NEUROPHARMACOLOGY OF NOVEL DOPAMINE MODULATORS

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CHAPTER 1

INTRODUCTION

The neurotransmitter dopamine is involved in various physiological central nervous system (CNS) functions as well as the pathogenesis of several neuropsychiatric disorders, including Parkinson's disease, schizophrenia, drug addiction and hyperprolactinemia. Pharmacological methods to alter dopamine neurotransmission currently have only limited efficacy in alleviating the symptoms of these disorders, but adverse side effects can be debilitating. Thus, improvement of dopaminergic pharmacotherapy remains a high priority. This thesis describes several early phase drug development trials with novel compounds that aim to improve dopaminergic pharmacotherapy by various strategies. This introductory chapter provides an overview of dopamine neurotransmission and the various disorders associated with dopaminergic systems. Also, several pharmacological strategies for improvement of dopaminergic pharmacotherapy as well as the specific aims of the following chapters of this thesis are outlined.

DOPAMINE NEUROTRANSMISSION

Dopamine is a monoamine neurotransmitter, which is produced by neurons in the midbrain and hypothalamus. The synthesis of dopamine starts with the uptake of tyrosine from blood by amino acid transporters[1]. Once inside the neuron, tyrosine is converted to L-dopa by the cytosolic enzyme tyrosine hydroxylase (TH), which is the rate-limiting enzyme in dopamine synthesis[1]. In turn, L-dopa is converted to dopamine by the cytosolic enzyme aromatic L-amino acid decarboxylase (AAAD)[1]. Subsequently, dopamine is transported from the cytoplasm into storage vesicles. Upon arrival of an action potential, the storage vesicles discharge their dopamine contents into the synaptic cleft by means of exocytosis[1]. Dopamine traverses the synaptic cleft and binds to receptors on the postsynaptic neuron. Five different types of dopamine receptors have been characterized, termed the D_1, D_2, D_3, D_4 and D_5 receptor, all of which are G protein-coupled receptors. D_1-like receptors (which include the D_1 and D_5 receptor subtypes) are stimulatory G_s-coupled receptors and D_2-like receptors (which include D_2, D_3 and D_4 receptor subtypes) are inhibitory G_i-coupled receptors[2,3]. Two major isoforms of the D_2 receptor exist due to alternative splicing, termed D_2-short (or D_{2S}) and D_2-long (or D_{2L}) receptors[2,3]. The D_{2L} receptor appears to be expressed mostly on postsynaptic membranes, while the D_{2S} receptor appears to be expressed mostly on presynaptic membranes and to be involved in autoreceptor function[2,4]. Neurotransmitter action is terminated by the reuptake of dopamine by dopamine transporters (DAT) that actively pump extracellular dopamine from the synapse back into the nerve terminal[5].

The perikarya of most dopaminergic neurons are located in the substantia nigra and ventral tegmental area of the mesencephalon and in the periventricular and arcuate nuclei of the hypothalamus. The dopaminergic neurons project to various brain structures through several anatomically organized pathways[6–9] (see Table 1 and Figure 1), including:

- The nigrostriatal pathway originates in the substantia nigra and projects to the striatum and acts to modulate the output activity of the striatum. This pathway is involved in various motor and cognitive functions[10–12].
- The mesocortical pathway originates in the ventral tegmental area and projects to the prefrontal, insular, motor and sensory cortices. This pathway is involved in encoding and use of working memory information by prefrontal cortex circuits[13,14].
- The mesolimbic pathway originates in the ventral tegmental area and projects to the limbic cortices, hippocampus, nucleus accumbens and amygdala. This pathway is involved in reward and reinforcement mechanisms and appears to encode prediction of reward and facilitate learning of reward associations[2,3,15,16].
- The tuberoinfundibular pathway originates in the periventricular and arcuate nuclei of the hypothalamus and projects to the external zone of the median eminence, and is involved in hypothalamic inhibitory control of prolactin secretion[17,18].
- Dopaminergic neurons in the area postrema and nucleus tractus solitarius are involved in the control of emesis[7,19].

Dopamine neurotransmission is under stimulatory and inhibitory control of various other neurotransmitters. Furthermore, the presence of various feedback mechanisms, modulatory influence of interneurons and interactions between neurotransmitters ultimately result in very complex neurocircuits. In general, dopamine neurotransmission is stimulated by glutamate[20] and inhibited by γ-aminobutyric acid (GABA)[21] (see Figure 2), with further modulatory roles of serotonin[22], acetylcholine[23], tachykinin neuropeptides[24], as well as several others[16,25].

DISORDERS ASSOCIATED WITH DOPAMINE NEUROTRANSMISSION

Abnormalities in dopamine neurotransmission contribute to the pathogenesis of several neuropsychiatric disorders, including Parkinson's disease, schizophrenia, drug addiction and hyperprolactinemia. Accordingly, a large number

TABLE 1 Dopamine neuron systems

Dopaminergic pathway	Site of origin	Site of termination	Functions
Nigrostriatal	Substantia nigra	Nucleus caudatus, putamen, globus pallidus	Extrapyramidal motor control
Mesocortical	Ventral tegmental area	Prefrontal, insular, motor and sensory cortices	Cognitive processes, motivation, encoding and use of working memory information
Mesolimbic	Ventral tegmental area	Limbic cortices, hippocampus, nucleus accumbens and amygdala	Reward and reinforcement mechanisms. Motivational, emotional, contextual and affective influence on behavioral processes
Tuberoinfundibular	Periventricular nucleus Arcuate nucleus	Median eminence	Inhibition of prolactin secretion
Incerto-hypothalamic	Zona incerta, posterior hypothalamus	Hypothalamus, septum	Autonomic and neuroendocrine responses
Periventricular	Periaqueductal and periventricular grey, dorsal motor nucleus of the vagus nerve, nucleus tractus solitarius	Periventricular and periaqueductal gray, tegmentum, tectum, thalamus, hypothalamus	Autonomic function. Control of emesis.
Diencephalospinal	Dorsal and posterior hypothalamus	Intermedio-lateral cell columns of spinal cord	Sensorimotor integration and nociception
Olfactory bulb	Periglomerular cells	Glomeruli (mitral cells)	Processing of odorant sensory information
Retina	Interplexiform cells	Inner and outer plexiform layers of retina	Light adaptation

FIGURE 1 Major dopaminergic pathways

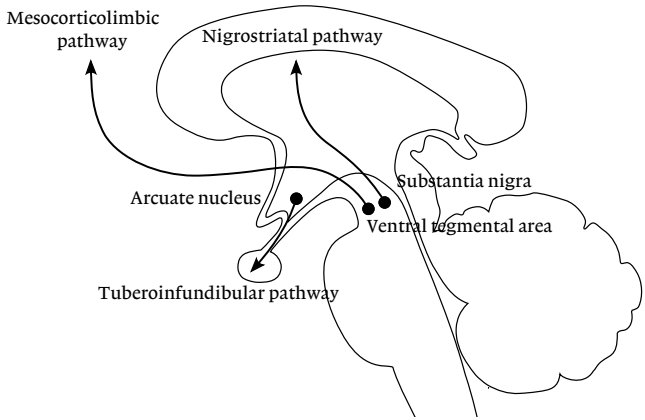
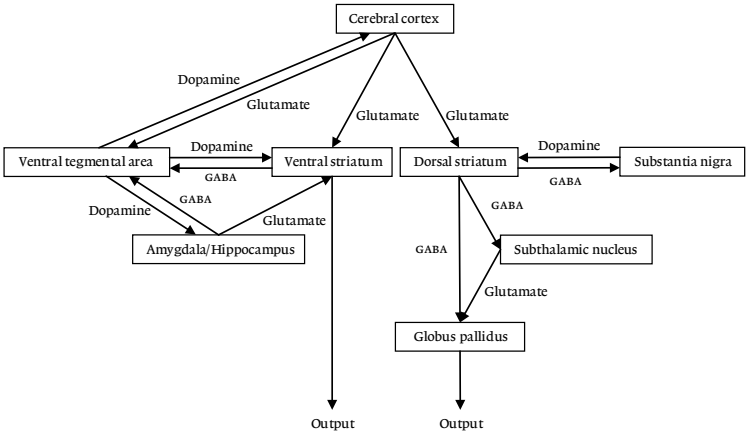


FIGURE 2 Simplified basic dopaminergic regulatory scheme of forebrain structures and basal ganglia. The ventral tegmental area projects to the prefrontal, insular, motor and sensory cortices (mesocortical pathway) and to the limbic cortices, hippocampus, amygdala and ventral striatum, including the nucleus accumbens (mesolimbic pathway). The substantia nigra projects to the dorsal striatum, including nucleus caudatus and putamen (nigrostriatal pathway). Dopamine binds to D_1-like receptors, which stimulate second messenger systems, or D_2-like receptors, which inhibit second messenger systems. Dopamine neurotransmission itself is stimulated by glutamate and inhibited by GABA, with further modulatory roles of serotonin, acetylcholine, tachykinin neuropeptides, and several others.



of well-established direct dopaminergic treatments are available, that either support the actions of dopamine (precursors and agonists) or inhibit dopaminergic activity (full or partial antagonists), depending on the disease. However, these drugs currently have only limited efficacy in alleviating the symptoms of these disorders, but adverse side effects can be debilitating. Thus, there is still a considerable need for new drugs with improved therapeutic windows or more selective modes of action.

Parkinson's disease

The pathological hallmark of Parkinson's disease is a selective loss of dopaminergic neurons from the pars compacta of the substantia nigra, which leads to symptoms such as limb resting tremor, bradykinesia, rigidity, postural instability and gait disorder[26–29]. Restoration of dopamine levels is the primary aim of pharmacotherapy. The dopamine precursor L-dopa (levodopa) is currently the most effective and preferred treatment[30]. Aromatic L-amino acid decarboxylase (AAAD) inhibitors, such as carbidopa, are usually added to inhibit peripheral metabolism of levodopa[30–32]. Also, dopamine receptor agonists such as pergolide, bromocriptine, apomorphine, pramipexole and ropirinol, are often used[30–32]. However, not all features of Parkinson's disease are adequately alleviated by levodopa or dopamine agonists and patterns of response can change over time[30]. Motor complications are frequent and disabling, and include dyskinesia and motor fluctuations (wearing off, on-off phenomena)[30,31].

Schizophrenia

The involvement of dopamine in the pathophysiology of schizophrenia was originally suggested by the observation that dopamine-enhancing drugs can have psychotogenic effects and also by the correlation between D_2 receptor blocking potency of antipsychotic drugs and their dosage for clinical antipsychotic effect[33–38]. It has been suggested that mesocortical dopaminergic projections to the prefrontal cortex might be hypoactive (resulting in hypostimulation of D_1 receptors and emergence of negative symptoms and cognitive impairments), while subcortical mesolimbic dopaminergic projections might be hyperactive (resulting in hyperstimulation of D_2 receptors and emergence of positive symptoms)[35,36,39,40]. A recent hypothesis proposes that the locus of dopamine dysregulation is primarily at the presynaptic level[41]. Pharmacological treatment options include first generation antipsychotic drugs (also known as classic or typical antipsychotics) such as chlorpromazine and haloperidol. Side effects related to dopamine blockade in the extrapyramidal system include dystonia, akathisia, bradykinesia, tremor and tardive dyskinesia[42,43]. In addition, hyperprolactinemia can result from dopamine blockade in the

tuberoinfundibular dopaminergic pathway[44,45]. Other side effects include prolonged QT interval and torsade de pointes arrhythmia, related to off-target effects on cardiac potassium channels[46], and neuroleptic malignant syndrome. Second-generation antipsychotic drugs (also known as atypical antipsychotics), such as clozapine, risperidone, quetiapine, olanzapine, ziprasidone, aripiprazole and amisulpride, generally have a lower risk of extrapyramidal symptoms and tardive dyskinesia[47–49] and may also be effective against negative symptoms, but can cause metabolic syndrome[50]. Meta-analyses and large clinical trials have demonstrated either roughly similar clinical efficacy or moderate superiority of second-generation drugs compared with first-generation antipsychotic drugs[51–58]. Clozapine has superior clinical efficacy in treatment-resistant schizophrenia[59], and carries a lower risk of extrapyramidal symptoms or hyperprolactinemia, but also has an increased incidence of agranulocytosis, requiring frequent monitoring of leukocyte counts. The exact mechanism of action of antipsychotic drugs is unknown, but all clinically effective antipsychotic drugs have been shown to attenuate dopamine D_2 receptor function[33,38,60,61].

Substance abuse and drug addiction

A large body of evidence indicates that the mesolimbic dopaminergic pathway is one of the major neuronal circuits involved in the acute rewarding effects of drugs of abuse[62–66]. Although addictive drugs interact with many different neurotransmitter systems, most addictive drugs ultimately cause an acute increase in synaptic dopamine in the nucleus accumbens and mesolimbic dopaminergic system[67–69], as demonstrated by microdialysis studies in rats[70] and positron emission tomography (PET) studies in humans[71–75]. Alcohol, nicotine, opiates, cocaine, amphetamine (and derivates such as methamphetamine, methylphenidate and MDMA, also known as ecstacy), benzodiazepines and barbiturates all increase dopamine transmission in the mesolimbic pathway by various mechanisms[76–78]. However, although addictive drugs initially lead to dopamine release in the nucleus accumbens (i.e. signaling reward), with repeated administration and as habits develop, increases of dopamine become associated with conditioned responses linked with administering the drugs, rather than the pharmacological effects of the drug per se[15,79]. In addicted subjects, the drug-induced dopamine release in the striatal regions becomes significantly reduced, while conditioned cues associated with drug use increase dopamine levels[15,79]. Despite the major role of dopamine neurotransmission in drug addiction, no dopaminergic agents have yet been demonstrated to be uniformly effective for substance abuse, which may be (at least partly) due to lack of selectivity for the mesolimbic pathway[80]. Thus, current pharmacotherapeutic strategies primarily aim to desensitize or partially antagonize the reward system by interacting

with other neurotransmitter systems. Current treatments for alcohol abuse include the opioid-antagonists naltrexone and nalmefene, the glutamate receptor modulator acamprosate, and sensitizers to the adverse effects of ethanol like disulfiram and calcium carbimide[77,80,81]. Current treatments for nicotine abuse include cholinergic drugs like the partial nicotinic acetylcholine receptor agonist varenicline and nicotine-replacement therapy, or monoaminergic therapy such as the dopamine- and norepinephrine-reuptake inhibitor bupropion, the tricyclic antidepressant nortriptyline and the α_2 adrenergic agonist clonidine[77]. Therapies for heroin addiction include the opioid agonists methadone and levo-α-acetylmethadol, the partial agonist buprenorphine combined with naloxone, and the opioid antagonist naltrexone[77,80]. No effective pharmacotherapeutic options for addiction to cocaine, cannabis, methamphetamine or other stimulants exist[77,80].

Hyperprolactinemia

Dopamine neurotransmission is also involved in the pathophysiology of hyperprolactinemia. Hyperprolactinemia can result from a large variety of disorders, including prolactin-secreting pituitary tumors (prolactinomas), lesions to the hypothalamus and pituitary gland that interfere with neuroendocrine control mechanisms, as well as diseases that can lead to decreased clearance of prolactin, such as liver cirrhosis and chronic renal failure[82,83]. Hyperprolactinemia can also be caused by use of certain drugs. The most common drugs that cause hyperprolactinemia are drugs that inhibit dopamine neurotransmission, such as dopamine D_2 receptor antagonists (e.g. antipsychotic drugs and domperidone)[44,45,84]. Pharmacological treatment of prolactinomas include dopamine receptor agonists, such as bromocriptine, cabergoline and quinagolide, whereas management of drug-induced hyperprolactinemia includes dose reduction of the causative drug or switching to a different drug with lower potential for prolactin elevation, if this can be achieved safely[83,85,86].

Emesis

Finally, dopaminergic neurons in the area postrema and nucleus tractus solitarius are involved in the control of emesis[7,19]. Neurocircuitry in the area postrema and nucleus tractus solitarius involves many neurotransmitters[87], but dopamine, serotonin and the tachykinin neuropeptide substance P are thought to play the largest roles[88]. Accordingly, several dopamine D_2 receptor antagonists (including metoclopramide, domperidone and olanzapine), serotonin 5-HT_3 receptor antagonists, as well as the tachykinin NK_1 receptor antagonist aprepitant, have demonstrated antiemetic efficacy[87,89,90].

STRATEGIES FOR IMPROVEMENT OF DOPAMINERGIC PHARMACOTHERAPY

In this thesis, several pharmacological strategies are presented that aim to improve dopaminergic pharmacotherapy by allowing more subtle manipulations of dopaminergic systems. Several early phase clinical trials are described that are part of larger drug development programs which explore various stategies, including improvement of receptor kinetics and receptor selectivity, as well as targeting dopaminergic control mechanisms as a means to indirectly modulate dopamine neurotransmission.

Improvement of receptor kinetics

It has been proposed that competitive receptor antagonists that dissociate quickly from the receptor (i.e. a fast k_{off} constant) are more accommodating to physiological fluctuations of endogenous ligand concentrations (i.e. dopamine) than drugs with a slow k_{off}[91]. Fast dissociation might therefore allow for a drug effect to occur with an appropriate functioning of physiological systems and a substantially lower risk for side effects associated with persistent strong activity at the receptor[91]. This pharmacological concept was originally proposed to explain the differential effects of typical and atypical antipsychotic drugs[91–93], but was later criticized because several exceptions were identified[94]. Although the fast dissociation hypothesis may thus not fully qualify as a general model for atypical antipsychotic drug action, it does provide a theoretical way to achieve reduction of side effects and may thus serve as a strategy for novel drug development. Using receptor dissociation rates as a means to screen novel compounds for antipsychotic drug candidates, the novel fast dissociating dopamine D_2 receptor antagonist JNJ-37822681 was developed[95]. In **CHAPTER 2**, the pharmacokinetics and central nervous system (CNS) effects of JNJ-37822681 in healthy volunteers are described. In **CHAPTER 3**, the binding characteristics of JNJ-37822681 in vivo are investigated using positron emission tomography (PET) and the radioligand [^{11}C]raclopride to evaluate if JNJ-37822681, despite its high dissociation rate, is able to achieve significant levels of dopamine D_2 receptor occupancy.

Improvement of receptor selectivity

Increased receptor selectivity may minimize side effects related to drug action at receptor sites other than the primary target. Despite the major role of dopamine neurotransmission in the acute rewarding effects of addictive drugs, no dopaminergic agents have been demonstrated to be uniformly effective for

drug addiction, which may be (at least partly) due to lack of pharmacological and functional selectivity for the mesolimbic pathway, as well as poor tolerability[80]. It has been suggested that selective dopamine D_3 receptor antagonism may be an effective strategy in pharmacotherapy of addiction[96–98]. Preclinical models have shown that selective dopamine D_3 receptor antagonists do not affect the primary reinforcing effects of drugs of abuse, but can influence the motivation to self-administer drugs under certain schedules of reinforcement[99]. In addition, selective dopamine D_3 receptor antagonists appear to disrupt the responsiveness to drug-associated stimuli that play a key role in reinstatement of drug-seeking behavior triggered either by re-exposure to the drug itself, re-exposure to environmental cues that had been previously associated with drug-taking behavior, or stress[99]. Thus, selective dopamine D_3 receptor antagonists might be efficacious in decreasing craving and preventing relapse[99,100]. GSK598809 is a novel selective dopamine D_3 receptor antagonist[101]. CHAPTER 4 describes the pharmacokinetics and central nervous system effects of GSK598809. In addition, possible interactions between GSK598809 and alcohol were evaluated. Evaluation of interactions with alcohol is important for candidate drugs for pharmacotherapy of addiction, because the target population of patients will have substance dependence as primary disorder, and are thus likely to co-administer prescription drugs together with alcohol and other drugs of abuse.

Modulation of the tachykinergic control of dopamine neurotransmission

An alternative to direct pharmacological modulation of dopamine neurotransmission is modulation of the control mechanisms of dopamine neurotransmission. Among the many control mechanisms, the peptide neuromodulators have been proposed as suitable targets for novel drug candidates, perhaps even advantageous over antagonists to classic monamine neurotransmitters[102], for several reasons. First, the effects of peptide neurotransmitters are milder than those of monoamines[102,103]. Second, much evidence indicates that neuropeptides are preferentially released after stressful and noxious stimuli, challenges or pathological conditions[102–104]. Thus, antagonists may only act on pathological systems with increased peptide release and have limited effects under normal conditions[102,103]. These characteristics together might result in clinical efficacy with less pronounced side effects[102].

Tachykinins (also known as neurokinins) are a group of related peptide neurotransmitters that includes substance P, neurokinin A and neurokinin B[24,105]. Tachykinins control and activate dopaminergic neurons in all major dopaminergic pathways, including substantia nigra, ventral tegmental area and

hypothalamus[24,106–108]. Tachykinins interact with three types of receptors, the neurokinin 1 (NK_1), neurokinin 2 (NK_2) and neurokinin 3 (NK_3) receptor, all of which are G protein-coupled receptors[24,105]. The three tachykinin receptors are recognized with moderate selectivity by the endogenous tachykinins. Substance P shows the highest affinity for the NK_1 receptor, neurokinin A exhibits the highest affinity for the NK_2 receptor and neurokinin B has the highest affinity for the NK_3 receptor[105]. Several clinical trials have evaluated tachykinin receptor antagonists for possible antipsychotic activity[109–111]. Results of an exploratory clinical trial with the NK_1 receptor antagonist aprepitant in schizophrenia patients demonstrated no significant antipsychotic efficacy[110]. In addition, despite an early positive clinical trial with osanetant[112], most clinical trials with the NK_3 receptor antagonists osanetant, talnetant and AZD2624 did not demonstrate convincing antipsychotic efficacy, although suboptimal pharmacokinetic characteristics of osanetant and talnetant may partly account for their poor efficacy[113,114].

Recently, interest in tachykinins has focused on a potential role in the treatment of drug addiction and substance abuse disorders[115,116]. Substance P may play a role in addiction-related behavior by acting directly on NK_1 receptors in brain areas associated with drug reward, such as the nucleus accumbens and ventral pallidum, and on dopaminergic neurons in the ventral tegmental area, but also by influencing other neurotransmitters such as serotonin, acetylcholine and noradrenalin[117]. Therefore, it has been suggested that NK_1 receptor antagonists may modulate stress- and reward-related processes and may contribute in altering drug reward[115]. **CHAPTER 5** describes the pharmacokinetics and central nervous system effects of the NK_1 receptor antagonist aprepitant. Also, possible interactions between aprepitant and alcohol were evaluated. To confirm and to extend the findings obtained with aprepitant, the hypothesis that tachykinin receptor antagonists may alter drug reward was investigated further. **CHAPTER 6** describes the pharmacokinetics and central nervous system effects of GSK1144814, which has antagonist action at both NK_1 and NK_3 receptors, in a subgroup of alcohol-intoxicated volunteers, to evaluate if dual tachykinin receptor antagonism can modulate the CNS effects of alcohol.

Modulation of the GABAergic control of dopamine neurotransmission

GABA is one of the major inhibitors of dopamine neurotransmission (see Figure 2). Accordingly, drugs that modulate GABA neurotransmission may indirectly influence dopamine neurotransmission. Indeed, the $GABA_B$ receptor agonist baclofen has demonstrated promising results in the treatment of

alcohol addiction, probably by inhibiting activity of the mesolimbic dopaminergic pathway through activation of $GABA_B$ receptors in the ventral tegmental area[118,119]. Modulation of the GABAergic control of dopamine neurotransmission has also been suggested to underlie the therapeutic potential of benzodiazepines in the treatment of schizophrenia[120]. Benzodiazepines were proposed to exert antipsychotic effects because of their agonist effects on GABAergic inhibition of dopamine neurotransmission[120]. However, although the sedative and anxiolytic effects of benzodiazepines can be useful in the clinical management of acutely agitated patients, no significant effects on more specific psychotic manifestations of schizophrenia have been demonstrated[121].

Benzodiazepines are nonselective allosteric modulators of α_1, α_2, α_3 and α_5 subunit-containing $GABA_A$ receptors[122]. Recently, studies in animal models have suggested that $GABA_A$ receptor subtypes are associated with different aspects of GABAergic drug action, like sedation (α_1 subtype), anxiolysis (α_2 and α_3 subtypes) and memory impairment (α_5 subtype)[122,123]. Although GABA generally causes a reduction of dopaminergic neuronal activity[21], benzodiazepines have been shown to increase dopamine levels in the mesolimbic pathway[76]. This increase probably results from serial inhibition of coupled GABAergic interneurons in the mesolimbic pathway, which leads to disinhibition of dopaminergic neurons, which outweighs the direct inhibitory influence of benzodiazepines on those dopaminergic neurons[123–125]. The disinhibition of dopaminergic neurons and the resulting increase in dopamine levels appears to be mediated by the α_1 receptor subtype[124,125]. It has been suggested that selective agonists at α_3 receptor subtypes without efficacy at α_1 receptor subtypes, may attenuate dopamine neurotransmission in the mesolimbic pathway, without counteractive disinhibition from GABAergic interneurons[123]. Accordingly, $GABA_A$ receptor subunit-selective agonists may differ significantly from nonselective benzodiazepines in their effects on dopaminergic pathways, and may have therapeutic potential for (some aspects of) schizophrenia[123].

A targeted selection of the proper subtype-selective $GABA_A$ agonist would require a good understanding of the exact role of the various receptor subtypes in the regulation of different dopaminergic activities. To explore the exact role of the various receptor subtypes in the regulation of dopamine neurotransmission, CHAPTER 7 describes the effects of two novel positive modulators of α_2 and α_3 subunit-containing $GABA_A$ receptors, AZD7325 and AZD6280[126], and the nonselective benzodiazepine lorazepam on circulating prolactin levels as a marker for activity of the tuberoinfundibular pathway, which is the most readily accessible dopaminergic pathway for evaluation in vivo.

CONCLUSION AND AIM OF THESIS

The neurotransmitter dopamine is involved in the pathogenesis of several neuropsychiatric disorders. Despite the large number of registered dopamine receptor agonists and antagonists, pharmacological methods to alter dopamine neurotransmission currently have only limited efficacy in alleviating the symptoms of these disorders, while adverse side effects can be debilitating. This thesis describes early phase drug development studies that are part of larger drug development programs which explore various stategies for improvement of dopaminergic pharmacotherapy. These strategies include improvement of receptor kinetics (CHAPTER 2 AND 3) and receptor selectivity (CHAPTER 4), as well as modulation of dopamine control mechanisms (CHAPTER 5, 6 AND 7). This thesis aims to evaluate pharmacokinetics and dose-effect relationships of several drug candidates on various neurophysiological parameters in healthy volunteers in order to show penetration through the blood-brain barrier, target engagement in vivo and differentiation of pharmacodynamic effects on several functional CNS domains.

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CHAPTER 2

PHARMACOKINETICS AND CENTRAL NERVOUS SYSTEM EFFECTS OF THE NOVEL DOPAMINE D_2 RECEPTOR ANTAGONIST JNJ-37822681

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ABSTRACT

Using the rate of dissociation from the D_2 receptor as a means to screen novel compounds for antipsychotic drug candidates, the centrally acting and fast-dissociating selective dopamine D_2 receptor antagonist JNJ-37822681 was developed. In a blinded, placebo-controlled, randomized first-in-human study, JNJ-37822681 was administered orally to 27 healthy male volunteers at doses of 0.5, 2, 5, 10, 15 and 20 mg. Safety, pharmacokinetics and central nervous system effects were evaluated by measuring prolactin levels, eye movements, adaptive tracking, visual analogue scales, body sway, finger tapping and electroencephalography. JNJ-37822681 was well tolerated and somnolence was the most frequently reported adverse effect. Peak plasma concentrations increased more than proportional to dose, but increases in AUC were dose-proportional. Prolactin elevations started at doses of 5 mg, while small decreases in adaptive tracking were demonstrated at 10 mg doses. At higher doses, JNJ-37822681 caused a small decrease in saccadic peak velocity, smooth pursuit, alertness, finger tapping and EEG activity, and an increase in body sway. This effect profile is likely the result of the selectivity of JNJ-37822681 for the D_2 receptor, leading to strong D_2 receptor-mediated elevations in serum prolactin, but fewer effects on more complex CNS functions, which are likely to involve multiple neurotransmitters.

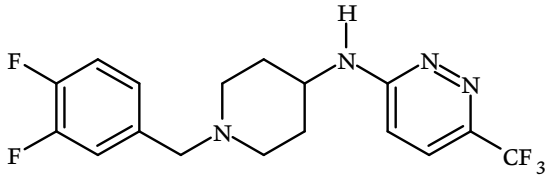
INTRODUCTION

Current treatment options for schizophrenia and psychosis include a variety of drugs, all of which act by attenuating dopamine D_2 receptor function[1–3]. However, these drugs vary considerably in clinical effect and side effect profile. Typical antipsychotic drugs are effective against positive symptoms but also have a high incidence of extrapyramidal side effects, whereas atypical antipsychotic drugs have far fewer extrapyramidal side effects and may also be effective against negative signs[4]. Several different pharmacological concepts have been proposed to account for the distinct effect profile of atypical antipsychotic drugs compared with typical antipsychotic drugs. These include, among others, a high ratio of serotonin 5-HT_{2A} receptor to dopamine D_2 receptor antagonism[5], preferential mesolimbic dopamine D_2/D_3 receptor binding[6] and rapid dissociation from the dopamine D_2 receptor[7–9]. The latter hypothesis proposes that fast dissociation rates of an antipsychotic drug from the dopamine D_2 receptor may provide sufficient receptor occupancy for an antipsychotic effect to occur, yet allow accommodation to physiological dopamine release with a substantially lower risk for extrapyramidal symptoms, hyperprolactinaemia or secondary negative signs[8–12]. However, this hypothesis has been criticized on several grounds. It has been argued that this hypothesis can only apply to drugs such as clozapine and quetiapine, which have low affinity for the dopamine D_2 receptor, and may not be generalized as a model for atypical antipsychotic drug action[13]. Also, this hypothesis may not fully explain the improved neurocognitive effects of atypical drugs, nor why clozapine is more effective in treatment-resistant schizophrenia[4,13–16]. However, these limitations do not necessarily preclude usefulness of this hypothesis as the basis for a drug development strategy by selecting novel candidate drugs on their respective dissociation rates. Moreover, development of a novel compound with selectivity for the dopamine D_2 receptor and a fast rate of dissociation can provide an opportunity to study this hypothesis in a prospective clinical setting.

Using receptor dissociation rates as a means to screen novel compounds for antipsychotic drug candidates, the centrally acting dopamine D_2 receptor antagonist JNJ-37822681 (see Figure 1) was developed[17]. JNJ-37822681 is a novel chemical entity that combines specificity for the dopamine D_2 receptor and a fast rate of dissociation. In vitro receptor binding studies demonstrated that JNJ-37822681 displays moderate affinity for the dopamine D_{2L} receptor (K_i = 158 nM) and low affinity for dopamine D_1 (K_i > 3000 nM) and D_3 receptors (K_i = 1159 nM), serotonin 5-HT_{2A} (K_i = 2896 nM) and 5-HT_{2C} (K_i >4000 nM) receptors, histamine H_1 receptors (K_i = 4931 nM) and adrenergic α_{1A} receptors (K_i >5000

nM). JNJ-37822681 also binds to the σ_1 receptor (K_i = 8.9 nM)[17]. When tested in parallel, the time for 50% dissociation of [^{3}H]JNJ-37822681 from the dopamine D_{2L} receptor was 6.5 seconds at room temperature and 3.7 seconds at 37°C (k_{off} = 0.108 and 0.191 s^{-1}, respectively), which was similar to the dissociation rate of [^{3}H]clozapine (14.5 seconds at room temperature and 5.8 seconds at 37°C) and significantly faster than those of [^{3}H]haloperidol, [^{3}H]risperidone and [^{3}H] paliperidone[17]. In animal models, JNJ-37822681 antagonizes the behavior induced by various stimulants in the following potency order: apomorphine-induced stereotypy (ED_{50} = 0.194 mg/kg), *d*-amphetamine-induced stereotypy (ED_{50} = 0.294 mg/kg), *d*-amphetamine hyperlocomotion (ED_{50} = 1.02 mg/kg), β-phenylethylamine-induced stereotypy (ED_{50} = 1.17 mg/kg) and phencyclidine-induced hyperlocomotion (ED_{50} = 4.7 mg/kg)[17]. Using the crossed-leg position test in rats, the ED_{50} for inducing catalepsy was higher, at 8.7 mg/kg. These data suggest that JNJ-37822681 is a potent, specific and fast dissociating dopamine D_2 antagonist with a relatively low potential for extrapyramidal effects within the projected therapeutic dose range. Furthermore, specificity for the dopamine D_2 receptor by JNJ-37822681 may also minimize potential for side effects related to interactions with other receptors, such histamine H_1, adrenergic α_1 and serotonin 5-HT_{2C}, which are thought to contribute to major side effects associated with second generation antipsychotics[18].

FIGURE 1 Chemical structure of JNJ-37822681



The present study was performed to obtain a pharmacokinetic profile of single oral doses of JNJ-37822681 in healthy volunteers, using a blinded, randomized, placebo-controlled study design. In addition, central nervous system (CNS) effects were evaluated using a battery of quantitative tests, sensitive to the central effects of various drugs, including antipsychotic drugs (dopamine D_2 receptor antagonists)[19], such as haloperidol[20,21] and risperidone[22].

METHODS

Study design

Twenty-four healthy male volunteers, between 18 and 55 years of age and with a body mass index between 18 and 30 kg/m^2, were planned to participate in an alternating panel, blinded, placebo-controlled, randomized, variable dose study of six volunteers per dosing group. The study was approved by the medical ethics review board of the Leiden University Medical Center. Prior to medical screening, all volunteers gave written informed consent. All volunteers underwent training sessions for the pharmacodynamic tests in order to make them acquainted with the procedures and to reduce possible learning effects.

Treatments

Volunteers were randomly assigned to a treatment sequence consisting of three study periods with administration of single ascending oral doses of JNJ-37822681 and one interspersed study period with administration of placebo. Volunteers were randomly divided over two alternating panels. Twelve volunteers were assigned to the first panel and received 0.5 mg, 5 mg, 15 and 20 mg of JNJ-37822681 or matching placebos. Twelve other volunteers were assigned to the second panel and received 2 mg, 10 mg, 15 mg and 20 mg of JNJ-37822681 or matching placebos. All periods were separated by a washout time of two weeks. Dose escalation steps were guided by the observed safety, tolerability and the pharmacokinetic and pharmacodynamic data of the previous dose levels. JNJ-37822681 was administered in a liquid (1 mg/mL; only for the 0.5 mg dose) or solid (capsules of 1 and 10 mg) dosage formulation after a 10 hour fasting period.

Safety monitoring

Evaluation of adverse events, 12-lead electrocardiograms (ECG), blood pressure, heart rate, body temperature measurements, urinalysis and blood sampling for haematology and chemistry was performed at regular time points after each dose administration. Blood pressure was measured using automated oscillometric monitors. ECGs were obtained with Cardiofax V equipped with ECAPS12 analysis program (Nihon-Kohden, Tokyo, Japan). In addition, continuous ECG monitoring was performed from 30 minutes prior to dosing until 12 hours after dose administration using one-lead telemetric ECG. Volunteers were evaluated for akathisia and extrapyramidal symptoms, using the Barnes akathisia rating scale[23] and Simpson-Angus scale[24].

Pharmacokinetics

Venous blood samples for JNJ-37822681 plasma concentration analysis were collected prior to dose administration and at 15 and 30 minutes and 1, 1½, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48 and 72 hours after dose administration. The concentration of JNJ-37822681 in plasma samples was determined using liquid chromatography-mass spectrometry with a lower limit of quantification of 0.2 ng/mL.

Pharmacodynamic testing

Pharmacodynamic measurements were performed as described previously[20,25], prior to dose administration and at fixed time intervals around 1, 1½, 2, 3, 4, 6, 8 and 12 hours after dose administration. Volunteers were tested individually in a quiet room with ambient illumination. Quantitative tests, sensitive to single oral doses of antipsychotic drugs[19] such as haloperidol[20,21] and risperidone[22] in healthy volunteers, included measurements of smooth pursuit and saccadic eye movements, adaptive tracking, body sway, finger tapping, visual analogue scales, electroencephalography and serum prolactin levels.

Analysis of eye movements

To evaluate oculomotor performance and sedation, smooth pursuit and saccadic eye movements were recorded as described previously[26–29], using a microcomputer-based system for data recording and analysis (Cambridge Electronic Design Ltd., Cambridge, UK), Nihon-Kohden equipment for stimulus display, signal collection and amplification (Nihon-Kohden, Tokyo, Japan), and disposable surface electrodes (Medicotest N-00-S, Olstykke, Denmark). For smooth pursuit eye movements, a target light source moves sinusoidally over 20° eyeball rotation at frequencies ranging from 0.3 to 1.1 Hz. The time in which the eyes were in smooth pursuit was calculated for each frequency and expressed as the percentage of stimulus duration. The average percentage of smooth pursuit for all frequencies was used as parameter. For saccadic eye movements, the target light source jumps from side to side. Peak velocity (degrees per second), reaction time and inaccuracy (%) was calculated of all artifact-free saccades.

Adaptive tracking

To evaluate visuo-motor coordination, the adaptive tracking task was performed as described previously[28–32], using customized equipment and software developed by K.W. Hobbs (Hertfordshire, UK). Adaptive tracking is a pursuit tracking task in which a circle moves randomly over a computer screen and the

volunteer must try to keep a dot inside the moving circle using a joystick. If this effort is successful, the speed of the moving circle is increased and if the effort is unsuccessful, the speed is reduced. Performance was scored over a fixed period of three minutes.

Body sway

Postural stability in the sagittal plane was measured with an apparatus similar to the Wright ataxiameter[33], using a string attached to the waist of the subject. Movements over a period of two minutes, while standing still with eyes closed, were integrated and expressed as mm sway.

Finger tapping

The finger tapping test was adapted from the Halstead-Reitan test battery[34] to evaluate motor activation and fluency. The volunteer is instructed to rest the wrist of the dominant hand on a table and to tap as quickly as possible with the index finger onto the space bar of a key board. The mean tapping rate is used for statistical analysis.

Visual analogue scales

Subjective effects were quantified using a Dutch translation of the visual analogue scales (VAS), originally described by Norris[35], to derive three composite factors corresponding to alertness, mood (contentedness) and calmness, as described by Bond & Lader[36]. Additional psychedelic effects were monitored using an adapted version of the visual analogue scales as described by Bowdle[37].

Electroencephalography

Electroencephalography (EEG) recordings were obtained at leads Fz-Cz and Pz-Oz, according to the international 10/20 system, using disposable silver-silver chloride electrodes (Medicotest N-00-S, Olstykke, Denmark). Signals were amplified by use of a Grass 15LT series amplifier systems with a time constant of 0.3 seconds and a low pass filter at 100 Hz. Data collection and analyses were performed using customized CED and Spike2 for Windows software (Cambridge Electronics Design, Cambridge, UK). For each lead, fast Fourier transform analysis was performed to obtain the sum of amplitudes in the δ (0.5-3.5 Hz), θ (3.5-7.5 Hz), α (7.5-11.5 Hz) and β (11.5-30 Hz) frequency ranges. Measurements were performed with eyes closed in sessions of 1 minute, consisting of 8 consecutive blocks of 8 seconds. Data blocks containing artifacts were identified by visual inspection and excluded from analysis.

Serum prolactin levels

Blood samples for measurement of prolactin levels were collected in plain tubes and serum was separated by centrifugation (2000 g at 4°C for 10 minutes). Prolactin levels were determined using an electrochemiluminescence immunoassay (ECLIA) on a Modular Analytics E170 (Elecsys module) immunoassay analyzer.

Statistical analysis

Pharmacokinetic parameters of JNJ-37822681 were determined based on the individual plasma concentration-time data, using WinNonlin software version 5.0 (Pharsight, Mountain View, California, USA), and included peak plasma concentration (C_{max}), time to reach peak plasma concentration (t_{max}), area under the plasma concentration-time curve extrapolated to infinity (AUC_{∞}) and terminal half-life ($t_{½}$).

Pharmacodynamic parameters were compared using a mixed model analysis of variance (using SAS PROC MIXED) with treatment, period, time and treatment by time as fixed factors, and with subject, subject by treatment and subject by time as random factors, and with the average pre-value (average over all measurements prior to dosing) as covariate. Body sway, PEEG and prolactin data were log-transformed prior to analysis to correct for the expected log-normal distribution of the data. Contrasts relative to placebo treatment were calculated in original measurement unit with 95% confidence intervals and the associated *p*-value, except for the log-transformed parameters, which were calculated as a percentage relative to placebo. Following the observation that pharmacodynamic effects were maximal between 1 and 3 hours after dosing, data analysis was repeated for all measurements up to 5 hours. Using a restricted time range for analysis has the benefit that medication-related effects are detected more easily. All calculations were performed using SAS for Windows version 9.1.2 (SAS Institute Inc., Cary, North Carolina, USA).

RESULTS

Subjects

Participants had a mean age of 26.6 years (range 18-53), weight of 74.6 kg (range 57-93) and body mass index of 22.9 kg/m² (range 20-30). After inclusion, three volunteers were discontinued later (one volunteer because of acute appendicitis and two volunteers for personal reasons) and were replaced. Therefore, data was obtained from 27 volunteers in total. However, pharmacodynamic data from one volunteer was excluded from analysis, prior to data review, after additional

information about an undeclared psychiatric history had surfaced after study completion, which violated the in- and exclusion criteria of the study. In total, 14 volunteers were able to complete all four study periods. The remaining 13 volunteers missed one or more study periods for reasons unrelated to treatment with JNJ-37822681. A total of 18 volunteers received placebo, 9 volunteers received 0.5 mg, 7 volunteers received 2 mg, 9 volunteers received 5 mg, 8 volunteers received 10 mg, 16 volunteers received 15 mg and 16 volunteers received 20 mg of JNJ-37822681.

Clinical observations

Twenty-four volunteers (89%) reported at least one adverse event (see Table 1). One volunteer was hospitalized for acute appendicitis three days after having received 5 mg of JNJ-37822681. The most frequently reported adverse effect was somnolence (21 volunteers, 78%). Other common events were headache (9 volunteers, 33%), dizziness (4 volunteers, 15%) and fatigue (4 volunteers, 15%). Adverse effects were mild and occasionally moderate and all disappeared spontaneously within a few hours. Transient mild restlessness (akathisia) was reported by one volunteer after 20 mg and by another volunteer after 15 and 20 mg of JNJ-37822681. The volunteer with an undeclared psychiatric history reported mild restlessness after administration of placebo. All events, except the event following placebo administration, were associated with very small increases on the Barnes akathisia rating scale, started within one hour after dosing and resolved within 3 hours after dosing. Mild musculoskeletal stiffness was reported by one volunteer after 10 mg and by another volunteer after 15 mg, without concomitant changes on the Simpson-Angus scale. There were no clinically relevant changes in heart rate, blood pressure, body temperature, haematology, biochemistry, urinalysis or any of the ECG intervals.

Pharmacokinetic and pharmacodynamic parameters

Pharmacokinetic parameters are summarized in Table 2. At the lowest dose of 0.5 mg, derived pharmacokinetic parameters could not be estimated reliably, because most values were below the lower limit of quantification. JNJ-37822681 was rapidly absorbed followed by a mean terminal half-life of approximately 38 hours after 2 mg and 24 to 27 hours after doses between 5 and 20 mg (see Figure 2). With increasing doses, absorption gradually increased, with t_{max} changing from 3.47 hours after 0.5 mg to 1.35 hours after 20 mg. Peak plasma concentration (C_{max}) increased more than dose-proportional within the dose range of 0.5 to 20 mg, whereas increases in the extent of exposure (AUC_{∞}) were dose-proportional.

Pharmacodynamic parameters are summarized in Table 3. In line with the pharmacokinetic profile of JNJ-37822681, pharmacodynamic effects were maximal between 1 and 3 hours after dosing and disappeared within 8 hours. Statistically significant elevations of serum prolactin levels were found at doses of 5 mg and higher (see Figure 3), increasing up to 758% in estimated mean at 20 mg over the first five hours. At doses of 10 mg, JNJ-37822681 significantly decreased adaptive tracking. At doses of 15 and 20 mg, JNJ-37822681 significantly decreased saccadic peak velocity, smooth pursuit eye movements, finger tapping rate, EEG α and β activity, and increased body sway. No consistent and statistically significant changes were found on any of the visual analogue scales, except a small decrease of VAS alertness following 20 mg (difference -2.7 mm; 95% confidence interval -4.4/-1.0; $p = 0.0034$).

TABLE 1 Summary of common adverse events, with an incidence higher than 5%. Incidence is based on the number of subjects (with percentages between brackets), not the number of events.

Adverse event	Placebo	0.5 mg	2 mg	5 mg	10 mg	15 mg	20 mg
Somnolence	1 (6)	1 (11)	1 (14)	5 (56)	2 (25)	11 (69)	14 (88)
Headache	1 (6)	1 (11)	1 (14)	2 (22)	0	4 (25)	2 (13)
Dizziness	0	0	0	0	0	1 (6)	3 (19)
Akathisia	1 (6)	0	0	0	0	1 (6)	2 (13)
Paraesthesia	0	0	1 (14)	1 (11)	0	1 (6)	0
(Vasovagal) syncope	1 (6)	0	0	0	1 (13)	0	1 (6)
Fatigue	0	0	1 (14)	3 (33)	0	0	0
Nausea	1 (6)	0	0	0	1 (13)	1 (6)	1 (6)
Nasopharyngitis	0	0	0	2 (22)	1 (13)	0	0
Musculoskeletal stiffness	0	0	0	0	1 (13)	1 (6)	0

TABLE 2 Pharmacokinetic parameters of JNJ-37822681. All data are presented as means (with standard deviation). Pharmacokinetic parameters of the 0.5 mg dose could not be estimated reliably.

Parameter	2 mg	5 mg	10 mg	15 mg	20 mg
C_{max} (ng/mL)	2.84 (0.91)	10.5 (5.51)	31.5 (5.14)	47.6 (13.9)	73.3 (26.4)
t_{max} (h)	2.76 (1.11)	2.46 (0.99)	1.44 (0.18)	1.47 (0.53)	1.35 (0.6)
AUC_{∞} (ng.h/mL)	57.5 (15.9)	123 (40)	303 (32.7)	432 (81.4)	598 (141)
Terminal half life (h)	37.9 (14.4)	26.7 (4.8)	27.3 (5.7)	24.4 (3.7)	26.3 (5.9)

FIGURE 2 Time course of plasma concentration of JNJ-37822681 following administration of 2 mg (closed triangles), 5 mg (open triangles), 10 mg (closed circles), 15 mg (open circles) and 20 mg (closed diamonds). Means are presented with standard deviations as error bars.

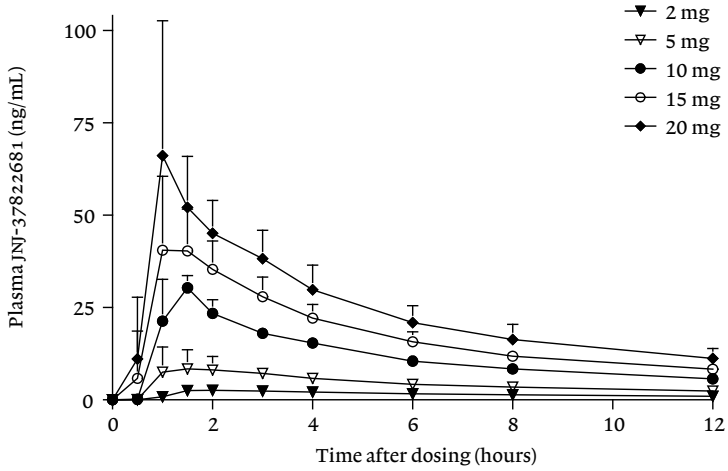


FIGURE 3 Serum prolactin levels following administration of placebo (closed squares), 0.5 mg (open squares), 2 mg (closed triangles), 5 mg (open triangles), 10 mg (closed circles), 15 mg (open circles) and 20 mg (closed diamonds) of JNJ-37822681. Means are presented with standard deviations as error bars.

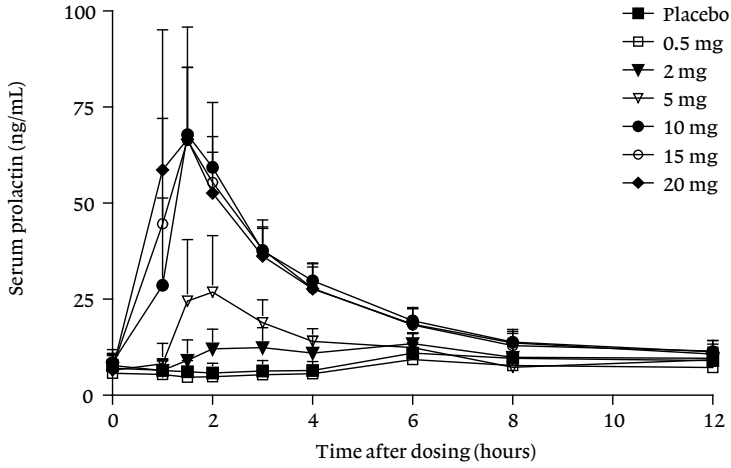


TABLE 3 Pharmacodynamic parameters of JNJ-37822681. Treatment differences in least square means are shown from 0 to 5 hours relative to baseline. Statistically significant results are indicated in bold. Results of the 0.5 mg and 2 mg doses are not shown because these doses did not show any statistically significant difference compared with placebo on the pharmacodynamic parameters.

Parameter		5 mg	10 mg	15 mg	20 mg
Prolactin (ng/mL)	Contrast	**220.5%**	**478.3%**	**710.8%**	**757.8%**
	95% CI	**153.4/305.3%**	**351.3/641.0%**	**576.2/872.1%**	**621.2/920.3%**
	p-value	**≤0.0001**	**≤0.0001**	**≤0.0001**	**≤0.0001**
Saccadic peak velocity (deg/sec)	Contrast	-15.1	-12.7	**-20.9**	**-24.7**
	95% CI	-36.4/6.1	-35.0/9.6	**-37.7/-4.1**	**-40.6/-8.9**
	p-value	0.1583	0.2598	**0.0157**	**0.0029**
Smooth pursuit (%)	Contrast	-1.9	-1.5	**-4.1**	**-5.2**
	95% CI	-7.0/3.2	-6.8/3.9	**-8.1/-0.2**	**-9.0/-1.4**
	p-value	0.4605	0.5841	**0.0413**	**0.0080**
Adaptive tracking (%)	Contrast	-2.46	**-3.66**	**-2.32**	**-3.59**
	95% CI	-5.24/0.32	**-6.57/-0.75**	**-4.48/-0.16**	**-5.67/-1.50**
	p-value	0.0821	**0.0146**	**0.0355**	**0.0011**
Body sway (mm)	Contrast	9.05%	-3.01%	15.08%	**21.17%**
	95% CI	-11.2/33.9%	-21.2/19.3%	-1.2/34.1%	**4.7/40.3%**
	p-value	0.4010	0.7688	0.0705	**0.0114**
Finger tapping (taps/10 sec)	Contrast	-1.5	-0.2	**-2.8**	**-2.9**
	95% CI	-4.9/1.9	-3.8/3.4	**-5.5/-0.1**	**-5.5/-0.2**
	p-value	0.3887	0.9113	**0.0393**	**0.0344**
EEG α Fz-Cz (mV)	Contrast	-4.09%	3.13%	**-19.9%**	**-22.9%**
	95% CI	-22.2/18.2%	-17.1/28.3%	**-32.3/-5.1%**	**-34.7/-9.0%**
	p-value	0.6915	0.7793	**0.0108**	**0.0026**
EEG α Pz-Oz (mV)	Contrast	-12.4%	-5.46%	**-18.2%**	**-24.9%**
	95% CI	-30.4/10.4%	-25.7/20.4%	**-32.0/-1.6%**	**-37.2/-10.2%**
	p-value	0.2576	0.6444	**0.0333**	**0.0022**
EEG β Fz-Cz (mV)	Contrast	6.28%	3.79%	-4.61%	-1.86%
	95% CI	-9.1/24.2%	-11.9/22.3%	-16.2/8.5%	-13.4/11.2%
	p-value	0.4389	0.6525	0.4675	0.7647
EEG β Pz-Oz (mV)	Contrast	-11.7%	-5.32%	**-17.4%**	**-20.4%**
	95% CI	-24.8/3.7%	-19.9/12.0%	**-27.3/-6.0%**	**-29.8/-9.7%**
	p-value	0.1266	0.5174	**0.0043**	**0.0006**

DISCUSSION

The present study evaluated pharmacokinetics and central nervous system (CNS) effects of single oral doses of JNJ-37822681 in healthy volunteers. The main effect of JNJ-37822681 was dose-related elevation of serum prolactin, which normalized within 8 hours, while other subjective, neuropsychological and neurophysiological effects were generally small. The time course of CNS effects corresponded well with the pharmacokinetics of JNJ-37822681 with a t_{max} of approximately 1-3 hours.

The elevation of serum prolactin levels is most likely the result of blockade of dopamine D_2 receptors in the pituitary gland by JNJ-37822681. Prolactin release by the lactotroph cells in the anterior pituitary gland is under inhibitory control by dopamine, released from hypothalamic tracts, which binds to the lactotrophic dopamine D_2 receptors[38,39]. Pharmacologic blockade of the D_2 receptors on the lactotrophs removes the inhibitory control of dopamine, resulting in hyperprolactinaemia[40]. All antipsychotics (D_2 antagonists) cause increases in plasma prolactin, although the extent and duration can vary[41–43]. As a result, only some agents cause clinically relevant hyperprolactinaemia (prolactin-elevating agents), whereas others do not (prolactin-'sparing' agents)[44]. The highest rates of hyperprolactinaemia are observed with the atypical antipsychotic drugs risperidone and amisulpride[45]. It has been suggested that prolactin-sparing antipsychotics lack clinically relevant hyperprolactinaemia, because they are associated with a short-lasting transient prolactin increase immediately after dosing, without accumulation over time, as the prolactin level has returned to baseline prior to the next dose[42,43]. Also, it has been proposed that fast dissociation may explain this effect, because this may lead to rapidly declining rates of pituitary D_2 receptor occupancy over time[42]. Indeed, the fast dissociating antipsychotic drugs clozapine and quetiapine are not associated with clinically relevant hyperprolactinaemia[45–48]. The currently measured prolactin levels after administration of JNJ-37822681 are higher than those after single oral doses of 2 mg of risperidone[22], 3 mg of haloperidol[20], 50 mg of clozapine[49] or 150 mg of quetiapine[50] in healthy volunteers. However, as demonstrated in Figure 3, serum prolactin has largely returned to baseline levels within 8 hours after administration of JNJ-37822681, which may prevent accumulation during chronic treatment. Future clinical trials with repeated dosing regimes are needed to determine whether JNJ-37822681 produces sustained and clinically relevant hyperprolactinaemia with therapeutic use.

The decreases in smooth pursuit performance and saccadic peak velocity and the results of body sway after administration of JNJ-37822681 are indicative of decreased attention and slight sedation[19,28,29]. Similar effects have been demonstrated following single dose administration of 2 mg of risperidone[22] and 3 mg of haloperidol in healthy volunteers[20]. In fact, sedation is a common effect of most registered antipsychotic drugs and occurs particularly with drugs that show significant histamine H_1 receptor antagonism[51]. JNJ-37822681 exhibits low affinity in vitro for this receptor (K_i = 4931 nM). Reduced attention after administration of JNJ-37822681 therefore seems to be related to dopamine D_2 receptor antagonism per se. However, the somnolence reported by the volunteers was generally very mild, without subjective distress, and disappeared sponta-

neously within a few hours. The results of adaptive tracking and finger tapping are indicative of slightly impaired (visuo-)motor performance[19]. These effects have also been previously demonstrated after single oral doses of risperidone[22] and haloperidol in healthy volunteers[20]. At the highest doses, a few volunteers reported transient mild restlessness (akathisia) with an accompanying small increase in scores on the Barnes akathisia rating scale. However, these effects were very mild and resolved spontaneously and rapidly, without need for anticholinergic (or other) treatment. No other motor symptoms were observed.

The observed psychomotor effects of JNJ-37822681 are relatively small in comparison with the elevations in serum prolactin. These findings could theoretically be related to insufficient penetration through the blood-brain barrier. If a compound does not easily cross the blood-brain barrier, the concentration in the pituitary gland (which is situated outside the blood-brain barrier) may rise much higher than the concentration within the brain[52,53]. However, preclinical studies in rodents have demonstrated much higher levels in brain tissue compared to plasma, resulting in brain-to-plasma ratios of 10 to 16, indicating good blood-brain barrier penetration (unpublished results). Also, in a separate positron emission tomography (PET) study using [^{11}C]raclopride in healthy volunteers, it was demonstrated that JNJ-37822681 produces striatal dopamine D_2 receptor occupancy up to 60-74% after oral doses of 20 mg[54]. Therefore, the small central effects of JNJ-37822681 seem unlikely to result from lack of penetration through the blood-brain barrier. A more likely explanation of these findings may be the selectivity of JNJ-37822681 for the dopamine D_2 receptor. Prolactin release is controlled primarily by dopamine acting on dopamine D_2 receptors, whereas the other pharmacodynamic tests measure more complex CNS functions, which are likely to involve multiple neurotransmitter receptor systems. Accordingly, specific dopamine D_2 antagonism by JNJ-37822681 significantly affects prolactin release, while other pharmacodynamic tests show only small effects.

Hyperprolactinaemia-inducing dose equivalencies of a wide range of registered antipsychotic drugs are correlated with affinity for D_2 receptors, and also with the lowest therapeutic maintenance dose, indicating a close D_2 mediated relationship between prolactin response and therapeutic efficacy[19]. Therefore, it has been suggested that the effects of novel antipsychotic compounds may be compared with established antipsychotic agents by using the prolactin response to estimate dose equivalencies and to estimate a likely therapeutic starting dose, provided that penetration through the blood-brain barrier is sufficient[19]. By plotting the dose of haloperidol that produces a prolactin reponse equivalent to 5 mg of JNJ-37822681 onto the curve describing the relationship between

the dose equivalency and the lowest therapeutic maintenance dose (Figure 2 in De Visser et al[19]), an estimated dose of 5-10 mg of JNJ-37822681 is predicted as the lowest therapeutic maintenance dose (ignoring any possible accumulation after multiple dosing). Preliminary results of a recently completed multicenter, double blind, placebo-controlled trial with JNJ-37822681 (10 mg, 20 mg or 30 mg BID) in patients with schizophrenia indicate antipsychotic efficacy of all doses superior to placebo with low frequency of extrapyramidal symptoms[55]. Confirmation of these findings may support the 'fast dissociation' hypothesis as a means to achieve an atypical antipsychotic effect profile. Despite the shortcomings of the 'fast dissociation' hypothesis as a general model for atypical drug action, screening of novel candidate drugs on their respective dopamine D_2 receptor dissociation rates may be a useful strategy for novel antipsychotic drug development.

In conclusion, the present study demonstrates the prolactin response and small CNS effects of single oral doses of JNJ-37822681, attributable to its selective dopamine D_2 receptor antagonism. During the further process of development of JNJ-37822681, studies using multiple dosing regimes in patients with schizophrenia or psychosis are needed to evaluate antipsychotic efficacy, but also to evaluate specifically the potential for inducing akathisia, sedation or sustained hyperprolactinaemia.

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CHAPTER 3

IN VIVO QUANTIFICATION OF STRIATAL DOPAMINE D_2 RECEPTOR OCCUPANCY BY JNJ-37822681 USING [^{11}C]RACLOPRIDE AND POSITRON EMISSION TOMOGRAPHY

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ABSTRACT

JNJ-37822681 is a novel, fast-dissociating dopamine D_2 receptor antagonist, currently in development as an antipsychotic drug candidate. A previous first-in-human study demonstrated mild central nervous system effects of JNJ-37822681 in healthy male volunteers. Significant but transient serum prolactin elevations were demonstrated, whereas other neurophysiological effects were relatively small. To investigate striatal dopamine D_2 receptor occupancy by variable single doses of JNJ-37822681, an open-label [^{11}C]raclopride positron emission tomography study was performed in twelve healthy male volunteers, using the simplified reference tissue model with cerebellum as reference tissue. Oral administration of JNJ-37822681 resulted in dose-dependent dopamine D_2 receptor occupancy. Receptor occupancy increased from 9-19% at 2 mg doses to 60-74% at 20 mg doses of JNJ-37822681. Therefore, single oral doses of JNJ-37822681 can produce occupancy levels that are generally associated with clinical efficacy for registered antipsychotic drugs.

INTRODUCTION

The theory that rapid dissociation rates of an antipsychotic drug from the dopamine D_2 receptor is a principal pharmacological characteristic that determines the distinct effect profile of atypical antipsychotic drugs, has been proposed specifically to explain the lower incidence of extrapyramidal side effects, hyperprolactinaemia or secondary negative signs compared with typical antipsychotic drugs[1–6]. However, this theory may not fully explain the efficacy of clozapine in treatment-resistant schizophrenia[7,8]. In addition, it has been argued that this hypothesis does not apply to all atypical antipsychotic drugs but only to drugs such as clozapine and quetiapine, which have low affinity for the dopamine D_2 receptor[7,9–11]. Nevertheless, despite the limitations of the 'fast dissociation hypothesis' as a general model for atypical drug action, screening of novel compounds by their respective dissociation rates may be a useful means to select novel antipsychotic drug candidates with an improved side effect profile. Moreover, development of a novel compound with selectivity for the dopamine D_2 receptor and a fast rate of dissociation can provide an opportunity to study the 'fast dissociation hypothesis' in a prospective clinical setting.

Recently, the novel chemical entity JNJ-37822681 was developed, which combines selectivity for the dopamine D_2 receptor with a fast rate of dissociation[12]. JNJ-37822681 has moderate affinity for the dopamine D_{2L} receptor and low affinity for dopamine D_1 and D_3 receptors, serotonin 5-HT_{2A} and 5-HT_{2C} receptors, histamine H_1 receptors and adrenergic α_{1A} receptors[12]. JNJ-37822681 also binds to the σ_1 receptor. When tested in parallel, the time for 50% dissociation of [^{3}H]JNJ-37822681 from the dopamine D_{2L} receptor was similar to that of [^{3}H]clozapine and significantly faster than that of [^{3}H]haloperidol, [^{3}H]risperidone and [^{3}H]paliperidone[12]. In animal models, JNJ-37822681 antagonized apomorphine-induced behavior in rats with a low potential for catalepsy[12]. In a separate study, pharmacokinetics and central nervous system (CNS) effects of JNJ-37822681 were evaluated in healthy volunteers[13]. The main pharmacodynamic effect was a dose-related elevation of serum prolactin starting at doses of 5 mg, whereas other subjective and neurophysiological effects were small and only observed at higher doses. Somnolence was the most frequent reported adverse event. No significant extrapyramidal effects were noted, although transient mild restlessness (akathisia) was reported occasionally after higher doses. The purpose of the present study was to characterize the relationship between plasma concentration following single oral doses of JNJ-37822681 and striatal dopamine D_2 receptor occupancy in vivo.

METHODS

Study design

An open-label study was performed to obtain 16 positron emission tomography (PET) scans using [^{11}C]raclopride, after administration of various dosages of JNJ-37822681 in healthy male volunteers, in order to characterize the saturation curve of JNJ-37822681 within tolerable dose levels. To enable calculation of dopamine D_2 receptor occupancy, baseline PET scans without prior administration of JNJ-37822681 were also performed in each volunteer. The study was approved by the medical ethics review committee of the VU University Medical Center in Amsterdam. Prior to medical screening, all volunteers gave written informed consent. Medical screening included medical history, physical examination, urinalysis, routine haematology and chemistry, 12-lead electrocardiography and an MRI scan to exclude cerebral pathology. Up to three [^{11}C]raclopride PET scans were performed per individual volunteer: one baseline scan and up to two scans following a single oral dose of JNJ-37822681. The postdose scans were initiated 2 hours (±30 minutes) after dosing. This time point was chosen to coincide with the expected t_{max} of the plasma concentrations of JNJ-37822681 (which varied between approximately 4 hours after 0.5 mg to 1.5 hours after 20 mg) and the presence of central nervous system effects (which were maximal between 1 and 3 hours after dosing)[13]. The first two volunteers were scanned following administration of 10 mg of JNJ-37822681. This 10 mg dose was expected to result in a dopamine D_2 receptor occupancy of about 75%, based on previous PET studies of JNJ-37822681 in Cynomolgus monkeys and pharmacokinetic data from healthy volunteers[13]. Subsequent doses were chosen based on the outcomes of the previous PET scans. The maximum dose was set to 20 mg JNJ-37822681, because safety data above 20 mg were not available at the time of study execution. Moreover, preclinical studies suggested that this dose would result in significant occupancy of striatal dopamine D_2 receptors and allow for reasonable estimation of the dose-occupancy relationship. Blood samples for pharmacokinetic analyses of JNJ-37822681 were taken before dosing and prior to, at the midpoint of and immediately after each scan. Plasma concentrations of JNJ-37822681 were determined using liquid chromatography-mass spectrometry. At several time points, development of akathisia or other extrapyramidal symptoms was evaluated using the Barnes akathisia rating scale and the Simpson-Angus scale[14,15]. In addition, blood pressure and heart rate measurements, 12-lead electrocardiograms, urinalysis, alcohol breath test and routine blood chemistry and haematology were performed on the days before and after administration of JNJ-37822681. Administration of first and second doses of JNJ-37822681 was always separated by a washout time of at least 7 days.

Magnetic resonance imaging (MRI)

T1-weighted gradient echo pulse MRI scans were obtained using a Philips 3 Tesla Achieva scanner (Philips Healthcare Nederland, Eindhoven, The Netherlands). These scans were used to exclude cerebral pathology and to define regions of interest (ROI).

Positron emission tomography (PET)

PET scans were performed on an ECAT EXACT HR+ scanner (Siemens/CTI, Knoxville, Tennessee, USA), equipped with a neuro-insert to reduce the contribution of scattered photons. This scanner enables the acquisition of 63 transaxial planes over a 15.5 cm axial field of view[16]. First, using three retractable rotating line sources, a 10 minute transmission scan was performed in 2D acquisition mode. This scan was used to correct the subsequent emission scan for photon attenuation. Next, a dynamic emission scan in 3D acquisition mode was performed. Data acquisition comprised of 21 frames (6×5, 3×10, 4×60, 2×150, 2×300 and 4×600 seconds) with a total duration of 60 minutes. At the start of this scan, 196 ± 13 MBq [^{11}C]raclopride with specific activity (SA) in the range of 32-111 GBq/μmol and a total volume of 12 mL was administered intravenously using an infusion pump (MEDRAD, Beek, The Netherlands) at a rate of 0.8 mL/sec, followed by a flush of 42 mL saline at 2.0 mL/sec. The total injected amount of raclopride did not differ significantly between baseline and postdose PET scans (1.33 ± 0.52 μg and 1.09 ± 0.98 μg respectively, $p = 0.38$) and all amounts were less than 1% of a clinically active raclopride dose (range 0.48-4.43 μg). The scanning protocol was identical for baseline and postdose scans. [^{11}C]raclopride was produced in the government licensed GMP facility of the department of Nuclear Medicine & PET Research (no. 108897F) according to current GMP guidelines (EudraLex volume 4) using a previously reported method[17].

Image analysis

All PET sinograms were corrected for dead time, scatter, decay, randoms and tissue attenuation and reconstructed using filtered back projection with a 0.5 Hanning filter, resulting in a transaxial spatial resolution of ~ 7 mm full width at half maximum in the centre of the field of view. Images were then transferred to ULTRASPARC workstations (Sun Microsystems Inc., Santa Clara, California, USA) for further analysis. For each subject, all scans were co-registered to the corresponding individual MRI. Left and right putamen, together with cerebellum, ROI were defined manually on the MRI scan and then projected onto the co-registered PET scans, guaranteeing identical ROI for all successive scans of the same subject. Total putamen was obtained as the volume weighted average of

left and right putamen. Putamen time-activity curves were analysed using the simplified reference tissue model (SRTM) with cerebellum as reference tissue[18]. This provides an estimate of the nondisplaceable binding potential BP_{ND}[19]. For each [^{11}C]raclopride scan following administration of JNJ-37822681, dopamine D_2 receptor occupancy in putamen was derived by relating its BP_{ND} (BP_{ND}^{drug}) to the corresponding baseline BP_{ND} ($BP_{ND}^{baseline}$):

$$\text{Receptor occupancy}\,(\%) = \left[1 - \frac{BP_{ND}^{drug}}{BP_{ND}^{baseline}}\right] \times 100\,\%$$

This approach assumes that affinity is not affected by a pharmacological dose of JNJ-37822681. For illustrative purposes, the simplified reference tissue model was also applied at the voxel level using a basis function implementation of SRTM, generating parametric images of BP_{ND}[20]. To describe the induced D_2 receptor occupancy as a function of plasma concentration of JNJ-37822681, data were fitted to the following equation:

$$\text{Receptor occupancy}\,(\%) = \frac{100 \times C_P}{C_P + EC_{50}}$$

where C_p stands for the plasma concentration of JNJ-37822681 and EC_{50} for the estimated plasma concentration of JNJ-37822681 that results in 50% receptor occupancy. C_p was calculated as the mean of plasma concentrations of JNJ-37822681, measured prior to, at midpoint of and immediately after each PET scan.

Data were further analyzed using PVE-lab, a software program using a probability map of 35 delineated ROI that has been validated previously[21], in order to evaluate receptor occupancy in the caudate nucleus, putamen and striatum (left, right and total).

RESULTS

Subjects

All participants were healthy males, aged 18 to 34 years, with a body mass index ranging from 20 to 29 kg/m^2. Four included volunteers dropped out before administration of JNJ-37822681 and their first planned postdose PET scan for reasons unrelated to the study. Data obtained in these volunteers were not used for analysis. Four volunteers completed one baseline [^{11}C]raclopride scan and two scans following administration of different doses of JNJ-37822681. Eight additional volunteers underwent one baseline and only one postdose scan. Therefore, in total, data were obtained in 12 volunteers, consisting of 12 baseline scans and 16 postdose scans with six different doses of JNJ-37822681 ranging from 2 to 20 mg.

Clinical observations

All reported adverse events were mild in severity. The most commonly reported adverse event was somnolence, occurring three times after 15 mg and three times after 20 mg of JNJ-37822681. A mild restless feeling after administration of 20 mg was reported by one volunteer. There were no consistent and clinically relevant abnormalities in blood pressure, heart rate, 12-lead electrocardiogram, blood chemistry and haematology. In addition, no consistent and clinically relevant changes were observed on the Barnes akathisia rating scale and the Simpson-Angus scale.

Plasma concentration of JNJ-37822681

Plasma concentrations of JNJ-37822681, measured prior to, at midpoint of and immediately after each PET scan, are shown in Table 1.

TABLE 1 Plasma concentration of JNJ-37822681 in all individual subjects. The mean plasma concentration of JNJ-37822681 was determined from plasma samples taken immediately before, at midpoint, and immediately after PET scanning.

Dose (mg)	Subject number	Plasma JNJ-37822681 (ng/mL)			
		Prior to PET scan	At midpoint of PET scan	After PET scan	Mean
2	1006	1.92	1.63	1.59	1.71
2	1102	1.41	1.71	2.93	2.02
2	1105	1.96	1.84	1.65	1.82
5	1002	10.9	7.34	6.06	8.10
5	1007	8.05	5.92	5.29	6.42
7	1107	11.9	8.82	11.2	10.6
10	1001	19.4	16.8	15.0	17.1
10	1002	19.8	14.0	11.6	15.1
15	1003	25.8	20.6	22.3	22.9
15	1005	32.5	27.1	21.7	27.1
15	1106	33.1	18.8	16.4	22.8
20	1003	10.6	13.1	17.6	13.8
20	1004	49.5	37.9	36.4	41.3
20	1005	74.5	52.7	39.2	55.5
20	1007	30.7	24.0	20.5	25.1
20	1101	57.7	49.8	42.6	50.0

Binding potential and D_2 receptor occupancy

BP_{ND} values and D_2 receptor occupancies in the manually defined left and right putamen are shown in Table 2. Average baseline BP_{ND} was 2.85 ± 0.21 (range 2.38 to 3.06). A decrease in BP_{ND} and corresponding increase in receptor occupancy was seen with increasing doses of JNJ-37822681. Examples of reconstructed parametric BP_{ND} images are shown in Figure 1. Receptor occupancy increased from 9-19% after an oral dose of 2 mg to 60-74% after an oral dose of 20 mg of JNJ-37822681, as illustrated in Figure 2. Receptor occupancy as a function of plasma concentration of JNJ-37822681 provided an estimated EC_{50} of 14.5 ng/mL (coefficient of variation 5.4%). The associated hyperbolic function is shown in Figure 3.

Calculated D_2 receptor occupancies in the caudate nucleus, putamen and striatum using PVE-lab, are shown in Table 3. In general, occupancy levels by JNJ-37822681 in caudate nucleus and putamen were similar.

FIGURE 1 Transaxial (left), coronal (middle) and sagittal (right) parametric BP_{ND} images of subject 1007, co-registered to corresponding MRI data. Top row represents baseline images, obtained prior to administration of medication. Middle row represents images following administration of 5 mg of JNJ-37822681, resulting in striatal D_2 receptor occupancy of 30%. Bottom row represents images following administration of 20 mg of JNJ-37822681, resulting in striatal D_2 receptor occupancy of 65%.

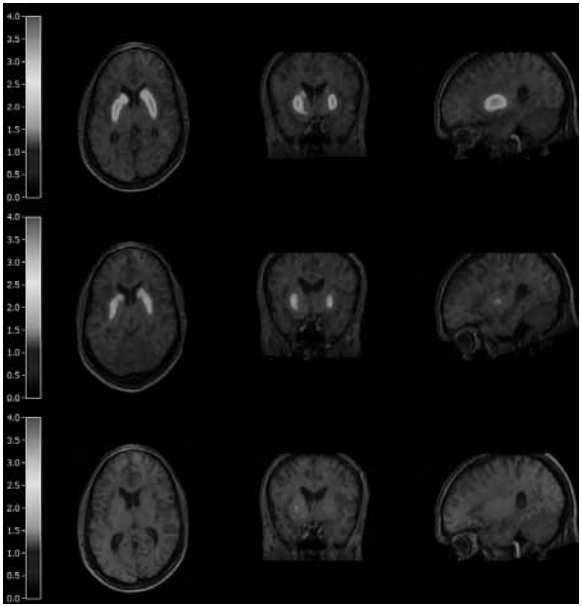

FIGURE 2 Dopamine D_2 receptor occupancy as function of administered dose of JNJ-37822681 for manually defined putamen (volume weighted average of left and right putamen).

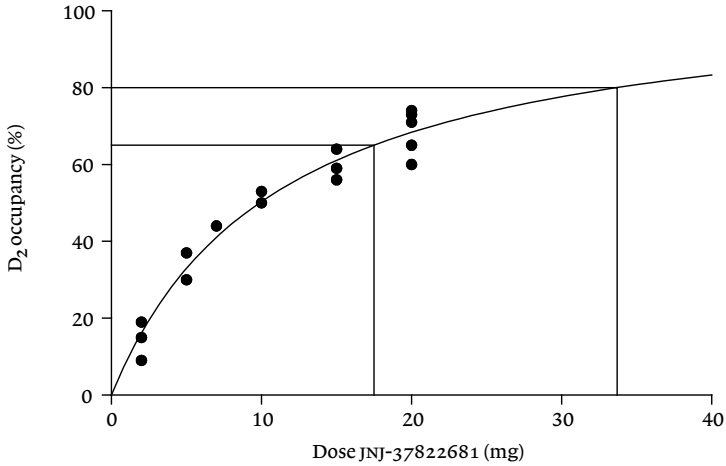

FIGURE 3 Dopamine D_2 receptor occupancy as function of mean plasma concentration of JNJ-37822681 for manually defined putamen (volume weighted average of left and right putamen). The fitted curve indicates that receptor occupancy between 65 and 80% is achieved by plasma concentrations between 27 and 58 ng/mL.

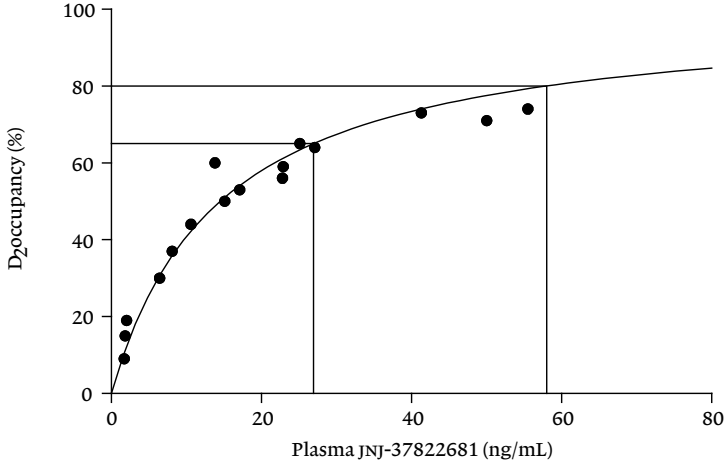

TABLE 2 Binding potential (BP_{ND}) and dopamine D_2 receptor occupancy levels in all subjects for manually defined putamen (volume weighted average of left and right putamen).

Dose (mg)	Subject number	Baseline BP_{ND}	Postdose BP_{ND}	D_2 receptor occupancy (%)
2	1006	2.38	2.17	9
2	1102	3.06	2.48	19
2	1105	3.01	2.56	15
5	1002	3.01	1.90	37
5	1007	2.69	1.87	30
7	1107	2.85	1.61	44
10	1001	3.01	1.41	53
10	1002	3.01	1.52	50
15	1003	2.90	1.20	59
15	1005	3.06	1.09	64
15	1106	2.74	1.21	56
20	1003	2.90	1.15	60
20	1004	2.80	0.77	73
20	1005	3.06	0.78	74
20	1007	2.69	0.93	65
20	1101	2.64	0.76	71

TABLE 3 Dopamine D_2 receptor occupancy in all subjects for PVE-lab defined caudate nucleus, putamen and striatum (left and right).

Dose (mg)	Subject number	Caudate nucleus	Putamen	Whole striatum
2	1006	13,6	11,9	12,8
2	1102	12,1	15,5	14,3
2	1105	22,3	19,3	20,2
5	1002	32,5	34,1	33,2
5	1007	41,3	29,9	34,3
7	1107	47,6	44,3	45,5
10	1001	57,5	48,7	51,8
10	1002	50,0	49,3	49,5
15	1003	60,8	59,2	59,7
15	1005	63,4	63,5	63,4
15	1106	62,1	55,9	57,7
20	1003	62,3	59,0	60,2
20	1004	76,8	74,5	75,4
20	1005	75,8	73,5	74,4
20	1007	73,3	65,2	67,9
20	1101	71,4	70,7	71,1

DISCUSSION

The present study was performed to characterize striatal dopamine D_2 receptor occupancy over a range of oral dosages of JNJ-37822681. Dosages from 2 to 20 mg resulted in receptor occupancy levels ranging from 9 to 74%, with a hyperbolic function providing a good description of the saturation curve (see Figure 3).

PET studies following single dose administration of novel compounds in healthy volunteers are widely used in early phase drug development to evaluate binding characteristics in vivo and to guide dose selection for future clinical trials. However, the predictive value of single dose PET studies for dose selection in clinical trials is somewhat limited because single dose estimates may differ from steady state estimates after multiple dosing. For example, single dose PET studies with ziprasidone[22,23] have predicted higher dopamine D_2 receptor occupancy than multiple dose studies[23,24]. On the other hand, PET studies in healthy volunteers after single doses of olanzapine[25] and risperidone[26], even in spite of very small sample sizes and limited dose ranges, have provided rather accurate predictions of receptor occupancy in schizophrenic patients following subchronic treatment[27-31]. Preliminary results of a separate PET study with JNJ-37822681 demonstrate that, although refinement after multiple dose administration enhances predictive value to some extent, measurements of D_2 receptor occupancy following single doses appear to provide reasonable estimates for guidance and interpretation of clinical studies[32,33].

Several PET studies using [^{11}C]raclopride have consistently demonstrated that, following haloperidol treatment, a striatal dopamine D_2 receptor occupancy higher than 65% is associated with clinical response, whereas occupancy above 80% is associated with extrapyramidal side effects[34-39]. Similar occupancy levels have been found with other typical agents such as chlorpromazine[40], perphenazine[40] and loxapine[41] and with atypical agents such as risperidone[29-31] and olanzapine[28,29,42]. Accordingly, it has been suggested that 65-80% receptor occupancy is optimal for most registered antipsychotic agents in terms of antipsychotic effect and adverse (i.e. extrapyramidal) events in clinical practice[37,39,43]. The present data indicate that 65 to 80% receptor occupancy is associated with plasma concentrations of 27 to 58 ng/mL of JNJ-37822681 (see Figure 3). However, target levels of 65 to 80% striatal dopamine D_2 receptor occupancy do not always represent absolute thresholds for antipsychotic activity and exceptions have been identified. First, PET studies with long-acting depot injections of haloperidol decanoate[44] and risperidone[45] in schizophrenic patients have demonstrated dopamine D_2 receptor occupancy levels below 60% without clinical relapse, suggesting that sustained dopamine D_2 receptor occupancy be-

tween 65 and 80% (although associated with acute clinical response) may not be necessary to maintain clinical effect. It is possible that lower levels of dopamine D_2 receptor occupancy offer some protection against psychotic relapses, or that long-term adaptive changes contribute to the stable clinical situation in chronically well-treated schizophrenic patients. Second, the partial D_2 receptor agonist aripiprazole is associated with occupancy levels over 80% at therapeutic doses, while the risk for extrapyramidal symptoms seems to increase only at occupancy levels of 90% or higher[46,47]. This apparent discrepancy with dopamine D_2 receptor antagonists, however, could reflect the distinct pharmacological characteristics of aripiprazole. Third, PET studies have demonstrated lower occupancy levels with clozapine[29,35,40] and quetiapine[48] at clinically effective doses. It has been suggested that the occupancy levels of clozapine and quetiapine may have been underestimated because these drugs, compared with other antipsychotics, can rather easily be displaced from the D_2 receptor by endogenous dopamine release[5]. Further studies with shorter time intervals between dosing of quetiapine and PET scanning showed higher occupancy levels, reflecting a rapid reduction in occupancy after transiently high levels[49,50].

Despite a fast k_{off}, JNJ-37822681 is able to achieve relatively high dopamine D_2 receptor occupancy levels. If fast dissociation is the reason why clozapine and quetiapine are therapeutically active at relatively low striatal occupancy levels, this could also be the case for JNJ-37822681. Preliminary results of a recently completed multicenter, double blind, placebo-controlled trial with twice daily dosing of 10, 20 and 30 mg of JNJ-37822681 in patients with schizophrenia indicate clinical efficacy superior to placebo with low frequency of extrapyramidal symptoms with all three dosing regimes[51]. The present study demonstrates that doses of 10 mg of JNJ-37822681 are associated with merely 50-53% receptor occupancy two hours after dose administration. It would be of interest to further study the time course of D_2 receptor occupancy, in order to assess whether JNJ-37822681, similar to quetiapine, produces only transiently high levels of receptor occupancy with a rapid reduction over time.

Central neurophysiological and neuropsychological effects of single doses up to 20 mg of JNJ-37822681 are generally quite small with the exception of increases in serum prolactin[13]. Small decreases in adaptive tracking were observed after doses of 10 mg, whereas small reductions in saccadic peak velocity, smooth pursuit eye movements, alertness, finger tapping and α and β activity on the EEG and an increase in body sway were observed at higher doses. The present data demonstrate that 20 mg doses of JNJ-37822681 can induce receptor occupancy levels that are generally associated with clinical response (i.e. 65 to 80%) for registered antipsychotic drugs. Therefore, the mild CNS effects of

JNJ-37822681, compared with the increase in serum prolactin, do not seem to result from lack of D_2 receptor occupancy. A more likely explanation of these findings may be the selectivity of JNJ-37822681 for the dopamine D_2 receptor. Prolactin release is controlled primarily by dopamine acting on dopamine D_2 receptors, whereas the other pharmacodynamic tests measure more complex CNS functions, which are likely to involve multiple neurotransmitter receptor systems. Accordingly, specific dopamine D_2 antagonism by JNJ-37822681 significantly increases prolactin release, while only moderately affecting the other pharmacodynamic tests.

Within the present small group of healthy volunteers, none of the investigated dosages of JNJ-37822681 was associated with receptor occupancy levels above 80%. Accordingly, akathisia and other extrapyramidal side effects were absent. However, in a previous study with similar doses in healthy volunteers, transient mild restlessness was reported occasionally following the highest dosages[13]. Especially at the higher doses, peak plasma concentrations are reached somewhat earlier than two hours after dosing[13]. Striatal dopamine D_2 receptor occupancy could therefore have increased transiently above 80%, with an associated higher risk for peak dose-related extrapyramidal symptoms. To further characterize the relationship between dopamine D_2 receptor occupancy by JNJ-37822681 and the emergence of extrapyramidal side effects, PET studies at higher dosages of JNJ-37822681 would be needed. The present study was limited to a maximum dose level of 20 mg JNJ-37822681, because no safety data above 20 mg were available at the time of study execution.

Several imaging studies have demonstrated a non-uniform blockade of striatal D_2/D_3 receptors, with higher occupancy levels in the head of the caudate nucleus than in the putamen, by amisulpride[52], risperidone[52], clozapine[53] and aripiprazole[46], although this finding was not replicated for risperidone[54]. To explore this issue, PVE-lab[21] was used in the present study to evaluate occupancy levels by JNJ-37822681 in caudate nucleus and putamen. No clear differences in occupancy levels were found, but sample sizes in the different dosing groups may have been too small.

Postulated clinical importance of dopamine D_2 receptor antagonism in extrastriatal limbic and neocortical regions has been a matter of some controversy. Preferential extrastriatal dopamine D_2 receptor binding was first demonstrated with clozapine[55] and later with olanzapine[56] using single photon emission computed tomography (SPECT) and [^{123}I]epidepride, although PET studies with [^{11}C]raclopride and [^{11}C]FLB457 did not confirm preferential extrastriatal binding by clozapine[57,58]. However, all these studies have been criticized on methodological grounds[59–61]. Subsequently, other PET studies using [^{76}Br]FLB457 or

[^{18}F]fallypride have demonstrated preferential extrastriatal binding by clozapine[53,62,63], quetiapine[62,64] and ziprasidone[23], but not haloperidol[63,65]. Contradictory results have been obtained with risperidone and olanzapine, as SPECT using [^{123}I]epidepride and PET using [^{76}Br]FLB457 demonstrated preferential extrastriatal binding[63,66], whereas PET using [^{18}F]fallypride or [^{11}C]FLB457 and [^{11}C]raclopride in the same subjects, did not[54,65,67]. A recent meta-analysis of SPECT and PET in vivo receptor imaging data[68] demonstrated that both typical and atypical antipsychotic drugs produce high D_2 receptor occupancy in temporal cortex, whereas only the typical antipsychotic drugs produced high D_2 receptor occupancy in the striatum. The present study using [^{11}C]raclopride does not allow for accurate estimation of limbic and neocortical binding characteristics of JNJ-37822681. Future PET studies with high affinity ligands, such as [^{11}C]FLB457 and [^{18}F]fallypride, are needed to quantify extrastriatal binding by JNJ-37822681. Such studies would also enable evaluation of pituitary dopamine D_2 receptor occupancy, as demonstrated in recent PET studies[69,70].

In conclusion, the present results provide guidance for dose selection in future clinical trials using JNJ-37822681 in patients with an acute exacerbation of schizophrenia. Preliminary results of the recently completed multicenter, double blind, placebo-controlled trial with JNJ-37822681 in patients with schizophrenia indicate clinical efficacy superior to placebo with low frequency of extrapyramidal symptoms[51]. Confirmation of these findings may support the usefulness of dissociation rates as a strategy for novel antipsychotic drug development.

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CHAPTER 4

PHARMACOKINETICS AND CENTRAL NERVOUS SYSTEM EFFECTS OF THE NOVEL DOPAMINE D_3 RECEPTOR ANTAGONIST GSK598809 AND INTRAVENOUS ALCOHOL INFUSION AT PSEUDO-STEADY STATE

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ABSTRACT

GSK598809 is a novel selective dopamine D_3 receptor antagonist, currently in development for treatment of substance abuse and addiction. In a blinded, randomized, placebo-controlled study, effects of single oral doses of 175 mg GSK598809 were evaluated in healthy volunteers. Pharmacokinetics, central nervous system (CNS) effects and potential for interactions with alcohol were evaluated, using an alcohol infusion paradigm and analysis of eye movements, adaptive tracking, visual analogue scales, body sway, serum prolactin and verbal visual learning test. Adverse effects of GSK598809 included headache, dizziness and somnolence. Plasma concentration of GSK598809 was maximal 2-3 hours postdose and decreased with a half life of roughly 20 hours. CNS effects were limited to prolactin elevation and decreased adaptive tracking. Co-administration of GSK598809 and alcohol did not affect alcohol pharmacokinetics, but caused a 9% decrease of C_{max} and a 15% increase of AUC of GSK598809. CNS effects of co-administration were mainly additive, except a small supra-additive increase in saccadic reaction time and decrease in delayed word recall. In conclusion, GSK598809 causes elevation of serum prolactin and a small decrease in adaptive tracking performance. After co-administration with alcohol, effects of GSK598809 are mainly additive and the combination is well tolerated in healthy volunteers.

INTRODUCTION

A large body of evidence indicates that the mesolimbic dopaminergic pathway, which includes dopaminergic neurons in the ventral tegmental area projecting to the nucleus accumbens and other limbic forebrain structures, is one of the major neuronal circuits involved in the acute rewarding effects of drugs of abuse[1–5]. Although addictive drugs interact with many different neurotransmitter systems, most drugs ultimately cause an acute increase in synaptic dopamine in the nucleus accumbens and the mesolimbic dopaminergic system[6–8], as demonstrated by microdialysis studies in rats[9] and positron emission tomography (PET) studies in humans[10–14]. Several important observations have suggested that dopamine D_3 receptors may play a significant role in the effects of drugs of abuse and the pathophysiology of drug addiction[15,16]. First, dopamine D_3 receptors are located primarily in mesolimbic regions such as nucleus accumbens and ventral striatum[17–21]. Second, studies in animal models have demonstrated that dopamine D_3 receptor activation may be involved in the reinforcing effects and self-administration of cocaine[22]. Third, long term drug exposure appears to cause upregulation of dopamine D_3 receptors as demonstrated in postmortem studies of cocaine overdose fatalities[23,24]. Accordingly, it has been suggested that dopamine D_3 antagonism may be an effective strategy in pharmacotherapy of addiction[15,25,26].

GSK598809 is a novel, potent and selective dopamine D_3 receptor antagonist[27], which is being developed as a novel treatment for substance dependence disorders. Functional assays showed that GSK598809 has greater than 100-fold selectivity for dopamine D_3 receptors over dopamine D_2, histamine H_1, muscarinic M_1, M_2, M_3, M_4, serotonin 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors (data on file). Conditioned place preference (CPP) experiments in animal models indicated that GSK598809 significantly reduced nicotine- and cocaine-seeking behavior in a dose-dependent manner (data on file). In addition, GSK598809 significantly prevented relapse to nicotine-seeking behavior, although no effect was observed on reducing alcohol consumption in rats (data on file).

The present study was performed to evaluate the pharmacokinetics and central nervous system (CNS) effects of single oral doses of GSK598809 in healthy volunteers. Special emphasis was given to evaluating possible interactions with alcohol, because the target population of patients will have alcohol dependence as primary disorder, or may abuse alcohol as comorbidity next to another substance abuse disorder. Pharmacokinetic interactions between alcohol and GSK598809 are theoretically possible, because a metabolite of GSK598809 shows in vitro to have a potential for inhibiting CYP2E, which is one of the main enzymes involved in alcohol metabolism[28]. Also, pharmacodynamic interactions

are theoretically possible as both compounds are centrally active and influence the dopamine system. However, apart from these theoretical possibilities, there are no reasons to assume a priori that any specific pharmacodynamic interaction will occur between GSK598809 and alcohol. Currently, no validated human pharmacodynamic markers for dopamine D_3 antagonism are available. For exploratory purposes, we used a battery of quantitative central nervous system tests, sensitive to various compounds, including alcohol[29] and antipsychotic drugs (dopamine D_2 receptor antagonists)[30], was used to evaluate pharmacodynamic effects. An oral dose of 175 mg GSK598809 was chosen because positron emission tomography using [^{11}C]-(+)-PHNO in healthy volunteers has demonstrated that this dose can induce high occupancy (near 100%) of dopamine D_3 receptors in the substantia nigra[27]. Also, previous studies in healthy volunteers demonstrated that this dose is generally well tolerated (data on file).

METHODS

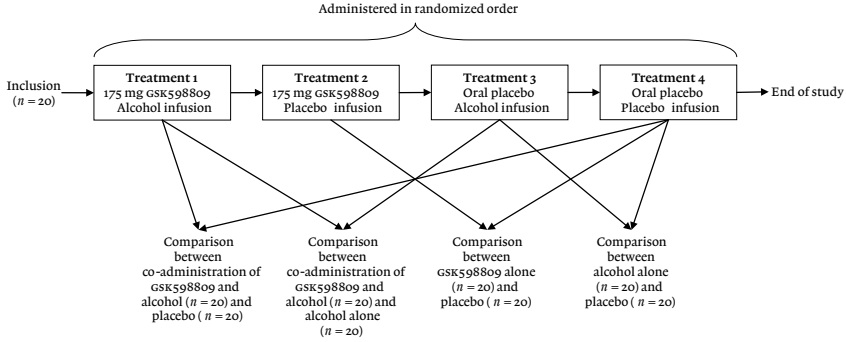
Study design

Twenty healthy volunteers, between 18 and 65 years of age and with a body mass index (BMI) between 18 and 30 kg/m^2, were planned to participate in a blinded, randomized, placebo-controlled, double-dummy, four-period cross-over study. The study was approved by the medical ethics review board of the Leiden University Medical Center and registered at the NIH database of clinical trials (website http://clinicaltrials.gov) with identifier NCT00887367 and GSK ID number 106591. Prior to medical screening, all volunteers gave written informed consent. All volunteers underwent training sessions for the pharmacodynamic tests in order to minimize possible learning effects.

Volunteers were assigned to a randomized treatment sequence (see Figure 1), consisting of one period of oral administration of 175 mg of GSK598809 combined with intravenous alcohol infusion (alcohol clamping, see below for further details), one period of oral administration of 175 mg of GSK598809 combined with intravenous placebo infusion, one period of oral placebo administration combined with intravenous alcohol infusion, and one period with oral placebo administration combined with intravenous placebo infusion. This study design enables analysis of the following comparisons (see Figure 1):

- Administration of 175 mg GSK598809 ($n = 20$) versus placebo ($n = 20$)
- Intravenous alcohol infusion ($n = 20$) versus placebo ($n = 20$)
- Co-administration of 175 mg GSK598809 and intravenous alcohol infusion ($n = 20$) versus placebo ($n = 20$)
- Co-administration of 175 mg GSK598809 and intravenous alcohol infusion ($n = 20$) versus intravenous alcohol infusion alone ($n = 20$)

FIGURE 1 Study design.



GSK598809 or matching placebo was administered orally 30 minutes after the start of the ethanol (or placebo) infusion. The alcohol (or placebo) infusion continued for 5 hours in total to cover the main part of the plasma concentration curve of GSK598809. Each study period consisted of five study days. The randomized treatment was administered in the morning of the first study day, followed by pharmacokinetic and pharmacodynamic measurements at regular time points. All periods were separated by a wash-out time of at least five days.

Occasional (non-daily) smokers were eligible to participate in the study. Subjects were excluded from participation if they smoked on a daily basis. Also, subjects were excluded if they had an average daily intake of greater than 2 units (in case of females) or 3 units (in case of males) or an average weekly alcohol intake of greater than 14 units (in case of females) or 21 units (in case of males). One unit is equivalent to a half-pint (220 mL) of beer or 1 (25 mL) measure of spirits or 1 glass (125 mL) of wine. Subjects were instructed to abstain from smoking and alcoholic drinks on the day preceding all study periods and all subsequent study days. In addition, use of illicit drugs was not permitted. In all study periods, breath alcohol measurements were performed to ascertain non-use of alcohol. Also, urine drug screening for cocaine, amphetamines, opiates (morphine), benzodiazepines, barbiturates and THC (Innovacon, Inc., San Diego, California, USA) was performed to ascertain non-use of illicit drugs.

Alcohol clamping

The method for attaining constant alcohol levels has been described in detail elsewhere[29,31]. In brief, alcohol (ethanol 10% w/v solution in 5% glucose) was infused intravenously over a period of five hours, guided by breath alcohol measurements to maintain a pseudo-steady state alcohol serum level of 0.6 g/L. This target level was chosen because this level produces significant central nervous

system effects without causing too many inadvertent effects and is considered safe, since it is only just above the legal driving limit in the Netherlands (i.e. 0.5 g/L). Alcohol infusion started 30 minutes prior to administration of GSK598809. The infusion rate for the first ten minutes was determined using demographic data of the volunteer (weight, height, age and gender). Infusion rates were subsequently adjusted, guided by breath alcohol measurements at baseline and at every five minutes for the first 30 minutes after the start of the infusion, every 10 minutes for the next 30 minutes and then every half hour until the end, using two calibrated Alco-Sensor IV Intoximeters (Honac, Apeldoorn, the Netherlands), which were alternated to avoid fatigue of the sensors. To prevent local pain at the beginning of the alcohol infusion, an additional diluting glucose 5% infusion at 100 mL/h was given to all participants during the first 10 minutes after the start of the alcohol infusion over the same infusion line. Alcohol clamping was performed in a randomized, double-blind, placebo-controlled fashion by an infusion assistant, who was not a member of the study team. A sham procedure, consisting of saline infusion in a manner similar to the alcohol infusion, including repeated breath alcohol measurements and subsequent infusion rate adjustments, was used to maintain blinding of the subject and the rest of the team. The mock infusion rate adjustments were provided by the clamping program.

Safety monitoring

Evaluation of adverse events, 12-lead electrocardiograms (ECG), blood pressure, heart rate, alcohol breath test, urinalysis and blood sampling for haematology and chemistry was performed at regular time points after each dose administration. Automated oscillometric blood pressures were measured using a Nihon-Kohden BSM-1101K monitor or a Colin Pressmate BP 8800. ECGs were obtained with Cardiofax V equipped with ECAPS12 analysis program (Nihon-Kohden, Tokyo, Japan). In addition, telemetry monitoring was started at the beginning of alcohol infusion and was continued for six hours. Volunteers were evaluated for akathisia and extrapyramidal symptoms, using the Barnes Akathisia Rating Scale[32], Simpson-Angus Scale[33] and Abnormal Involuntary Movement Scale[34].

Pharmacokinetics

Venous blood samples for GSK598809 concentration analysis were collected prior to dose administration and at 15 and 30 minutes and 1, 2, 3, 4, 6, 8, 10, 12, 24, 48 and 72 hours after dose administration. Concentration of GSK598809 in plasma samples was determined using protein precipitation followed by HPLC/MS analysis with a lower limit of quantification (LLQ) of 0.5 ng/mL. Pharmacokinetic

parameters of GSK598809 include the maximum observed plasma concentration (C_{max}), time to reach maximum plasma concentration (t_{max}), area under the plasma concentration-time curve extrapolated to infinity (AUC_{∞}) and terminal phase half life (t½).

Venous blood samples for pharmacokinetic analysis of serum alcohol were taken prior to start of infusion and at 15, 30, 45, 60, 90, 150, 210, 270 and 390 minutes after start of alcohol infusion. Serum alcohol levels were measured with an enzymatic assay (Roche Diagnostics, Mannheim, Germany) using a Hitachi 911 (Boehringer Mannheim, Mannheim, Germany). In this enzymatic assay, alcohol and nicotinamide adenine dinucleotide (NAD+) are converted to acetaldehyde and NADH by alcohol dehydrogenase (ADH). The NADH formed during the reaction, measured photometrically as a rate of change in absorbance, is directly proportional to the alcohol concentration.

Pharmacodynamic testing

All pharmacodynamic measurements were performed as described previously[35,36]. Volunteers were tested individually in a quiet room with ambient illumination. Quantitative tests, sensitive to the effects of alcohol[29] and single oral doses of antipsychotic drugs (dopamine D_2 receptor antagonists)[30] such as haloperidol[36,37] and risperidone[38] in healthy volunteers, included measurements of smooth pursuit and saccadic eye movements, adaptive tracking, body sway, visual analogue scales, the visual verbal learning test (VVLT) and serum prolactin levels. Previous studies using the alcohol clamping paradigm and this pharmacodynamic test battery[29] demonstrated that smooth pursuit eye movements and body sway were the most sensitive pharmacodynamic parameters for the effects of alcohol. In order to obtain accurate time profiles of the effects of alcohol and GSK598809, smooth pursuit eye movements and body sway were recorded at a high frequency. The other pharmacodynamic tests could not be performed as frequently due to limitations in time and logistics.

Analysis of eye movements

To evaluate oculomotor performance and sedation, smooth pursuit and saccadic eye movements were recorded as described previously[39-42], using a microcomputer-based system for data recording and analysis (Cambridge Electronic Design Ltd., Cambridge, UK), Nihon-Kohden equipment for stimulus display, signal collection and amplification (Nihon-Kohden, Tokyo, Japan), and disposable surface electrodes (Medicotest N-00-S, Olstykke, Denmark). For smooth pursuit eye movements, a target light source moves sinusoidally over 20° eyeball rotation at frequencies ranging from 0.3 to 1.1 Hz. The time in which the eyes

were in smooth pursuit was calculated for each frequency and expressed as the percentage of stimulus duration. The average percentage of smooth pursuit for all frequencies was used as parameter. Smooth pursuit eye movements were recorded prior to dose administration and at 10, 20, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 390, 420 and 450 minutes after dose administration. For saccadic eye movements, the target light source jumps from side to side. Peak velocity (degrees per second), reaction time and inaccuracy (%) was calculated of all artifact-free saccades. Saccadic eye movements were recorded prior to dose administration and at 30, 90, 150, 210, 270, 330, 390 and 450 minutes after dose administration.

Adaptive tracking

To evaluate visuo-motor coordination, the adaptive tracking task was performed as described previously[41-45], using customized equipment and software developed by K.W. Hobbs (Hertfordshire, UK). Adaptive tracking is a pursuit tracking task in which a circle moves randomly over a computer screen and the volunteer must try to keep a dot inside the moving circle using a joystick. If this effort is successful, the speed of the moving circle is increased and if the effort is unsuccessful, the speed is reduced. The adaptive tracking task was performed prior to dose administration and at 30, 90, 150, 210, 270, 330, 390 and 450 minutes after dose administration and performance was scored over a fixed period of three minutes. Average performance and standard deviation of scores were used for analysis.

Body sway

Postural stability in the sagittal plane was measured with an apparatus similar to the Wright ataxiameter[46], using a string attached to the waist of the volunteer. Measurements were performed prior to dose administration and at 10, 20, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 390, 420 and 450 minutes after dose administration. Movements over a period of two minutes, while standing still with eyes closed, were integrated and expressed as mm sway.

Visual analogue scales

Subjective effects were quantified prior to dose administration and at 30, 90, 150, 210, 270, 330, 390 and 450 minutes after dose administration using a Dutch translation of the visual analogue scales (VAS), originally described by Norris[47], to derive three composite factors corresponding to alertness, mood (contentedness) and calmness, as described by Bond & Lader[48]. In addition, a visual analogue scale was used to quantify the subjective effects of alcohol.

Visual verbal learning test

The visual verbal learning test (VVLT)[49] is an adapted version of the auditory verbal learning test[50] and was performed 150 minutes after dose administration. Three trials of 30 words are presented on a computer screen in the same sequence. The volunteer is requested to reproduce as many words as possible at the ending of each trial (immediate recall) and after 30 minutes (delayed recall). The number of correctly reproduced words is analyzed for each trial. Also, a recognition test is performed, consisting of 15 previously presented words and 15 new words, in which the volunteer has to indicate recognition of the word (delayed recognition) as quickly as possible. Response time and the number of correctly recognized words are analyzed.

Serum prolactin levels

Blood samples for measurement of prolactin levels were collected at baseline and at 60, 90, 120, 210, 390, 720 and 1320 minutes after study drug administration and serum was separated by centrifugation (2000 g at 4°C for 10 minutes). Prolactin levels were determined using an electrochemiluminescence immunoassay (ECLIA) on a Modular Analytics E170 (Elecsys module) immunoassay analyzer.

Statistical analysis

Analysis of variance models were performed on the pharmacokinetic parameters, including the factors treatment and period as fixed effects and subject as random effect. AUC and C_{max} pharmacokinetic parameters were log-transformed prior to analysis. Comparisons were expressed as ratios of the pharmacokinetic parameters after GSK598809 combined with ethanol relative to those after alcohol alone or relative to those after GSK598809 alone.

Pharmacodynamic data were compared using a mixed model analysis of variance with treatment, gender, period, time, and treatment by time as fixed factors, and with subject, subject by treatment and subject by time as random factors. VVLT data were compared using a mixed model analysis of variance with treatment, gender and period as fixed factors, and with subject as random factor. The parameters body sway, prolactin, saccadic eye movements and the delayed word variables were log-transformed prior to analysis to correct for the expected log-normal distribution of the data. The following contrasts were calculated (see Figure 1): alcohol versus placebo, GSK598809 versus placebo, co-administration of GSK598809 and alcohol versus placebo, co-administration of GSK598809 and alcohol versus alcohol alone. Supra-additive effects (defined as effects, resulting from co-administration of two independent agents, being greater than the sum

of effects of each individual agent) were evaluated by analyzing the contrast of the effects of co-administration of GSK598809 and alcohol with subtraction of the effects of GSK598809 alone versus the effects of alcohol alone with subtraction of the effects of placebo:

$$\left[\left(\begin{array}{c}\text{effects of}\\ \text{GSK598809}\\ \text{and alcohol}\end{array}\right) - \left(\begin{array}{c}\text{effects of}\\ \text{GSK598809}\\ \text{alone}\end{array}\right)\right] \text{ versus } \left[\left(\begin{array}{c}\text{effects of}\\ \text{alcohol}\\ \text{alone}\end{array}\right) - \left(\begin{array}{c}\text{effects}\\ \text{of}\\ \text{placebo}\end{array}\right)\right]$$

After identifying gender effects on prolactin levels, analysis of prolactin data was repeated using a mixed model analysis of variance with treatment, gender, period, time, treatment by gender, treatment by time, gender by time and treatment by gender by time as fixed factors, and with subject, subject by treatment and subject by time as random factors. Contrasts were calculated in original measurement unit with 95% confidence intervals and the associated *p*-value, except for the log-transformed parameters, which were calculated as a percentage relative to placebo or alcohol. All calculations were performed using SAS for Windows version 9.1.3 (SAS Institute Inc., Cary, North Carolina, USA).

RESULTS

Subjects

Twenty volunteers (10 males and 10 females) were included in the study. Volunteers had a mean age of 32.8 years (range 18-55), weight of 73.5 kg (range 54-108) and body mass index (BMI) of 23.6 kg/m^2 (range 18.5-29.8). One female volunteer tested positive for benzodiazepines on the drug screen in study period 2 and thereby violated the exclusion criteria. She was subsequently withdrawn from the study and not replaced. This volunteer was administered alcohol infusion and placebo capsules in the first study period.

Clinical observations

All adverse events were transient and mild or moderate in severity and no serious adverse events occurred during the study. Overall, the most frequent adverse effect were headache, somnolence, feeling drunk, dizziness, fatigue, pain at infusion site, nausea and vomiting (see Table 1). Somnolence and fatigue were reported more frequently after GSK598809 administration combined with alcohol ($n = 18$), compared to alcohol alone ($n = 8$), GSK598809 alone ($n = 4$) or placebo ($n = 4$). There were no consistent and clinically relevant changes on the Barnes Akathisia Rating Scale, Simpson-Angus Scale and Abnormal Involuntary

Movement Scale. Mild short-lasting akathisia was reported spontaneously once after administration of GSK598809 combined with alcohol and once after GSK598809 alone, but these events were not verified objectively by the Barnes Akathisia Rating Scale, when this was performed as scheduled. There were no consistent and clinically relevant changes in vital signs, blood chemistry and haematology or any of the ECG intervals.

Pharmacokinetics of alcohol

Following intravenous infusion, serum alcohol concentration increased rapidly and remained constant at the target level all over the time of infusion, after which serum concentrations declined (see Figure 2).

Pharmacokinetics of GSK598809

Pharmacokinetic parameters are presented in Table 2. Oral administration of GSK598809 resulted in peak levels after roughly 2 to 3 hours (see Figure 3) with an apparent bi-exponential decline and a half life of roughly 20 hours.

TABLE 1 Summary of common adverse events, reported by two subjects or more. Incidence is based on the number of subjects, not the number of events.

Adverse event	Placebo *n*=19	Alcohol *n*=20	GSK598809 *n*=19	GSK598809 + Alcohol *n*=19
Headache	6 (32%)	9 (45%)	5 (26%)	7 (37%)
Somnolence	1 (5%)	4 (20%)	3 (16%)	11 (58%)
Dizziness	1 (5%)	6 (30%)	6 (32%)	5 (26%)
Akathisia	0	0	1 (5%)	1 (5%)
Feeling drunk	0	7 (35%)	0	6 (32%)
Fatigue	3 (16%)	4 (20%)	1 (5%)	7 (37%)
Infusion site pain	0	3 (15%)	0	2 (11%)
Catheter site related reaction	0	1 (5%)	1 (5%)	0
Nausea	0	1 (5%)	3 (16%)	3 (16%)
Vomiting	0	1 (5%)	4 (21%)	0
Dry mouth	0	0	0	2 (11%)
Upper respiratory tract infection	0	2 (10%)	0	0
Dysmenorrhoea	1 (5%)	0	0	1 (5%)
Oropharyngeal discomfort	1 (5%)	0	0	1 (5%)
Skin reaction	1 (5%)	1 (5%)	0	0

FIGURE 2 Serum alcohol levels after intravenous alcohol infusion starting at t=-0.5 hours and continuing until t=4.5 hours, in combination with oral administration (at t=0 hours) of GSK598809 (open circles) or placebo (closed circles). Means are presented with standard deviations as error bars.

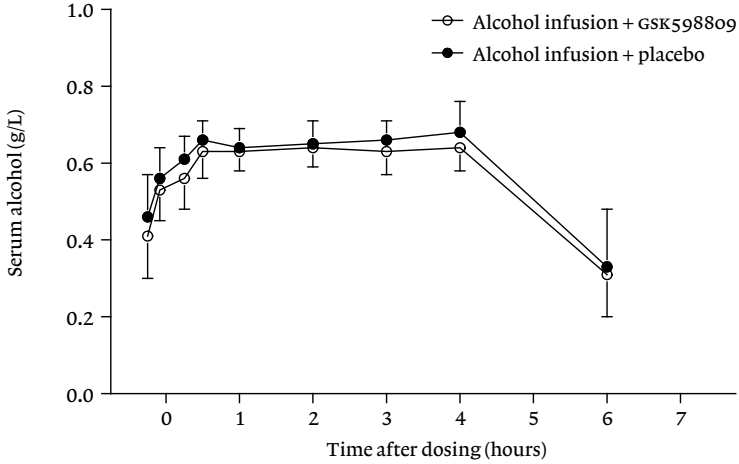
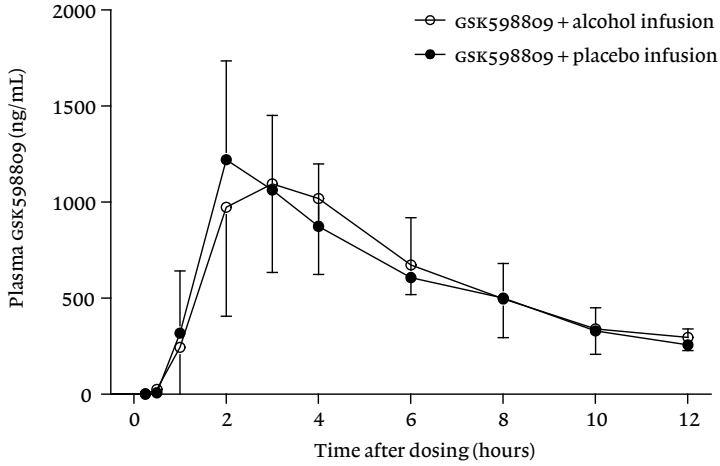


FIGURE 3 Plasma concentrations of GSK598809 after oral administration (at t=0 hours), in combination with intravenous alcohol infusion (open circles) or placebo infusion (closed circles) starting at t=-0.5 hours and continuing until t=4.5 hours. Means are presented with standard deviations as error bars.



Pharmacokinetics of GSK598809 combined with alcohol

No relevant effect of GSK598809 on ethanol pharmacokinetic parameters was observed. Regarding the effects of alcohol on GSK598809 pharmacokinetic parameters, an average 15% increase in AUC_{∞} of GSK598809 (ratio of LS geometric means 1.15; 90% confidence interval 1.02/1.30) and an average 9% decrease in C_{max} of GSK598809 (ratio of LS geometric means 0.91; 90% confidence interval 0.83/1.00) was observed after administration of GSK598809 combined with alcohol compared to GSK598809 alone. Other parameters were roughly similar compared to GSK598809 alone (see Table 2).

Pharmacodynamics of alcohol

Following alcohol infusion, a statistically significant decrease in adaptive tracking and smooth pursuit eye movements and increase in body sway were observed compared to placebo, but there were no effects on saccadic peak velocity, inaccuracy or reaction time (see Table 3 and Figures 4 to 7). Clear increases in the feeling of being drunk were noted. In addition, there was some decrease in alertness on the VAS Bond & Lader scales, compared with placebo (see Table 4 and Figure 8). Alcohol did not demonstrate any clear effect on VVLT performance (see Table 5).

Pharmacodynamics of GSK598809

Following administration of GSK598809, transient increases in serum prolactin were observed (see Figure 9). Peak prolactin levels, which increased much more in females than in males ($p < 0.0001$), were reached roughly 3 hours after study drug administration and normalized within 12 hours. Administration of GSK598809 also caused a decrease in adaptive tracking performance, which was maximal between 2 and 6 hours after dose administration (see Table 3 and Figure 6). No statistically significant effects were observed on any of the other pharmacodynamic parameters (see Tables 3 to 5 and Figures 4, 5, 7 and 8).

Pharmacodynamics of GSK598809 combined with alcohol

Co-administration of GSK598809 and alcohol resulted in additive effects on several pharmacodynamic parameters, compared to either treatment alone (see Tables 3 to 5 and Figures 4 to 8). While the effects of administration of GSK598809 alone or alcohol alone on saccadic eye movements did not reach statistical significance, co-administration of GSK598809 and alcohol resulted in a significant impairment. No significant supra-additive effects were found on any of the pharmacodynamic parameters, except a small increase in saccadic reaction time (see Table 3) and a small decrease in delayed word recall on the VVLT (see Table 5).

TABLE 2 Pharmacokinetic parameters of GSK598809 with and without co-administration of alcohol. Data are presented as geometric means (with coefficient of variation), except t_{max} which is presented as median (with range). Note: $n = 19$ for all calculated values, except t½ and AUC_{∞} of GSK598809 alone ($n = 11$) and combined with alcohol ($n = 15$), because these parameters could not be calculated reliably in 8 subjects and 4 subjects, respectively.

Parameter	GSK598809	GSK598809 + alcohol
C_{max} (ng/mL)	1320 (39)	1190 (39)
t_{max} (h)	2.07 (2.0-6.05)	3.03 (2.00-7.87)
AUC_{0-t} (ng.h/mL)	14000 (28)	15700 (27)
AUC_{∞} (ng.h/mL)	14000 (32)	16600 (23)
Terminal half life (h)	19.3 (33)	21.6 (27)

TABLE 3 Neurophysiological effects of administration of alcohol alone, GSK598809 alone and co-administration of GSK598809 and alcohol. Treatment differences in least square means are shown with statistically significant results indicated in bold.

Parameter		Alcohol compared with placebo	GSK598809 compared with placebo	GSK598809 + alcohol compared with placebo	GSK598809 + alcohol compared with alcohol alone	Supra-additive effects
Prolactin (ng/mL)	Contrast 95% CI p-value	5.33% -6.17/18.25% 0.3711	**118.4%** **94.49/145.1%** **≤0.0001**	**113.1%** **89.74/139.3%** **≤0.0001**	**102.3%** **80.26/127.1%** **≤0.0001**	-7.35% -21.3/9.10% 0.3525
Saccadic peak velocity (deg/sec)	Contrast 95% CI p-value	-2.53% -5.01/0.01% 0.0505	-1.98% -4.45/0.57% 0.1239	**-7.15%** **-9.51/-4.72%** **≤0.0001**	**-4.73%** **-7.18/-2.22%** **0.0005**	-2.81% -6.30/0.80% 0.1229
Saccadic inaccuracy (%)	Contrast 95% CI p-value	-0.23% -8.67/9.00% 0.9592	3.96% -4.82/13.56% 0.3805	**10.17%** **0.79/20.42%** **0.0334**	**10.42%** **0.97/20.75%** **0.0306**	6.21% -6.34/20.45% 0.3405
Saccadic reaction time (sec)	Contrast 95% CI p-value	2.51% -0.43/5.54% 0.0937	2.09% -0.83/5.09% 0.1584	**9.20%** **6.05/12.44%** **≤0.0001**	**6.53%** **3.43/9.72%** **≤0.0001**	**4.35%** **0.11/8.76%** **0.0443**
Smooth pursuit (%)	Contrast 95% CI p-value	**-6.9** **-10.1/-3.7** **≤0.0001**	-0.9 -4.1/2.3 0.5930	**-6.1** **-9.3/-2.9** **0.0004**	0.8 -2.4/4.0 0.6210	1.7 -2.9/6.2 0.4674
Adaptive tracking (%)	Contrast 95% CI p-value	**-2.0** **-3.7/-0.3** **0.0207**	**-2.0** **-3.7/-0.3** **0.0227**	**-5.6** **-7.3/-3.9** **≤0.0001**	**-3.5** **-5.2/-1.8** **0.0001**	-1.5 -3.9/0.9 0.2099
Body sway (mm)	Contrast 95% CI p-value	**31.83%** **9.96/58.06%** **0.0035**	4.80% -12.6/25.70% 0.6066	**41.31%** **17.69/69.67%** **0.0004**	7.18% -10.7/ 28.67% 0.4493	2.27% -21.0/32.33% 0.8618

TABLE 4 Visual analogue scales (VAS) results after administration of alcohol alone, GSK598809 alone and co-administration of GSK598809 and alcohol. Treatment differences in least square means are shown with statistically significant results indicated in bold.

Parameter		Alcohol compared with placebo	GSK598809 compared with placebo	GSK598809 + alcohol compared with placebo	GSK598809 + alcohol compared with alcohol alone	Supra-additive effects
VAS alertness (mm)	Contrast 95% CI p-value	**-6.9** **-11.4/-2.4** **0.0031**	-3.3 -7.9/1.2 0.1428	**-8.4** **-12.9/-3.9** **0.0005**	-1.5 -6.0/3.0 0.5134	1.9 -4.5/8.2 0.5574
VAS calmness (mm)	Contrast 95% CI p-value	-1.5 -3.8/0.8 0.1911	-0.3 -2.6/2.0 0.7964	-0.9 -3.2/1.4 0.4288	0.6 -1.7/2.9 0.6024	0.9 -2.4/4.2 0.5820
VAS mood (mm)	Contrast 95% CI p-value	-2.0 -5.0/0.9 0.1761	-0.9 -3.9/2.1 0.5537	-0.9 -3.9/2.1 0.5415	1.1 -1.9/4.1 0.4542	2.0 -2.2/6.2 0.3442
VAS alcohol effects (mm)	Contrast 95% CI p-value	**23.1** **13.4/32.8** **≤0.0001**	2.1 -7.6/11.8 0.6650	**24.6** **14.9/34.3** **≤0.0001**	1.5 -8.2/11.2 0.7588	-0.6 -14.3/13.1 0.9280

TABLE 5 Visual verbal learning test (VVLT) results after administration of alcohol alone, GSK598809 alone and co-administration of GSK598809 and alcohol. Treatment differences in least square means are shown with statistically significant results indicated in bold.

Parameter		Alcohol compared with placebo	GSK598809 compared with placebo	GSK598809 + alcohol compared with placebo	GSK598809 + alcohol compared with alcohol alone	Supra-additive effects
Immediate recall 1st trial	Contrast 95% CI p-value	-0.3 -1.6/1.1 0.6812	0.3 -1.1/1.7 0.6725	-0.8 -2.1/0.6 0.2516	-0.5 -1.8/0.8 0.4554	-0.8 -2.7/1.1 0.4115
Immediate recall 2nd trial	Contrast 95% CI p-value	-1.3 -2.8/0.3 0.1047	-0.1 -1.7/1.5 0.8822	-1.3 -2.9/0.2 0.0970	-0.0 -1.6/1.5 0.9640	0.1 -2.1/2.3 0.9408
Immediate recall 3rd trial	Contrast 95% CI p-value	-1.1 -2.9/0.7 0.2210	-1.0 -2.8/0.8 0.2702	**-2.7** **-4.5/-1.0** **0.0034**	-1.6 -3.4/0.1 0.0704	-0.6 -3.2/1.9 0.6206
Delayed recall	Contrast 95% CI p-value	-9.90% -26.6/10.65% 0.3135	11.31% -9.44/36.80% 0.3020	**-25.5%** **-39.4/-8.42%** **0.0061**	-17.3% -32.7/1.56% 0.0692	**-25.7%** **-44.5/-0.60%** **0.0456**
Word recognition (correct)	Contrast 95% CI p-value	-0.82% -12.1/11.91% 0.8923	2.24% -9.49/15.48% 0.7169	-8.62% -19.1/3.22% 0.1436	-7.87% -18.3/3.95% 0.1790	-9.88% -24.1/6.97% 0.2288
Word recognition (incorrect)	Contrast 95% CI p-value	9.81% -24.6/59.91% 0.6193	15.24% -21.9/70.03% 0.4670	39.26% -4.69/103.5% 0.0855	26.82% -12.3/83.41% 0.2017	10.05% -35.6/88.10% 0.7212
Reaction time (correct)	Contrast 95% CI p-value	1.97% -4.70/9.11% 0.5649	1.72% -4.98/8.90% 0.6172	0.54% -6.08/7.64% 0.8736	-1.40% -7.85/5.50% 0.6775	-3.07% -11.9/6.70% 0.5176

FIGURE 4 Time course of smooth pursuit eye movements following administration of GSK598809 capsules combined with alcohol infusion (open circles), GSK598809 capsules combined with placebo infusion (closed circles), placebo capsules combined with alcohol infusion (open triangles) and placebo capsules combined with placebo infusion (closed triangles). Least square means are presented with 95% confidence intervals as error bars.

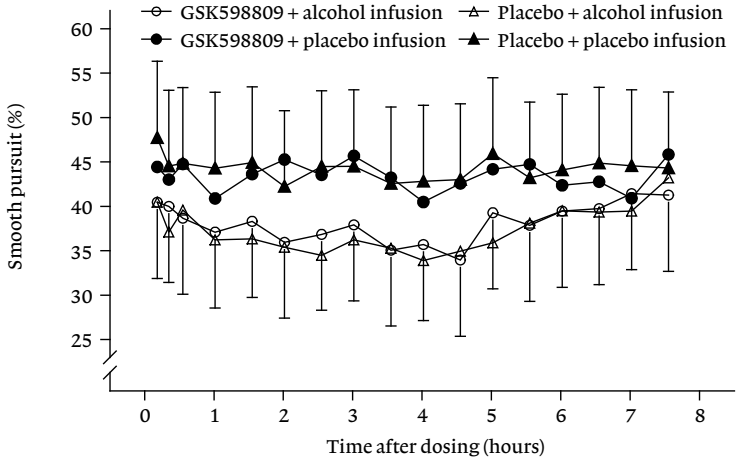


FIGURE 5. Time course of saccadic peak velocity following administration of GSK598809 capsules combined with alcohol infusion (open circles), GSK598809 capsules combined with placebo infusion (closed circles), placebo capsules combined with alcohol infusion (open triangles) and placebo capsules combined with placebo infusion (closed triangles). Least square means are presented with 95% confidence intervals as error bars.

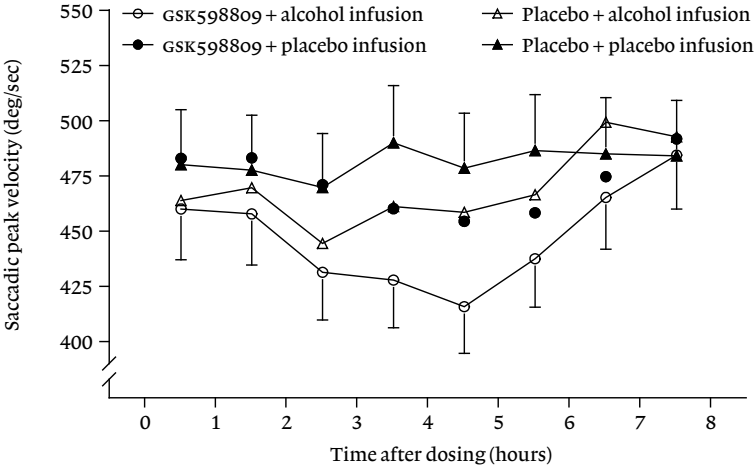


FIGURE 6 Time course of adaptive tracking performance following administration of GSK598809 capsules combined with alcohol infusion (open circles), GSK598809 capsules combined with placebo infusion (closed circles), placebo capsules combined with alcohol infusion (open triangles) and placebo capsules combined with placebo infusion (closed triangles). Least square means are presented with 95% confidence intervals as error bars.

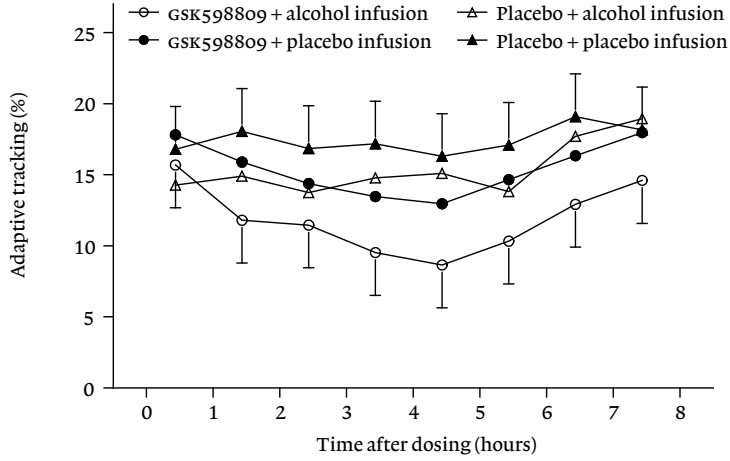


FIGURE 7 Time course of body sway following administration of GSK598809 capsules combined with alcohol infusion (open circles), GSK598809 capsules combined with placebo infusion (closed circles), placebo capsules combined with alcohol infusion (open triangles) and placebo capsules combined with placebo infusion (closed triangles). Least square means are presented with 95% confidence intervals as error bars.

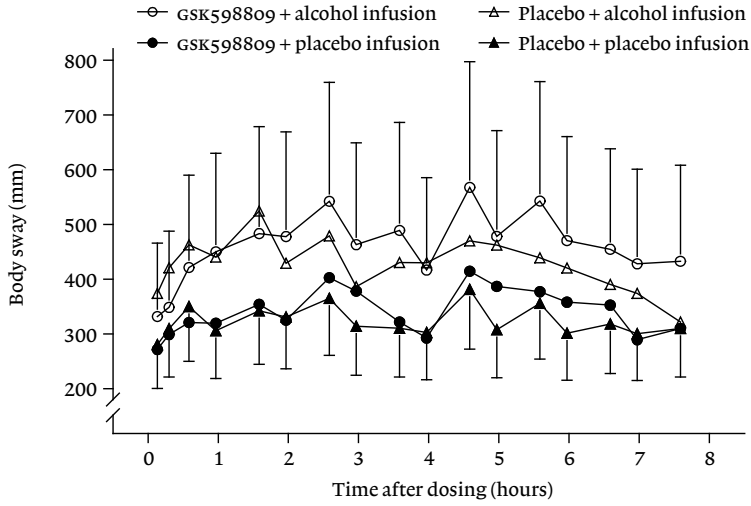


FIGURE 8 Time course of alertness (visual analogue scales of Bond & Lader) following administration of GSK598809 capsules combined with alcohol infusion (open circles), GSK598809 capsules combined with placebo infusion (closed circles), placebo capsules combined with alcohol infusion (open triangles) and placebo capsules combined with placebo infusion (closed triangles). Least square means are presented with 95% confidence intervals as error bars.

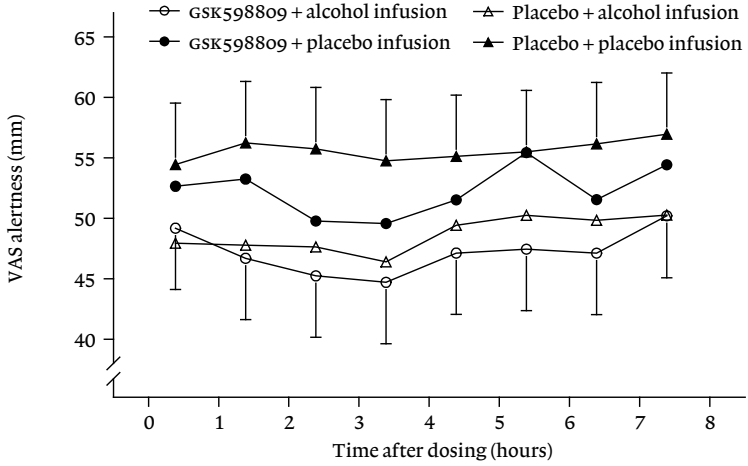
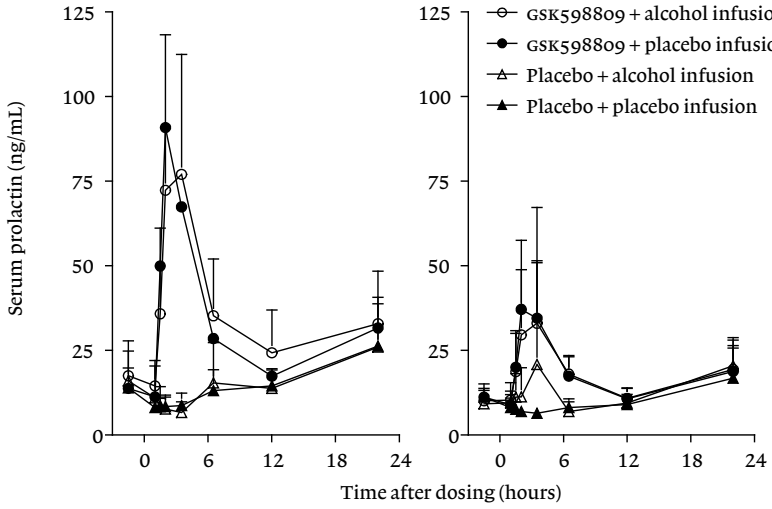


FIGURE 9 Time course of serum prolactin in female (left panel) and male subjects (right panel), following administration of GSK598809 capsules combined with alcohol infusion (open circles), GSK598809 capsules combined with placebo infusion (closed circles), placebo capsules combined with alcohol infusion (open triangles) and placebo capsules combined with placebo infusion (closed triangles). Means are presented with standard deviations as error bars.



DISCUSSION

The present study was performed to evaluate the pharmacokinetics and central nervous system (CNS) effects of single oral doses of 175 mg of the novel dopamine D_3 receptor antagonist GSK598809 in healthy volunteers and possible interactions with alcohol. Within the present group of healthy volunteers, single doses of GSK598809 were generally well tolerated. The most frequent adverse effects were mild headache, dizziness, somnolence, nausea and vomiting. GSK598809 did not induce any significant extrapyramidal symptoms. Mild short-lasting akathisia was reported spontaneously once after administration of GSK598809, although this was not verified objectively by the Barnes Akathisia Rating Scale, when this was performed according to protocol. Plasma concentration of GSK598809 increased rapidly after oral administration (t_{max} of roughly 2 to 3 hours) and subsequently decreased in an apparent bi-exponential manner (terminal half life of roughly 20 hours). No effect of GSK598809 on the pharmacokinetics of alcohol was observed, but alcohol decreased C_{max} and increased the AUC of GSK598809 to a limited extent, which is not considered to be of any clinical significance.

The CNS effects of GSK598809 alone were limited to an elevation of serum prolactin and a small decrease in adaptive tracking performance, with a time course that corresponds well with the observed pharmacokinetics. This study represents the first use of this pharmacodynamic test battery to evaluate the effects of a selective dopamine D_3 antagonist in healthy volunteers. As a result, no data of other dopamine D_3 receptor antagonists are available for comparison with the effects of GSK598809. Antipsychotic drugs (dopamine D_2 receptor antagonists) have been evaluated extensively with this pharmacodynamic test battery[30,36–38], but differences in tissue expression of D_2 and D_3 receptors and differences in receptor affinity profiles of the various drugs significantly limit the comparison of their effects to those of GSK598809.

Prolactin secretion by the lactotroph cells of the pituitary gland is under inhibitory control by dopamine, released predominantly from tuberoinfundibular dopaminergic neurons, acting on lactotrophic dopamine D_2 receptors[51,52]. Pharmacological blockade of dopamine D_2 receptors removes this inhibitory influence and subsequently increases prolactin levels. However, the role of dopamine D_3 receptor antagonism in the control of prolactin secretion is unknown. An autoradiographic study has demonstrated presence of D_3 receptors in the pituitary gland[18], but the density was quite low and any possible role for dopamine D_3 receptors in the pituitary gland in endocrine function remains unclear. Alternatively, dopamine D_3 antagonism may cause prolactin elevation by acting

at the level of the hypothalamus. The periventricular and arcuate nuclei of the hypothalamus constitute the origin of the tuberoinfundibular dopaminergic pathway, which projects to the median eminence, where dopamine is released into the hypophyseal portal vessels[51,53,54]. Hypothalamic expression of dopamine D_3 receptors has not yet been examined in full detail, but one study found no detectable levels in the arcuate nucleus, whereas the periventricular nucleus was not investigated[17]. Therefore, any possible effect of dopamine D_3 antagonism on the hypothalamus, leading to prolactin elevation, also remains unclear. Another theoretical possibility is that GSK598809 could be acting on extra-dopaminergic mechanisms of prolactin control. However, a more likely explanation is that, despite a greater than 100-fold selectivity for D_3 receptors over D_2 receptors, GSK598809 at doses of 175 mg might cause enough D_2 receptor antagonism to modestly increase prolactin secretion.

The increases in serum prolactin following GSK598809 administration were much larger in female volunteers than in male volunteers (see Figure 9). Similar gender differences in prolactin levels have been previously demonstrated after administration of typical antipsychotic drugs[55–59] and atypical antipsychotic drugs[60–62], which have been attributed to an enhanced responsiveness of lactotrophs to prolactin-releasing stimuli by females, compared to males, due to the effects of estrogens[51,63–65].

The other pharmacodynamic tests used in this study measure complex CNS functions. The neurophysiological and neurochemical mechanisms underlying these CNS functions have not yet been fully characterized, but are likely to involve multiple neurotransmitter receptor systems. The decrease in adaptive tracking performance after administration of GSK598809 indicates slight impairment in visuo-motor performance. Similar impairments in adaptive tracking performance have also been observed after administration of single doses of antipsychotic drugs (dopamine D_2 antagonists) such as haloperidol[36] and risperidone[38] in healthy volunteers. However, unlike haloperidol and risperidone, GSK598809 did not affect smooth pursuit and saccadic eye movements, memory performance or any of the visual analogue scales. This clearly demonstrates the pharmacological distinctions between GSK598809 and antipsychotic drugs, but it is not necessarily an argument for D_3 receptor selectivity, since most antipsychotic drugs affect other neurotransmitter systems in addition to D_2 receptors. Recently, however, we examined the novel selective dopamine D_2 receptor antagonist JNJ-37822681 in healthy volunteers using similar pharmacodynamic tests[66]. Single oral doses of 15 mg JNJ-37822681 caused a reduction in adaptive tracking performance of about 2%, comparable to 175 mg doses of GSK598809. Also, this dose of JNJ-37822681 caused about 60% D_2 receptor oc-

cupancy[67] and produced prolactin elevations of more than 700%[66], much larger than the 117% increase found with GSK598809 in this study. JNJ-37822681 also impaired saccadic and smooth pursuit eye movements[66], which were unaffected by GSK598809. Although these indirect comparisons have their limitations, they provide at least some support for the in vivo selectivity of GSK598809 for dopamine D_3 receptors.

In addition to obtaining a dopamine D_3 receptor-mediated profile of CNS effects, our study was specifically designed to evaluate potential pharmacokinetic and pharmacodynamic interactions between GSK598809 and alcohol. An intravenous alcohol clamping paradigm was used to achieve pseudo-steady state levels of alcohol, which produced clear and expected CNS effects, similar to previously reported results of this alcohol clamping paradigm[29,31]. Co-administration of GSK598809 with intravenous alcohol levels at pseudo-steady state was generally well tolerated. However, somnolence and fatigue were reported more frequently, compared with the other treatments. Mild akathisia was reported spontaneously once, which was not verified objectively by the Barnes Akathisia Rating Scale, similar to the event after administration of GSK598809 alone. Co-administration of GSK598809 and alcohol generally produced additive CNS effects, without clear signs of supra-additive amplification of the effects of each treatment alone. Both GSK598809 and alcohol caused slight impairments of saccadic eye movements that failed to reach statistical significance by themselves, but the combination clearly differed from placebo. There was a small supra-additive increase in saccadic reaction time (see Table 3) and there were also some indications that memory might be affected more by the combination than by each drug individually. These findings suggest that caution may be needed in the use of GSK598809 in individuals who consume alcohol moderately or excessively, although the effects will probably be dominated by alcohol.

In conclusion, the present study demonstrates elevation of serum prolactin and a small decrease in adaptive tracking performance after administration of the novel selective dopamine D_3 receptor antagonist GSK598809 within a small group of healthy volunteers. An interaction with intravenous alcohol infusion at pseudo-steady state was demonstrated, resulting in a decreased C_{max} and increased AUC of GSK598809 and mainly additive effects on several CNS parameters. Although somnolence and fatigue were reported more frequently, the combination was generally well tolerated by healthy volunteers.

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CHAPTER 5

PLACEBO- AND AMITRIPTYLINE-CONTROLLED EVALUATION OF CENTRAL NERVOUS SYSTEM EFFECTS OF THE NK_1 RECEPTOR ANTAGONIST APREPITANT AND INTRAVENOUS ALCOHOL INFUSION AT PSEUDO-STEADY STATE

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ABSTRACT

Recent interest in NK_1 receptor antagonists has focused on a potential role in the treatment of drug addiction and substance abuse. In the present study, the potential for interactions between the NK_1 receptor antagonist aprepitant and alcohol, given as an infusion at a target level of 0.65 g/L, was evaluated. Amitriptyline was included as positive control to provide an impression of the profile of central nervous system (CNS) effects. In a double-blind, randomized, placebo- and amitriptyline-controlled study, the pharmacokinetics and CNS effects of aprepitant and alcohol were investigated in 16 healthy volunteers. Cognitive and psychomotor function tests included the visual verbal learning test (VVLT), Bond and Lader visual analogue scales (VAS), digit symbol substitution test (DSST), visual pattern recognition, binary choice reaction time, critical flicker fusion (CFF), body sway, finger tapping and adaptive tracking. Alcohol impaired finger tapping and body sway. Amitriptyline impaired DSST performance, VAS alertness, CFF, body sway, finger tapping and adaptive tracking. No impairments were found after administration of aprepitant. Co-administration of aprepitant with alcohol was generally well tolerated and did not cause significant additive CNS effects, compared with alcohol alone. Therefore, our study found no indications for clinically relevant interactions between aprepitant and alcohol.

INTRODUCTION

The peptide neurotransmitter substance P and its preferred receptor, the neurokinin 1 (NK_1) receptor have been the focus of several different drug development programs. Recently, interest in NK_1 receptor antagonists has focused on a potential role in the treatment of drug addiction and substance abuse disorders[1]. Substance P may play a role in addiction-related behavior by acting directly on NK_1 receptors in brain areas associated with drug reward, such as the nucleus accumbens and ventral pallidum, and on dopaminergic neurons in the ventral tegmental area, but also by influencing other neurotransmitters such as serotonin, acetylcholine and noradrenalin[2]. Studies in animals have demonstrated that pharmacological blockade of NK_1 receptors dose-dependently suppresses alcohol intake[3] and stress-induced reinstatement of alcohol seeking behavior[4]. Also, the rewarding effects of opiates (but not cocaine) are absent in NK_1 receptor knockout mice[5,6] and in mice with bilateral ablation of NK_1 receptor-expressing neurons in the amygdala[7]. A recent case-control association study identified two haplotypes and a single nucleotide polymorphism (SNP) in the NK_1 receptor gene (NK1R) that were significantly associated with the development of alcohol dependence[8]. Furthermore, a recent clinical trial with the NK_1 receptor antagonist LY686017 in detoxified alcoholic inpatients demonstrated suppression of spontaneous alcohol cravings and improved overall well-being[9].

The present study was performed to evaluate possible interactions between the NK_1 receptor antagonist aprepitant and alcohol. Pharmacokinetic interactions are not expected because aprepitant is metabolized primarily by CYP3A4[10], whereas alcohol is metabolized by a pathway that involves alcohol dehydrogenase, catalase and CYP2E1[11]. However, pharmacodynamic interactions are theoretically possible as both compounds are centrally active and may influence several neurotransmitters, including dopamine[2,12]. A battery of quantitative tests, sensitive to the central effects of various compounds, including alcohol[13], was used to evaluate pharmacodynamic central nervous system (CNS) effects. Similar to the phase III trials with aprepitant for the indication of major depressive disorder[14], an oral dose of 160 mg of aprepitant had been chosen for this study, because this dose was generally well tolerated in the depression program and was expected to result in high occupancy of central NK_1 receptors. Positron emission tomography (PET) using [^{18}F]SPA-RQ in healthy volunteers has demonstrated that daily doses of 100 mg aprepitant or higher achieve high levels (>90%) of NK_1 receptor occupancy in the striatum[15]. The effects of co-administration of aprepitant and alcohol were primarily compared with those of alcohol alone and aprepitant alone. At the time of study execution, no other studies evaluating NK_1 receptor antagonists with this

pharmacodynamic test battery were avaliable. As a consequence, no a priori estimation of effect size of aprepitant with or without alcohol could be made. To set a clinical benchmark for the effect size of CNS effects of co-administration of aprepitant and alcohol, we included amitriptyline as a comparator drug. Amitriptyline shows a wide range of significant CNS effects in healthy volunteers, which have been well characterized previously using this pharmacodynamic test battery[16].

METHODS

Study design

Sixteen healthy male or female volunteers, between 18 and 55 years of age were planned to participate in a double-blind, randomized, placebo- and active comparator-controlled, triple-dummy, four treatment, two-period crossover study to investigate the psychomotor and cognitive effects of aprepitant and ethanol in healthy volunteers. The study was approved by the medical ethics committee of the Leiden University Medical Center. Prior to medical screening, all volunteers gave written informed consent. Medical screening included a medical history, physical examination, urinalysis, routine haematology and chemistry and 12-lead electrocardiography (ECG). All volunteers underwent training sessions for the pharmacodynamic tests in order to minimize possible learning effects.

This study was designed primarily to compare the effects of co-administration of aprepitant and alcohol with those of alcohol alone and aprepitant alone. To optimize the likelihood of detecting possible pharmacodynamic effects, aprepitant was administered daily for 7 days, after which plasma concentrations of aprepitant can be expected to be maximal (Merck & Co., data on file). As stated, amitriptyline was included in this study as an pharmacodynamic comparator, which is expected to exert its maximum tolerable effects after a single dose.

The study consisted of study periods of 10 days (see Figure 1). On day 1, all volunteers were administered 50 mg amitriptyline (or placebo capsules) and a placebo-ethanol infusion (consisting of a 5% glucose solution). On day 2 and 3, all volunteers were administered placebo capsules to allow a complete washout of amitriptyline. On days 4 to 10, all volunteers took 160 mg aprepitant once daily as nanoparticle capsules (or placebo capsules). On day 10, all volunteers received an active alcohol infusion (5% alcohol in a 5% glucose solution, see below for details). Psychomotor and cognitive testing was performed on day 1, 9 and 10 (see Figure 2). This study design minimizes carry-over effects of amitriptyline administration at day 1 on the pharmacodynamic measurements on days 9 and 10, as the half life of amitriptyline is roughly 20 hours[17]. On days 2, 4, 6 and 8, the volunteers reported to the clinic for administration of study medication. On

days 3, 5 and 7, the volunteers administered study medication at home, which was confirmed by telephone. Each volunteer participated in two study periods in a randomized, blinded crossover fashion (see Figure 1). Both study periods were separated by a washout period of at least 14 days.

FIGURE 1 Study design. Active treatments are indicated in bold.

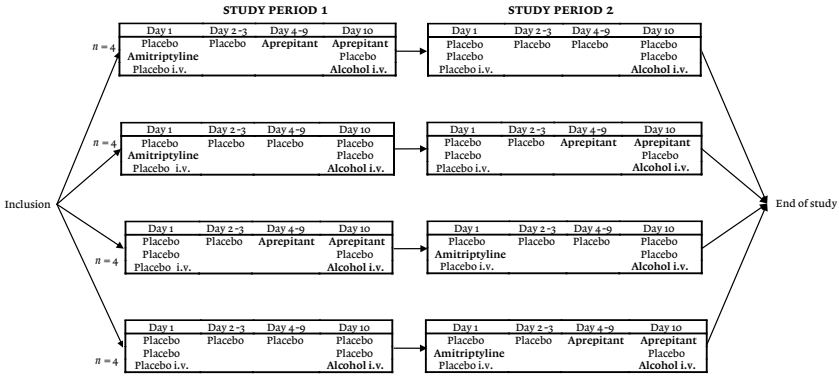
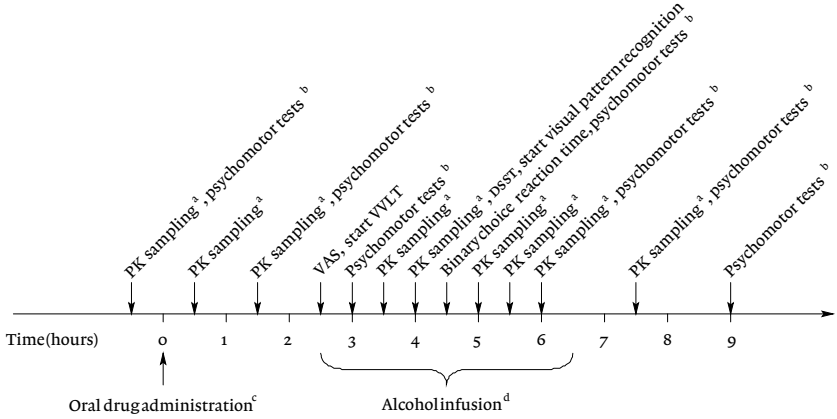


FIGURE 2 Schedule of pharmacokinetic and pharmacodynamic tests performed on days 1, 9 and 10 of both study periods. [a] Pharmacokinetic (PK) sampling includes blood sampling for aprepitant concentration analysis on study days 9 and 10, and breath sampling for alcohol concentration analysis on study day 1 and 10. [b] Psychomotor tests include the adaptive tracking test, critical flicker fusion, finger tapping, and body sway. [c] Amitriptyline (or placebo) on study day 1, aprepitant (or placebo) on study day 9 and 10. [d] Alcohol infusion on study day 10 and placebo infusion on day 1



This rather complex study design enables, after combining both study periods, analysis of the following comparisons:

- On day 1, single doses of amitriptyline (or placebo) are administered, followed by pharmacokinetic and pharmacodynamic measurements. These data enable analysis of the effects of single oral doses of amitriptyline ($n=16$) compared with placebo ($n=16$).
- On days 4-9, single daily doses of aprepitant (or placebo) are administered. On day 9, pharmacokinetic and pharmacodynamic measurements are performed. These data enable analysis of the effects of aprepitant ($n=16$) compared with placebo ($n=16$).
- On day 1, a placebo-ethanol infusion (consisting of a 5% glucose solution) and placebo capsules (or amitriptyline) are administered, followed by pharmacokinetic and pharmacodynamic measurements. On day 10, an alcohol infusion and placebo capsules (or aprepitant) are administered, followed by pharmacokinetic and pharmacodynamic measurements. These data enable analysis of the effects of alcohol infusion ($n=16$) compared with placebo infusion ($n=16$), although it is recognized that the estimated effects are confounded with day.
- On day 10, an alcohol infusion and aprepitant (or placebo capsules) are administered, followed by pharmacokinetic and pharmacodynamic measurements. These data enable analysis of co-administration of aprepitant and alcohol ($n=16$), compared with co-administration of placebo and alcohol ($n=16$).

Alcohol infusion paradigm

The procedure for attaining pseudo-steady state alcohol levels was based on the method of Hartmann et al[18] and performed as described earlier[19–21]. In brief, alcohol (ethanol 5% w/v solution in 5% glucose) was administered intravenously to achieve a target blood alcohol concentration of 0.65 g/L, beginning 2½ hours after administration of aprepitant (or placebo) and ending 6½ hours after study drug administration in order to coincide with the expected maximal plasma concentration of aprepitant (t_{max} of the marketed 80 mg and 125 mg capsules is roughly 4 hours)[22]. Breath alcohol concentrations were determined at 0, 60, 90, 150, 180, 210 and 300 minutes after start of the alcohol infusion using a calibrated hand-held Alco-Sensor IV Intoximeter (Honac, Apeldoorn, the Netherlands), which has a limit of quantification (LOQ) of 0.01 g/L. Alcohol infusion was performed at a constant rate for the first hour, followed by a slower constant rate over the next 3 hours to maintain the target level.

Rates of infusion were set individually, based on measured alcohol kinetics in each volunteer during a separate alcohol infusion, after inclusion in the study

but prior to the first study period. During this pre-study infusion, 50 gram of alcohol was administered intravenously over 1 hour (500 mL of 100 g/L ethanol solution in 5% glucose). Serum alcohol and breath alcohol concentrations were determined prior to infusion and at 30, 55, 75, 90, 120, 180, 240, 300 and 360 minutes after start of the alcohol infusion. Individual pharmacokinetic parameters were calculated by fitting the serum alcohol concentrations to a two-compartment open model with Michaelis-Menten kinetics, derived from previous studies[18-20]. Population parameters were used as priors in a Bayesian nonlinear regression analysis to generate pharmacokinetic parameters of individual infusion regimes. The regime that approaches and stays at 0.65 g/L was applied on subsequent alcohol infusions. Pseudo-steady state alcohol levels were attained after approximately 90 minutes. To avoid an overshoot in the alcohol levels, the infusion of alcohol was terminated whenever the level of 1.00 g/L was reached. Pharmacokinetic modeling and simulation was performed using NONMEM (software version V, University of California, San Francisco, USA).

Aprepitant pharmacokinetics

Venous blood samples for aprepitant concentration analysis were collected prior to study drug administration on days 1, 9 and 10 and at ½, 1½, 3½, 4, 5, 5½, 6 and 7½ hours after study drug administration of aprepitant (or placebo) on days 9 and 10 (see Figure 2). The concentration of aprepitant in plasma samples was determined using HPLC-MS/MS with a lower limit of quantification (LLQ) of 10 ng/mL, using a previously reported method[23,24].

Safety monitoring

Evaluation of adverse events, 12-lead electrocardiograms (ECG), blood pressure, heart rate, body temperature, urinalysis and blood sampling for haematology and chemistry was performed at regular time points during the study.

Pharmacodynamic testing

Volunteers were tested on days 1, 9 and 10 of each study period individually in a quiet room with ambient illumination. Cognitive function tests included the visual verbal learning test (VVLT) and Bond and Lader visual analogue scales (VAS), which were performed roughly 2½ hours after study drug administration (see Figure 2), and the digit symbol substitution test (DSST), visual pattern recognition with immediate and delayed recall and binary choice reaction time, which were performed roughly 4 hours after study drug administration (see Figure 2). Psychomotor function tests were performed prior to dose administration and at 1½, 3, 4½, 6, 7½ and 9 hours after drug administration (see Figure

2) and included critical flicker fusion (CFF), body sway, finger tapping and adaptive tracking. The primary endpoint of this study was the digit symbol substitution test (DSST). The visual verbal learning test (VVLT), Bond and Lader visual analogue scales (VAS), pattern recognition, binary choice reaction time, critical flicker fusion (CFF), finger tapping, adaptive tracking, and body sway were secondary endpoints. Change from baseline critical flicker fusion (CFF), finger tapping, adaptive tracking and body sway were exploratory endpoints.

Digit-symbol substitution test

During the digit-symbol substitution test (DSST)[25], the volunteer is asked to assign symbols to random digits using a substitution key that is presented on the worksheet. Each digit-symbol association constitutes one response and volunteers are instructed to complete as many responses as possible within 90 seconds. The number of correct substitutions is analyzed.

Visual verbal learning test

During the visual verbal learning test (VVLT)[26], three trials of 30 words are presented on a computer screen in the same sequence. The volunteer is requested to reproduce as many words as possible at the ending of each trial (immediate recall) and after 30 minutes (delayed recall). The number of correctly reproduced words is analyzed for each trial. Also, a recognition test is performed, consisting of 15 previously presented words and 15 new words, in which the volunteer has to indicate recognition of the word (delayed recognition) as quickly as possible. Response time (msec) and the number of correctly recognized words are analyzed.

Visual pattern recognition

A trial of 14 abstract visual patterns is presented on a computer screen for a duration of 3 seconds per pattern. A recognition test is performed, consisting of the same 14 previously presented patterns along with new patterns, in which the volunteer has to indicate recognition of the pattern as quickly as possible at the ending of the trial (immediate recall) and after 30 minutes (delayed recall). Response time (msec) and the number of correctly recognized patterns are analyzed.

Binary choice reaction time

Choice reaction time[27] is measured by displaying either a red or green block on either the left side or right side of a computer screen in random order. The volunteer reacts by pushing a button on either side of the keyboard, corresponding to

the position of the colored block on the screen. Sixty stimuli are presented and response time (msec) and the number of correct responses are analyzed.

Visual analogue scales

Subjective effects were quantified using a Dutch translation of the visual analogue scales (VAS), originally described by Norris[28], to derive three composite factors corresponding to alertness, mood (contentedness) and calmness, as described by Bond & Lader[29].

Critical flicker fusion

An intermittent light source is used with an increasing and decreasing frequency. The frequency at which the flickering light is perceived as a steady light source is termed the critical flicker fusion (CFF) threshold, which is a measure for CNS activation[30]. Volunteers are requested to respond by pressing a button at the moment they see fusion of flickering (when frequency is increased) or when the light starts to flicker (when frequency is decreased). Average response (threshold frequency in Hz) during four sequences is calculated.

Body sway

Postural stability in the sagittal plane was measured with an apparatus similar to the Wright ataxiameter[31], using a string attached to the waist of the volunteer. Movements over a period of two minutes, while standing still with eyes closed, were integrated and expressed as mm sway.

Finger tapping

The finger tapping test was adapted from the Halstead-Reitan test battery[32] to evaluate motor activation and fluency. The volunteer is instructed to rest the wrist of the dominant hand on a table and to tap as quickly as possible with the index finger onto the space bar of a key board. The mean tapping rate is used for statistical analysis.

Adaptive tracking

To evaluate visuo-motor coordination, the adaptive tracking task was performed as described previously[33], using customized equipment and software developed by K.W. Hobbs (Hertfordshire, UK). Adaptive tracking is a pursuit tracking task in which a circle moves randomly over a computer screen and the volunteer must try to keep a dot inside the moving circle using a joystick. If this effort is successful, the speed of the moving circle is increased and if the effort is unsuccessful, the speed is reduced. Performance was scored over a fixed period of 10 minutes.

Statistical analysis

Evaluation of the numbers of correct substitutions from the digit symbol substitution test (DSST) was performed using an analysis of variance (ANOVA) model appropriate for a two-period crossover design, with factors for sequence, subject-within-sequence, period, treatment and within subject error. A two-sided 90% confidence interval (equivalent to a one-sided 95% confidence interval) for the true mean treatment difference was computed from the ANOVA using the mean squared error and referencing a *t*-distribution. Data from day 1 were used for comparison of amitriptyline and placebo. Data from day 9 were used for comparison of aprepitant and placebo. Data from day 10 were used for comparison of alcohol and co-administration of aprepitant and alcohol. An ANOVA with factors for subjects and treatment was used to compare alcohol (data from day 10) and placebo for alcohol (data from day 1), although the estimated alcohol effect was confounded with day. VVLT results, visual pattern recognition with immediate and delayed recall, binary choice reaction time and visual analogue scales were each analyzed with the same methods used for the DSST. It was determined post-hoc that due to observed statistically significant differences between treatment groups at baseline, critical flicker fusion (CFF), finger tapping, adaptive tracking and body sway should be baseline adjusted. The change from baseline values were also analyzed with the same methods used for the DSST, with the addition of factors for hour and the interaction of treatment-by-hour to the ANOVA model. The measurements performed 1½ hours after study drug administration were not included in the change from baseline analyses, because this time point was prior to alcohol infusion. For body sway data, the fold change from baseline was calculated, because the data were distributed as log-normal. All tests were performed at a significance level of 0.05 (two-tailed). No corrections for multiple comparisons were made.

In addition, to get an impression of possible effects of alcohol infusion on the pharmacokinetics of aprepitant after multiple doses, the repeated measured aprepitant plasma concentrations after co-administration of alcohol and aprepitant on day 10, were compared to those after administration of aprepitant alone on day 9, using a mixed model analysis of variance with treatment, time and treatment by time as fixed factors and subject, subject by treatment and subject by time as random factors. Because the alcohol infusion started 2½ hours after administration of aprepitant (or placebo), only the blood samples taken after $t = 2½$ hours were included in the analysis. Furthermore, because on days 9 and 10, aprepitant had already been administered for 6 and 7 days, respectively, the prevalues (i.e. the plasma concentration measurements before aprepitant administration on days 9 and 10, respectively) were included in the model as a covariate.

It is recognized that, in this analysis, treatments are sequential and not randomized. Data were natural log-transformed prior to analysis and the log scale estimates of the treatment difference (plasma concentration of aprepitant with or without alcohol) and 95% confidence interval for the treatment difference were exponentiated to obtain the geometric mean ratio and 95% confidence interval.

RESULTS

Subjects

A total of 17 healthy volunteers (9 males and 8 females) were included and randomized. Participants had a mean age of 27 years (range 18-53), weight of 77.1 kg (range 52.6-95) and body mass index of 24.9 kg/m^2 (range 19.8-30.2). One volunteer was withdrawn after completing the first study period, because of phlebitis on both arms on day 11. This volunteer was randomized to receive treatments B and C and was replaced by a new volunteer who completed both study periods. Safety data from all 17 volunteers is reported below. Statistical analysis was subsequently performed on all pharmacokinetic and pharmacodynamic data sets of all volunteers who completed both study periods (i.e. $n = 16$).

Clinical observations

Adverse events were generally mild and occasionally moderate in severity. No serious adverse events occurred during the study. The most frequently reported adverse events after administration of aprepitant were tiredness or somnolence (7), headache (2) and dizziness (2). The most frequently reported adverse events after administration of amitriptyline were tiredness or somnolence (15) and dizziness (2). The most frequently reported adverse events after alcohol infusion were feeling drunk (13), tiredness or somnolence (6), altered taste (2) and local infusion reactions (which generally consisted of pain at the infusion site) (9). The combination of aprepitant with alcohol infusion did not affect the frequency or intensity of feeling drunk and local infusion reactions. The most frequently reported adverse events after co-administration of aprepitant and alcohol were feeling drunk (13), local infusion reactions (10), tiredness or somnolence (9), headache (3), nausea (2), altered taste (2) and dry mouth (2). There were no clinically relevant changes in heart rate, blood pressure, haematology, biochemistry, urinalysis or ECG.

Pharmacokinetics of alcohol

Following intravenous infusion, breath alcohol levels increased rapidly and remained fairly constant at the target level all over the time of infusion (see Figure 3).

FIGURE 3 Breath alcohol levels after intravenous alcohol infusion starting at t = 2½ hours and continuing until t = 6½ hours on day 10, in combination with oral administration (at t = 0 hours) of aprepitant (open circles) or placebo (closed circles). Means are presented with standard deviations as error bars.

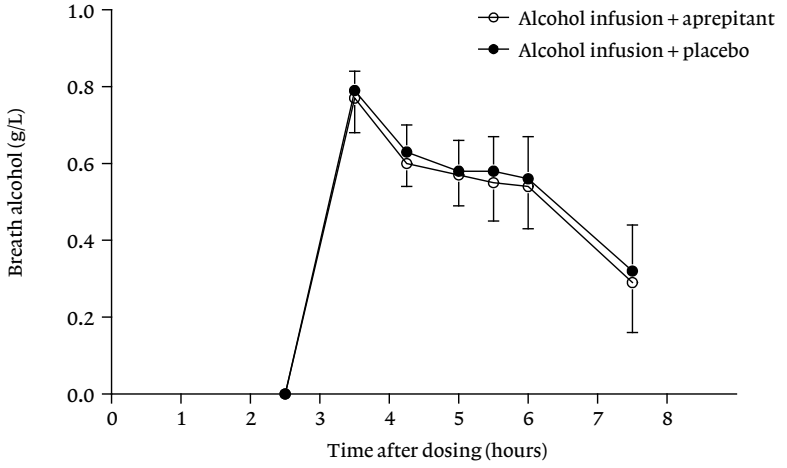
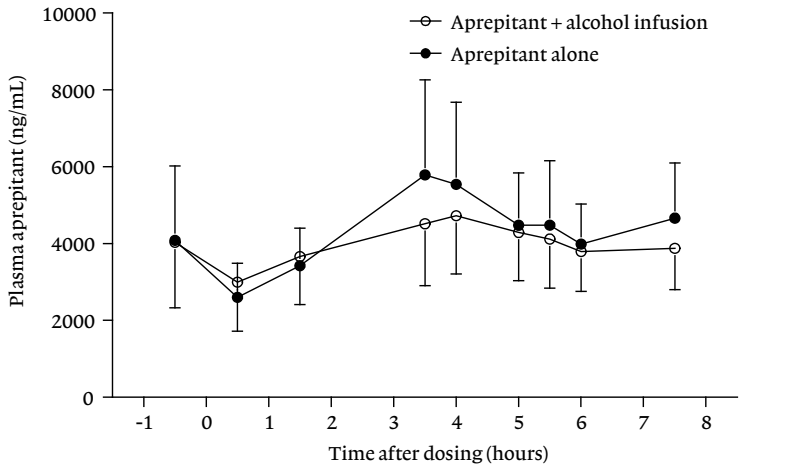


FIGURE 4 Plasma levels of aprepitant after oral administration at t = 0 hours, either alone on day 9 (closed circles) or in combination with alcohol infusion on day 10 (open circles), starting at t = 2½ hours and ending at t = 6½ hours. Means are presented with standard deviations as error bars.



Pharmacokinetics of aprepitant

As expected, plasma concentrations of aprepitant during study days 9 and 10 were generally quite stable, showing only a small increase at 3.5 to 4 hours after dose administration (see Figure 4), consistent with its t_{max}.

Pharmacokinetics of co-administration of alcohol and aprepitant

Plasma concentrations of aprepitant (see Figure 4) were higher when administered alone on day 9, compared with co-administration of alcohol on day 10 (estimate of difference in mean time-profile in percents was 11.3%; 95% confidence interval 3.9/18.2%; $p = 0.0065$).

Pharmacodynamics of alcohol

No statistically significant differences in the results of DSST, binary choice reaction time or any of the visual analogue scales were observed, as demonstrated in Table 1. Despite a small decrease in immediate recall after the first and third trial ($p = 0.049$ and $p = 0.026$, respectively) and shorter reaction time (for incorrect answers) for word recognition ($p = 0.012$), no consistent effects of alcohol on VVLT performance was observed. Also, despite a shorter reaction time (for incorrect answers) for immediate recognition ($p = 0.042$), no consistent effects of alcohol administration on visual pattern recognition were observed.

As demonstrated in Table 2, body sway and finger tapping were significantly impaired ($p = 0.029$ or lower), whereas critical flicker fusion did not demonstrate any clear effect of alcohol administration compared to placebo. Adaptive tracking performance was significantly decreased at 7.5 hours postdose ($p = 0.012$), while decreases at 4.5 hours and 6 hours approached (but failed to reach) significance level.

Pharmacodynamics of amitriptyline

As demonstrated in Table 1, amitriptyline significantly decreased DSST performance compared to placebo ($p = 0.008$). Despite a small decrease in immediate recall (numbers correct) after the third trial ($p = 0.014$), no consistent effects of amitriptyline on VVLT performance were observed. Visual pattern recognition and binary choice reaction time remained unaffected. VAS alertness was significantly decreased ($p = 0.005$), but no differences were found in any of the other visual analogue scales.

As demonstrated in Table 2, critical flicker fusion, body sway, finger tapping and adaptive tracking were significantly reduced after administration of amitriptyline compared to placebo ($p = 0.044$ and lower).

TABLE 1 Results of cognitive tests ($n=16$). All results are expressed as differences in treatment means (with 90% confidence intervals and p-values). Statistically significant results are indicated in bold.

		Alcohol versus placebo		Amitriptyline versus placebo		Aprepitant versus placebo		Co-administration of alcohol and aprepitant versus alcohol alone	
		Difference (90% CI)	p-value	Difference (90% CI)	p-value	Difference (90% CI)	p-value	Difference (90% CI)	p-value
DSST	Number (correct)	-0.75 (-5.75/4.25)	0.796	**-6.62 (-10.39/-2.86)**	**0.008**	**3.00 (0.55/5.45)**	**0.049**	-1.94 (-5.72/1.85)	0.382
Word learning	Immediate recall 1st trial	**-1.81 (-3.30/-0.33)**	**0.049**	-0.94 (-2.19/0.31)	0.207	1.00 (-0.36/2.36)	0.215	1.19 (-0.49/2.87)	0.233
	Immediate recall 2nd trial	-1.38 (-3.01/0.26)	0.160	-0.81 (-2.54/0.92)	0.422	1.06 (-0.03/2.15)	0.108	-0.04 (-2.20/2.12)	0.976
	Immediate recall 3rd trial	**-2.06 (-3.52/-0.60)**	**0.026**	**-2.81 (-4.58/-1.05)**	**0.014**	1.38 (-0.13/2.88)	0.130	1.16 (-1.72/4.03)	0.490
	Delayed recall	-1.37 (-3.10/0.35)	0.182	-2.00 (-3.81/-0.19)	0.073	1.44 (0.21/2.66)	0.058	-0.25 (-2.88/2.38)	0.870
Word recognition	Number (correct)	0.06 (-1.14/1.27)	0.929	-2.00 (-4.28/0.28)	0.145	0.94 (-1.01/2.88)	0.410	0.06 (-1.76/1.87)	0.957
	Reaction time (correct)	-15.89 (-40.44/8.67)	0.274	-1.63 (-55.62/52.36)	0.958	4.08 (-43.46/51.62)	0.882	-6.90 (-45.03/31.23)	0.753
	Reaction time (incorrect)	**-105.82 (-170.12/-41.51)**	**0.012**	-34.23 (-98.84/30.39)	0.363	191.27 (22.75/359.78)	0.066	59.23 (-78.17/196.63)	0.412
Pattern recognition (immediate)	Number (correct)	0.72 (-0.01/1.45)	0.104	0.42 (-0.64/1.47)	0.498	-0.06 (-0.98/0.86)	0.906	0.13 (-1.03/1.30)	0.841
	Reaction time (correct)	-215.89 (-438.72/6.94)	0.110	154.32 (-53.54/362.18)	0.212	-85.62 (-196.13/24.89)	0.194	-17.04 (-194.59/160.50)	0.867
	Reaction time (incorrect)	**-507.22 (-896.54/-117.89)**	**0.042**	26.58 (-359.96/413.12)	0.900	-12.22 (-317.72/293.28)	0.943	**320.67 (87.17/554.18)**	**0.043**
Pattern recognition (delayed)	Number (correct)	-0.06 (-0.69/0.57)	0.864	-0.01 (-0.93/0.90)	0.984	1.12 (0.07/2.17)	0.082	0.75 (0.13/1.37)	0.052
	Reaction time (correct)	-72.82 (-331.63/186.00)	0.629	57.91 (-165.49/281.30)	0.654	-38.66 (-194.85/117.52)	0.668	44.03 (-189.69/277.75)	0.745
	Reaction time (incorrect)	-218.30 (-568.70/132.10)	0.291	-159.90 (-686.36/366.56)	0.598	-141.30 (-322.02/39.42)	0.177	412.19 (4.83/819.55)	0.097
Binary choice reaction time	Number (correct)	-0.19 (-1.49/1.11)	0.804	-0.50 (-2.10/1.10)	0.592	-0.26 (-0.99/0.48)	0.548	-1.62 (-3.31/0.06)	0.112
	Reaction time (correct)	-10.37 (-31.78/11.03)	0.409	25.56 (-0.50/51.63)	0.106	-14.35 (-37.38/8.69)	0.291	0.87 (-12.65/14.40)	0.911
	Reaction time (incorrect)	85.38 (3.75/167.00)	0.087	91.56 (-3.56/186.68)	0.112	22.03 (-65.02/109.08)	0.662	56.88 (-6.82/120.57)	0.138
Visual analogue scales	Factor 1: alertness	0.89 (-0.52/2.31)	0.285	**2.25 (1.07/3.43)**	**0.005**	-0.31 (-1.19/0.57)	0.545	0.38 (-0.91/1.67)	0.610
	Factor 2: contentedness	0.09 (-0.79/0.96)	0.865	0.31 (-0.62/1.23)	0.571	0.05 (-0.76/0.85)	0.923	0.38 (-0.68/1.44)	0.543
	Factor 3: calmness	0.02 (-0.57/0.60)	0.962	-0.46 (-0.93/0.00)	0.101	0.09 (-0.24/0.42)	0.646	0.30 (-0.22/0.82)	0.332

TABLE 2 Results of psychomotor tests ($n=16$) using change from baseline analysis. All results are expressed as differences in treatment means (with 90% confidence intervals and p-values), except body sway data which are expressed as geometric mean ratios (with 90% confidence intervals and p-values). Statistically significant results are indicated in bold.

		Alcohol versus placebo		**Amitriptyline versus placebo**		**Aprepitant versus placebo**		**Co-administration of alcohol and aprepitant versus alcohol alone**	
		Difference (90% CI)	p-value	Difference (90% CI)	p-value	Difference (90% CI)	p-value	Difference (90% CI)	p-value
Critical flicker fusion	3 hours	-0.59 (-1.32/0.13)	0.176	-0.12 (-1.07/0.83)	0.833	-0.59 (-1.47/0.29)	0.271	-0.05 (-0.91/0.81)	0.923
	4.5 hours	-0.43 (-1.16/0.29)	0.322	**-1.18 (-2.03/-0.33)**	**0.023**	-0.03 (-0.82/0.76)	0.953	0.64 (-0.13/1.41)	0.173
	6 hours	-0.29 (-1.01/0.43)	0.510	**-1.36 (-2.21/-0.51)**	**0.009**	0.40 (-0.39/1.19)	0.399	0.27 (-0.50/1.04)	0.561
	7.5 hours	-0.13 (-0.85/0.60)	0.775	**-1.44 (-2.29/-0.58)**	**0.006**	0.27 (-0.52/1.06)	0.575	-0.14 (-0.91/0.63)	0.763
	9 hours	0.42 (-0.30/1.14)	0.335	**-1.10 (-1.95/-0.25)**	**0.034**	0.84 (0.05/1.63)	0.079	-0.43 (-1.20/0.34)	0.357
Body sway (ratio)	3 hours	**1.46 (1.19/1.79)**	**0.002**	0.99 (0.79/1.24)	0.945	1.00 (0.86/1.17)	0.979	1.21 (0.97/1.52)	0.159
	4.5 hours	**1.44 (1.18/1.77)**	**0.004**	**1.60 (1.30/1.95)**	**<0.001**	1.08 (0.94/1.24)	0.337	1.05 (0.85/1.29)	0.712
	6 hours	**1.42 (1.16/1.74)**	**0.005**	**1.58 (1.29/1.93)**	**<0.001**	1.01 (0.88/1.16)	0.889	1.09 (0.89/1.33)	0.484
	7.5 hours	1.16 (0.95/1.42)	0.225	**1.29 (1.05/1.58)**	**0.040**	1.08 (0.94/1.24)	0.357	0.98 (0.80/1.19)	0.840
	9 hours	1.00 (0.81/1.22)	0.977	1.16 (0.95/1.42)	0.220	**1.18 (1.03/1.35)**	**0.047**	1.06 (0.87/1.30)	0.616
Finger tapping	3 hours	**-3.96 (-6.40/-1.52)**	**0.008**	**-0.30 (-2.64/2.04)**	**0.832**	0.90 (-1.26/3.05)	0.493	1.48 (-1.07/4.03)	0.338
	4.5 hours	**-4.07 (-6.51/-1.63)**	**0.007**	**-3.37 (-5.47/-1.27)**	**0.009**	0.00 (-1.93/1.93)	1.000	-0.34 (-2.62/1.94)	0.807
	6 hours	**-5.63 (-8.07/-3.18)**	**<0.001**	**-2.57 (-4.67/-0.47)**	0.044	0.29 (-1.64/2.22)	0.801	2.05 (-0.23/4.33)	0.138
	7.5 hours	**-3.99 (-6.43/-1.55)**	**0.008**	**-2.35 (-4.45/-0.25)**	**0.066**	-0.58 (-2.51/1.35)	0.619	0.20 (-2.12/2.52)	0.889
	9 hours	**-3.26 (-5.70/-0.82)**	**0.029**	**-2.81 (-4.91/-0.72)**	0.028	-0.07 (-2.00/1.86)	0.953	0.50 (-1.78/2.78)	0.717
Adaptive tracking	3 hours	0.18 (-2.84/3.19)	0.923	-2.90 (-6.66/0.86)	0.204	-0.58 (-2.49/1.32)	0.613	-0.80 (-3.66/2.06)	0.645
	4.5 hours	-3.32 (-6.33/-0.31)	0.070	**-8.20 (-11.56/-4.84)**	**<0.001**	0.97 (-0.76/2.71)	0.353	1.93 (-0.59/4.46)	0.206
	6 hours	-3.64 (-6.72/-0.57)	0.052	**-5.51 (-8.99/-2.03)**	**0.010**	1.44 (-0.27/3.14)	0.165	3.00 (0.48/5.52)	0.051
	7.5 hours	**-4.65 (-7.66/-1.64)**	**0.012**	**-4.50 (-7.86/-1.14)**	**0.028**	-0.17 (-1.87/1.53)	0.870	**3.10 (0.58/5.63)**	**0.043**
	9 hours	-0.52 (-3.58/2.54)	0.779	-1.88 (-5.24/1.48)	0.356	0.74 (-0.96/2.44)	0.472	2.48 (-0.09/5.05)	0.112

Pharmacodynamics of aprepitant

As demonstrated in Table 1, the CNS effects of aprepitant were limited. There was no impairment of DSST performance, VVLT performance, binary choice reaction time or any of the visual analogue scales, compared with placebo.

As demonstrated in Table 2, no clear effects on critical flicker fusion, body sway, finger tapping and adaptive tracking were demonstrated after administration of aprepitant compared to placebo.

Pharmacodynamics of co-administration of aprepitant and alcohol

No statistically significant effects were observed in the results of DSST, binary choice reaction time or any of the visual analogue scales after co-administration of alcohol and aprepitant compared to administration of alcohol alone, as demonstrated in Table 1. Despite a longer reaction time (for incorrect answers) for immediate recognition after co-administration of alcohol and aprepitant compared to administration of alcohol alone ($p = 0.043$), no consistent effects of co-administration of aprepitant and alcohol on visual pattern recognition was observed.

As demonstrated in Table 2, no clear effects on critical flicker fusion, body sway, finger tapping and adaptive tracking were demonstrated after co-administration of alcohol and aprepitant compared with alcohol alone.

DISCUSSION

This study had been performed primarily to evaluate central nervous system (CNS) effects of single oral doses of the now marketed NK_1 receptor antagonist aprepitant and possible interactions with alcohol in healthy volunteers, using an intravenous alcohol infusion paradigm to achieve pseudo-steady state levels of alcohol. Aprepitant was generally well tolerated in this study and adverse events were similar to those reported previously in healthy volunteers[22] and patients with major depressive disorder[14], although diarrhea was not reported in our group of volunteers. Co-administration of aprepitant with intravenous alcohol infusion at pseudo-steady state was also generally well tolerated. Adverse events were comparable with those after administration of alcohol alone.

Following administration of aprepitant alone, no significant impairments were observed with either the cognitive or the psychomotor tests. This study represents the first use of this pharmacodynamic test battery to evaluate the effects of a selective NK_1 antagonist in healthy volunteers. As a result, no data of other selective NK_1 receptor antagonists are available for comparison with the effects of aprepitant. Recently, the NK_3 receptor antagonist talnetant has been

evaluated with this pharmacodynamic test battery in healthy volunteers[34]. Single oral doses of 200 mg talnetant decreased VAS calmness and alpha power EEG and improved adaptive tracking performance. However, differences in tissue expression of NK_1 and NK_3 receptors and differences in receptor affinity profiles of aprepitant and talnetant significantly limit the comparison of their effects. Another recent study using a similar pharmacodynamic test battery evaluated possible interactions of alcohol and GSK1144814, a dual antagonist at both NK_1 and NK_3 receptors[35]. Co-administration of GSK1144814 and alcohol resulted in small additional impairments in saccadic reaction time and peak velocity, adaptive tracking performance, alertness, sleepiness, word recognition score and recognition reaction time at some point, compared with the effects of alcohol alone. The effects of GSK1144814 alone (without co-administration of alcohol) were not investigated, but the interaction with alcohol suggests that GSK1144814 either has small pharmacodynamic effects of its own or that GSK1144814 slightly modifies the effects of alcohol. However, the limited effect size of the interaction suggests that the pharmacodynamic effects of GSK1144814 are also limited. Therefore, antagonists at central NK_1 receptors seem to affect CNS performance of healthy volunteers to a rather limited extent.

Positron emission tomography (PET) using [^{18}F]SPA-RQ in healthy volunteers has demonstrated that daily doses of 100 mg aprepitant or higher can achieve high levels (>90%) of NK_1 receptor occupancy in the striatum[15], which provides support for sufficient CNS penetration and NK_1 receptor occupancy by aprepitant at the dose employed in this study. Therefore, the limited effect size and scope of the CNS effects of aprepitant in this study do not seem to result from lack of central NK_1 receptor occupancy. It has been suggested that antagonism of neuropeptide receptors may show less dramatic effects than antagonism of classic neurotransmitter receptors, because the neuromodulatory nature of substance P and other neuropeptides seems to result in milder effects than drugs that interfere directly with the levels of monoamines and amino acid transmitters[36]. In addition, much evidence indicates that neuropeptides are released after stressful and noxious stimuli[36–38]. Accordingly, it has been suggested that neuropeptides exert their main action after various types of challenges or pathological conditions[36,38]. Neuropeptide receptor antagonists might therefore have significant effects in pathological conditions with increased peptide release, whereas effects in normal healthy volunteers are limited[36,38].

In addition to obtaining a NK_1 receptor mediated profile of CNS effects, our study was specifically designed to evaluate potential interactions between aprepitant and alcohol. Pharmacokinetic interactions between aprepitant and alcohol were not expected, given their separate pathways of metabolism. Aprepi-

tant is metabolized primarily by CYP3A4[10], whereas alcohol is metabolized by a pathway that involves alcohol dehydrogenase, catalase and CYP2E1[11], although alcohol has been suggested as a potential inducer of CYP3A4 activity[39]. Plasma concentrations of aprepitant (see Figure 4) were statistically significantly higher when administered alone on day 9, compared with co-administration of alcohol on day 10, but the difference was quite small, with a cumulative mean difference in plasma concentrations of 11.3% (95% confidence interval: 3.9-18.2%), which might be considered not clinically meaningful. However, this study was not specifically designed to evaluate the induction of aprepitant metabolism by alcohol and there is a limitation with the statistical analysis, because treatments are sequential and not randomized.

Pharmacodynamic interactions between aprepitant and alcohol are theoretically possible as both compounds are centrally active and may influence several neurotransmitters, including dopamine, at various sites in the brain[2,12]. The intravenous alcohol infusion produced clear and expected CNS effects, similar to previously reported results of this alcohol infusion paradigm[13,19,20]. Contrary to those investigations, our study did not find a clear decrease in VAS alertness, but the visual analogue scales were performed shortly after the start of the alcohol infusion at which time blood alcohol concentration probably had not yet reached significant levels. Co-administration of aprepitant and alcohol did not result in significant additive effects, compared to the effects of alcohol alone, on any of the pharmacodynamic parameters, and is therefore not very likely to result in a clinically relevant interaction. To get a further impression of the effect size of pharmacodynamic effects, amitriptyline was included in this study as a pharmacodynamic comparator. Amitriptyline is one of the first 'reference' tricyclic antidepressant drugs[40] and its CNS effects have long been known in the literature[16]. Amitriptyline demonstrated significant and expected impairments in almost all pharmacodynamic parameters, in contrast with the limited effects of aprepitant. These findings generally confirmed the large functional CNS effects of a single dose of 50 mg amitriptyline, which were generally larger than those of alcohol levels above the legal driving limit in most Western countries.

In conclusion, our study demonstrates no significant CNS impairments after administration of aprepitant in healthy volunteers. Furthermore, co-administration of aprepitant with intravenous alcohol levels at pseudo-steady state was generally well tolerated and did not result in significant additive CNS effects, compared to the effects of alcohol alone. Therefore, our study found no indications for clinically relevant additive effects when alcohol is co-administered with aprepitant.

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CHAPTER 6

PHARMACOKINETICS AND CENTRAL NERVOUS SYSTEM EFFECTS OF THE NOVEL DUAL NK_1/NK_3 RECEPTOR ANTAGONIST GSK1144814 IN ALCOHOL-INTOXICATED VOLUNTEERS

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ABSTRACT

AIMS: Antagonism of both NK_1 and NK_3 receptors may be an effective strategy in pharmacotherapy of schizophrenia, drug addiction or depression. GSK1144814 is a novel selective dual NK_1/NK_3 receptor antagonist. The potential influence of GSK1144814 on the effects of alcohol was investigated.

METHODS: In a blinded, randomized, placebo-controlled, two-period cross-over study, the pharmacokinetics and central nervous system (CNS) effects of single oral doses of 200 mg GSK1144814 were evaluated in 20 healthy volunteers, using a controlled alcohol infusion paradigm to maintain stable alcohol levels with subsequent analysis of eye movements, adaptive tracking, body sway, visual analogue scales, Epworth sleepiness scale and the verbal visual learning test.

RESULTS: Frequent adverse effects were mild somnolence, fatigue and headache. Plasma concentration of GSK1144814 in the presence of alcohol was maximal 1.5 hours after dose administration. GSK1144814 did not affect alcohol pharmacokinetics. Co-administration of GSK1144814 and alcohol impaired saccadic reaction time and peak velocity, adaptive tracking, alertness, sleepiness, word recognition and recognition reaction time compared with administration of alcohol alone, but the size of the interaction was small.

CONCLUSIONS: Administration of GSK1144814 in the presence of alcohol is generally well tolerated and not likely to produce clinically relevant additional impairments after alcohol consumption.

INTRODUCTION

Antagonists at the neurokinin-1 (NK_1) and neurokinin-3 (NK_3) receptors have been evaluated in several clinical trials for possible antidepressant and antipsychotic activity, respectively[1–3]. Clinical trials with the NK_1 receptor antagonists casopitant, aprepitant and L-759274 have demonstrated antidepressant efficacy[4–6], although these findings were not replicated for the latter two compounds[7,8]. An early clinical trial with the NK_3 receptor antagonist osanetant has demonstrated antipsychotic efficacy in schizophrenic patients[9], but further clinical development of this compound has been discontinued because of poor pharmacokinetic characteristics[3,10]. Development of another NK_3 receptor antagonist, talnetant, also has been discontinued, due to rather low penetration of the blood-brain barrier[3,10]. Recently, interest in NK_1 and NK_3 receptor antagonists has focused on a potential role in the treatment of drug addiction and substance abuse disorders. Involvement of neurokinin receptors in the etiology of substance abuse disorders has been suggested by recent studies, which identified haplotypes and single nucleotide polymorphisms (SNP) in the NK1R gene[11] and TACR3 gene[12], encoding the NK_1 and NK_3 receptor respectively, that were significantly associated with the development of alcohol dependence. Pre-clinical studies in various animal models have demonstrated that pharmacological blockade of NK_1 receptors dose-dependently suppresses alcohol intake[13] and stress-induced reinstatement of alcohol seeking behavior[14], while pharmacological blockade of NK_3 receptors attenuates the behavioural effects of cocaine[15,16] and prevents behavioral sensitization to cocaine[17]. Furthermore, a recent clinical trial with the NK_1 receptor antagonist LY686017 in detoxified alcoholic inpatients has demonstrated suppression of spontaneous alcohol cravings and improved overall well-being[18]. Together, these data suggest that antagonism of both NK_1 and NK_3 receptors may be an effective strategy in pharmacotherapy of schizophrenia, drug addiction or depression, especially in patients with co-morbid schizophrenia and substance abuse disorder, which is quite common[19,20] and is associated with poor clinical outcome[21,22].

GSK1144814 is a novel selective high affinity ligand for recombinant human NK_1 and NK_3 receptors, that is being developed as a novel treatment for schizophrenia, depression and substance abuse disorders (data on file). Pre-clinical in vitro studies demonstrated that GSK1144814 was selective for the human NK_1 and NK_3 receptors, versus 88 other non-tachykinin human receptors, enzymes and transporters (data on file). Previous studies of GSK1144814 in healthy volunteers demonstrated peak levels within one hour and a terminal elimination half life of roughly 15 hours (data on file).

A potential role for GSK1144814 in pharmacotherapy of drug abuse and addiction necessitates evaluation of possible interactions with drugs of abuse, because the target population of patients will have alcohol dependence as primary disorder or co-morbidity. Pharmacodynamic interactions are theoretically possible as both compounds are centrally active and may potentially influence several neurotransmitters, including dopamine, at various sites in the brain[23,24]. NK_1 receptor antagonists with chemical structures similar to GSK1144814, such as casopitant[25] and aprepitant[26], are metabolized primarily by CYP3A4. In contrast, alcohol is metabolized by a pathway that involves alcohol dehydrogenase, catalase and CYP2E[27]. Accordingly, pharmacokinetic interactions between GSK1144814 and alcohol are not expected. Therefore, in the present study, we investigated whether single oral doses of GSK1144814 can modulate the central nervous system (CNS) effects of alcohol in healthy volunteers. An intravenous alcohol infusion paradigm[28] was used to achieve pseudo-steady state alcohol levels, while either single oral doses of GSK1144814 or placebo were co-administered. A recent literature review[29] identified the most sensitive and useful functional biomarkers for the acute CNS effects of alcohol, which included divided attention, focused attention, visuomotor control, visual analogue scales for subjective effects, reaction time, working memory and inhibition, digit-symbol substitution, motor control, postural stability and immediate recall (auditory or verbal memory). A previous study using the alcohol clamping method[30] demonstrated significant effects of alcohol on smooth pursuit eye movements, adaptive tracking, body sway and visual analogue scales for alertness and the subjective effects of alcohol, while the peak velocity of saccadic eye movements also seemed to decrease somewhat, albeit not statistically significant. These quantitative tests were all included in the present study, while the visual verbal learning test (VVLT)[31] was added to evaluate effects on memory. To our knowledge, no other NK_1/NK_3 receptor antagonists have been evaluated using these tests and we are unaware of other CNS tests capable of demonstrating effects in healthy volunteers after single oral doses. Thus, our study can be regarded as exploratory in that regard. However, the quantitative tests used in this study have previously been demonstrated to be sensitive to the central effects of various compounds, including antipsychotic drugs[32], antidepressant drugs[33] and the NK_3 receptor antagonist talnetant[34]. Finally, also for exploratory purposes, we included an adapted version of the Epworth sleepiness scale[35,36] in this study to evaluate its usefulness for assessing drug-induced sleepiness in early phase clinical trials. An oral dose of 200 mg GSK1144814 was chosen based on observed pharmacokinetics in previous studies in healthy volunteers and on AUC and NOAEL estimations derived from animal models (data on file). This dose is generally well tolerated in healthy volunteers and can produce high levels of receptor occupancy (>99%

NK_1 receptor occupancy in frontal cortex), as demonstrated by positron emission tomography (PET) using [^{11}C]GR205171 in healthy volunteers (data on file). To demonstrate that the effects of alcohol combined with GSK1144814 do not significantly differ from the effects of alcohol alone, we applied generally accepted statistical criteria for bioequivalence.

METHODS

Study design

Twenty healthy volunteers, between 18 and 65 years of age and with a body mass index between 19 and 30 kg/m^2, were planned to participate in a blinded, randomized, placebo-controlled, two-period cross-over study. The study was approved by the medical ethics review board of the Leiden University Medical Center and registered at the NIH database of clinical trials (website http://clinicaltrials.gov) with identifier NCT01181908 and GSK ID number 113476. Prior to medical screening, all volunteers gave written informed consent. Medical screening included a medical history, physical examination, urinalysis, routine hematology and chemistry, 12-lead electrocardiography (ECG) and 24-hour Holter-ECG recording. All volunteers underwent training sessions for the pharmacodynamic tests in order to minimize possible learning effects.

Volunteers were assigned to a randomized treatment sequence, consisting of one study period of intravenous alcohol infusion (alcohol clamping, see below for further details) combined with oral administration of 200 mg of GSK1144814 and one study period of intravenous alcohol infusion combined with oral placebo administration. GSK1144814 or matching placebo was administered orally 30 minutes after the start of the alcohol infusion. The alcohol infusion continued until 5 hours after study drug administration, in order to coincide with the expected t_{max} of GSK1144814. Subjects remained in the clinic until 48 hours after study drug administration. The first study period was preceded by a baseline study day in which all study-related activities were performed following unblinded intravenous saline infusion without drug administration, to familiarize all volunteers with the study-related procedures. All periods were separated by a wash-out time of approximately 7 days.

Subjects were excluded if they had an average weekly alcohol intake of greater than 14 units (in case of females) or 21 units (in case of males). Subjects with a past history of alcohol abuse or dependence were excluded. Subjects were also excluded if they had a history of regular use of tobacco- or nicotine-containing products within 6 months prior to screening, as indicated by urinary cotinine levels. Subjects were instructed to abstain from alcoholic drinks on the day preceding all study periods and all subsequent study days. In addition, the use of to-

bacco products or illicit drugs was not permitted. In all study periods, breath alcohol measurements and urinary cotinine analysis were performed to ascertain non-use of alcohol and tobacco. Also, urine drug screening for cocaine, amphetamine, methamphetamine, opiates (morphine), benzodiazepines, barbiturates, MDMA and THC (Innovacon, Inc., San Diego, California, USA) was performed to ascertain non-use of illicit drugs.

Alcohol clamping

The method for attaining constant alcohol levels has been described in detail elsewhere[28,30]. In brief, alcohol (ethanol 10% w/v solution in 5% glucose) was infused intravenously over a period of 5½ hours in total, guided by breath alcohol measurements to achieve a pseudo-steady state alcohol serum level of 0.6 g/L. This target level was chosen because it produces significant central nervous system effects without causing too many inadvertent effects and is considered safe, since it is only just above the legal driving limit in the Netherlands (i.e. 0.5 g/L). The alcohol infusion started 30 minutes prior to administration of GSK1144814. The infusion rate for the first ten minutes was determined using individual demographic characteristics (weight, height, age and sex). Infusion rates were subsequently adjusted, guided by breath alcohol measurements at baseline and at every five minutes for the first 30 minutes after the start of the infusion, every 10 minutes for the next 30 minutes and then every half hour until the end, using two calibrated Alco-Sensor IV Intoximeters (Honac, Apeldoorn, the Netherlands), which were alternated to avoid any fatigue of the apparatus. To prevent local pain at the beginning of the alcohol infusion, an additional diluting glucose 5% infusion at 100 mL/h was administered to all participants during the first 10 minutes after the start of the alcohol infusion through the same infusion line. The time profiles of the individually adjusted infusion rates necessary to maintain a target alcohol serum level of 0.6 g/L provide an indirect measure of individual alcohol pharmacokinetics and were subsequently used for statistical comparison between treatments.

Safety monitoring

Evaluation of adverse events, 12-lead electrocardiograms (ECG), blood pressure, heart rate, body temperature, urinalysis and blood sampling for hematology and chemistry was performed at regular time points after dose administration. Automated oscillometric blood pressures were measured using a Nihon-Kohden BSM-1101K monitor or a Colin Pressmate BP 8800. ECGS were obtained with Cardiofax V equipped with ECAPS12 analysis program (Nihon-Kohden, Tokyo, Japan).

Pharmacokinetics

Venous blood samples for concentration analysis of GSK1144814 were collected prior to dose administration and at 30 minutes and 1, 1½, 2, 2½, 3, 3½, 4, 5, 6, 8, 12 and 24 hours after dose administration. Plasma concentrations of GSK1144814 were determined using protein precipitation followed by HPLC/MS/MS analysis with a lower limit of quantification (LLQ) of 1.5 ng/mL.

Pharmacodynamic testing

Pharmacodynamic measurements were performed as described previously[34,37], prior to dose administration and at fixed time intervals at 1, 2, 3, 4½ and 8 hours after dose administration. Volunteers were tested individually in a quiet room with ambient illumination. Quantitative tests included measurements of smooth pursuit and saccadic eye movements, adaptive tracking, body sway, visual analogue scales and an adapted version of the Epworth sleepiness scale. In addition, the visual verbal learning test (VVLT) was performed at 2 and 4½ hours after dose administration.

Analysis of eye movements

To evaluate oculomotor performance and sedation, smooth pursuit and saccadic eye movements were recorded as described previously[38-41], using a computer-based system for signal collection (Cambridge Electronic Design Ltd., Cambridge, UK) and amplification (Grass-Telefactor, An Astro-Med, Inc. Product Group, Braintree, USA), and disposable surface electrodes (Medicotest N-00-S, Olstykke, Denmark). For smooth pursuit eye movements, a target light source moves sinusoidally over 20° eyeball rotation at frequencies ranging from 0.3 to 1.1 Hz. The time in which the eyes were in smooth pursuit was calculated for each frequency and expressed as the percentage of stimulus duration. The average percentage of smooth pursuit for all frequencies was used as parameter. For saccadic eye movements, the target light source jumps from side to side. Peak velocity (degrees per second), reaction time and inaccuracy (%) was calculated of all artifact-free saccades.

Adaptive tracking

To evaluate visuo-motor coordination, the adaptive tracking task was performed as described previously[40-44], using customized equipment and software developed by K.W. Hobbs (Hertfordshire, UK). Adaptive tracking is a pursuit tracking task in which a circle moves randomly over a computer screen and the volunteer must try to keep a dot inside the moving circle using a joystick. If this effort is successful, the speed of the moving circle is increased and if the effort is

unsuccessful, the speed is reduced. Performance was scored over a fixed period of three minutes.

Body sway

Postural stability in the sagittal plane was measured with an apparatus similar to the Wright ataxiameter[45], using a string attached to the waist of the volunteer. Movements over a period of two minutes, while standing still with eyes closed, were integrated and expressed as mm sway.

Visual analogue scales

Subjective effects were quantified using a Dutch translation of the visual analogue scales (VAS), originally described by Norris[46], to derive three composite factors corresponding to sedation, mood (contentedness) and calmness, as described by Bond & Lader[47]. In addition, a visual analogue scale was used to quantify the subjective effects of alcohol.

Epworth sleepiness scale

For exploratory purposes, an adapted version of the Epworth sleepiness scale[35,36] was included in this study. The Epworth sleepiness scale is a self-administered scale for assessing subjective daytime sleepiness persisting from week to week, independent of changes with the time of day and from day to day. Eight specific situations are presented in a questionnaire and subjects are instructed to rate the chance they would have dozed when those situations occur in daily life in recent time, on a scale from 0 to 3 with increasing chance of dozing. Scores of individual items are summed to produce a total score. In this study, the volunteers were instructed to rate the chance of dozing in the recent hour instead of the recent days to weeks, to assess if the Epworth sleepiness scale is sensitive to single dose drug effects over the course of a few hours.

Visual verbal learning test

The visual verbal learning test (VVLT)[31] is an adapted version of the auditory verbal learning test[48]. Three trials of 30 words are presented on a computer screen in the same sequence. The volunteer is requested to reproduce as many words as possible at the ending of each trial (immediate recall) and after 30 minutes (delayed recall). The number of correctly reproduced words is analyzed for each trial. Also, a recognition test is performed, consisting of 15 previously presented words and 15 new words, in which the volunteer has to indicate recognition of the word (delayed recognition) as quickly as possible. Response time and the number of correctly recognized words are analyzed.

Statistical analysis

Pharmacokinetic parameters of GSK1144814 in the presence of alcohol were determined based on the individual plasma concentration-time data, using WinNonlin professional edition software version 5.2 (Pharsight Corporation, Mountain View, California, USA) and included the maximum observed plasma concentration (C_{max}), time to reach maximum plasma concentration (t_{max}) and area under the plasma concentration-time curve from time zero extrapolated to the last time of quantifiable concentration (AUC_{0-t}) and to 24 hours post-dose ($AUC_{0-24\ hours}$).

Effects of GSK1144814 administration on the pharmacokinetics of alcohol were evaluated by comparing the rates of alcohol infusion necessary to maintain a pseudo-steady state alcohol serum level of 0.6 g/L, using a mixed model analysis of variance with treatment, period, time and treatment by time as fixed factors and with subject, subject by treatment and subject by time as random factors.

Pharmacodynamic parameters were compared using a mixed model analysis of variance with treatment, period, time and treatment by time as fixed factors and with subject and subject by period as random factors. Saccadic eye movement data (reaction time, peak velocity and inaccuracy) and body sway data were log-transformed prior to analysis. VVLT data were compared using a mixed model with treatment and period as fixed effects and subject as random effect. Treatment differences with corresponding 90% confidence intervals were calculated. All calculations were performed using SAS for Windows version 9.1.2 (SAS Institute Inc., Cary, North Carolina, USA).

RESULTS

Subjects

Twenty healthy male volunteers were included in the study. Participants had a mean age of 27.6 years (range 18-62), weight of 75.9 kg (range 60-90) and body mass index of 22.8 kg/m^2 (range 19.9-27.6). All volunteers completed both study periods and a follow-up visit, except one volunteer who completed both study periods but was subsequently lost to follow-up. Pharmacokinetic and pharmacodynamic data from this volunteer were included in the statistical analysis.

Clinical observations

All adverse events were transient and mild or moderate in severity and no serious adverse events occurred during the study. Overall, the most common adverse events were somnolence and fatigue (see Table 1). Somnolence, headache,

infusion reaction (generally consisting of redness of skin and burning feeling at infusion site), nausea, phlebitis, diarrhea, dizziness and light headedness were reported more frequently after co-administration of alcohol and GSK1144814, whereas fatigue and vasovagal reaction were reported more frequently after administration of alcohol alone. There were no consistent and clinically relevant changes in vital signs, blood chemistry and hematology or any of the ECG intervals.

TABLE 1 Summary of common adverse events, reported by two subjects or more. Incidence is based on the number of subjects, not the number of events.

Adverse event	Alcohol infusion with placebo	Alcohol infusion with GSK1144814
Somnolence	7 (35%)	13 (65%)
Fatigue	6 (30%)	4 (20%)
Headache	5 (25%)	7 (35%)
Feeling drunk	6 (30%)	8 (40%)
Infusion reaction	3 (15%)	5 (25%)
Nausea	2 (10%)	3 (15%)
Phlebitis	1 (5%)	2 (10%)
Diarrhoea	0	2 (10%)
Dizziness	1 (5%)	2 (10%)
Light headedness	1 (5%)	2 (10%)
Vasovagal reaction	2 (10%)	1 (5%)

Pharmacokinetics

Following intravenous alcohol infusion, breath alcohol levels increased rapidly and remained constant at the target level all over the time of infusion (see Figure 1). The rate of alcohol infusion necessary to maintain a pseudo-steady state alcohol serum level of 0.6 g/L (see Figure 2) was not significantly different between administration of GSK1144814 and placebo capsules ($p = 0.5105$).

Following co-administration of 200 mg tablets of GSK1144814 and intravenous alcohol infusion, GSK1144814 was rapidly absorbed (see Figure 3). Median time to peak concentration (t_{max}) was 1.5 hours (range 0.97-3.48). The geometric mean C_{max} was 1500 ng/mL (coefficient of variation 21.6%, 95% confidence interval 1360-1660). The geometric mean $AUC_{0\text{-}t}$ and $AUC_{0\text{-}24\ hours}$ were 7680 ng.h/mL (coefficient of variation 29.7%, 95% confidence interval 6700-8800) and 7630 ng.h/mL (coefficient of variation 30.5%, 95% confidence interval 6610-8810), respectively.

FIGURE 1 Breath alcohol levels after intravenous alcohol infusion starting at t=-0.5 hours and continuing until t=5 hours, in combination with oral administration (at t=0 hours) of GSK1144814 (open circles) or placebo (closed circles). Means are presented with standard deviations as error bars.

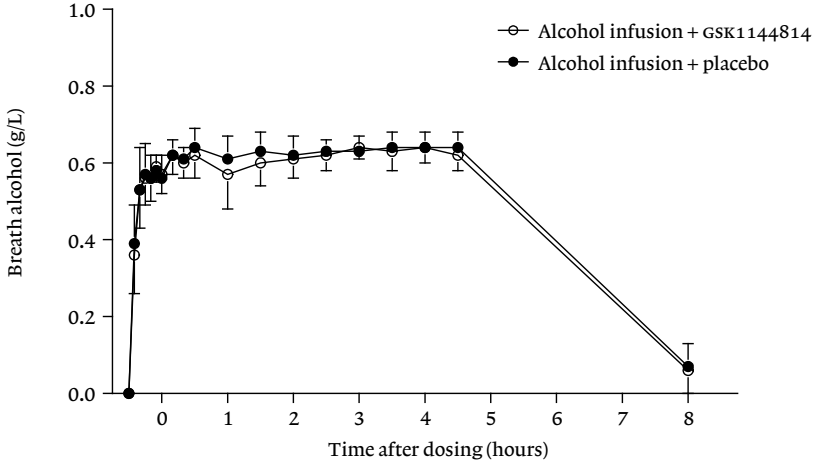


FIGURE 2 Alcohol infusion rates necessary to maintain a pseudo-steady state alcohol serum level of 0.6 g/L, starting at t=-0.5 hours and continuing until t=5 hours, in combination with oral administration (at t=0 hours) of GSK1144814 (open circles) or placebo (closed circles). Means are presented with standard deviations as error bars.

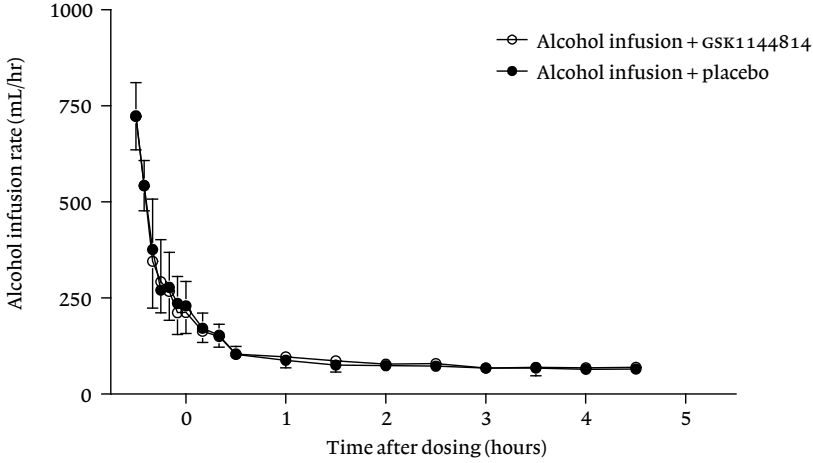


FIGURE 3 Plasma levels of GSK1144814 after oral administration at t = 0 hours, in combination with intravenous alcohol infusion starting at t = -0.5 hours and continuing until t = 5 hours. Means are presented with standard deviations as error bars.

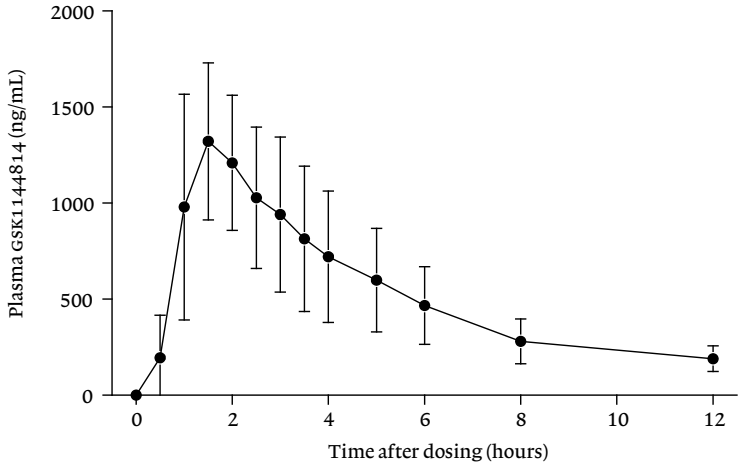
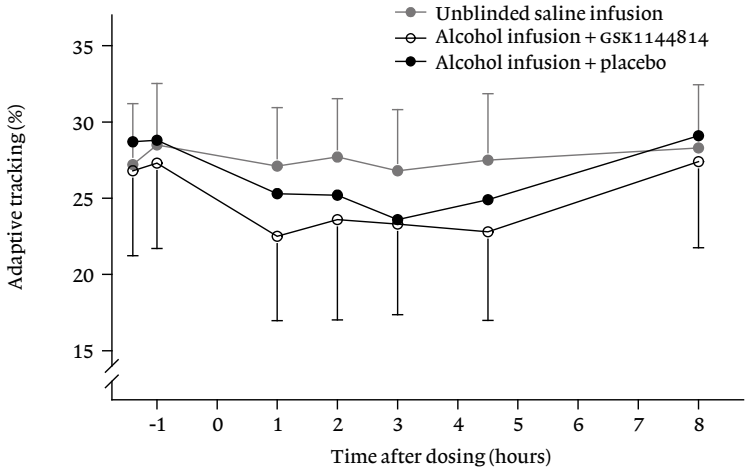


FIGURE 4 Adaptive tracking performance after intravenous alcohol infusion combined with oral administration (at t = 0 hours) of either GSK1144814 (open circles) or placebo (closed circles). The grey curve represents measurements following unblinded intravenous saline infusion (without drug administration) during a baseline study day preceding the first study period, which is included in the figure for reference. Means are presented with standard deviations as error bars.



Pharmacodynamics

Neurophysiological parameters are summarized in Table 2 and Figures 4, 5, 6 and 7. There was a statistically significant increase in saccadic reaction time at 1 hour and a decrease in saccadic peak velocity at 4½ hours after co-administration of GSK1144814 and alcohol compared with administration of alcohol alone. A clear reduction of overall adaptive tracking performance was observed after co-administration of GSK1144814 and alcohol compared with alcohol alone, although the time course of effects was not very consistent. Effects were statistically significant at 1, 4½ and 8 hours, while effects at 2 and 3 hours were not statistically significant. There were no statistically significant differences in saccadic inaccuracy, smooth pursuit eye movements and body sway.

Subjective effects are summarized in Table 3 and Figures 8, 9 and 10. An increase in sedation was observed at 3 and 4½ hours after co-administration of GSK1144814 and alcohol compared with alcohol alone. There were no statistically significant differences in contentedness, calmness or the feeling of being drunk. The Epworth sleepiness scale demonstrated a clear increase of sleepiness in the first 3 hours after co-administration of GSK1144814 and alcohol compared with alcohol alone.

TABLE 2 Neurophysiological parameters. Treatment differences between co-administration of alcohol and GSK1144814 compared with co-administration of alcohol and placebo, are expressed as differences in treatment means (with 90% confidence intervals and p-values) or as geometric mean ratios (with 90% confidence intervals and p-values). Statistically significant results are indicated in bold. The alcohol infusion started 30 minutes prior to administration of GSK1144814 (at t = 0) and continued over a period of 5½ hours in total.

Parameter		1 hour Postdose	2 hours postdose	3 hours postdose	4,5 hours postdose	8 hours postdose	Overall
Saccadic peak velocity (deg/sec)	Ratio 90% CI p-value	0.97 0.93/1.00 0.093	1.00 0.96/1.04 0.914	0.96 0.92/1.00 0.084	**0.95** **0.92/0.99** **0.030**	0.97 0.94/1.01 0.191	**0.97** **0.95/0.99** **0.035**
Saccadic inaccuracy (%)	Ratio 90% CI p-value	1.04 0.89/1.21 0.663	1.13 1.00/1.27 0.104	1.10 0.95/1.26 0.277	1.10 0.95/1.26 0.269	1.04 0.90/1.20 0.652	1.08 1.01/1.15 0.058
Saccadic reaction time (sec)	Ratio 90% CI p-value	**1.06** **1.02/1.10** **0.017**	1.01 0.96/1.06 0.693	1.01 0.97/1.06 0.642	1.05 1.01/1.09 0.052	1.05 1.00/1.10 0.100	**1.04** **1.01/1.07** **0.045**
Smooth pursuit (%)	Difference 90% CI p-value	2.04 -0.75/4.83 0.224	-0.32 -2.76/2.12 0.824	1.90 -0.51/4.30 0.190	2.40 0.26/4.54 0.067	-1.83 -5.05/1.39 0.325	0.84 -0.94/2.62 0.413
Adaptive tracking (%)	Difference 90% CI p-value	**-2.78** **-4.24/-1.32** **0.004**	-1.63 -3.41/0.16 0.132	-0.24 -1.73/1.26 0.788	**-2.02** **-3.42/-0.63** **0.022**	**-1.77** **-2.97/-0.57** **0.019**	**-1.69** **-2.65/-0.72** **0.007**
Body sway (mm)	Ratio 90% CI p-value	1.28 0.99/1.64 0.107	1.18 0.96/1.44 0.187	1.14 0.95/1.36 0.235	1.09 0.95/1.25 0.278	1.06 0.92/1.23 0.466	1.15 1.00/1.31 0.097

FIGURE 5 Body sway after intravenous alcohol infusion combined with oral administration (at t = 0 hours) of either GSK1144814 (open circles) or placebo (closed circles). The grey curve represents measurements following unblinded intravenous saline infusion (without drug administration) during a baseline study day preceding the first study period, which is included in the figure for reference. Means are presented with standard deviations as error bars.

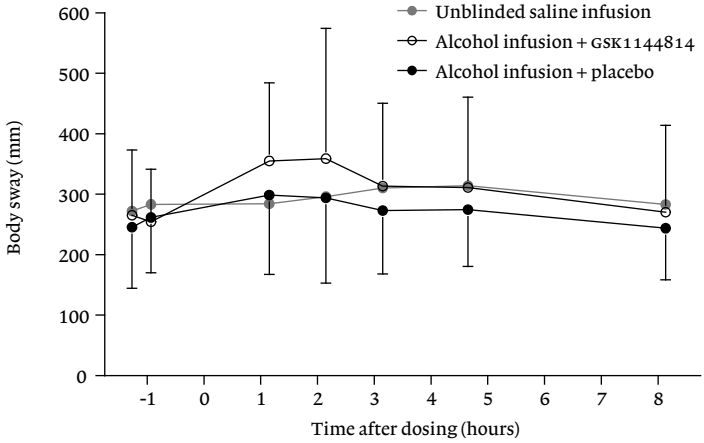


FIGURE 6 Saccadic peak velocity after intravenous alcohol infusion combined with oral administration (at t = 0 hours) of either GSK1144814 (open circles) or placebo (closed circles). The grey curve represents measurements following unblinded intravenous saline infusion (without drug administration) during a baseline study day preceding the first study period, which is included in the figure for reference. Means are presented with standard deviations as error bars.

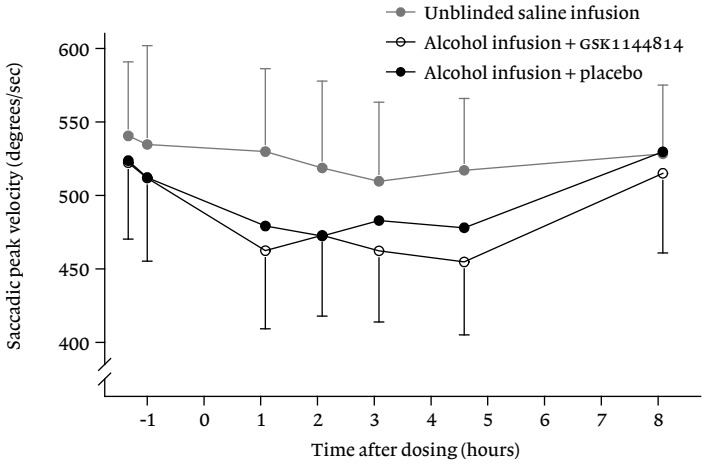


FIGURE 7 Smooth pursuit measurements after intravenous alcohol infusion combined with oral administration (at t = 0 hours) of either GSK1144814 (open circles) or placebo (closed circles). The grey curve represents measurements following unblinded intravenous saline infusion (without drug administration) during a baseline study day preceding the first study period, which is included in the figure for reference. Means are presented with standard deviations as error bars.

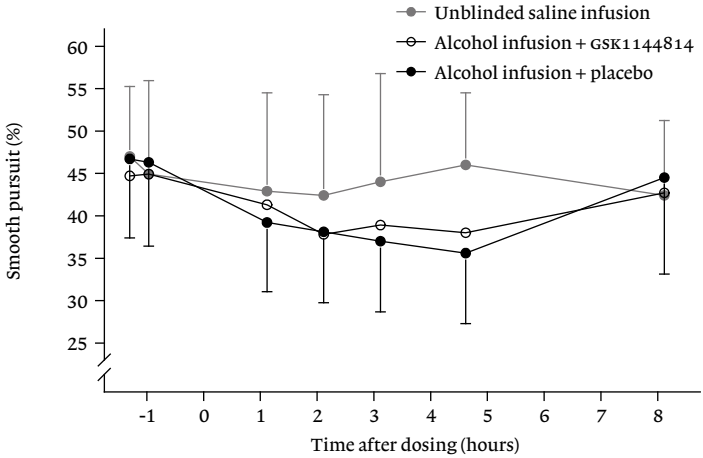


FIGURE 8 Visual analogue scale (VAS) sedation scores after intravenous alcohol infusion combined with oral administration (at t = 0 hours) of either GSK1144814 (open circles) or placebo (closed circles). The grey curve represents scores following unblinded intravenous saline infusion (without drug administration) during a baseline study day preceding the first study period, which is included in the figure for reference. Means are presented with standard deviations as error bars.

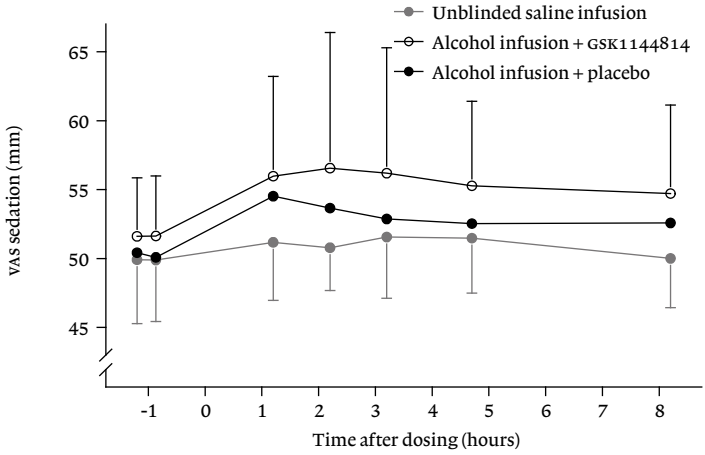


FIGURE 9 Visual analogue scale (VAS) scores for the subjective effects of alcohol after intravenous alcohol infusion combined with oral administration (at t = 0 hours) of either GSK1144814 (open circles) or placebo (closed circles). The grey curve represents scores following unblinded intravenous saline infusion (without drug administration) during a baseline study day preceding the first study period, which is included in the figure for reference. Means are presented with standard deviations as error bars.

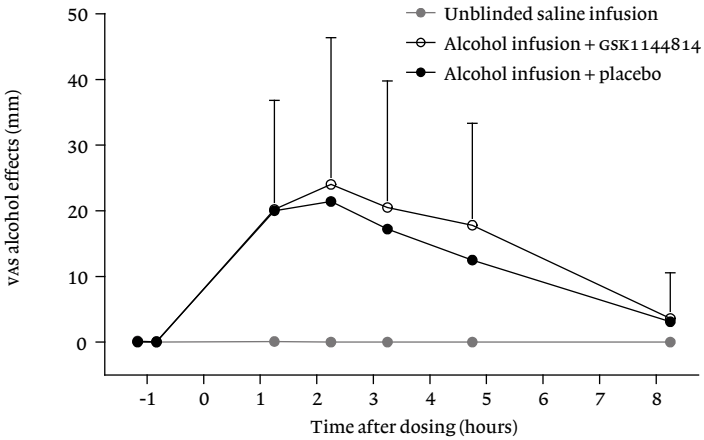


FIGURE 10 Epworth sleepiness scale scores after intravenous alcohol infusion combined with oral administration (at t = 0 hours) of either GSK1144814 (open circles) or placebo (closed circles). The grey curve represents scores following unblinded intravenous saline infusion (without drug administration) during a baseline study day preceding the first study period, which is included in the figure for reference. Means are presented with standard deviations as error bars.

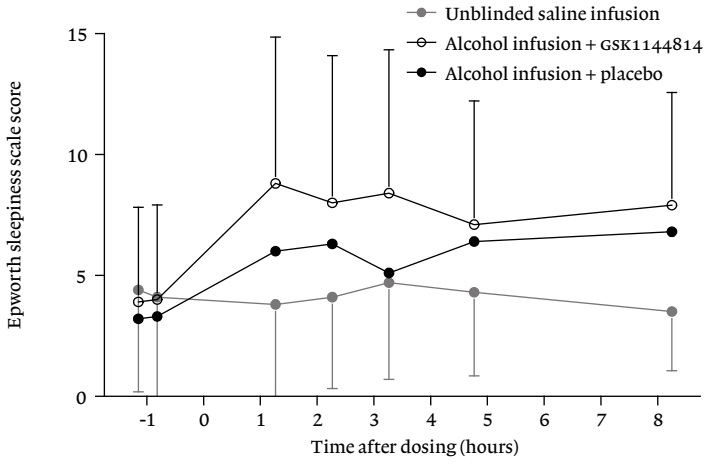


TABLE 3 Subjective effects. Treatment differences between co-administration of alcohol and GSK1144814 compared with co-administration of alcohol and placebo, are expressed as differences in treatment means (with 90% confidence intervals and p-values). Statistically significant results are indicated in bold. The alcohol infusion started 30 minutes prior to administration of GSK1144814 (at t = 0) and continued over a period of 5½ hours in total.

Parameter		1 hour Postdose	2 hours postdose	3 hours postdose	4 hours postdose	8 hours postdose	Overall
VAS sedation (mm)	Difference 90% CI p-value	1.97 -0.22/4.16 0.134	2.90 -0.31/6.11 0.133	**3.32** **0.91/5.73** **0.028**	**2.74** **0.59/4.89** **0.041**	2.14 -1.64/5.92 0.343	**2.61** **0.82/4.41** **0.022**
VAS contentedness (mm)	Difference 90% CI p-value	0.81 -1.31/2.93 0.517	-0.19 -2.86/2.48 0.903	-0.90 -2.56/0.76 0.355	-0.15 -1.79/1.49 0.876	1.64 -0.25/3.53 0.151	0.24 -1.30/1.78 0.783
VAS calmness (mm)	Difference 90% CI p-value	-1.19 -4.34/1.97 0.520	-0.95 -4.67/2.77 0.665	-3.43 -6.47/-0.38 0.066	-0.15 -2.12/1.82 0.897	0.73 -1.41/2.86 0.567	-1.00 -3.09/1.09 0.410
VAS alcohol effects (mm)	Difference 90% CI p-value	1.24 -7.73/10.21 0.816	2.55 -8.50/13.60 0.698	3.30 -5.56/12.16 0.532	5.30 -2.00/12.60 0.227	0.50 -1.61/2.61 0.688	2.58 -4.00/9.15 0.509
Epworth sleepiness scale	Difference 90% CI p-value	**3.23** **1.53/4.93** **0.004**	**1.75** **0.33/3.17** **0.047**	**3.25** **1.61/4.89** **0.002**	0.75 -0.64/2.14 0.355	1.10 -1.25/3.45 0.430	**2.02** **1.10/2.93** **0.001**

TABLE 4 Results of the visual verbal learning test (VVLT). Treatment differences between co-administration of alcohol and GSK1144814 compared with co-administration of alcohol and placebo, are expressed as differences in treatment means (with 90% confidence intervals and p-values). Statistically significant results are indicated in bold.

Parameter	Difference (90% CI)	p-value
Immediate recall 1st trial (correct)	-0.20 (-1.24/0.84)	0.742
Immediate recall 2nd trial (correct)	-0.65 (-1.89/0.59)	0.377
Immediate recall 3rd trial (correct)	0.40 (-1.11/1.91)	0.652
Delayed recall (correct)	-0.50 (-2.10/1.10)	0.594
Relative recall (%)	-7.96 (-17.85/1.93)	0.180
Word recognition (correct)	**-2.33 (-4.19/-0.47)**	**0.043**
Recognition time (correct)	**68.44 (28.09/108.80)**	**0.009**

The results of the visual verbal learning test (VVLT) are summarized in Table 4. There were no significant differences in immediate, delayed or relative recall, but word recognition score was reduced and recognition reaction time was increased after co-administration of GSK1144814 and alcohol compared with alcohol alone.

DISCUSSION

A potential role of NK_1 and NK_3 antagonism in the treatment of substance abuse disorders and psychosis necessitates evaluation of possible pharmacokinetic and pharmacodynamic interaction with drugs of abuse. The present study was performed primarily to evaluate if single oral doses of GSK1144814 can modulate the CNS effects of alcohol in healthy volunteers, because the target population of patients will have alcohol dependence as primary disorder or co-morbidity.

In general, administration of GSK1144814 in the presence of alcohol was well tolerated. Median time to peak concentration of GSK1144814 (t_{max}) in the presence of alcohol was 1.5 hours while the mean C_{max} was 1500 ng/mL, which were in line with results from previous studies with GSK1144814 in healthy volunteers. Administration of GSK1144814 did not notably affect alcohol pharmacokinetics, as the infusion rates necessary to maintain stable alcohol levels were similar between treatment groups. However, administration of GSK1144814 did affect several alcohol-induced pharmacodynamic impairments. Saccadic reaction time, saccadic peak velocity and adaptive tracking performance, alertness, sleepiness, word recognition score and recognition reaction time were all impaired to a small extent at some point following co-administration of GSK1144814 and alcohol, compared with administration of alcohol alone. These interactions suggest either that GSK1144814 has mild pharmacodynamic effects of its own that are superimposed on those of alcohol or that GSK1144814 slightly modifies the effects of alcohol. However, the additional CNS effects were very limited in extent and are therefore not very likely to produce clinically relevant impairments on top of those of alcohol alone.

For exploratory purposes, we included an adapted version of the Epworth sleepiness scale in this study. The Epworth sleepiness scale[35,36] was designed to measure only those components of daytime sleepiness that persist from week to week and longer in a given subject, independent of changes with the time of day and from day to day. However, by rating the subjective sleepiness in recent hours, instead of recent days to weeks, we used this scale to assess sleepiness over the course of a few hours following administration of GSK1144814. This adapted version of the Epworth sleepiness scale clearly demonstrated an increased chance of sleepiness and was one of the most sensitive parameters for the effects of GSK1144814. These results indicate that the Epworth sleepiness scale may be a sensitive tool not only for long-term assessment of drug-induced sleepiness, but also short-term effects after a single dose.

In a recent alcohol interaction study[49], the CNS effects of the NK_1 receptor antagonist aprepitant were studied, with or without co-administration of alcohol,

using pharmacodynamic tests similar to our present study. A therapeutic dose of 160 mg aprepitant did not significantly impair performance on the digit-symbol substitution test (DSST), VVLT, binary choice reaction time, visual analogue scales, critical flicker fusion, body sway, finger tapping and adaptive tracking, nor were there any signs of a significant interaction with alcohol. In another recent study, the CNS effects of the NK_3 receptor antagonist talnetant were investigated, at a dose of 200 mg, which is at the low end of the range that is investigated in clinical trials[34]. Talnetant improved adaptive tracking performance, decreased α power electroencephalography (EEG), and reduced calmness, while VVLT performance, body sway, finger tapping, saccadic and smooth pursuit eye movements were not affected[34]. These studies suggest that single dose administration of NK_1 and NK_3 receptor antagonists affect CNS performance of healthy volunteers to a rather limited extent. The present study was not designed to examine the CNS effects of GSK1144814 alone. Therefore, it cannot be excluded that GSK1144814 by itself can cause limited CNS effects. It is theoretically conceivable that alcohol, which is an allosteric modulator of different transmembrane receptors, can change the properties of the ligand-receptor complex formed by GSK1144814 with its target receptors. However, the effects of GSK1144814 alone are unlikely to be large considering the limited extent of CNS effects observed with talnetant and aprepitant alone and the lack of significant interactions between alcohol and aprepitant. Moreover, in the present study, the addition of GSK1144814 to alcohol caused only a limited increase of a few CNS parameters, which also argues against large CNS effects of GSK1144814 by itself.

The relative lack of effects of NK_1 and NK_3 receptor antagonists in healthy volunteers seems to contrast with the rather widespread expression of NK_1 receptors[50–55] and NK_3 receptors[55,56] in the human central nervous system, and with the many interactions of these targets with dopamine, serotonin, noradrenalin, acetylcholine and GABA[1,3,23]. It has been suggested that antagonism of neuropeptide receptors may show less dramatic effects than antagonism of classic neurotransmitter receptors, because the neuromodulatory nature of neuropeptides seems to result in milder effects than monoamines and amino acid transmitters and their direct agonists and antagonists[57]. In addition, much evidence indicates that neuropeptides are released after stressful and noxious stimuli[52,57,58]. Accordingly, it has been suggested that neuropeptides exert their main action after various types of challenges or pathological conditions[57,58]. Neuropeptide receptor antagonists might therefore have significant effects in pathological conditions with increased peptide release, while effects in normal healthy volunteers are limited[57,58]. However, it cannot be excluded that effects could have been detected more clearly after multiple dosing with GSK1144814, af-

ter testing of other functional CNS domains, or after challenge tests of relevant functional or pharmacological systems.

In conclusion, our study demonstrated that administration of the novel dual NK_1/NK_3 receptor antagonist GSK1144814 in the presence of alcohol was generally well tolerated. Administration of GSK1144814 did not notably affect alcohol pharmacokinetics, but did affect alcohol-induced impairments in several CNS parameters. However, differences between the treatment groups were quite small and not very likely to produce clinically relevant additional impairments after alcohol consumption.

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CHAPTER 7

THE EFFECTS OF THE NONSELECTIVE BENZODIAZEPINE LORAZEPAM AND THE α_2/α_3 SUBUNIT-SELECTIVE $GABA_A$ RECEPTOR MODULATORS AZD7325 AND AZD6280 ON PLASMA PROLACTIN LEVELS

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ABSTRACT

OBJECTIVE: The effect of GABA and GABAergic drugs on prolactin secretion has been evaluated in many studies, often with inconsistent or opposing results. Moreover, little is known about the $GABA_A$ receptor subtypes that could be involved with prolactin release. The present study aimed to provide additional data by evaluating the effects of the nonselective benzodiazepine lorazepam, as well as two novel α_2/α_3 subunit-selective $GABA_A$ receptor modulators AZD7325 and AZD6280, on prolactin levels.

METHODS: Plasma prolactin concentrations were measured in 32 healthy male volunteers after administration of single oral doses of 2 mg lorazepam, 2 mg or 10 mg AZD7325, 10 mg or 40 mg AZD6280 or placebo.

RESULTS: Following administration of lorazepam at 2 mg doses and AZD6280 at 10 mg and 40 mg doses, prolactin levels increased significantly compared with placebo (difference 42.0%, 19.8% and 32.8% respectively). The increases in prolactin levels after administration of AZD7325 at 2 mg and 10 mg doses (difference 7.6% and 10.5% respectively) did not reach statistical significance.

CONCLUSIONS: The nonselective benzodiazepine lorazepam and the novel α_2/α_3 subunit-selective $GABA_A$ receptor modulator AZD6280 increase prolactin levels in healthy subjects, suggesting that the α_2 and/or α_3 receptor subtypes are involved in GABAergic modulation of prolactin secretion, although possible roles of the α_1 and α_5 receptor subtypes are not excluded. Prolactin release following AZD7325 was smaller and did not reach statistical significance, suggesting that doses of AZD7325 or intrinsic efficacy at the α_2 and α_3 receptor subtypes may have been too low.

INTRODUCTION

Prolactin secretion by the lactotroph cells of the pituitary gland is primarily controlled by the inhibitory influence of dopamine, released predominantly from the hypothalamic tuberoinfundibular dopaminergic neurons[1,2]. A variety of other neurotransmitters, amino acids and neuropeptides have also been demonstrated to influence prolactin secretion. Many of these agents have multiple levels of action, often with opposing effects[2]. The effect of γ-aminobutyric acid (GABA) on prolactin secretion has been evaluated in a large number of in vitro studies, as well as in vivo studies in animals, which have reported both stimulatory and inhibitory effects[3–7]. In contrast, only a few studies have evaluated the direct effects of GABAergic drugs on circulating basal prolactin levels in healthy subjects. Diazepam[8,9] and bromazepam[10] did not significantly affect prolactin levels, while temazepam was found to increase prolactin levels only to a small extent[11]. Alprazolam at high doses increased prolactin levels[12], while lower doses had no significant effect[13]. Zolpidem and bretazenil stimulated nocturnal secretion of prolactin[14,15], while sodium valproate decreased prolactin levels[16]. The effect sizes in these studies, if any, were very small, especially compared with the potent prolactin-elevating effects of dopamine D_2 receptor antagonists. However, all these studies used small group sizes (6-10 subjects in most studies) and it cannot be excluded that effects might have reached statistical significance if larger study groups had been used.

It has been proposed that GABA exerts a dual control of prolactin secretion, one inhibitory and the other stimulatory[17]. The inhibitory control of prolactin secretion by GABA is thought to occur peripherally at the level of the anterior pituitary. GABA is released from the median eminence into the hypophyseal portal vessels to act at GABA receptors in the anterior pituitary gland[2,18]. Through interaction with GABA receptors on the lactotrophs, GABA inhibits prolactin secretion and also prolactin gene expression[19–21]. However, while there is little doubt that GABA has an inhibitory effect on prolactin release in vitro, a direct in vivo inhibitory effect is less clear[2,22]. The stimulatory control of prolactin secretion by GABA is thought to occur at the level of the central nervous system. GABAergic neurons are present throughout the hypothalamus, including the arcuate nucleus and periventricular area, which constitute the origin of the tuberoinfundibular dopaminergic pathway[23–26]. Within the arcuate nucleus, GABA may directly inhibit the activity of the tuberoinfundibular dopaminergic pathway, with a resulting decrease of pituitary dopamine levels and a concomitant increase in prolactin secretion[2,18,23,27,28].

The counteracting peripheral and central effects of GABA could explain the limited net effect of benzodiazepines on prolactin secretion in healthy volunteers. In addition, although GABA generally causes a reduction of dopaminergic neuronal activity[29], benzodiazepines have also been shown to increase dopamine levels in the mesolimbic pathway[30]. This increase probably results from serial inhibition of coupled GABAergic interneurons in the mesolimbic pathway, which leads to disinhibition of dopaminergic neurons, which outweighs the direct inhibitory influence of benzodiazepines on those dopaminergic neurons[31–33]. Benzodiazepines are allosteric modulators of α_1, α_2, α_3 and α_5 subunit-containing $GABA_A$ receptors[34]. Studies in animal models have provided indications that certain effects of benzodiazepines can be attributed to specific receptor subtypes, such as sedation (α_1 receptor subtype[35,36]), anxiolysis (α_2 and α_3 receptor subtypes[37–40]) and learning and memory (α_5 receptor subtype[41,42]). The disinhibition of mesolimbic dopaminergic neurons and the resulting increase in dopamine levels appear to be mediated by the α_1 receptor subtype[32,33]. It has been suggested that selective agonists at α_3 receptor subtypes without efficacy at α_1 receptor subtypes, may attenuate dopamine neurotransmission in the mesolimbic pathway, without counteractive disinhibition from GABAergic interneurons[31]. Accordingly, $GABA_A$ receptor subunit-selective agonists may differ significantly from nonselective benzodiazepines in their effects on dopaminergic pathways[31].

Little is known about the $GABA_A$ receptor subtypes that could be involved with prolactin secretion. The α_1, α_2, α_3, α_4 and α_5 receptor subtypes are all expressed to some extent in the nucleus accumbens and hypothalamus[43]. To explore the exact role of the various receptor subtypes in the regulation of prolactin secretion, the present study evaluated the effects of two novel α_2/α_3 subunit-selective $GABA_A$ receptor modulators, AZD7325 and AZD6280[44], and a therapeutic dose of lorazepam on prolactin levels in an adequately powered group of 32 healthy volunteers. In vitro receptor binding studies demonstrated that AZD7325 and AZD6280 have high affinity for the α_1 receptor subtype (mean $K_i = 0.5$ nM for both compounds), α_2 receptor subtype (mean $K_i = 0.3$ and 21 nM, respectively) and α_3 receptor subtype (mean $K_i = 1.3$ and 31 nM, respectively), and lower affinity for the α_5 receptor subtype (mean $K_i = 230$ and 1680 nM, respectively) (AstraZeneca data on file). Electrophysiological assays to evaluate the potentiation of GABA-induced current relative to the maximal response of diazepam (set at 100%) demonstrated potentiation by AZD7325 and AZD6280 at the α_2 receptor subtype (18% and 32%, respectively) and α_3 receptor subtype (15% and 34%, respectively), but neutral antagonism at the α_1 receptor subtype (6% and 8%, respectively) and α_5 receptor subtype (8% and 7%, respectively) (Astra-

Zeneca data on file). These preclinical data suggest that AZD7325 and AZD6280 are selective modulators of α_2 and α_3 subunit-containing $GABA_A$ receptors. Based on positron emission tomography (PET) using [^{11}C]flumazenil in healthy volunteers, AZD7325 at 2 and 10 mg doses and AZD6280 at 10 and 40 mg doses are expected to result in substantial occupancy of $GABA_A$ receptors in the brain (e.g., ›50% occupancy of the maximal displaceable [^{11}C]flumazenil binding in occipital cortex) (AstraZeneca data on file). Previous studies have demonstrated that sleep-inducing doses of classical benzodiazepines such as clonazepam[45], diazepam[46], midazolam[47] and lorazepam[48-50], as well as the nonbenzodiazepine GABA receptor agonist zolpidem[51] are associated with relatively low receptor occupancy levels (up to approximately 30%), whereas pharmacologically active doses of the α_2/α_3 subunit-selective $GABA_A$ partial agonists TPA023[48] and TPA023B[52] are associated with higher occupancy levels (i.e. approximately 50%). Also, doses of 2 and 10 mg of AZD7325 and 10 and 40 mg of AZD6280 are predicted to lead to peak plasma concentrations above minimally efficacious concentrations in animal models of anxiety (AstraZeneca data on file). These data demonstrate that AZD7325 and AZD6280 cross the blood-brain barrier, interact with the target receptor, and have the potential to produce anxiolytic activity in humans.

METHODS

Study design

In total, 32 healthy male volunteers were planned to participate in two parallel double-blind, placebo-controlled, randomized, cross-over studies. To be eligible for inclusion in both studies, subjects were required to be aged between 18 and 55 years, with a body mass index (BMI) of 18 to 30 kg/m^2 and refrain from alcoholic beverages, smoking and caffeine-containing products during study days. Both studies were approved by the medical ethics review board of the Leiden University Medical Center. Prior to medical screening, all subjects gave written informed consent. Both studies had an identical design, except the administered drugs. In the first study, 16 subjects were administered single oral doses of 2 mg lorazepam, 2 mg AZD7325, 10 mg AZD7325 or placebo, during four separate study periods. In the second study, 16 subjects were administered single oral doses of 2 mg lorazepam, 10 mg AZD6280, 40 mg AZD6280 or placebo, during four separate study periods. Study periods were scheduled in randomized order using a Williams Latin square design and were separated by a washout time of at least 7 days. On study days, subjects fasted for minimally 2.5 hours after a light standard breakfast until dose administration (which generally occured between 11h00m and 12h00m AM) and continued fasting until 4 hours after dose administration.

Power calculation

A power calculation using data from a previous study[53] indicated that the present study ($n = 32$ subjects receiving lorazepam, power 80% and alpha 0.05) was powered to detect an increase of 12.5% or a decrease of 11% in prolactin concentration after administration of lorazepam, compared with placebo.

Plasma concentration of lorazepam and prolactin

Venous blood samples for analysis of lorazepam and prolactin concentration were collected prior to study drug administration and at ½, 1, 1¼, 1½, 2, 2½, 3¼, 4, 4½, 6, 8, 12 and 21 hours after study drug administration. Plasma concentrations of lorazepam were determined using LC-MS/MS with a lower limit of quantification of 0.3 ng/mL. Plasma concentrations of AZD7325 were determined using LC-MS/MS with a lower limit of quantification of 0.05 ng/mL. Plasma concentrations of AZD6280 were determined using LC-MS/MS with a lower limit of quantification of 0.15 ng/mL. Plasma concentrations of prolactin were determined using an electrochemiluminescence immunoassay (ECLIA) with a lower detection limit of 0.047 ng/mL.

Statistical analysis

Prolactin measurements up to 8 hours after administration of lorazepam or placebo were compared with a mixed model analysis of variance with treatment, period, time and treatment by time as fixed factors, and subject, subject by treatment and subject by time as random factors, and the pre-value (measurement prior to study drug administration) as covariate. Prolactin measurements were log-transformed prior to analysis to correct for the log-normal distribution of the data. Estimates of treatment differences and back-transformed estimates of the difference in percentage with corresponding 95% confidence intervals (95% CI) and *p*-values were calculated. All calculations were performed using SAS for Windows version 9.1.3 (SAS Institute Inc., Cary, North Carolina, USA).

RESULTS

Subjects

Subjects had a mean age of 28.1 years (range 18-54), weight of 76.1 kg (range 62.0-89.5) and body mass index (BMI) of 23.0 kg/m^2 (range 19.1-26.7). Two subjects withdrew informed consent after completion of study period 1 for reasons unrelated to study drug administration. Another subject tested positive for THC in study period 2 and was excluded from participation. Pharmacodynamic data from these subjects were not used for further analysis. All three subjects were replaced. Therefore, in total, 32 subjects completed the study.

Plasma concentration of lorazepam, AZD7325 and AZD6280

Plasma concentrations of lorazepam, AZD7325 and AZD6280 are shown in Figures 1, 2 and 3, respectively. The pharmacokinetics of lorazepam were similar to those reported in literature[54].

FIGURE 1 Time course of plasma concentration of lorazepam after administration of single oral doses of 2 mg lorazepam (at t = 0 hours). Means are presented with standard deviations as error bars.

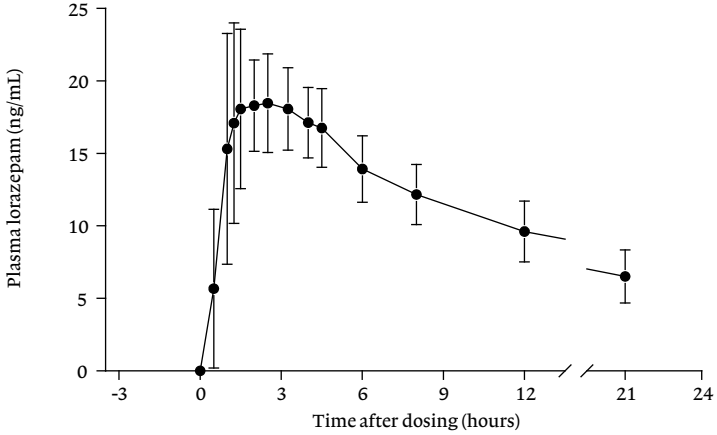

FIGURE 2 Time course of plasma concentration of AZD7325 after administration of single oral doses of 2 mg and 10 mg AZD7325 (at t = 0 hours). Means are presented with standard deviations as error bars.

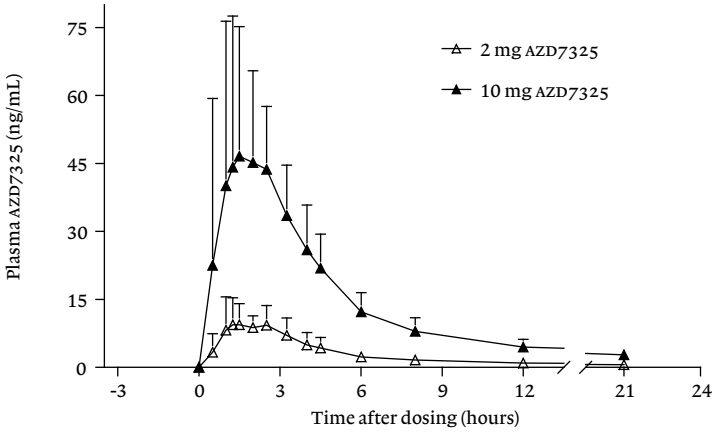

FIGURE 3 Time course of plasma concentration of AZD6280 after administration of single oral doses of 10 mg and 40 mg AZD6280 (at t = 0 hours). Means are presented with standard deviations as error bars.

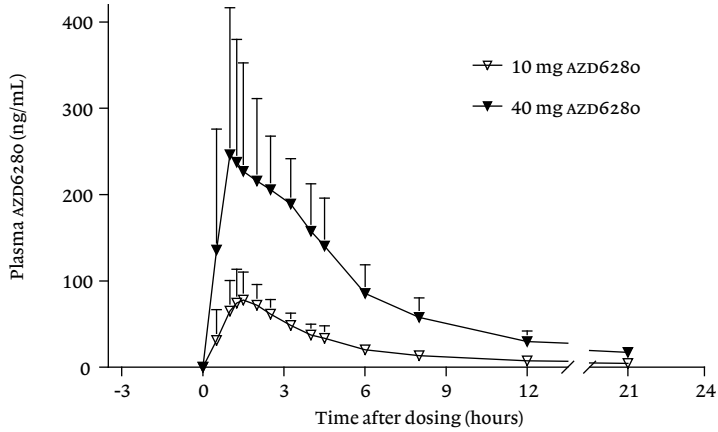

FIGURE 4 Time course of plasma concentration of prolactin after administration of single oral doses of 2 mg lorazepam, 2 mg AZD7325, 10 mg AZD7325, 10 mg AZD6280 and 40 mg AZD6280 (at t = 0 hours). Means are presented with standard deviations as error bars.

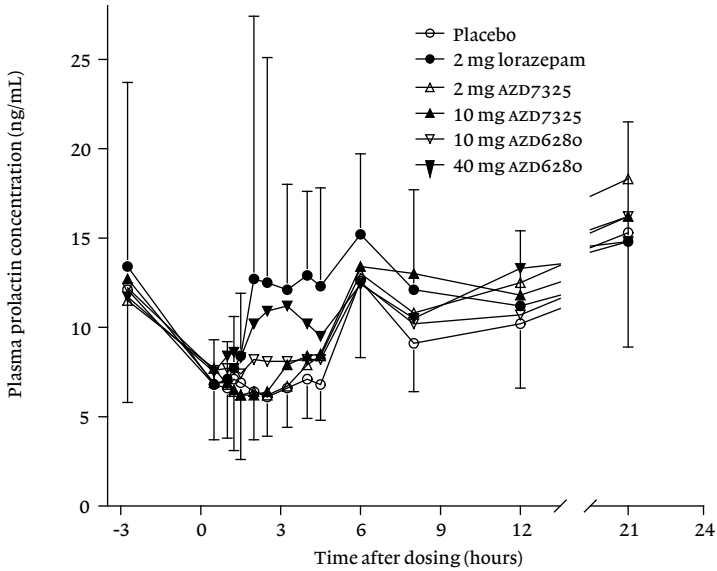

TABLE 1 Comparison of prolactin levels after administration of lorazepam, AZD7325 and AZD6280 compared with placebo. Treatment differences are expressed as percentages with 95% confidence intervals and p-values.

Treatment comparisons	Percentage difference (95% CI)	p-value
Lorazepam versus placebo	42.0 (31.4/53.5)	<0.0001
AZD7325 2 mg versus placebo	7.6 (-2.8/19.1)	0.1566
AZD7325 10 mg versus placebo	10.5 (-0.2/22.3)	0.0536
AZD6280 10 mg versus placebo	19.8 (8.2/32.6)	0.0007
AZD6280 40 mg versus placebo	32.8 (20.0/47.0)	<0.0001
AZD7325 2 mg versus lorazepam	-24.2 (-31.6/-16.1)	<0.0001
AZD7325 10 mg versus lorazepam	-22.2 (-29.7/-13.9)	<0.0001
AZD6280 10 mg versus lorazepam	-15.7 (-23.8/-6.7)	0.0012
AZD6280 40 mg versus lorazepam	-6.4 (-15.5/3.6)	0.1957

Plasma concentration of prolactin

Plasma concentrations of prolactin after administration of lorazepam, AZD7325 and AZD6280 are shown in Figure 4 and Table 1. Following administration of 2 mg lorazepam, prolactin levels increased with 42.0% compared with placebo (95% CI 31.4/53.5%, $p < 0.001$), which remained elevated until at least 8 hours after dose administration. Following administration of 2 mg and 10 mg AZD7325, prolactin levels increased with 7.6% and 10.5%, respectively, compared with placebo. Both increases did not reach statistical significance, although the 10.5% increase after the 10 mg dose has a *p*-value of 0.0536. Following administration of 10 mg AZD6280, prolactin levels increased significantly compared with placebo (difference 19.8% versus placebo, 95% CI 8.2/32.6%, $p = 0.0007$). A larger increase was observed after administration of 40 mg of AZD6280 (difference 32.8% versus placebo, 95% CI 20.0/47.0%, $p < 0.0001$). Prolactin levels after administration of lorazepam were significantly higher than those after AZD7325 at 2 and 10 mg doses and AZD6280 at 10 mg doses, but were not significantly different from those after AZD6280 at 40 mg doses.

DISCUSSION

The present study was performed to evaluate the effects of the GABAergic drugs on circulating prolactin levels in healthy subjects, compared with placebo. After administration of placebo, prolactin levels showed an initial decrease with a return to baseline values at the end of the study day, which is consistent with a normal circadian rhythm[2,55]. Also, a peak in prolactin levels was observed 6 hours

after dose administration, which probably reflects normal prolactin release following food consumption[56,57]. After administration of a single oral dose of 2 mg lorazepam, an increase of 42.0% in prolactin levels was observed. The magnitude of the effects of lorazepam on prolactin levels was rather small, especially in comparison to the much more potent prolactin-elevating effects of dopamine D_2 receptor antagonists. Haloperidol at 3 mg doses increases prolactin levels with 130.9%[58]. Thus, the effects of lorazepam administration on prolactin secretion are not likely to produce clinically relevant hyperprolactinaemia. However, our studies showed clear results in comparison with other studies that evaluated the effects of GABAergic drugs on basal prolactin levels in healthy subjects. The benzodiazepines diazepam and bromazepam showed no significant effects on prolactin levels under resting conditions[8–10], whereas temazepam caused a small increase in prolactin levels of roughly 21.4 mU/L (which would correspond to roughly 1 ng/mL), but only at a single time point 1 hour after dose administration[11]. In contrast, our study demonstrates that lorazepam increases prolactin levels with roughly 5-6 ng/ml, which remain elevated until at least 8 hours after dose administration. The dose of lorazepam (2 mg) used in our study is roughly equipotent with the doses of diazepam (10 mg), bromazepam (3 mg) and temazepam (20 mg) used in these earlies studies, although estimates of benzodiazepine dose equivalencies differ somewhat between various authors[59–61]. Dose dependency of the effects on prolactin secretion has been demonstrated with the benzodiazepine alprazolam, which causes an increase of prolactin levels with roughly 9-10 ng/mL at relatively high doses (3 mg)[12], while doses in the lower therapeutic range (0.5 mg) demonstrated no effects[13]. The different findings might be explained by the small sample sizes used in the earlier studies (6-10 subjects in most studies) and statistical power may thus have been too small.

The increase in prolactin levels following administration of the GABA agonist lorazepam in our study suggests that the postulated stimulatory effect of GABA transmission (by suppressing the tuberoinfundibular dopaminergic neurons in the hypothalamus) exceeds the postulated inhibitory effect of GABA transmission (either directly at the anterior pituitary gland or by stimulating GABA release from the median eminence into the hypophyseal portal vessels). The preferential effect of lorazepam on the tuberoinfundibular dopaminergic neurons might result from differences in affinity for the pituitary and hypothalamic GABA binding sites, as has been shown for the GABA agonist muscimol and antagonist bicuculline[62], both of which have higher affinity for the binding sites in the mediobasal hypothalamus than for the binding sites in the anterior pituitary. However, effects of benzodiazepines on the activity of the tuberoinfundibular dopaminergic neurons have not been confirmed in vivo in

man. A recent positron emission tomography (PET) study using the dopamine D_2 receptor ligand [^{11}C]FLB457 in healthy subjects has demonstrated that single oral doses of 2.5 mg lorazepam induce a statistically significant decrease in dopamine D_2 receptor binding potential (BP_{ND}) in the medial temporal and dorsolateral prefrontal cortex[63], but effects on the hypothalamus were not reported. Although a decrease in BP_{ND} (i.e. suggesting dopamine release) in the cerebral cortex does not imply that lorazepam inhibits the tuberoinfundibular dopaminergic pathway in the hypothalamus, it does confirm that lorazepam can alter dopamine levels in extrastriatal areas in humans in vivo.

The present study also evaluated the effects of two novel α_2/α_3 subunit-selective $GABA_A$ receptor modulators, AZD7325 and AZD6280, on prolactin levels. Administration of 2 mg and 10 mg AZD7325 produced small increases in prolactin levels, which did not reach statistical significance, although the 10.5% increase after the 10 mg dose has a *p*-value of 0.0536. Administration of 10 mg and 40 mg AZD6280 produced statistically significant increases in prolactin levels of 19.8% and 32.8%, respectively. These findings suggest that the α_2 and/or α_3 receptor subtypes are involved in GABAergic modulation of the tuberoinfundibular dopaminergic pathway. Indeed, α_2 and α_3 subunit-containing $GABA_A$ recepors have been shown to be expressed in the arcuate nucleus and hypothalamus[43]. However, it is not excluded that α_1 or α_5 receptor subtypes, which are also expressed in the arcuate nucleus and hypothalamus[43], are also involved in the control of prolactin secretion. The nonbenzodiazepine GABA agonist zolpidem (10 mg), which has modest selectivity for α_1 receptor subtypes[64], increased nocturnal prolactin levels by two-fold[15]. Furthermore, despite functional selectivity for α_2 and α_3 receptor subtypes, AZD7325 and AZD6280 also have limited efficacy at α_1 receptor subtypes (AstraZeneca data on file).

The effects of AZD7325 on prolactin secretion were less clear than those of AZD6280. Similarly, in other studies (Chen et al 2013, submitted; Chen et al 2013, in preparation), AZD7325 also caused fewer effects than AZD6280 on peak velocity of saccadic eye movements, which is one of the most consistent and sensitive biomarkers for the effects of nonselective benzodiazepines[65] and α_2/α_3 subtype-selective compounds[66] in healthy volunteers. These differences may be related to the lower dosages of AZD7325 used. Although the dosages of both compounds were expected to result in relatively high levels of $GABA_A$ receptor occupancy, AZD7325 is less effective in potentiation of GABA-induced current than AZD6280 at the α_2 receptor subtype (18% and 32%, respectively, compared to the maximal response of diazepam) and α_3 receptor subtype (15% and 34%, respectively), whereas efficacy is very low and roughly similar at the α_1 and α_5 receptor subtypes (AstraZeneca data on file).

In conclusion, our study demonstrates that single oral doses of 2 mg lorazepam increase plasma prolactin levels in healthy male subjects. Our findings contrast with the inconsistent results obtained in earlier studies with other GABAergic drugs. The sample sizes or the administered doses used in these earlier studies may have been too small to adequately demonstrate statistically significant differences, whereas our present results were obtained in an adequately powered group of 32 healthy volunteers, although it is not excluded that the different outcomes are caused by pharmacological differences between the various drugs. Our study also evaluated the effects of two novel α_2/α_3 subunit-selective $GABA_A$ receptor modulators, AZD7325 and AZD6280, on prolactin levels. AZD6280 produced significant increases in prolactin levels, which may indicate that the α_2 and/or α_3 receptor subtypes are involved in GABAergic modulation of prolactin secretion, although contributions of the α_1 and α_5 receptor subtypes are not excluded. The increases in prolactin levels after administration of AZD7325 did not reach statistical significance, which may be related to the lower dosages of AZD7325 used.

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CHAPTER 8

DISCUSSION

This thesis describes early phase drug development studies that are part of larger drug development programs which explore various stategies for improvement of dopaminergic pharmacotherapy. These strategies include improvement of receptor kinetics (CHAPTER 2 and 3) and receptor selectivity (CHAPTER 4), as well as targeting tachykinergic (CHAPTER 5 and 6) and GABAergic (CHAPTER 7) control mechanisms as a means to modulate dopamine neurotransmission. This thesis aims to evaluate pharmacokinetics and dose-effect relationships of several drug candidates on various neurophysiological parameters in healthy volunteers in order to show penetration through the blood-brain barrier, target engagement in vivo and differentiation of pharmacodynamic effects on several functional CNS domains. This chapter reviews the use of these different pharmacological approaches in the preceding chapters of this thesis and correlates the findings to currently available clinical data obtained with these drug candidates in patients.

Improvement of receptor kinetics

It has been proposed that competitive dopamine receptor antagonists that dissociate quickly from the receptor (i.e. a fast k_{off} constant) are more accommodating to physiological fluctuations of dopamine concentrations than drugs with a slow k_{off}[1]. Fast dissociation of an antipsychotic drug from the dopamine D_2 receptor might therefore allow for a drug effect to occur with a lower risk for D_2 receptor-mediated side effects[1]. Using the rate of dissociation from the D_2 receptor as a means to screen novel compounds for antipsychotic drug candidates, the novel fast dissociating selective dopamine D_2 receptor antagonist JNJ-37822681 was developed[2]. CHAPTER 2 describes the pharmacokinetics and central nervous system (CNS) effects of JNJ-37822681 in healthy volunteers. JNJ-37822681 was rapidly absorbed with a t_{max} of approximately 1–3 hours followed by a mean terminal half-life of 24–38 hours. The main CNS effect of JNJ-37822681 was dose-related elevation of serum prolactin, which increased with approximately 750% after 20 mg doses. Other subjective, neuropsychological and neurophysiological effects were generally small. This effect profile is likely the result of the selectivity of JNJ-37822681 for the D_2 receptor, leading to strong D_2 receptor-mediated elevations in serum prolactin, but fewer effects on more complex CNS functions, which are likely to involve multiple neurotransmitters. However, serum prolactin largely returned to baseline levels within 8 hours after administration of JNJ-37822681, which may prevent accumulation during chronic treatment. CHAPTER 3 evaluated the dopamine D_2 receptor occupancy by JNJ-37822681, which increased from 9-19% at 2 mg doses to 60-74% at 20 mg doses, with an estimated EC_{50} of 14.5 ng/mL. It has been suggested that 65–80% receptor occupancy is

optimal for most registered antipsychotic agents in terms of antipsychotic effect and adverse (i.e. extrapyramidal) events in clinical practice[3–5]. The present data indicate that 65–80% receptor occupancy is associated with plasma concentrations of 27–58 ng/mL of JNJ-37822681.

Based on **CHAPTER 2** and **CHAPTER 3** and the results of another [^{11}C]raclopride PET study[6], which evaluated D_2 receptor occupancy levels after multiple doses of 10 mg JNJ-37822681 twice daily, the dosing regimens of 10, 20, and 30 mg twice daily were selected for further study. A phase II multicentre, double blind, placebo- and olanzapine-controlled trial with JNJ-37822681 in patients with acute exacerbation of schizophrenia was performed, which indicated antipsychotic efficacy of all three dosing regimens superior to placebo to a roughly comparable extent[7]. Incidences of extrapyramidal symptoms were similar after administration of placebo, 15 mg olanzapine once daily, and 10 mg JNJ-37822681 BID, whereas incidence was higher after 20 or 30 mg JNJ-37822681 BID[7]. Average prolactin levels were similar after administration of placebo and 10 mg JNJ-37822681 BID, but were numerically higher after administration of 20 and 30 mg JNJ-37822681 BID and 15 mg olanzapine once daily[7]. Regarding metabolic effects, which are of particular concern with the use of atypical antipsychotic drugs[8], none of the JNJ-37822681 dosing regimens showed a significant change in triglycerides, cholesterol, free fatty acids, glucose, HbA1c or insulin, whereas the lipid profile deteriorated significantly with olanzapine[7]. In addition, all JNJ-37822681 dosing regimens showed lesser weight gain compared with olanzapine[7]. Although the lowest effective dose for JNJ-37822681 has not yet been established, the safety profile in the clinical studies suggests that the 10 mg BID dose may have the most positive benefit to risk ratio (i.e. efficacy with minimal to no weight gain, minimal metabolic effects or liability for extrapyramidal symptoms and no prolactin-elevating effects)[7].

Many studies have attempted to correlate serum prolactin concentrations with clinical response to dopamine D_2 antagonists in schizophrenia patients, which yielded inconsistent results[9–17]. Reasons for failure to establish this correlation include the use of high dosages (higher than required for maximal release of prolactin), failure to examine male and female patients separately (despite clear evidence that females develop higher prolactin levels), the use of clinician-adjusted dosages, varying time intervals between onset of antipsychotic therapy and assessment of clinical response, and differences in blood-brain barrier kinetics of the various drugs used[12,13,18]. Alternatively, several PET and SPECT studies have reported correlations between striatal D_2 receptor occupancy and prolactin levels[3,13,19–21] and demonstrated that increases in prolactin levels were associated with striatal D_2 receptor occupancy

higher than 50%[13] or higher than 72-73%[3,20]. However, other studies found no clear correlation between D_2 receptor occupancy and hyperprolactinemia[22-24], which may be attributable to sample sizes and differences in binding affinities and blood-brain barrier kinetics. The correlation between prolactin levels and striatal D_2 receptor occupancy by JNJ-37822681 can be inferred from **CHAPTER 2** and **CHAPTER 3**. The relationship between plasma concentration of JNJ-37822681 and striatal dopamine D_2 receptor occupancy was described in **CHAPTER 3**, as the following hyperbola:

$$\text{Receptor occupancy}\,(\%) = \frac{100 \times C_P}{C_P + 14{,}5}$$

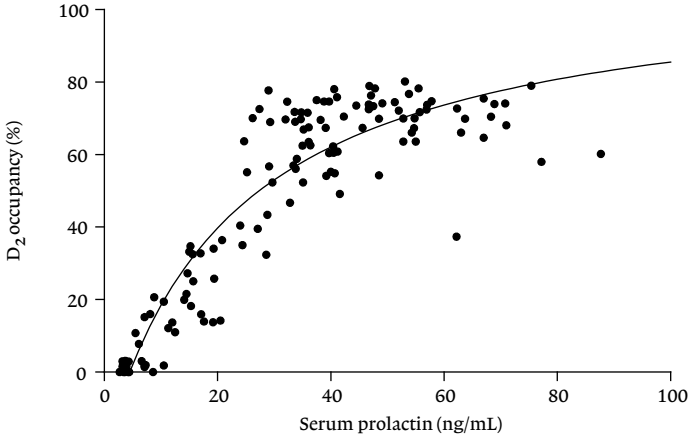
where C_p stands for the plasma concentration of JNJ-37822681. This relationship was determined using PET between 2 and 3 hours after dose administration. Application of this relationship to the plasma concentrations of JNJ-37822681 obtained within the same time interval in **CHAPTER 2**, which were measured together with prolactin concentrations, allows evaluation of the correlation between striatal D_2 receptor occupancy estimates and the associated serum prolactin concentrations. This yields a curvilinear relationship (see Figure 1), which demonstrates that prolactin values can be used to estimate striatal dopamine D_2 receptor occupancy by JNJ-37822681.

A review of CNS effects of antipsychotic drugs in healthy volunteers demonstrated that hyperprolactinaemia-inducing dose equivalencies of a wide range of registered antipsychotic drugs are correlated with affinity for the dopamine D_2 receptor and also with the lowest therapeutic daily maintenance dose[25]. Accordingly, it has been suggested that the effects of novel antipsychotic compounds may be compared with registered antipsychotic agents by using the prolactin response to estimate dose equivalencies and to estimate a likely therapeutic starting dose, provided that penetration through the blood-brain barrier is sufficient[25]. Using this strategy, an estimated dose of 5–10 mg of JNJ-37822681 was predicted as the lowest therapeutic maintenance dose, as described in **CHAPTER 2**. Although the phase II study was not able to identify the lowest effective dose for JNJ-37822681, it has confirmed antipsychotic efficacy for the 10 mg BID dose[7]. However, as demonstrated in **CHAPTER 3**, single oral doses of 10 mg produced D_2 receptor occupancy levels of merely 50-53%, which is well below the occupancy levels associated with clinical response for most registered antipsychotic drugs (i.e. 65-80%)[3-5]. In addition, the multiple dose PET study[6] demonstrated that D_2 receptor occupancy levels did not exceed 62% after multiple doses of 10 mg JNJ-37833681 BID. Therefore, significant antipsychotic efficacy of the 10 mg dose JNJ-37822681 BID was not immediately expected from these PET

data[6,7]. However, previous PET studies have also demonstrated relatively low occupancy levels with clozapine[26–28] and quetiapine[29] at clinically effective doses, which has been attributed to fast dissociation from the D_2 receptor[30]. Further studies with shorter time intervals between dosing of quetiapine and PET scanning showed higher occupancy levels, reflecting a rapid reduction in occupancy after transiently high levels[31,32]. If fast dissociation is the reason why clozapine and quetiapine are therapeutically active at relatively low striatal occupancy levels, this could also be the case for JNJ-37822681.

The phase II study demonstrated that JNJ-37822681 at 10 mg BID doses has antipsychotic efficacy with low incidence of extrapyramidal side effects, metabolic side effects and hyperprolactinemia, although the incidence of extrapyramidal side effects as well as circulating prolactin levels seem to increase somewhat with higher dosing regimens[7]. However, the lowest effective dose of JNJ-37822681 has not yet been established. Therefore, the full therapeutic window of clinical antipsychotic efficacy of JNJ-37822681 remains to be defined. Confirmation of the effect profile of JNJ-37822681 in large phase III trials may further support the 'fast dissociation' hypothesis as a means to achieve signifcant antipsychotic effect with lower potential for side effects and also corroborate the use of prolactin dose equivalencies as an antidopaminergic predictor of antipsychotic activity.

FIGURE 1 Correlation between serum concentration of prolactin and striatal dopamine D_2 receptor occupancy by JNJ-37822681, limited to a time interval between 2 and 3 hours after administration of doses between 0.5 and 20 mg.



Improvement of receptor selectivity

Despite the major role of dopamine neurotransmission in the acute rewarding effects of addictive drugs, no dopaminergic agents have been demonstrated to be uniformly effective for drug addiction[33], which may be (at least partly) due to lack of pharmacological and functional selectivity for the mesolimbic pathway, as well as poor tolerability. Given the primarily mesolimbic expression pattern of dopamine D_3 receptors, it has been suggested that selective dopamine D_3 receptor antagonism may be an effective strategy in pharmacotherapy of addiction by decreasing craving and preventing relapse[34]. GSK598809 is a novel selective dopamine D_3 receptor antagonist[35]. **CHAPTER 4** describes the pharmacokinetics and CNS effects of single oral doses of 175 mg GSK598809 in healthy volunteers. In addition, possible interactions between GSK598809 and alcohol were evaluated, because the target population of patients will have alcohol dependence as primary disorder or may abuse alcohol as comorbidity next to another substance abuse disorder. Plasma concentration of GSK598809 was maximal 2-3 hours postdose and decreased with a half life of roughly 20 hours. CNS effects of GSK598809 were limited to prolactin elevation and a decrease in adaptive tracking performance. The cause of prolactin elevation by GSK598809 is not known and could reflect antagonist effects at dopamine D_3 receptors in the tuberoinfundibular dopaminergic pathway, but could also be due to off-target antagonist effects at dopamine D_2 receptors, despite a greater than 100-fold selectivity of GSK599809 for D_3 receptors over D_2 receptors. The increases in serum prolactin following GSK598809 administration were much larger in female volunteers (approximately 900%) than in male volunteers (approximately 350%), probably due to the effects of estrogens. Co-administration of GSK598809 and alcohol did not affect alcohol pharmacokinetics, but caused a 9% decrease of C_{max} and a 15% increase of AUC of GSK598809, which was not considered to be of any clinical significance. CNS effects of co-administration were mainly additive, except a small supra-additive increase in saccadic reaction time and decrease in delayed word recall. GSK598809 did not significantly affect the subjective feelings of drunkenness after alcohol administration. Thus, dopamine D_3 receptor antagonism does not appear to inhibit the neurophysiological effects of alcohol. These findings are in line with preclinical models which have shown that selective dopamine D_3 receptor antagonists do not affect the primary reinforcing effects of drugs of abuse, but rather regulate the motivation to self-administer drugs under certain schedules of reinforcement[34]. No validated biomarkers for drug addiction or (challenge) models in healthy volunteers exist, which precludes any means for prediction of pharmacodynamic effects in patients.

In a study in healthy male and female smokers[36], a single dose of 75 mg GSK598809, giving submaximal levels of dopamine D_3 receptor occupancy (72–89%), transiently alleviated craving after overnight abstinence. Although the findings also suggested that GSK598809 does not affect the rewarding properties of nicotine per se (in line with preclinical findings showing that selective D_3 receptor antagonists do not interfere with the primary reinforcing properties of drugs of abuse), there was a slightly increase in cigarette consumption and puffs/cigarette when subjects were allowed to smoke freely in the natural environment after the experimental session, which the authors attributed to a compensatory mechanism[36]. These data provide the first clinical evidence of potential efficacy of a selective D_3 receptor antagonist for the treatment of substance abuse disorders[36]. However, further studies with long term abstinent smokers motivated to quit, with repeated dose treatment designed to achieve higher and more sustained levels of D_3 receptor occupancy, are needed to further investigate the role of GSK598809 or other dopamine D_3 receptor antagonists in human craving[36].

Modulation of the tachykinergic control of dopamine neurotransmission

An alternative to direct pharmacological modulation of dopamine neurotransmission is modulation of the control mechanisms of dopamine neurotransmission. Among the many control mechanisms, the peptide neuromodulators have been proposed as suitable targets for novel drug candidates, perhaps even advantageous compared with antagonists to classic monamine neurotransmitters[37]. Tachykinins (also known as neurokinins) are a group of related peptide neurotransmitters that control and activate dopaminergic neurons in all major dopaminergic pathways[38]. Accordingly, it has been suggested that antagonists at tachykinin (NK_1) receptors may modulate stress- and reward-related processes and may contribute in altering drug reward[39]. In **CHAPTER 5**, the pharmacokinetics and CNS effects of the NK_1 receptor antagonist aprepitant, and possible interactions with alcohol, were investigated in healthy volunteers. Aprepitant did not significantly affect CNS performance and there were no indications for clinically relevant interactions between aprepitant and alcohol. In **CHAPTER 6**, the pharmacokinetics and CNS effects of the dual NK_1/NK_3 receptor antagonist GSK1144814 were investigated in alcohol-intoxicated volunteers. Co-administration of GSK1144814 and alcohol caused only small additional CNS effects, compared with administration of alcohol alone, which are not likely to cause clinically relevant impairments. GSK1144814 did not significantly affect the subjective feelings of drunkenness after alcohol administration. The doses of aprepitant and GSK1144814 used in **CHAPTER 5** and **6** can produce high levels

of receptor occupancy, as demonstrated with positron emission tomography using [^{18}F]SPA-RQ[40] and [^{11}C]GR205171 (GlaxoSmithKline data on file), respectively. Thus, effects of antagonists at central NK_1 and NK_3 receptors on CNS performance in healthy volunteers appear to be very limited or absent.

It has been previously argued that the poor results obtained with tachykinin antagonists in clincial trials might be attributable to redundancy in expression of tachykinins and NK receptor subtypes[41]. Substance P and neurokinin A are both encoded by the preprotachykinin A (PPT-A or TAC1) gene, whereas neurokinin B is encoded by the preprotachykinin B (PPT-B or TAC3) gene[38,41]. These two PPT genes exhibit marked structural similarity and therefore possibly evolved from a common ancestor gene by duplication[38]. Also, the three known NK receptor genes originate from duplication of a common ancestral gene[41]. Duplicated genes are common in genomes and the products may have different functions but in many occasions they share the same function[41]. A main consequence of redundancy could be that the loss of function of a particular gene product (e.g. tachykinins or NK receptors) might not have noticeable effects due to functional substitution by the duplicate gene product[41]. Indeed, considerable overlap exists between the expression patterns of NK_1 and NK_3 receptors in the brain[38]. Also, the neuropeptides substance P, neurokinin A and neurokinin B all have demonstrated affinity to all three NK receptors to some extent[38,41]. Functional substitution could theoretically explain the lack of effect of the selective NK_1 receptor aprepitant in healthy volunteers in **CHAPTER 5**. However, as demonstrated in **CHAPTER 6**, simultaneous antagonism of both NK_1 and NK_3 receptors with GSK1144814 in alcohol-intoxicated volunteers caused only small additional CNS effects, compared with administration of alcohol alone. Although the CNS effects of GSK1144814 alone were not investigated, comparisons with reference data suggested that these are unlikely to be large. In addition, a recent study of the selective NK_3 receptor antagonist talnetant in healthy volunteers demonstrated improved adaptive tracking performance, decreased α power electroencephalography (EEG) and reduced feelings of calmness, all to a small extent, while VVLT performance, body sway, finger tapping, saccadic and smooth pursuit eye movements were not affected[42]. Taken together, these studies suggest that the effects of selective NK_1 antagonists or NK_3 antagonists are limited or absent, and a dual NK_1/NK_3 receptor antagonist probably also has limited CNS effects in healthy volunteers. This argues against significant functional substitution of NK_1 receptor-mediated events by NK_3 receptors.

A more likely explanation for the relative lack of effect of tachykinin receptor antagonists in healthy volunteers may be the fact that much evidence indicates

that neuropeptides are released after stressful and noxious stimuli, challenges or pathological conditions[37,43,44]. Neuropeptide receptor antagonists might therefore have significant effects in pathological conditions with increased peptide release, whereas effects in normal healthy volunteers are limited[37,43]. This would imply that measurements of neurophysiological parameters in healthy volunteers are not an adequate model for pharmacodynamic effects in patients. An alternative would be to increase peptide release by functional or pharmacological challenge tests, provided that this can be achieved in a safe manner. Currently, no readily applicable and validated pharmacological challenges or models exist in humans. To this end, pharmacological challenges such as amphetamine or other drugs of abuse, which are known to induce dopamine release, could be evaluated for possible induction of tachykinin release. Alcohol has also been shown to increase dopamine levels in the mesolimbic pathway[45,46]. Thus, intravenous alcohol infusion might constitute a mild dopaminergic challenge. No indications for clinically relevant interactions between aprepitant and alcohol were found, as demonstrated in CHAPTER 5, whereas GSK1144814 caused only small additional CNS effects on top of those of alcohol alone, as demonstrated in CHAPTER 6. However, the target alcohol serum concentration of 0.6-0.65 g/L may have been too small to detect any modifications by tachykinin antagonists.

The NK_1 receptor antagonist aprepitant has been shown to be effective against postoperative and chemotherapy-induced nausea and vomiting[47]. At present, the effects on other dopaminergic disorders are unclear. A clinical trial with the NK_1 receptor antagonist LY686017 in detoxified alcoholic inpatients (n = 25) demonstrated suppression of spontaneous alcohol cravings and improved overall well-being[48]. Preliminary findings from another small pilot study in patients maintained on methadone[49] demonstrated that aprepitant slightly diminished the desire to use methadone, compared with placebo, but the effect did not reach statistical significance, possibly due to the study design and the small sample size (n = 15). However, aprepitant also appeared to increase the positive subjective effects of methadone[49]. Similarly, another recent study evaluated the effects of aprepitant on the response to oxycodone in opioid abusers (n = 8)[50], which demonstrated that aprepitant significantly enhanced the subjective and observer-rated effects of euphoria and liking. Although these findings do not clearly confirm therapeutic utility of aprepitant in the treatment of substance abuse disorders, they do suggest a biologically meaningful interaction between NK_1 receptors and drug reward-related processes, worthy of further exploration[50].

Modulation of the GABAergic control of dopamine neurotransmission

GABA is one of the major inhibitors of dopamine neurotransmission. Accordingly, drugs that modulate GABA neurotransmission may indirectly influence dopamine neurotransmission. It has recently been suggested that subunit-selective $GABA_A$ receptor agonists, in contrast to the nonselective benzodiazepines, may have therapeutic potential in schizophrenia by selectively inhibiting dopamine neurotransmission in the mesolimbic pathway[51]. Selective agonists at α_3 receptor subtypes without efficacy at α_1 receptor subtypes may attenuate dopamine neurotransmission in the mesolimbic pathway which, unlike benzodiazepines, is not counteracted by disinhibition from GABAergic interneurons[51]. Thus, $GABA_A$ receptor subunit-selective compounds may differ significantly from nonselective benzodiazepines in their effects on dopaminergic pathways. To explore the exact role of the various receptor subtypes in the regulation of different dopaminergic activities, CHAPTER 7 describes the effects of two novel positive modulators of α_2 and α_3 subunit-containing $GABA_A$ receptors, AZD7325 and AZD6280[52], and the nonselective benzodiazepine lorazepam on circulating prolactin levels as a marker for activity of the tuberoinfundibular pathway, which is the most readily accessible dopaminergic pathway for evaluation in vivo. Prolactin levels increased significantly after administration of AZD6280 and lorazepam, whereas increases in prolactin levels after administration of AZD7325 did not reach statistical significance. This difference may be related to the lower dosages of AZD7325 used, which was also suggested in another study that demonstrated a relative lack of pharmacodynamic effects of the same dose of AZD7325 (Chen et al 2013, submitted). These findings suggest that the α_2 and/or α_3 receptor subtypes are involved in GABAergic modulation of the tuberoinfundibular dopaminergic pathway, but do not exclude that α_1 or α_5 receptor subtypes are also involved. These results clearly confirm modulating effects of GABAergic drugs on prolactin levels in human in vivo, which contrasts with the inconsistent results obtained in many earlier studies with other GABAergic drugs.

Recently, GABAergic drugs have been suggested as possible treatments for several diseases, such as (aspects of) Parkinson's disease[53], drug addiction[54], schizophrenia and other neuropsychiatric disorders[51,55,56]. None of these strategies have yet found general application in clinical practice, which may reflect the complex role of GABAergic mechanisms in the regulation of dopaminergic circuits and the lack of specific compounds. The modulatory effects of $GABA_A$ receptor subtype-selective compounds on the tuberoinfundibular pathway, as demonstrated in CHAPTER 7, offers the possibility that these compounds are selective enough for targeted modification of other dopaminergic pathways with

fewer off-target effects on other $GABA_A$ receptor subtypes. However, given the complex control mechanisms of prolactin secretion[57,58] and GABAergic neurocircuitry[59,60], effects of GABAergic drugs on the tuberoinfundibular pathway, as demonstrated in CHAPTER 7, cannot readily be extrapolated to the mesocorticolimbic and striatonigral dopaminergic pathways. Future PET studies using [^{11}C]raclopride and [^{18}F]fallypride are needed to evaluate the indirect effects of subunit-selective $GABA_A$ receptor modulators on dopaminergic neurotransmission in striatal and extrastriatal areas.

Conclusion

The neurotransmitter dopamine is involved in the pathogenesis of several neuropsychiatric disorders, but pharmacological methods to alter dopamine neurotransmission currently have only limited efficacy in alleviating the symptoms of these disorders, while adverse side effects can be debilitating. The findings in this thesis and clinical follow-up studies demonstrate that modulation of kinetics at the level of the dopamine receptors, as well as improvement of selectivity for dopamine receptor subtypes, are promising strategies to increase efficacy and reduce side effects of dopaminergic pharmacotherapy. Modulation of tachykinergic and GABAergic control of dopamine neurotransmission as a strategy to improve dopaminergic pharmacotherapy currently have not yet shown clear indications of therapeutic utility, but the findings do suggest biologically meaningful effects of these indirect dopamine modulators that are worthy of further exploration. These lines of research demonstrate that, despite the large number of registered dopamine receptor agonists and antagonists, the therapeutic potential of dopaminergic agents is far from exhausted.

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SUMMARY

The neurotransmitter dopamine is involved in various neurophysiological functions as well as the pathogenesis of several neuropsychiatric disorders, including Parkinson's disease, schizophrenia, drug addiction and hyperprolactinemia. A large number of registered dopamine agonists and antagonists is available for treatment of these disorders, but these have only limited efficacy in alleviating the symptoms of these disorders, while adverse side effects can be debilitating. Thus, improvement of dopaminergic pharmacotherapy remains a high priority. The introduction of this thesis (CHAPTER 1) provides a detailed overview of the physiological role of dopamine, the involvement in the pathophysiology of several neuropsychiatric disorders, and the specific aims of this thesis. The following chapters describe several strategies for improvement of dopaminergic pharmacotherapy, including improvement of receptor kinetics (CHAPTER 2 and 3) and receptor selectivity (CHAPTER 4), as well as targeting control mechanisms as a means to modulate dopamine neurotransmission (CHAPTER 5, 6 and 7). This thesis aims to evaluate pharmacokinetics and dose-effect relationships of several drug candidates on various neurophysiological parameters in healthy volunteers in order to show penetration through the blood-brain barrier, target engagement in vivo and differentiation of pharmacodynamic effects on several functional CNS domains. In the discussion chapter of this thesis (CHAPTER 8) the findings in the preceding chapters of this thesis are correlated to currently available clinical data obtained with these drug candidates in patients, which have appeared in the literature.

Improvement of receptor kinetics

It has been proposed that competitive dopamine receptor antagonists that dissociate quickly from the receptor (i.e. a fast k_{off} constant) are more accommodating to physiological fluctuations of dopamine concentrations than drugs with a slow k_{off}. This characteristic might lead to a significant pharmacotherapeutic effect, with an appropriate functioning of normal dopaminergic neurotransmission and a substantially lower risk for side effects associated with persistent strong activity at the D_2 receptor. Using this hypothesis, the novel selective dopamine D_2 receptor antagonist JNJ-37822681, which has a fast k_{off} constant, was developed. CHAPTER 2 describes the pharmacokinetics of JNJ-37822681 in healthy volunteers. In addition, pharmacodynamic effects of JNJ-37822681 on several neurophysiological functions were evaluated. The main effect of JNJ-37822681 was elevation of serum prolactin. Other neurophysiological effects were generally small, compared with the effects on prolactin secretion. This effect profile is likely the result of the selectivity of JNJ-37822681 for the D_2 receptor, leading to strong D_2 receptor-mediated elevations in serum prolactin, but

fewer effects on more complex CNS functions, which are likely to involve multiple neurotransmitters. Using prolactin concentrations, dose equivalencies of new and registered antipsychotics can be estimated. Accordingly, 5-10 mg JNJ-37822681 was predicted as the lowest therapeutic maintenance dose.

CHAPTER 3 evaluated if JNJ-37822681, despite its fast rate of dissociation, is able to achieve significant levels of dopamine D_2 receptor occupancy. Dopamine D_2 receptor occupancy in the corpus striatum was evaluated with positron emission tomography (PET) using [^{11}C]raclopride in healthy volunteers. Receptor occupancy increased from 9-19% at 2 mg doses to 60-74% at 20 mg doses of JNJ-37822681. Receptor occupancy following therapeutic doses of most registered antipsychotic agents is roughly 65-80%. Thus, despite the fast k_{off}, JNJ-37822681 is able to occupy a significant percentage of receptors.

In the discussion chapter of the thesis, **CHAPTER 8**, the results of the first clinical trial evaluating the efficacy of JNJ-37822681 in the treatment of schizophrenia patients are reviewed. Twice daily dosing with 10 mg, 20 mg and 30 mg all demonstrated antipsychotic efficacy to a roughly comparable extent. Although the lowest effective dose for JNJ-37822681 has not yet been established, these results confirm therapeutic efficacy of the 10 mg BID dose, which is in line with the prediction in **CHAPTER 2** that 5-10 mg may be the lowest therapeutic maintenance dose. Receptor occupancy of the 10 mg dose, as demonstrated in **CHAPTER 3**, is lower than the receptor occupancy of most other antipsychotic drugs, but is more comparable to the receptor occupancy of clozapine, which may be explained by the fast rate of dissociation of both JNJ-37822681 and clozapine. Further studies on the effects of JNJ-37822681 are needed, but the present results indicate that modulation of receptor kinetics may be a promising strategy for drug development.

Improvement of receptor selectivity

Improvement of selectivity of a drug for a receptor may reduce side effects associated with interactions with receptors other than the primary target. Despite the major role of dopamine in the neurobiological effects of addictive drugs and the pathophysiology of drug addiction, no dopaminergic agents have been demonstrated to be uniformly effective for drug addiction, which may be (at least partly) due to lack of functional selectivity. It has been suggested that selective dopamine D_3 receptor antagonists, contrary to nonselective antagonists, may have therapeutic efficacy in the treatment of drug addiction. In theory, dopamine D_3 antagonists do not affect the primary reinforcing properties of drugs of abuse, but may decrease craving and prevent relapse. **CHAPTER 4** describes the pharmacokinetics of 175 mg doses of the novel selective dopamine D_3 receptor

antagonist GSK598809, as well as the pharmacodynamic effects on several neurophysiological functions in healthy volunteers. In addition, possible interactions between GSK598809 and alcohol were evaluated, because the target population of patients will have alcohol dependence as primary disorder or may abuse alcohol as comorbidity next to another substance abuse disorder. Also, alcohol has small stimulating effects on mesolimbic dopaminergic neurotransmission. Thus, interactions between GSK598809 and the dopaminergic reward system might also be evaluated. The main effect of GSK598809 was elevation of prolactin concentration. The cause of prolactin elevation by GSK598809 is not known and could reflect antagonist effects at dopamine D_3 receptors in the tuberoinfundibular dopaminergic pathway, but could also be due to off-target antagonist effects at dopamine D_2 receptors, despite a greater than 100-fold selectivity of GSK599809 for D_3 receptors over D_2 receptors. The increases in serum prolactin following GSK598809 administration were much larger in female volunteers than in male volunteers, probably due to the effects of estrogens. GSK598809 had no other neurophysiological effects except for a small impairment of eye-hand coordination. Co-administration of GSK598809 and alcohol did not affect alcohol pharmacokinetics and caused only small effects on the pharmacokinetics of GSK598809. CNS effects of co-administration were mainly additive. Thus, dopamine D_3 receptor antagonism does not appear to inhibit the neurophysiological effects of alcohol. These findings are in line with preclinical models which have shown that selective dopamine D_3 receptor antagonists do not affect the primary reinforcing effects of drugs of abuse, but rather regulate the motivation to self-administer drugs.

In the discussion chapter of this thesis, **CHAPTER 8**, the results of the first clinical trial evaluating the efficacy of GSK598809 in male and female smokers are reviewed. In that study, GSK598809 transiently alleviated craving, which constitutes the first clinical evidence of potential efficacy of a selective D_3 receptor antagonist for the treatment of substance abuse disorders, although further studies are needed to confirm these findings.

Modulation of the tachykinergic control of dopamine neurotransmission

An alternative to direct pharmacological modulation of dopamine neurotransmission is modulation of the control mechanisms of dopamine neurotransmission. Tachykinins are peptide neurotransmitters that control and activate dopaminergic neurons in all major dopaminergic pathways, and are thus suitable targets for novel drug development. Antagonists at tachykinin NK_1 and NK_3 receptors may have therapeutic efficacy in the treatment of drug addiction and schizophrenia. In **CHAPTER 5**, the pharmacodynamic effects of the NK_1 receptor

antagonist aprepitant on several neurophysiological functions were evaluated in healthy volunteers, as well as possible interactions with alcohol. In addition, amitriptyline was included as a positive control to obtain a further impression of the profile of neurophysiological effects. Aprepitant did not cause any neurophysiological impairments and there were no indications for clinically relevant interactions between aprepitant and alcohol. In **CHAPTER 6**, the effects of the novel dual NK_1/NK_3 receptor antagonist GSK1144814 onto the neurophysiological effects of alcohol were investigated in healthy volunteers. GSK1144814 caused only small additional effects on the effects of alcohol, which are not likely to cause clinically relevant impairments. The lack of effects of tachykinin antagonists in healthy volunteers is likely the result of the fact that neuropeptides (including tachykinins) are mainly released after stressful and noxious stimuli, challenges or pathological conditions. As a result, neuropeptide receptor antagonists might therefore have significant effects in pathological conditions with increased peptide release, whereas effects in normal healthy volunteers are limited. Following mild dopaminergic stimulation by alcohol infusion, there were no indications of clinically relevant interactions between aprepitant or GSK1144814 and alcohol. However, the serum concentration of alcohol may have been too low to show effects of tachykinin receptor antagonists.

In the discussion chapter of this thesis, **CHAPTER 8**, the effects of aprepitant in disorders characterized by dopaminergic stimulation are reviewed. Aprepitant is registered for clinical use to prevent nausea and vomiting induced by chemotherapy. Recently, the first preliminary results of studies evaluating the effects of aprepitant in the treatment of drug addicted patients have been published. Although aprepitant caused a small, statistically non-significant decrease in the desire to use methadone, aprepitant also appeared to increase the positive subjective effects of methadone and oxycodon. Although these findings do not clearly confirm therapeutic utility of aprepitant in the treatment of substance abuse disorders, they do suggest a biologically meaningful interaction between NK_1 receptors and drug reward-related processes, worthy of further exploration.

Modulation of the GABAergic control of dopamine neurotransmission

GABA is one of the major inhibitors of dopamine neurotransmission. Accordingly, drugs that modulate GABA neurotransmission may indirectly influence dopamine neurotransmission. Compounds with functional selectivity for $GABA_A$ receptor subtypes may have selective modulatory effects on dopaminergic systems. The exact role of the $GABA_A$ receptor subtypes in the regulation of different dopaminergic activities is not fully known. **CHAPTER 7** evaluates the

GABAergic control of the tuberoinfundibular dopamine system. The effects of two novel positive modulators of α_2 and α_3 subunit-containing $GABA_A$ receptors, AZD7325 and AZD6280, and the nonselective benzodiazepine lorazepam on circulating prolactin levels were evaluated in healthy male volunteers. Prolactin levels increased significantly after administration of AZD6280 and lorazepam, whereas increases in prolactin levels after administration of AZD7325 did not reach statistical significance, probably because the dosages were too low. These findings suggest that the α_2 and/or α_3 receptor subtypes are involved in GABAergic modulation of the tuberoinfundibular dopaminergic pathway, but do not exclude that α_1 or α_5 receptor subtypes are also involved. Thus, $GABA_A$ subtype-selective compounds are able to modulate dopaminergic systems. Absence of effects on other $GABA_A$ receptors might prevent other GABAergic side effects, such as sedation or instability. However, the effects of these compounds on other dopaminergic systems cannot be extrapolated from these data and need to be examined separately.

Conclusion

The neurotransmitter dopamine is involved in various neurophysiological functions and is involved in the pathogenesis of several neuropsychiatric disorders, including Parkinson's disease, schizophrenia, drug addiction and hyperprolactinemia. Current pharmacological methods to alter dopamine neurotransmission have only limited efficacy in alleviating the symptoms of these disorders, while adverse side effects can be debilitating. Thus, improvement of dopaminergic pharmacotherapy remains a high priority. The findings in this thesis and clinical follow-up studies demonstrate that modulation of kinetics at the level of the dopamine receptors, as well as improvement of selectivity for dopamine receptor subtypes, are promising strategies to increase efficacy and reduce side effects of dopaminergic pharmacotherapy. Modulation of tachykinergic and GABAergic control mechanisms currently have not yet shown clear indications of therapeutic utility, but the findings do suggest biologically meaningful effects of these indirect dopamine modulators that are worthy of further exploration. These lines of research demonstrate that, despite the large number of registered dopamine receptor agonists and antagonists, the therapeutic potential of dopaminergic agents is far from exhausted.

SAMENVATTING

De neurotransmitter dopamine speelt een essentiële rol in diverse neurofysiologische functies en is betrokken bij de pathofysiologie van diverse neuropsychiatrische aandoeningen, waaronder de ziekte van Parkinson, schizofrenie, drugsverslaving en hyperprolactinemie. Er is een groot aantal geregistreerde dopamine receptor agonisten of antagonisten beschikbaar voor de behandeling van deze ziekten, maar deze hebben slechts beperkt effect in vermindering van de symptomen, terwijl hinderlijke bijwerkingen kunnen optreden. Derhalve heeft verbetering van de farmacotherapie van deze ziekten een hoge prioriteit. De inleiding van dit proefschrift (**HOOFDSTUK 1**) geeft een gedetailleerd overzicht van de fysiologische rol van dopamine, de betrokkenheid bij de pathofysiologie van diverse neuropsychiatrische aandoeningen, en de doelstellingen van dit proefschrift. De verdere hoofdstukken beschrijven een aantal strategieën die momenteel worden toegepast in diverse onderzoekslijnen ter verbetering van dopaminerge farmacotherapie, namelijk verbetering van de kinetiek van het geneesmiddel ter plaatse van de receptor (**HOOFDSTUK 2** en **3**), verbetering van de selectiviteit van het geneesmiddel (**HOOFDSTUK 4**), en beïnvloeding van de controle mechanismen van dopamine (**HOOFDSTUK 5**, **6** en **7**). Dit proefschrift onderzoekt de farmacokinetiek en dosis-effect relaties van een aantal experimentele geneesmiddelen op verschillende neurofysiologische functies in gezonde vrijwilligers. Aldus wordt gepoogd passage door de bloed-hersenbarrière aan te tonen, binding aan de receptoren in de hersenen te bevestigen en farmacodynamische effecten op diverse functionele domeinen van het centrale zenuwstelsel te beschrijven. In de discussie van dit proefschrift (**HOOFDSTUK 8**) worden de uitkomsten van deze studies gecorreleerd aan de resultaten van onderzoeken naar de therapeutische effecten van behandeling van patiënten met deze experimentele geneesmiddelen, die inmiddels in de literatuur zijn verschenen.

Verbetering van de receptor kinetiek

Enkele jaren geleden werd de hypothese geformuleerd dat competitieve receptor antagonisten met een snelle dissociatie na binding aan de receptor (d.w.z. een snelle k_{off} constante) een flexibeler effect hebben met betrekking tot fysiologische fluctuaties in concentraties van dopamine dan geneesmiddelen met een langzame k_{off}. Deze eigenschap zou zodoende kunnen leiden tot een significant farmacotherapeutisch effect, terwijl de normale dopaminerge neurotransmissie zo weinig mogelijk wordt verstoord, met aanzienlijk minder bijwerkingen (door langdurig sterk receptor antagonisme) tot gevolg. Op basis van deze hypothese werd de selectieve dopamine D_2 receptor antagonist JNJ-37822681, welke een snelle k_{off} constante heeft, ontwikkeld. **HOOFDSTUK 2** beschrijft de

farmacokinetiek van JNJ-37822681 in gezonde vrijwilligers. Tevens werden de farmacodynamische effecten van JNJ-37822681 op diverse neurofysiologische functies onderzocht. Het belangrijkste effect van JNJ-37822681 was stijging van de plasma concentratie van prolactine. De overige neurofysiologische effecten van JNJ-37822681 waren slechts bescheiden in vergelijking met de effecten op prolactine secretie. Dit profiel van effecten is waarschijnlijk het gevolg van hoge selectiviteit van JNJ-37822681 voor de dopamine D_2 receptor. Dit leidt tot sterke effecten op prolactine secretie, wat normaliter voornamelijk gereguleerd wordt door dopamine en dopamine D_2 receptoren, maar kleinere effecten op complexere neurofysiologische functies, die veelal door meerdere neurotransmitters gereguleerd worden. Met behulp van prolactine concentraties kunnen schattingen van de equivalente doseringen van nieuwe en geregistreerde antipsychotica gemaakt worden. Zodoende werd een voorspelling gemaakt dat 5-10 mg JNJ-37822681 de laagste therapeutische onderhoudsdosering is.

In **HOOFDSTUK 3** werd onderzocht of JNJ-37822681, ondanks de hoge snelheid van dissociatie, in staat is om een significant percentage van de dopamine D_2 receptoren te bezetten. De dopamine D_2 receptor bezetting in het corpus striatum werd onderzocht door middel van positron emissie tomografie (PET) en [^{11}C]raclopride in gezonde vrijwilligers. De receptor bezetting nam toe van 9-19% bij 2 mg doseringen tot 60-74% bij 20 mg doseringen van JNJ-37822681. De receptor bezetting van therapeutische doseringen van de meeste geregisteerde antipsychotica bedraagt ongeveer 65-80%. Derhalve is JNJ-37822681, ondanks de snelle k_{off}, in staat een relevant percentage van receptoren te bezetten.

In het discussie hoofdstuk van dit proefschrift, **HOOFDSTUK 8**, werden de resultaten van het eerste klinische onderzoek naar behandeling van schizofrenie patiënten met JNJ-37822681 besproken. Tweemaal daags behandeling met 10 mg, 20 mg of 30 mg bleken allemaal een vergelijkbaar antipsychotisch effect in schizofrenie patiënten teweeg te brengen. Alhoewel de laagste therapeutische onderhoudsdosering vooralsnog niet bekend is, bevestigen deze resultaten therapeutisch effect van de 10 mg BID dosering, hetgeen in overeenstemming is met de voorspelling in **HOOFDSTUK 2** dat 5-10 mg JNJ-37822681 de laagste therapeutische onderhoudsdosering is. De receptor bezetting van de 10 mg dosering, zoals vastgesteld in **HOOFDSTUK 3**, is lager dan de receptor bezetting van de meeste andere antipsychotica, maar is meer vergelijkbaar met de receptor bezetting van clozapine, hetgeen mogelijk verklaard kan worden door de snelle dissociatie van JNJ-37822681 en clozapine. Verdere onderzoek naar de effecten van JNJ-37822681 is nodig, maar de huidige uitkomsten geven aan dat modulatie van receptor kinetiek een veelbelovende strategie is voor geneesmiddelontwikkeling.

Verbetering van de receptor selectiviteit

Verbetering van de selectiviteit van een geneesmiddel voor een receptor, kan leiden tot afname van bijwerkingen die veroorzaakt worden door interactie met andere receptoren. Ondanks de centrale rol die dopamine speelt in de neurobiologische effecten van drugs en de pathofysiologie van drugsverslaving, zijn er vooralsnog geen dopaminerge stoffen ontwikkeld die bewezen effectief zijn bij farmacotherapie van verslaving, waarschijnlijk ten gevolge van gebrek aan functionele selectiviteit. Op basis van eerdere onderzoeken werd de hypothese geformuleerd dat selectieve antagonisten van dopamine D_3 receptoren, in tegenstelling tot niet-selectieve antagonisten, therapeutisch effect tegen drugsverslaving zouden kunnen hebben. In theorie zouden dopamine D_3 antagonisten niet zozeer de euforische effecten van drugs tegengaan, maar vooral de motivatie om drugs in te nemen remmen. **HOOFDSTUK 4** beschrijft de farmacokinetiek van 175 mg doseringen van de nieuwe selectieve dopamine D_3 receptor antagonist GSK598809 in gezonde vrijwilligers, alsmede de farmacodynamische effecten op diverse neurofysiologische functies. Tevens werden mogelijke interacties met alcohol onderzocht, aangezien de populatie van patiënten waarvoor deze behandeling bedoeld is, veelal een alcoholverslaving heeft of alcohol misbruikt naast een andere drugsverslaving. Alcohol heeft bovendien een licht stimulerend effect op het dopaminerge mesolimbische systeem, zodat ook een indruk kon worden verkregen van de interacties van GSK598809 met dit dopaminerge beloningssysteem. Het belangrijkste effect van GSK599809 was stijging van de plasma concentratie van prolactine. De oorzaak van de stijging van prolactine is niet geheel duidelijk en kan mogelijk het gevolg zijn van antagonisme van dopamine D_3 receptoren in de tuberoinfundibulaire dopaminerge neuronen, maar kan ook het gevolg van antagonisme van dopamine D_2 receptoren. Ondanks dat GSK598809 een honderdvoudige selectiviteit heeft voor D_3 receptoren in vergelijking met D_2 receptoren, kan er bij 175 mg doseringen mogelijk toch voldoende D_2 antagonisme optreden om een stijging van plasma prolactine teweeg te brengen. De stijging van plasma prolactine was veel sterker in vrouwelijke vrijwilligers dan in mannelijke vrijwilligers, hetgeen waarschijnlijk het gevolg is van modulerende effecten van oestrogenen. GSK598809 had geen andere neurofysiologische effecten, behoudens een kleine verslechtering van oog-handcoordinatie. Gelijktijdige toediening van GSK598809 en alcohol had geen effect op de farmacokinetiek van alcohol en slechts geringe effecten op de farmacokinetiek van GSK598809. Na gelijktijdige toediening van GSK598809 en alcohol waren de farmacodynamische effecten voornamelijk additief. Antagonisme van dopamine D_3 receptoren lijken dus geen duidelijke vermindering van de neurofysiologische effecten van alcohol teweeg te brengen, hetgeen in overeenstemming is

met eerdere onderzoeken die suggereerden dat dopamine D_3 receptor antagonisten niet zozeer de euforische effecten van drugs tegengaan, maar de motivatie om drugs in te nemen tegengaan.

In het discussie hoofdstuk van dit proefschrift, **HOOFDSTUK 8**, werden de resultaten van het eerste klinische onderzoek naar de effecten van GSK598809 in mannelijke en vrouwelijke rokers besproken. In dat onderzoek verminderde GSK598809 het verlangen om te roken, hetgeen de eerste klinische aanwijzing vormt voor therapeutische waarde van dopamine D_3 antagonisten, alhoewel verder onderzoek nodig is om deze effecten te bevestigen.

Beïnvloeding van de dopaminerge controle door tachykinines

Als alternatief voor direct antagonisme van dopamine receptoren, werd beïnvloeding van de controle mechanismen onderzocht als methode om dopamine neurotransmissie te moduleren. Tachykinines zijn neuropeptides die activerende controle uitoefenen op alle belangrijke dopaminerge neuronen en zijn derhalve een geschikt doelwit voor nieuwe geneesmiddelen. Antagonisten van tachykinine receptoren, zoals de neurokinine NK_1 en NK_3 receptoren, zouden een rol kunnen spelen binnen de behandeling van drugsverslaving en schizofrenie. **HOOFDSTUK 5** beschrijft de farmacodynamische effecten van de NK_1 receptor antagonist aprepitant op diverse neurofysiologische functies in gezonde vrijwilligers, alsmede mogelijke interacties met alcohol. Tevens werd amitriptyline onderzocht als positieve controle om een indruk te krijgen van het profiel van neurofysiologische effecten. Aprepitant veroorzaakte geen enkele relevante verslechtering van neurofysiologische functies en er waren geen aanwijzingen voor klinisch relevante interacties tussen aprepitant en alcohol. **HOOFDSTUK 6** beschrijft de effecten van de nieuwe NK_1/NK_3 receptor antagonist GSK1144814 op de farmacodynamische effecten van alcohol in gezonde vrijwilligers. GSK1144814 had slechts geringe effecten of de neurofysiologische effecten van alcohol en er waren derhalve geen aanwijzingen voor klinisch relevante interacties tussen GSK1144814 en alcohol. Het gebrek aan effecten van tachykinine receptor antagonisten in gezonde vrijwilligers is waarschijnlijk het gevolg van het feit dat neuropeptides (waaronder tachykinines) vooral tot expressie komen tijdens stressvolle momenten, schadelijke omstandigheden of pathologische processen. Dit betekent dat tachykinine receptor antagonisten mogelijk significante effecten hebben bij pathologische processen, terwijl de effecten in gezonde vrijwilligers minimaal is. Na milde dopaminerge stimulatie door alcohol infusie, waren er geen aanwijzingen voor klinisch relevante interacties tussen aprepitant of GSK1144814 en alcohol. De serum concentratie van alcohol was echter misschien te laag om effecten van tachykinine receptor antagonisten aan te tonen.

In het discussie hoofdstuk van dit proefschrift, **HOOFDSTUK 8**, werden de effecten van aprepitant beschreven bij klinische aandoeningen die gekenmerkt worden door dopaminerge stimulatie. Aprepitant is geregistreerd ter voorkoming van misselijkheid en braken ten gevolg van hoog-emetogene chemotherapie. Recent zijn de eerste (voorlopige) resultaten van onderzoeken naar de effecten van aprepitant in drugsverslaafde patiënten geopenbaard. Alhoewel er een kleine, statistisch niet-significante, afname van het verlangen om methadon te gebruiken werd veroorzaakt door aprepitant, leken de subjectieve eufore effecten van methadon en oxycodon toe te nemen. Deze resultaten lijken geen therapeutisch nut van aprepitant aan te bevestigen, maar suggereren wel degelijk een effect van NK_1 antagonisme op de effecten van drugs. Verder onderzoek naar de rol van tachykinines binnen de neurobiologische effecten van drugs lijkt derhalve aangewezen.

Beïnvloeding van de dopaminerge controle door GABA

GABA is één van de belangrijkste dopamine-modulerende neurotransmitters. Geneesmiddelen met een effect op GABA transmissie kunnen indirect ook dopamine neurotransmissie beïnvloeden. Stoffen met functionele selectiviteit voor bepaalde $GABA_A$ receptor subtypes hebben mogelijk een selectief modulerend effect op dopaminerge systemen. De exacte rol van $GABA_A$ receptor subtypes in de regulering van dopaminerge systemen is echter niet volledig duidelijk. In **HOOFDSTUK 7** werd de GABAerge controle van het tuberoinfundibulaire dopamine systeem onderzocht. De effecten van twee nieuwe α_2/α_3 receptor subtype-selectieve agonisten, AZD7325 en AZD6280, en de niet-selectieve benzodiazepine lorazepam op de plasma concentratie van prolactine werd onderzocht in gezonde mannelijke vrijwilligers. Zowel lorazepam als AZD6280 veroorzaakten een significante stijging van plasma prolactine. De stijging van prolactine concentraties door AZD7325 was niet statistisch significant, vermoedelijk omdat de dosering te laag was. De resultaten suggereren dat α_2 en/of α_3 receptoren betrokken zijn bij GABAerge modulatie van prolactine secretie, alhoewel een rol van α_1 en/of α_5 niet uitgesloten is. $GABA_A$ receptor subtype-selectieve stoffen zijn dus in staat tot modulatie van dopaminerge systemen, waarbij de afwezigheid van effecten op andere $GABA_A$ receptoren ongewenste GABAerge neveneffecten, zoals sedatie of instabiliteit, zou kunnen voorkomen. De effecten van dit soort stoffen op andere dopaminerge systemen kunnen echter niet uit deze data worden afgeleid en dienen apart te worden onderzocht.

Conclusie

De neurotransmitter dopamine speelt een essentiële rol in diverse neurofysiologische functies en is betrokken bij de pathofysiologie van diverse neuropsychiatrische aandoeningen, waaronder de ziekte van Parkinson, schizofrenie, drugsverslaving en hyperprolactinemie. De huidige farmacotherapeutische methoden om dopaminerge neurotransmissie te beïnvloeden, hebben slechts een beperkt effect op de symptomen, terwijl hinderlijke bijwerkingen kunnen optreden. Derhalve heeft verbetering van de farmacotherapie van deze ziekten een hoge prioriteit. De bevindingen van studies in dit proefschrift en follow-up studies tonen aan dat verbetering van de kinetiek van het geneesmiddel ter plaatse van de receptor en verbetering van de selectiviteit van het geneesmiddel veelbelovende strategieën zijn. De resultaten van beïnvloeding van de controle mechanismen door tachykinines en GABA lijken vooralsnog minder therapeutisch nut op te leveren, maar geven wel indicaties voor biologische effecten die verder onderzoek verdienen. Deze onderzoekslijnen geven aan dat, ondanks de grote verscheidenheid aan beschikbare dopamine agonisten en antagonisten, de therapeutische mogelijkheden om dopamine neurotransmissie te beïnvloeden nog lang niet verzadigd zijn.

CURRICULUM VITAE

Erik te Beek was born in Naarden on September 26th in 1975. After graduation from medical school in 2003 at the University of Amsterdam, he worked as a resident in neurosurgery at the Slotervaartziekenhuis in Amsterdam and later as a resident in neurology at the Kennemer Gasthuis in Haarlem. In between, he also worked as a research physician at the neurogenetics laboratory of the Academic Medical Center in Amsterdam. From 2007 until 2010, he worked as a research physician at the Centre for Human Drug Research in Leiden. The research described in this thesis was performed in that period. During the same period, he was also trained as clinical pharmacologist. In January 2011, he started as a resident in nuclear medicine at the Leids Universitair Medisch Centrum in Leiden.

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