the cornea healthy by enabling a layer of moisture to form continuously. The rate of blinking is also closely related to psychological factors. If a task requires continuous visual attention, such as reading, the blink rate decreases. Due to strong emotional responses, stress (such as performing under time pressure) or having to perform when fatigued, the blink rate increases (Karson *et al.*, 1981B).

Patients suffering from parkinson's disease or supra nuclear palsy tend to have a decreased spontaneous eye blink rate compared to a healthy population (Karson *et al.*, 1984A,b). By contrast, patients with an acute psychotic episode often show an increased eye blink rate as well as an aberrant blinking pattern (Mackintosh *et al.*, 1983; Kitamura *et al.*, 1984; Helms *et al.*, 1985; Lovestone, 1992). These clinical observations of patients with diseases involving central dopaminergic neurotransmission have led to the suggestion of spontaneous eye blink rate as a putative marker for central dopamine activity.

Dopaminergic pharmacotherapy also appears to affect eye blink rate. Patients treated with dopamine precursors or agonists have an increased spontaneous eye blink rate. Dopamine antagonistic medication, such as several antipsychotics, decreases blinking frequency (Karson *et*

al., 1981A; Karson *et al.*, 1982; Kleinman *et al.*, 1984). Experiments with dopamine agonists in primates have confirmed these observations (Karson *et al.*, 1981C; Elsworth *et al.*, 1991; Lawrence *et al.*, 1991; Kleven *et al.*, 1996). Few studies in healthy volunteers have been performed to investigate the effect of dopaminergic drugs on spontaneous eye blink rate. Apomorphine demonstrates an increasing effect on blink-rate in healthy volunteers (Blin *et al.*, 1990). If a relationship exists, spontaneous eye blinking may provide a measure for the dopaminergic activity of new drugs, as well as a clinical tool for individual adjustment of dopaminergic drug dosage in patients with Parkinson's disease or schizophrenia.

Ideally, a useful marker should meet several requirements. First, a clear response of the biomarker to a therapeutic dose of a dopaminergic drug. Second, a clear, consistent response across studies (from different groups). Third, a dose (concentration)–response relationship of the drug and the marker. Finally, a plausible relationship between the biomarker and the pharmacology of the dopaminergic should also exist. The present study focuses on the first requirement.

The involvement of the D₂-receptor in spontaneous eye blinking is well documented, both by patient treatment data as well as in animal experiments (Karson *et al.*, 1981A; Karson *et al.*, 1982; Lawrence *et al.*, 1991; Kleven *et al.*, 1996). The drugs that were used in this study were matched on D₂-receptor selectivity. Sulpiride is a selective post-synaptic D₂-receptor antagonist (although, in lower doses, it can act as a pre-synaptic D₂-antagonist) and lisuride is a post-synaptic D₂-receptor agonist, with some weak D₁-antagonistic activity. The kinetic profiles of these drugs are fairly compatible. The drugs were administered at twice the clinically accepted starting dose, aimed at reaching clinically significant plasma concentrations.

Prolactin was measured as a reference marker for D₂ antagonism. Physiological pituitary prolactin secretion is inhibited by dopamine, and dopamine receptor antagonism induces a prolactin response. Indeed, a correlation can be observed between the affinity of a drug for the D₂ receptor and the prolactin inducing property (Visser *et al.*, 2001). The D₂ antagonist used in this study was expected to produce a maximal prolactin response of approximately 11 times baseline plasma concentrations (De Koning *et al.*, 1995). Lisuride was expected to lower prolactin levels slightly, depending on pre-treatment values. Eye blinking can also be affected by changes in attention. Although rarely used to evaluate dopaminergic drugs in healthy volunteers (Visser *et al.*, 2001), saccadic eye movements have been shown to be very sensitive to sedation, specifically saccadic peak velocity, in studies on sleep-deprivation and sedative

compounds (van Steveninck *et al.*, 1991; Harron *et al.*, 1995; Steveninck *et al.*, 1999) Smooth pursuit eye movements are altered in a number of neuropsychiatric diseases (e.g. schizophrenia and stroke), and are also affected by sedative agents such as barbiturates and alcohol (van Steveninck, 1993). Both types of eye movements were measured in this study.

Although not very sensitive, finger tapping has been used frequently in the evaluation of neuroleptic drugs in healthy volunteers (Visser *et al.*, 2001), and was also measured in the current study. In addition, subjective drug effects were rated with Visual Analogue Scales (VAS) for alertness, mood and calmness. Some sensitivity to dopaminergic effects, particularly neuroleptics, has been demonstrated (Visser *et al.*, 2001).

Methods

Subjects

After approval of the protocol by the Ethical Review Board of the Leiden University Medical Centre, 12 healthy volunteers were asked to participate in the study. After providing their written informed consent, subjects received a full medical examination before entering the study. The eye blink rate and eye-movement recording were practised to reduce learning effects during the study. Subjects refrained from alcohol, caffeine and xanthine containing foods and beverages from 12 h prior to the study day and during the study days. Concomitant medication other than paracetamol was not permitted during the study period.

Design and procedure

The study was designed as a double-blinded, completely counterbalanced within-subjects repeated measures experiment, with a minimum wash-out period of 2 days. Each study day at approximately 10.00 h, subjects received a capsule containing either sulpiride 400 mg, lisuride 0.2 mg or placebo. Eye blinks, eye movements, finger tapping and visual analogue scale scores were measured twice at baseline. Single measurements were performed at 45 min and each following hour until 12 h after drug administration. Blood samples for prolactin were drawn at 30 min and each following 30 min until 3 h, then every 1 h until 6 h and, finally, every 2 h until 12 h after drug administration. Safety assessments consisted of electrocardiograms, blood pressure and a general enquiry into the subjects' wellbeing pre-dose and at 3 h and 6 h after drug administration.

Eye blink recording

Eye blink rate was determined under tranquil conditions, with comfortable room temperature and normal humidity. The subject was measured in a comfortable position. Head movements were limited by instructing the subject to lean their heads against the headrest of the chair. The subject was instructed not to talk during the measurement, and to relax but not to fall asleep. Subjects were told that they could not close their eyes. The measurements were taken while the subjects were asked to look at a television monitor for a 5-min period, which was showing a virtual aquarium scene. The aquarium scene was used to provide a neutral eye focusing point. It does not require continuous visual attention and does not provoke strong emotional responses during the measurements, since these factors can influence the eye blink rate. The moving speed of fishes was low to avoid saccadic eye movements during gazing. A previous pilot study showed this to be the optimal measurement duration and circumstances. In this study, six subjects were measured under identical conditions over an 8-h period with 30-min intervals. Minimal effect sizes were calculated assuming a two-sided t-test with six subjects, a power of 80% and $\alpha = 0.05$. The 0–8-h values were used for a minimal effect size for a parallel study and the 0–4-h and 4–8-h (compared within subjects) to obtain a minimal effect size for a crossover study. A recording duration of more than 5 min did not decrease the minimal effect size.

Measurement electrodes were affixed to the upper and lower rim of the left orbita. The ground electrode was placed in the midline, between the eyebrows. Blinks were recorded electronically. Analysis of the recorded blinks was performed online with a microcomputer and specialized software. Blinks were recognized as peaks of bipolar waves with a duration of less than 1 s. The main parameters were the number of blinks over a 5-min period, the average blink interval and the SD of the blink interval.

Finger tapping

Tapping is one of the computerized tests for cognitive functions of the FePsy ('the iron psyche') (Alphers *et al.*, 1994), a system for automated (neuro)psychological testing. The test has been adapted from the Halstead Reitan Test Battery (Choca *et al.*, 1997), and evaluates motor activation and fluency. Speed of finger tapping is measured for the index finger for both the dominant and nondominant hand; a session

contains five performances of 10 s for each hand (e.g. 10 × 10 s). Feedback on performance is given by a counter in the centre of the screen, while the amount of taps of each 10-s trial is shown at the bottom of the screen. The space bar is used as tapping device. The volunteer is instructed to tap as quickly as possible. The mean tapping rate and the SDs for the dominant and non-dominant hand are used for statistical analysis.

Prolactin

Whole blood was drawn every study day in a Beckton & Dickinson (Erembodegem-Aalst, Belgium) Haemogard® EDTA-tube, tilted, and immediately stored on ice water (4 °C). Within 1 h, it was centrifuged at 4 °C for 10 min at 2000 g. Plasma was stored directly at -20 °C. Plasma prolactin was measured by enzyme assay using the Elisa System 300 (Minneapolis, MN, USA).

Eye movements

Recording and analysis of saccadic and smooth pursuit eye movements was performed with a microcomputer-based system (van Steveninck *et al.*, 1989). The equipment used for stimulus display, signal collection and amplification was from Nihon Kohden (Nihon Kohden Corporation, Tokyo, Japan). Saccadic eye movements were recorded for stimulus amplitudes of 15° to either side. Interstimulus intervals varied randomly between 3 s and 6 s, and 15 saccades were recorded. The average values of saccadic peak velocity, latency (reaction time) and inaccuracy were used as parameters. For smooth pursuit eye movements, the target moved sinusoidal at frequencies range from 0.3–1.1 Hz, increasing by steps of 0.1 Hz. The amplitude of target displacement corresponds to 20° eyeball rotations to both sides. Four cycles were recorded for each stimulus frequency. The parameter derived from these measurements is the percentage of time that the subjects' eyes are in smooth pursuit of the target.

Visual analogue scales

Visual analogue scales as originally described by Norris (1971) were used to quantify subjective effects. Three factors corresponding to alertness, mood and calmness were derived from these measurements (Bond *et al.*, 1976).

Statistical analysis

Treatment orders were balanced in a latin square design and assignment of subjects to treatment orders occurred at random. All withdrawals were replaced by subjects receiving the same randomization order. The pharmacodynamic analyses was performed in the per protocol population. The safety analysis was reported for all subjects who have finished at least one occasion with an active treatment.

All subjects who completed at least two occasions were investigated. A blinded data review of primary variables (eye blink number, interval and SD, prolactin, finger tapping) was performed after entry of all data, and decisions pertaining to missing, unused or spurious data were recorded.

Overall drug effects were quantified using time-corrected areas under the effect curves (AUECs divided by the corresponding time span) over 0–6 h and 0–12 h. These measures were compared between treatments using factorial analysis of variance with subject, treatment and occasion as factors and pre-treatment values as covariates. If a covariate was included, least square means were used to present the treatment response. The following contrasts were defined within the ANOVA model: (I) placebo versus sulpiride; (II) placebo versus lisuride and (III) lisuride versus sulpiride.

Contrasts are presented with 95% confidence intervals (CI); no corrections for multiple comparisons were implemented because the study was not designed to prove differences between treatments but to assess the size of effects.

Results

Thirteen male subjects (aged 19–40 years, mean 24 years), were enrolled in the study. Twelve subjects completed all three treatment periods, and were included in the dynamic analysis. One subject dropped out for non-study related reasons and was replaced. All subjects who had one or more treatments were included in the safety analysis.

Adverse events

The adverse events observed in this study were mainly somnolence ($n = 9$), fatigue ($n = 5$), headache ($n = 8$) and nausea ($n = 3$). In general, most adverse events occurred during the lisuride treatment. This was mainly

due to the higher incidence of somnolence (lisuride, n = 6; sulpiride, n = 4; placebo, n = 3) and nausea (solely during the lisuride treatment). The nausea resulted in moderate vomiting on two occasions. Most other adverse events were mild. All adverse events subsided before the subjects returned home at the end of a study day. No changes in vital signs or electrocardiogram were noted during any of the treatments.

Spontaneous eye blinking

As expected, the number of eye blinks showed a large interindividual variability. The raw number of eye blinks for the different treatments over time are presented in figure 1. Analysis of variance with pre-treatment values as covariate showed no treatment differences between the treatments or between treatments and placebo. The SD of the blink interval showed similar results.

Fig. 1 – p. 138

Prolactin

A clear and significant increase of the AUC compared to placebo was observed for sulpiride over the 0–6-h and 0–12-h periods: 917 U/ml (CI 773–1060) and 643 U/ml (CI 549–737), respectively (fig. 2, Top panel). The decrease of lisuride compared to placebo over these periods did not reach statistical significance: -74 U/ml (CI -217 to 69) and -39 U/ml (CI -133 to 54), respectively (fig. 2, Lower panel).

Fig. 2 – p. 138

Fig. 2 – p. 138

Finger tapping

No effect of any of the treatments was observed on finger tapping for the dominant or non-dominant hands, or on the SD of these measures.

Eye movements

The smooth pursuit percentages over time for the different treatments are presented in figure 3. No differences of the treatments compared to placebo or between treatments were observed for saccadic peak velocity, accuracy or latency. A decrease the AUC of percentage smooth pursuit occurred for lisuride over the 0–6-h and 0–12-h periods: -4.2% (CI -7.5 to -0.9) and -4.1% (CI -7.3 to -0.9), respectively.

Fig. 3 – p. 139

Visual analogue scales

Fig. 4 & 5 – p. 140

The VAS for alertness and mood both showed a treatment effect for lisuride. The scores for the different treatments over time are presented in figures 4 and 5. Lisuride decreased the VAS scores for mood over the 0–6-h and 0–12-h periods: -2.7 mm (CI -4.7 to -0.7) and -2.1 mm (CI -3.7 to -0.4), respectively. The decrease of VAS alertness scores over these periods did not reach statistical significance: -3.8 mm (CI -8.2 to 0.6) and -3.8 mm (CI -8.5 to 0.9), respectively.

Discussion

This study did not show any indication of effect of sulpiride or lisuride on the number of spontaneous eye blinks or on the SD of its interval. This is not in accordance with previous studies, indicating spontaneous eye blink rate as a putative marker of central dopamine activity (Karson *et al.*, 1981A,c, 1982, 1984; Blin *et al.*, 1990; Elsworth *et al.*, 1991; Lawrence *et al.*, 1991; Kleven *et al.*, 1996). Several explanations can be given for these findings. First, no influence of dopamine on spontaneous eye blink rate may exist. Publications of negative results could be underrepresented.

Second, in the present study, we used drugs that act mainly on the D₂-receptor. Although, from the literature, it appears that drug effects on eye blink rate are mediated mainly through the D₂-receptor, there are also indications that D₁ plays a role (Elsworth *et al.*, 1991). The specificity of the drugs could have prevented dopaminergic effects mediated through other dopamine receptor subtypes.

No drug concentrations were determined during this study. Thus, there is no absolute certainty that clinically relevant plasma drug concentrations were reached that could be expected to elicit an effect. However, it should be noted that, generally, there is no strong correlation between functional dopaminergic (therapeutic) effects and drug plasma concentrations. On the other hand, we can assume that relevant brain concentrations were reached. First, the dosages of both drugs were potent (twice the starting dose). Furthermore, sulpiride administration evoked a clear prolactin response (a maximum increase of approximately 10 times baseline), which indicates central penetration and D₂-antagonistic effect.

Fig. 2 – p. 138

Although apparent (fig. 2, lower panel, shows a clear decrease after lisuride), the decrease of prolactin after lisuride administration did not reach statistical significance. This could be due to the small

range of possible effects on physiologically low prolactin levels and the limited number of subjects. The subjective effects (decreases on VAS scales for mood and alertness and nausea/vomiting) also demonstrate pharmacologically active lisuride levels.

In conclusion, in spite of dopaminergically active drug levels, this study did not show evidence of any effects of lisuride or sulpiride on spontaneous eye blink rate in healthy male volunteers. There are no indications that spontaneous eye blink rate is a marker for central dopaminergic activity of drugs, mediated through the D_2 -receptors. Prolactin provides a good measure of D_2 -dopaminergic activity, particularly for an antagonist.

REFERENCES

- Alpers W C J, Aldenkamp A P (1994) *FePsy, The Iron Psyche*. Manual 5.0
- Blin O, Masson G, Azulay J P, Fondarai J, Serratrice G (1990) Apomorphine-induced Blinking And Yawning In Healthy Volunteers. *Br J Clin Pharmacol* 30: 769-773
- Bond A, Lader M (1976) Self-concepts In Anxiety States. *Br J Med Psychol* 49: 275-279
- Choca J P, Laatsch L, Wetzell A, Agresti A (1997) The Halstead Category Test: A 50 Year Perspective. *Neuropsychol Rev* 7: 61-75
- De Koning P, De Vries M H (1995) A Comparison Of The Neuro-endocrinological And Temperature Effects Of Du 29894, Flesinoxan, Sulpiride And Haloperidol In Normal Volunteers. *Br J Clin Pharmacol* 39: 7-14
- Elsworth J D, Lawrence M S, Roth R H, Taylor J R, Mailman R B, Nichols D E, Lewis M H, Redmond De Jr (1991) D₁ And D₂ Dopamine Receptors Independently Regulate Spontaneous Blink Rate In The Vervet Monkey. *J Pharmacol Exp Ther* 259: 595-600
- Harron D W, Hasson B, Regan M, McClelland R J, King D J (1995) Effects Of Rilmenidine And Clonidine On The Electroencephalogram, Saccadic Eye Movements, And Psychomotor Function. *J Cardiovasc Pharmacol* 26 (Suppl): S48-S54
- Helms P M, Godwin C D (1985) Abnormalities Of Blink Rate In Psychoses: A Preliminary Report. *Biol Psychiatry* 20: 103-106
- Karson C, Freed W J, Kleinman J E, Bigelow L B, Wyatt R J (1981a) Neuroleptics Decrease Blinking In Schizophrenic Subjects. *Biol Psychiatry* 16: 679-682
- Karson C N, Berman K F, Donnelly E F, Mendelson W B, Kleinman J E, Wyatt R J (1981b) Speaking, Thinking, And Blinking. *Psychiatry Res* 5: 243-246
- Karson C N, Bigelow L B, Kleinman J E, Weinberger D R, Wyatt R J (1982) Haloperidol-induced Changes In Blink Rates Correlate With Changes In Bprs Score. *Br J Psychiatry* 140: 503-507
- Karson C N, Berman K F, Kleinman J, Karoum F (1984a) Seasonal Variation In Human Central Dopamine Activity. *Psychiatry Res* 11: 111-117
- Karson C N, Burns R S, Lewitt P A, Foster N L, Newman R P (1984b) Blink Rates And Disorders Of Movement. *Neurology* 34: 677-678
- Karson C N, Staub R A, Kleinman J E, Wyatt R J (1981c) Blink Rates And Receptor Supersensitivity. *Neuropharmacology* 20: 91-93
- Kitamura T, Kahn A, Kumar R, Mackintosh J H (1984a) Blink Rate And Blunted Affect Among Chronic Schizophrenic Patients. *Biol Psychiatry* 19: 429-434
- Kleinman J E, Karson C N, Weinberger D R, Freed W J, Berman K F, Wyatt R J (1984b) Eye-blinking And Cerebral Ventricular Size In Chronic Schizophrenic Patients. *Am J Psychiatry* 141: 1430-1432
- Kleven M S, Koek W (1996) Differential Effects Of Direct And Indirect Dopamine Agonists On Eye Blink Rate In Cynomolgus Monkeys. *J Pharmacol Exp Ther* 279: 1211-1219
- Lawrence M S, Redmond D E Jr (1991) Mptp Lesions And Dopaminergic Drugs Alter Eye Blink Rate In African Green Monkeys. *Pharmacol Biochem Behav* 38: 869-874
- Lovestone S (1992) Periodic Psychosis Associated With The Menstrual Cycle And Increased Blink Rate. *Br J Psychiatry* 161: 402-404
- Mackintosh J H, Kumar R, Kitamura T (1983) Blink Rate In Psychiatric Illness. *Br J Psychiatry* 143: 55-57
- Norris H (1971) The Action Of Sedatives On Brain Stem Oculomotor Systems In Man. *Neuropharmacology* 10: 181-191
- Steveninck A L, Berckel, B N M, Schoemaker H C, Breimer D D, Gerven J M A, Cohen A F (1999) The Sensitivity Of Pharmacodynamic Tests For The Central Nervous System Effects Of Drugs On The Effects Of Sleep Deprivation. *J Psychopharm* 13: 10-17
- Van Steveninck A L (1993) *Methods Of Assessment Of Central Nervous System Effects Of Drugs In Man*. Thesis, State University Leiden
- Van Steveninck A L, Cohen A F, Ward T (1989) A Microcomputer Based System For Recording And Analysis Of Smooth Pursuit And Saccadic Eye Movements. *Br J Clin Pharmacol* 27: 712-713
- Van Steveninck A L, Schoemaker H C, Pieters M S, Kroon R, Breimer D D, Cohen A F (1991) 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* 50: 172-180
- Visser S J, Van Der Post J, Pieters M S M, Cohen A F, Gerven J M A (2001) Biomarkers For The Effects Of Antipsychotic Drugs In Healthy Volunteers *Br J Clin Pharmacol* 51: 119-132

FIGURE 1 Average number of blinks. Mean \pm SD time-effect profiles of blinks for placebo (●), lisuride (○) and sulpiride (■).

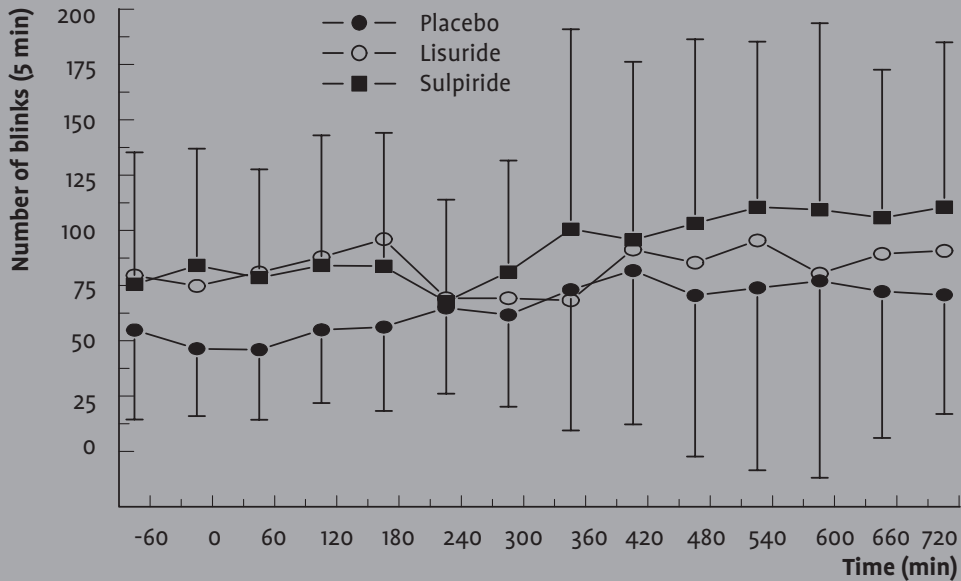


FIGURE 2 Average prolactin concentration. Mean \pm SD time-effect profiles of prolactin concentration for placebo (●), lisuride (○) and sulpiride (■).

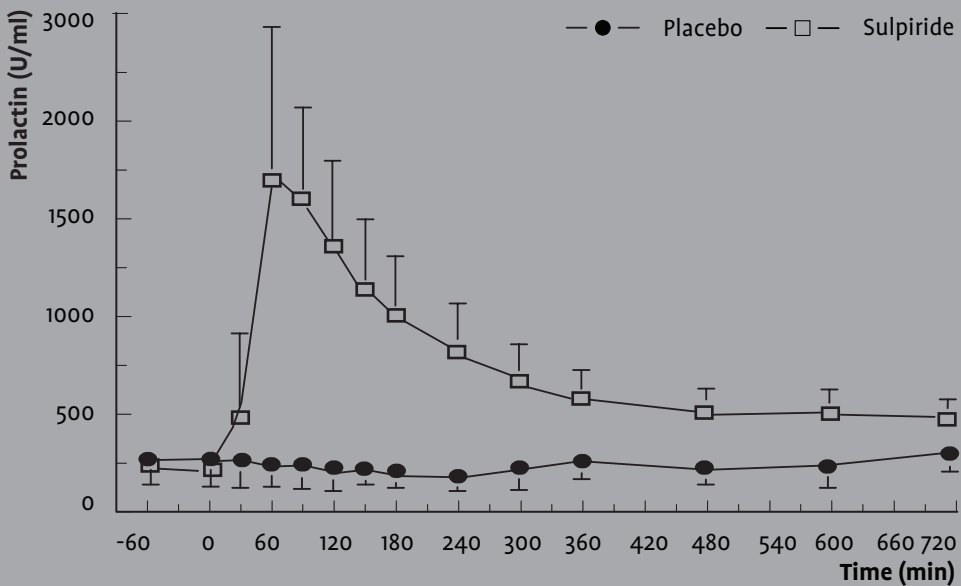


FIGURE 2

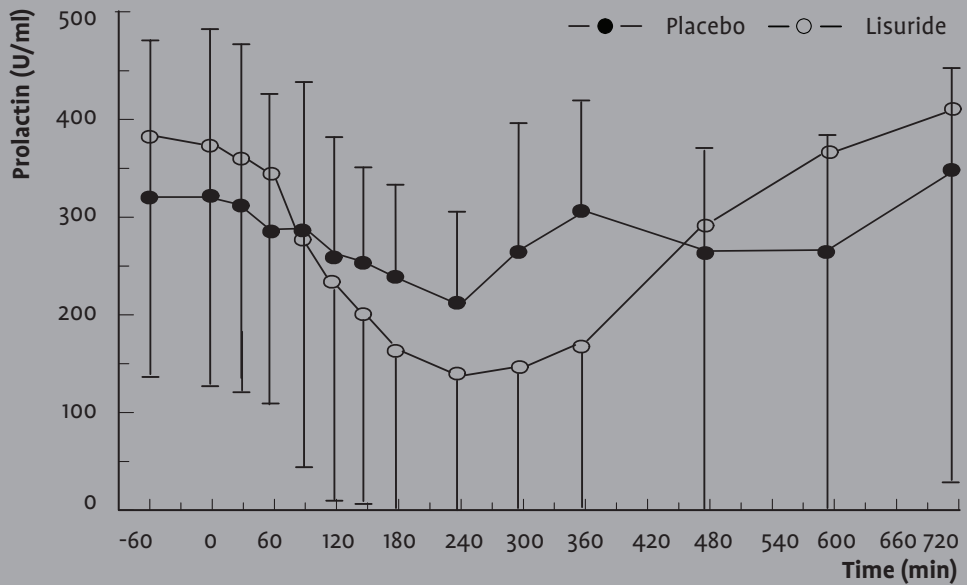


FIGURE 3 Average percentage smooth pursuit. Mean \pm SD time-effect profiles of percentage smooth pursuit for placebo (●), lisiride (○) and sulpiride (■).

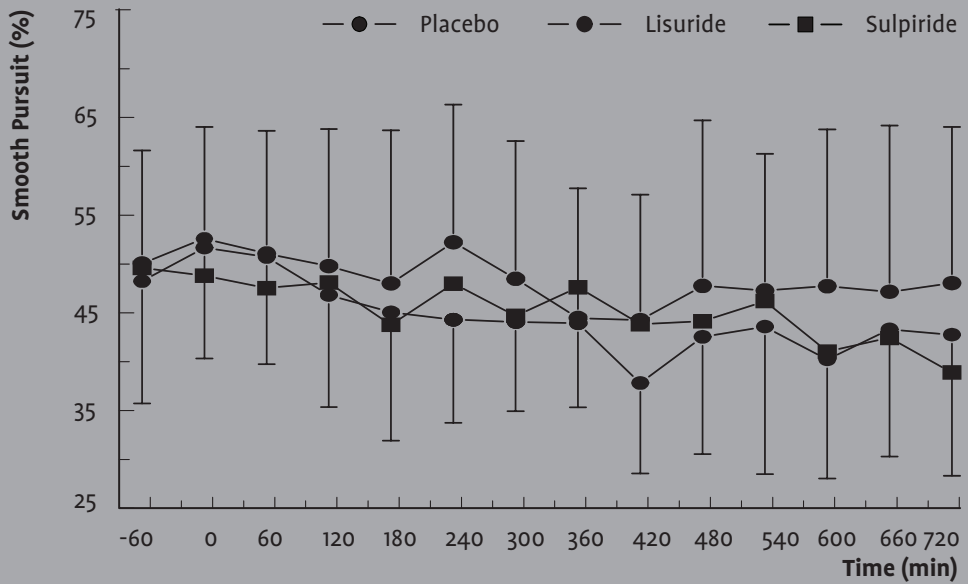


FIGURE 4 Average visual analogue scales for alertness. Mean \pm SD time-effect profiles of visual analogue scales for alertness for placebo (●), lisuride (○) and sulpiride (■)

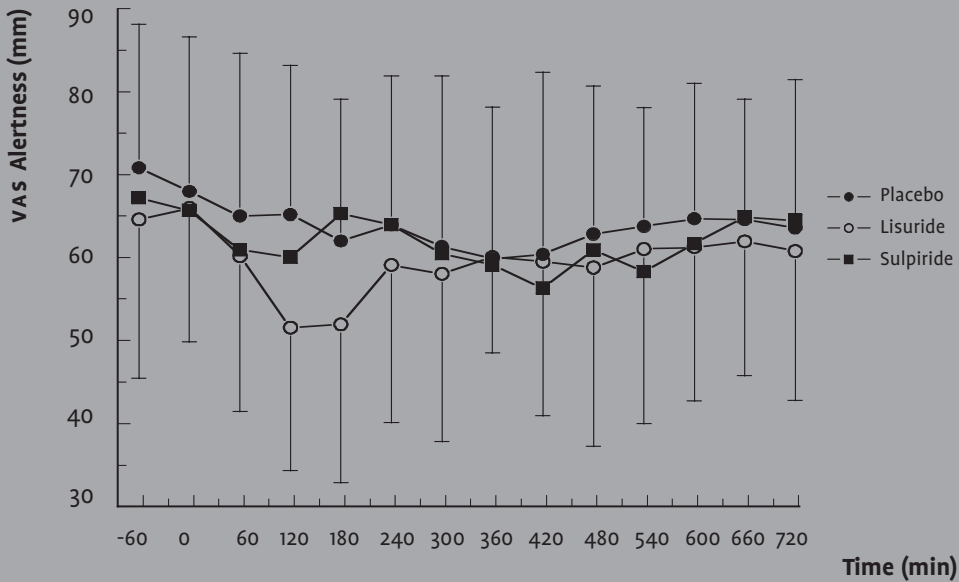
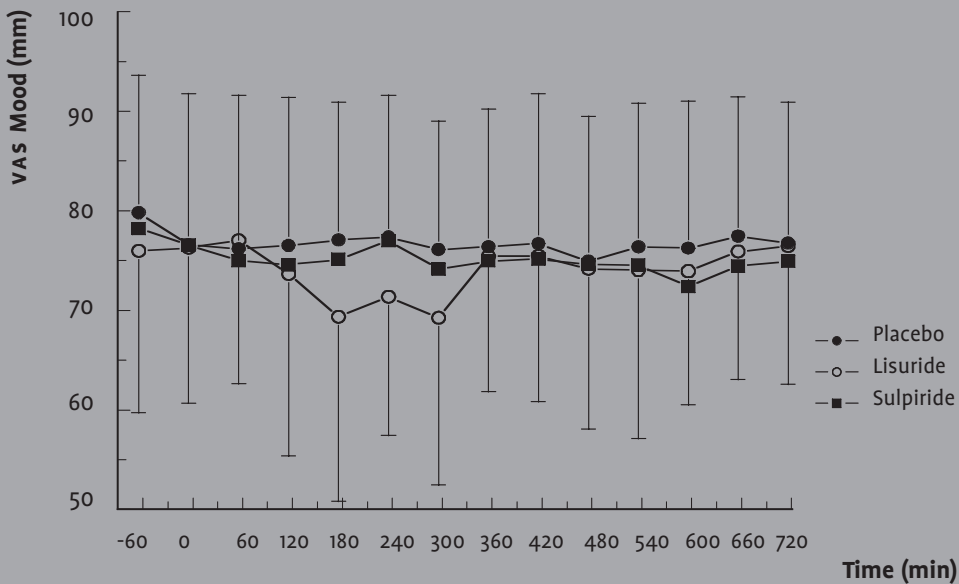
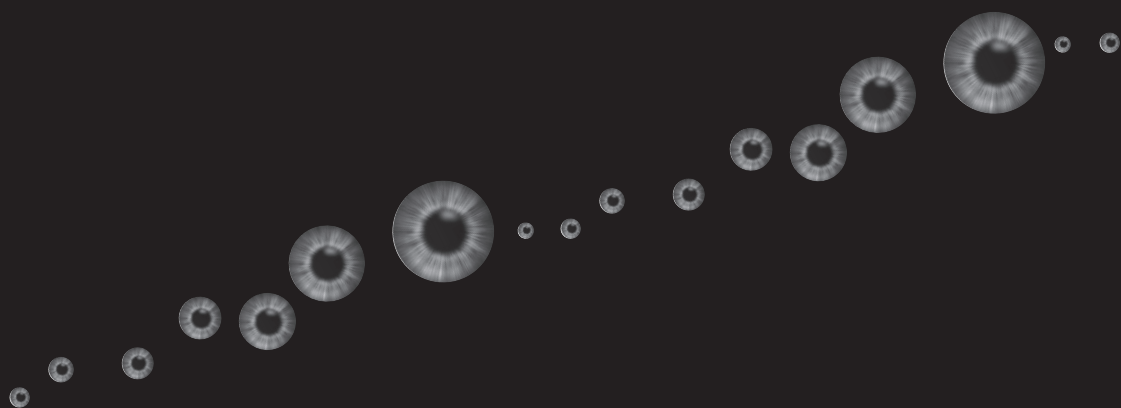


FIGURE 5 Average visual analogue scales for mood. Mean \pm SD time-effect profiles of visual analogue scales for mood for placebo (●), lisuride (○) and sulpiride (■).



CONCLUSIONS AND DISCUSSION



Conclusions and discussion

The main objective of this thesis is to provide a conceptual framework for the use of central nervous system (CNS) biomarkers in early phase clinical drug development. A classification into four groups of biomarkers was suggested, based on four general questions on which these markers should provide information on:

1. Proof of CNS-penetration and functional CNS effects, which can be sub-divided into:
 - A. Biomarkers of CNS pharmacokinetics
 - B. Biomarkers of adverse effects
2. Proof of pharmacodynamic principle (biomarkers of mechanism of action)
3. Proof of pathophysiological principle (biomarkers of effects on relevant pathophysiological process)
4. Proof of therapeutic principle (biomarkers of therapeutic effects)

Clinical studies with markers for functional CNS effects were presented in chapters 1-4. The approach of selecting sensitive markers for these functional CNS effects proved fruitful; not only in evaluating adverse effects (chapters 2: CNS effects of sumatriptan and rizatriptan, 3: PK/PD assessment of tolerance to central nervous system effects of a 3 mg sustained release tablet of rilmenidine in hypertensive patients and 4: CNS effects of moxonidine experimental sustained release formulation in patients with mild to moderate essential hypertension) but also in creating a CNS-profile of a novel drug (chapter 1: CNS effects, pharmacokinetics and safety of the NAALADase-inhibitor GPI 5693). In these studies, the profile of CNS-effects can be considered as a 'surrogate' of prevailing CNS drug levels, even though the effects themselves do not necessarily hold any relevance for the pharmacological or therapeutic effects of the drug. Although individually these CNS-effects are non-specific, they may in combination be unique for a particular drug class. An attempt to find a drug class specific combination of these individually non-specific markers was described in chapter 5. As far as data was available, it showed a unique drug class specific relationship between saccadic eye movements, visual analogue scales and EEG for diverse agents like benzodiazepines, imidazoline-receptor modulators and 5HT₁-agonists. Thus, biomarker combinations can be used to establish the similarities or differences between a novel agent and comparators within or outside its own class. However, this approach remains empirical, because there are

no clear explanations why a certain biomarker combination should show this relationship to a particular drug class. Chapter 6 described the first steps in the development of hypoxia as a potential disease model for cognitive impairment (proof of therapeutic principle), using markers for functional CNS-effects. In Chapter 7, another approach was explored, by investigating the response of a CNS-biomarker with an established pharmacological action mechanism, to drugs that act on the same mechanism. However, this receptor specific marker, eye blinking for central D₂-dopaminergic drug effect of mesocortical or mesolimbic pathways, failed to be validated. This would have been a useful adjunct to a pharmacological biomarker like prolactin, which in this study was confirmed to be a useful indicator for tuberoinfundibular D₂-dopaminergic activity. The interpretation of the results of these individual studies was discussed in each chapter. However, a few general remarks on the use of biomarkers in early CNS drug development can be made, based on these observations.

In the life of a CNS biomarker, its main ambition seems to be to become a surrogate marker for a clinical endpoint, and should thus provide information on how a patient “feels, functions or survives”. Consequently, its distance to a clinical endpoint determines the merits of a biomarker. This view of what a biomarker should be is especially prominent in phase III and IV drug development programs. In early CNS drug development, we are very short of such biomarkers. The only potential class of biomarkers that could be considered to be ‘clinically relevant’ is disease models, and such validated models are quite rare, and they hardly ever reflect all aspects of the disease. Nevertheless, biomarkers can provide valuable information even if they never become surrogate endpoints in this clinical sense.

To appreciate a biomarker, one should be very explicit as to what kind of information it can provide. The aim of the suggested categorization of biomarkers can help in this process. Figure 1 shows three of the four categories on an x, y and z-axis. Ideally, a biomarker would reflect the drugs pharmacological properties, its effects on pathophysiological pathways, and its therapeutic effects. This biomarker would lie in the upper right quadrant of the figure. However, most biomarkers used in early CNS drug development will provide information on the pharmacology of the drug (moving along the z-axis). Some biomarkers will also reflect disease related (patho)physiological systems (moving along the y-axis). Very few biomarkers in healthy volunteers, if any, will be related to therapeutic effects (moving along the x-axis). Of course, biomarkers could be placed anywhere in the cube. Figure 1 also shows the position of potential biomarkers presented in this thesis.

Fig. 1 – p. 149

If we look at the suggested classification of biomarkers, one could argue that the first category of “markers for functional CNS effects” isn’t useful at all (it is also not represented in the above figure). These biomarkers merely show that a drug has penetrated the CNS and has an effect, which could be desired or undesired and could be related to a therapeutic effect, but usually isn’t. This group basically constitutes a vast amount of tests that would, if we understood the pharmacology of the drug, CNS physiology and pathophysiology fully, be categorized under markers for a pharmacological principle, a (patho)physiological principle or even therapeutic principle, or a combination of the above. Then, should we abandon this category? Our lack of understanding of the CNS and related diseases still seems to necessitate the use of this category, and most biomarkers used in early CNS drug development fall in it. Within the limitations mentioned, an effort should be made to make use of these biomarkers to the full, and place them somewhere in the figure. How can we make the most of these “markers for functional CNS effects”? Here, the subdivision into “biomarkers of CNS kinetics” and “biomarkers of adverse effects” becomes important.

Can a biomarker be used to get an impression of CNS kinetics? Measuring drug concentrations in the CNS effect compartment is difficult. Pet-studies or drug concentrations in cerebrospinal fluid are often the closest we can get to CNS-pharmacokinetics. Plasma kinetics of a drug are much easier to assess. If a drug has an effect on the CNS, and this effect is somehow picked up by an effect on a biomarker, this is usually a direct consequence of the presence of the drug in the brain. Such a biomarker could be informative about the CNS pharmacokinetics, even if the pharmacodynamics of the drug are not fully understood. If plasma concentrations a drug can be related to functional CNS effects, these effects can thus provide information on CNS kinetics. Pharmacokinetic/pharmacodynamic (PK/PD) analysis provides a means to establish this relationship, and is often used in early drug development. For a biomarker to be useful in this manner, it should however be sensitive to a drug effect, and show some indication of a concentration-effect relationship. If these requirements are met, and subsequently a PK/PD relationship is found, it is often a sounder basis for establishing future dose regimens for the drug under investigation than plasma kinetics alone. However, some caution is needed when using a biomarker for CNS kinetics. Systemic drug effects can provoke a secondary CNS effect (eg nausea provoked by local gastric irritation can induce a prolactin release), and in this way lead to false conclusions on CNS kinetics.

Maybe the most frequent use of biomarkers in early CNS drug development is as indicators of adverse events. These biomarkers can provide valuable information on the general CNS properties of a drug. This is vital information in the early stages of drug development, which primarily aims to assess the tolerability of a drug. It allows us to identify for example dose limiting tolerability issues at a relatively early stage. This approach does require that sensitive biomarkers are being used and that these show at least a consistent and significant response across different studies and drugs in the same therapeutic class. Thus, the effects of related positive controls provide a benchmark for the (adverse) event profile of a novel agent from the same class. For these biomarkers to be useful, a consistent and significant response at therapeutic doses and a dose response relationship is also needed. The clinical relevance of these biomarkers for adverse events in daily practice, require further validation under different clinical circumstances (e.g. effects of sleep deprivation, circadian rhythms) for an effect to be attributed to a drug action.

More often than not, these basic requirements for fully validated effect biomarker are not met. Nevertheless, a test battery composed of tests fulfilling several of these requirements can be helpful in creating a CNS tolerability profile of drugs with a novel mechanism of action, especially since phase I drug development is constantly confronted with new drugs with only partly elucidated mechanisms of action, indicated for diseases with frequently only partially known pathophysiology. The inclusion of a positive control (i.e. a drug with a known effect on the biomarker) in studies with these drugs increases the validity of the observed effects.

What can biomarkers tell us about the pharmacodynamic properties of a drug, i.e. Its mechanism of action? It seems safe to assume that if a drug shows a CNS effect (in a manner described in the above paragraphs), it will be somehow related to its pharmacodynamic properties. It would therefore also be safe to assume that whatever is measured, this biomarker will reflect the pharmacodynamic properties of the investigated drug, and thus the biomarker would be proof of a “pharmacodynamic principle”. But what pharmacodynamic principle is proven? Does it demonstrate the unique pharmacodynamic properties of the drug under investigation? For most biomarkers this is not the case. As shown in this thesis, a decrease in saccadic peak velocity and a decrease of visual analogue scales for alertness can be observed after administration of a benzodiazepine (temazepam), a imidazoline receptor modulator (rilmenidine, moxonidine) as well as after a 5HT₁ agonist (sumatriptan, rizatriptan) and indeed several other drugs. Here an

important issue arises, that of specificity. Most ‘functional’ biomarkers are not very specific. The ‘CNS, to a large extent, is still a “black box” with motor function or subjective changes as its main outputs. This is true especially for most markers in the cognitive domains. And even for non-motor neurophysiologic measures, such as EEG or fMRI, it is uncertain if they have any additional relevance to the “motor output” of the CNS. Here we are confronted, again, with our lack of understanding and the complexity of even basic CNS-functions, such as attention.

An important question arises: if the same biomarkers are affected by drugs with different pharmacological properties, are they affected in a similar way? To put it in another way: can we identify a drug’s pharmacological action by combining different biomarkers that are affected by drugs with different pharmacological properties? If we could, this approach to the use of biomarkers has some advantages: even if the exact pharmacological pathway of a drug is unknown, and the relevance to the pathophysiology is unclear, a “pharmacological footprint” of a drug could be construed. In this approach, it would not matter if after taking a certain drug, a person would prefer the color red over blue and the taste of apples over pears, as long as it would be specific to a certain mechanism of action of the drug. To achieve this kind of “pharmacological footprints” it would be necessary to prospectively evaluate drugs with high receptor specificity with a combination of sensitive (non-specific) biomarkers, thus creating templates for drugs with unknown mechanisms of actions. In chapter 5 of this thesis, such an attempt was made to evaluate drugs with known mechanisms of action on their combined effect on sensitive but non-specific biomarkers.

The chances of a single biomarker to be specific to a certain drug (or even drug class) are slim. To create a specific pharmacological and adverse event profile of a drug, a combination of biomarkers will be required. If the above mentioned approach would provide a useful set of biomarkers, would this help in assessing the action of the drug on relevant pathophysiological pathways at which the drugs are targeted? This largely depends on our knowledge of the pathways involved in diseases affecting the CNS. For the majority of CNS diseases, these pathways are, at best, partly elucidated. However, some indications of an effect on a particular pharmacological system can often be obtained, because many such systems have well-established physiological relevance or well-known effects in experimental animals. For instance, a combination of increased alertness, rem sleep reduction and body temperature elevation would strongly point to serotonergic activation, even though none of the individual effects is very specific. The definite

answers most likely will come from physiological and clinical research in patients, not from biomarker research. Such research is important, because individual or combined biomarkers can only show to which degree a new drug is similar to well-known drugs. If we do not understand the physiological or pharmacological relevance of these biomarker, and if no new understanding of these pathways arises, the use of well-established functional biomarkers would simply facilitate the development of “me too drugs”, as has been the case, for example, with the increasing number of SSRI’s for the treatment of depression. The main distinguishing feature between these drugs seems to be their adverse effect profile, not clinical efficacy. However useful, biomarkers used in early drug development in healthy volunteers are primarily targeted at assessing the adverse effects, not to provide a pathophysiological proof of principle. But do we need to know the exact pathophysiology of a disease to find and use a biomarker related to it?

The use of disease models and challenge tests in drug development rely on the fact that we need not know the exact pathophysiology of a disease in order to use these models and the biomarkers derived from them. For example, the scopolamine model for memory impairment has greatly aided in the development of cholinesterase inhibiting anti-alzheimer drugs. Disease models, like the scopolamine model, often only model the part of the pathophysiology leading to identical symptoms, affecting a “final common pathway” (a decrease of acetylcholinergic neurotransmission). Models like these allow the evaluation of symptomatic drugs that affect the same physiological pathway. The scopolamine model will not necessarily be useful in the evaluation of a tau-tangle inhibitor for alzheimers disease.

In chapter 6 an attempt was made to develop a disease model for cognitive impairment (hypoxia). Cerebral (cortical) hypoxemia and the resulting impairment of function seem to be a “final common pathway” for several clinical cognitive impairment syndromes (e.g. Alzheimers disease, vascular dementia). The aim was to create a practical model that, if eventually used in drug development, does not have the drawback of pharmacological interaction of the scopolamine model, and can perhaps be applied more widely to other cognitive enhancers. In this study the effects on the biomarkers were small and bidirectional for different levels of hypoxia. Before this model can potentially be used in drug development, the physiology of cerebral hypoxia should be further investigated.

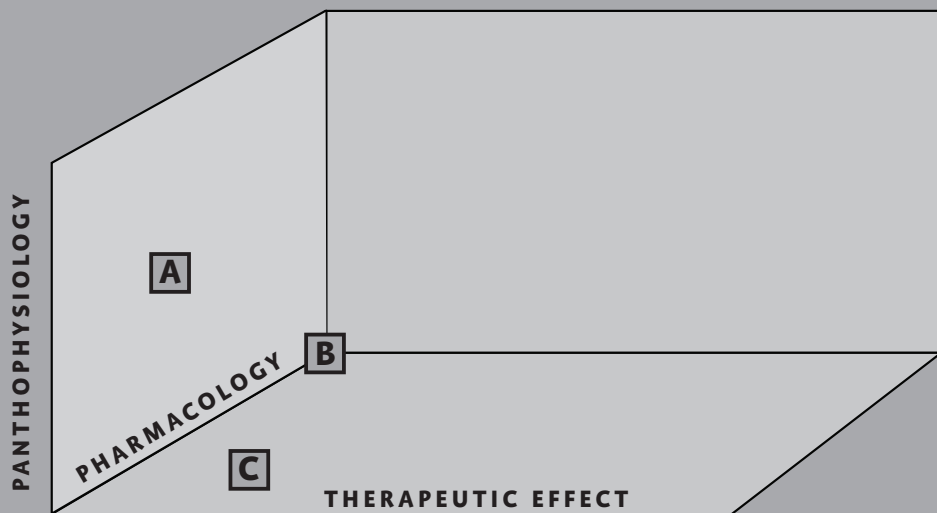
This attempt to validate a disease model does illustrate vital steps in it’s development: to have a conceptual idea of what part of disease

physiology is affected, to search for sensitive biomarkers to pick up these effects and to see if a dose-effect relationship is present. These steps should be taken carefully, prior to pharmacological intervention.

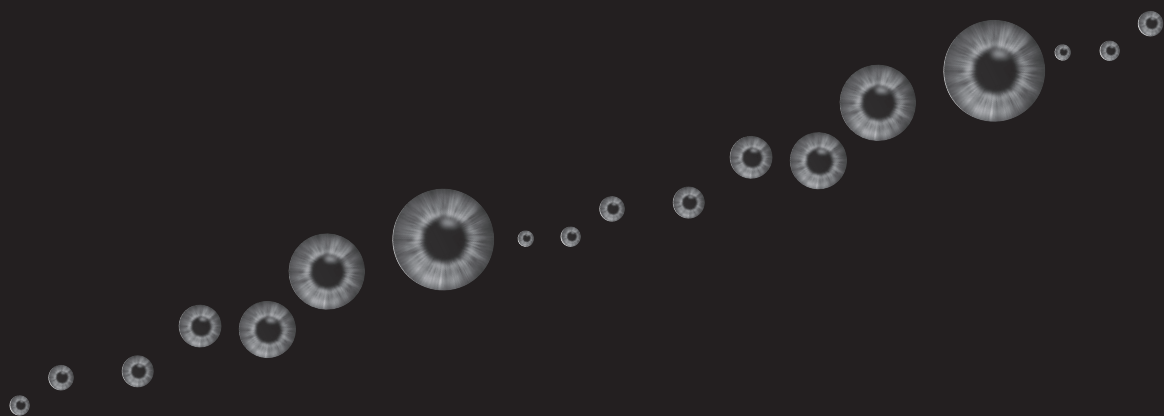
Disease models, and also challenge tests, are important in the development of CNS drugs even in the absence of complete knowledge of disease pathophysiology. This does require that they relate to a part of physiology that is affected by the disease and the drug under evaluation affects this particular part of physiology. Furthermore, biomarkers should be available that provide reliable information on that physiology. If these requirements are met, disease models and challenge tests are most likely to eventually provide biomarkers for therapeutic effects.

In conclusion, despite all the limitations, biomarkers can be of great value in early CNS drug development. But different biomarkers have different purposes. A critical evaluation of existing biomarkers, and positioning them in relation to drug pharmacology, disease pathophysiology and therapeutic effect, is a more fruitful approach than to increase their number. This seems particularly relevant for the evaluation of drug effects on cognitive function, where a large number of tests is being used that lack sensitivity and reproducibility. The development of challenge tests and disease models will not only increase our understanding of diseases, but may also provide us with biomarkers for therapeutic effects in healthy volunteers.

FIGURE 1 The position of potential biomarkers presented in this thesis in relation to pharmacological, pathophysiological and therapeutic parameters. A: eyeblinking (Chp 7), B: combined markers (Chp 5), C: hypoxia-model (Chp 6).



SAMENVATTING



Samenvatting

Het doel van deze dissertatie is het scheppen van een conceptueel raamwerk voor het gebruik van Centraal Zenuwstelsel (CZS) biomarkers in de vroege fase van klinisch geneesmiddelonderzoek. In de inleiding wordt een beeld geschetst van het huidige gebruik van biomarkers en de mogelijke tekortkomingen daar in. De noodzaak van een vraag gestuurde indeling in biomarkergroepen wordt bepleit. Er wordt een onderverdeling in vier groepen gesuggereerd, gebaseerd op vier algemene vragen waar een biomarker informatief over zou kunnen zijn:

1. Bewijs van CZS-penetratie en functionele CZS-effecten, die onderverdeeld kunnen worden in:
 - A. biomarkers van CZS farmacokinetiek
 - B. biomarkers van bijwerkingen
2. Bewijs van het farmacodynamisch principe (biomarkers van het werkingsmechanisme)
3. Bewijs van het pathofysiologisch principe (biomarkers van effecten op relevante pathofysiologische processen)
4. Bewijs van het therapeutisch principe (biomarkers van therapeutische effecten)

In hoofdstuk 1 t/m 4 worden vroege fase geneesmiddelstudies gepresenteerd waarin gebruik werd gemaakt van biomarkers van functionele CZS-effecten. Deze benadering van het selecteren van de meest sensitieve biomarkers voor deze functionele effecten bleek productief.

Hoofdstuk 1 “The central nervous system effects, pharmacokinetics and safety of the NAALADase-inhibitor GPI 5693” beschrijft een onderzoek waarin het CZS profiel van een nieuw geneesmiddel in gezonde vrijwilligers werd vastgesteld. Een deel van de gebruikte biomarkers (adaptive tracking, Visual Analogue Scales (VAS)) lieten effecten zien van de hoogste gebruikte dosis van dit geneesmiddel, die aantonen dat het middel doordringt in het centrale zenuwstelsel en zouden kunnen wijzen op klinisch relevante sedatie.

In Hoofdstuk 2 “CNS effects of sumatriptan and rizatriptan in healthy female volunteers” worden sensitieve biomarkers van o.a. sedatie gebruikt om de CZS effecten van twee triptanen te kwantificeren. De resultaten laten zien dat zowel sumatriptan en rizatriptan kleine

maar duidelijke effecten op het CZS hebben, die met deze biomarkers te onderscheiden zijn van de reeds bekende effecten van het benzodiazepine temazepam.

Hoofdstuk 3 “Pharmacokinetic/pharmacodynamic assessment of tolerance to central nervous system effects of a 3 mg sustained release tablet of rilmenidine in hypertensive patients” worden de sedatieve effecten onderzocht van rilmenidine, een in het CZS werkzaam antihypertensivum, bij patiënten met hypertensie. In deze studie wordt van deze sedatieve effecten het verloop in de tijd en de relatie met geneesmiddelconcentraties onderzocht, gedurende langdurige behandeling. De subjectief ervaren sedatie, vastgesteld met VAS-sen voor alertheid, nam in deze studie af na langdurige behandeling, hetgeen met name toe te schrijven was aan een toename van alertheid vóór inname van de dagdosis van het geneesmiddel. Hoofdstuk 4 beschrijft een vergelijkbaar onderzoek naar de CZS effecten van het qua werkingsmechanisme verwante antihypertensivum moxonidine. Deze studie liet met gebruik van identieke biomarkers vergelijkbare resultaten zien.

Een aantal van de biomarkers gebruikt in de onderzoeken beschreven in de Hoofdstukken 1 t/m 4 kan een indruk geven (door farmacokinetiek-farmacodynamiek (PK-PD) modellering) van geneesmiddel concentraties in het brein en zo een surrogaat vormen voor niet beschikbare geneesmiddelconcentraties op de plaats van werking. Daarbij is het niet strikt noodzakelijk dat de biomarker informatief is over de farmacodynamiek van het geneesmiddel of het eventuele therapeutische effect, als er tenminste geen belangrijke systemische effecten die secundair het zenuwstelsel beïnvloeden. Uit de onderzoeken beschreven in deze hoofdstukken blijkt tevens dat de meest sensitieve biomarkers elk voor zich weinig specifiek zijn voor een geneesmiddel of geneesmiddel klasse.

Hoofdstuk 5 “Drug class specific composite effects on saccadic peak velocity, visual analogue scales and electroencephalography” beschrijft een poging sensitieve maar non-specifieke biomarkers te combineren tot een nieuwe specifieke biomarker. Hiertoe werd gebruik gemaakt van data uit een aantal binnen het Centre for Human Drug Research verrichte studies. Er blijkt een unieke geneesmiddelklasse specifieke relatie te bestaan tussen saccadische oogbewegingen, visual analogue scales en EEG voor diverse geneesmiddelklassen zoals benzodiazepines, imidazoline-receptor modulators en 5HT₁-agonisten. Op deze wijze zouden biomarkercombinaties gebruikt worden om overeenkomsten

of verschillen aan te tonen tussen nieuwe geneesmiddelen en actieve controle geneesmiddelen binnen of buiten de geneesmiddelklasse. Deze aanpak blijft empirisch en verklaart niet waarom een specifieke combinatie van biomarkers gerelateerd is aan een bepaalde geneesmiddelklasse.

Hoofdstuk 6 “Evaluation of tests of central nervous system performance after hypoxemia for a model for cognitive impairment” beschrijft de ontwikkeling van een potentieel ziektemodel voor cognitieve functiestoornissen. Potentieel zou dit biomarkers voor therapeutische effecten van “cognitive enhancers” kunnen opleveren. Hoewel er effecten waren op diverse biomarkers, was de invloed van verschillende niveaus van hypoxemie hierop complex. Daardoor is dit model niet zonder meer bruikbaar voor geneesmiddelenonderzoek.

In Hoofdstuk 7 “No evidence of the usefulness of eye blinking as a marker for central dopaminergic activity” wordt een poging beschreven een receptor specifieke biomarker te ontwikkelen. Dit zou dan een biomarker voor (een deel van) het werkingsmechanisme zijn. Uit de literatuur waren aanwijzingen dat de frequentie van spontaan oogknipperen selectief beïnvloed zou worden door de centrale dopaminerge activiteit op de D_2 -receptor. Potentieel zou de frequentie van het oogknipperen een biomarker kunnen zijn voor de centrale D_2 activiteit van een geneesmiddel. Deze marker kon echter niet gevalideerd worden. In deze studie kon wel worden bevestigd, dat de afgifte van het hypofysehormoon prolactine onder invloed staat van D_2 -dopaminerge systemen.

In het Hoofdstuk “Conclusions and Discussion” wordt een kritische evaluatie gegeven van het gebruik van biomarkers in vroege fase geneesmiddelenonderzoek. Er wordt gesteld dat ondanks alle beperkingen het gebruik van biomarkers van groot nut kan zijn. Hiervoor is echter nodig dat bestaande biomarkers gepositioneerd worden in relatie tot de farmacologie van een geneesmiddel, tot de pathofysiologie van een ziekte waarvoor een middel geïndiceerd is en het therapeutisch effect dat verwacht wordt. Dit lijkt belangrijker dan om het aantal biomarkers te vergroten. Het ontwikkelen van challenge tests en ziektemodellen zal niet alleen onze kennis vergroten van een ziekte, maar zou tevens biomarkers voor de therapeutische effecten van een nieuw geneesmiddel kunnen opleveren.

CURRICULUM VITAE Jeroen-Paul van der Post was born in The Hague, the Netherlands on april 19, 1967. He obtained his Atheneum diploma (VWO) at the Haags Montessori Lyceum in 1985. After taking a degree in history at Leiden University, he studied medicine at Leiden University. He was conferred with an MD from Leiden University in 1996 and worked as a house officer at the cardiology department of the Leyenburg Hospital in The Hague. In 1997 he took on a position as a clinical researcher at the Centre for Human Drug Research (CHDR) in Leiden and registered as a clinical pharmacologist in 2004. During his time at the CHDR he also worked as a forensic physician for the GGZ Zuid-Holland Noord. In 2002 he began training as a psychiatrist which he completed in april, 2006. He currently holds a position as a psychiatrist at Rivierduinen, GGZ Leiden, division of Rivierduinen, organization for mental health care.

