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Novel approaches in clinical development of cannabinoid drugs

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NOVEL APPROACHES IN CLINICAL DEVELOPMENT OF CANNABINOID DRUGS

Novel approaches in clinical development of cannabinoid drugs

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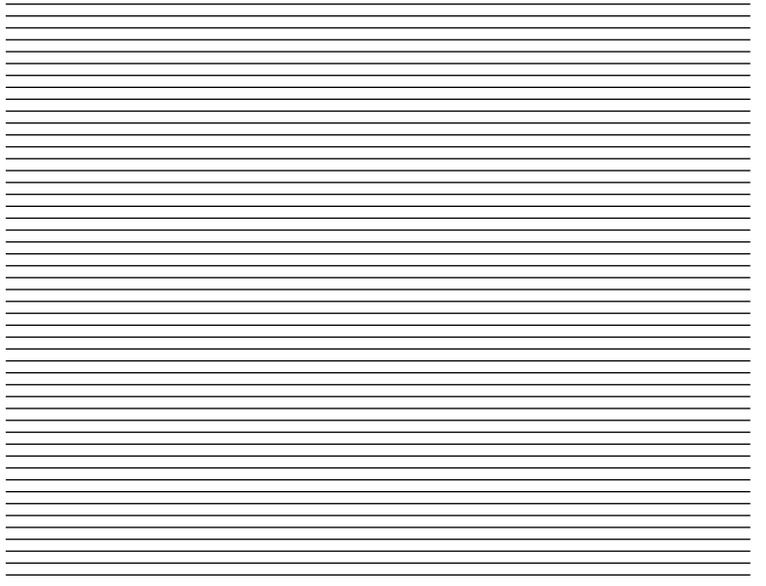
CHAPTER I

Introduction to the endocannabinoid system as a target for drug development



Mechanisms and functions of the endocannabinoid system

EVOLUTION OF THE ENDOCANNABINOID SYSTEM

Although a very ancient biological system, the endocannabinoid system was only discovered and explored over the previous five decades. Named after the plant *Cannabis sativa* L, which produces over 60 cannabinoid compounds, the system is widely distributed phylogenetically: it appears in very ancient, primitive invertebrate species, such as hydras, and in the most evolved mammals, such as humans. Already a few billion years ago the endocannabinoid precursor phosphatidylethanolamine (PEA) was expressed by the cytoplasmic membranes of bacteria. From there, the first molecules with cannabinoid receptor affinity were produced by cyanobacteria, which diverged from eukaryotes at least 2 billion years ago. After the cyanobacteria, endocannabinoids were produced by brown algae which diverged 1.5 billion years ago, again followed by sponges which diverged about 930 million years ago (for a review, see MacPartland (2004)). In absence of specific cannabinoid receptors the endocannabinoids initially had various other targets including 5-HT3A receptors and ion channels. About 790 million years ago, the primordial cannabinoid specific binding place evolved. The development of the endocannabinoid system has accompanied the evolution from monocellular organisms to higher animals, which is mirrored by its widespread involvement in intra- and intercellular signalling.

CANNABIS AND THC

Cannabis sativa L (or cannabis) is the most commonly illicit drug of abuse world-wide. Its major uses are for recreational and medicinal purposes, and the earliest evidence of cannabis use go back as far as 3000 years b.c. (World Health Organisation, 2013; Mechoulam, 1986). Δ^9-tetrahydrocannabinol (THC) is the most well-known active compound from cannabis and is generally held responsible for the well-known effects such as 'the munchies', a term used for hunger pangs after cannabis use, and central effects on consciousness, such as feeling high and altered time perception (Zuurman et al., 2009; Zuurman et al., 2008; Mathew et al., 1998; Plasse et al., 1991; Foltin et al., 1988). As a pharmaceutical substance, THC is mostly referred to as dronabinol, which is the generic name. Cannabis also contains many other cannabinoids such as cannabidiol, but for most of these compounds the pharmacologically activity is still unclear.

FUNCTIONS OF THE ENDOCANNABINOID SYSTEM

Currently, two cannabinoid receptors have been identified: CB_2 and CB_2 receptors, which have different functions and localisation patterns. CB_1 receptors are abundantly present in the nervous system, mostly located in cortical and limbic regions of the brain, as well as the cerebellum (Herkenham et al., 1991). In addition to the nervous system, CB_2 receptor MRNA has been found in the adrenal gland, bone marrow, heart, liver, kidney lung, prostate, ovary, and testicles of different species including humans (for review, see Pertwee (1997)). The CB_2 receptor is less widely expressed than the CB_2 receptor, and its MRNA is mainly present in various parts of the immune system, such as tonsils, spleen, thymus, bone marrow, and in B lymphocytes, monocytes, macrophages, mast cells and microglia in several species, including humans (for review, see Pertwee (1997)). CB_2 receptors are also expressed at lower densities in the brain, mainly on microglia (Gong et al., 2006; Nunez et al., 2004) (for an overview of the distribution of CB_2 and CB_2 receptors, see Figure 1).
The cannabinoid system mainly has a modulatory role in the regulation of complex physiological systems, such as metabolism (including digestive and endocrine systems), and the nervous system and immune system (for a review, see Melamede (2005)). Under normal physiological conditions, the endocannabinoid system is thought to generally have a low

activity, whereas the system can become overactive in pathological conditions or during stress. As earlier suggested by the late Ester Fride, this could be related to the numerous observations of biphasic cannabinoid effects (Fride, 2002). A clear example of biphasic characteristics following pharmacological intervention, include effects on anxiety (Rey et al., 2012): high doses of THC can induce panic attacks, whereas lower levels generally have a relaxing effect. This widespread involvement of endocannabinoids provides numerous opportunities for the development of new medicines for metabolic, neural or immune disorders, including Alzheimer's disease, multiple sclerosis, rheumatoid arthritis, diabetes mellitus, dyslipidemia and movement disorders.

PHARMACOLOGY OF THE ENDOCANNABINOID SYSTEM

In various mammal species, including humans, the endocannabinoid system includes two subtypes of G protein coupled cannabinoid receptors (CB_1 and CB_2) and endogenous messengers. The two most important messengers are anandamide (AEA) and 2-arachidonoyl glycerol (2-AG) (Figure 2) (Matsuda et al., 1990; Munro et al., 1993). AEA acts as a partial agonist with stronger binding affinity (Ki) and efficacy at the CB_1 receptor (Ki = 61-543 nM) compared to CB_2 (Ki = 279-1940 nM) (Pertwee, 2005). 2-AG has shown higher efficacy with similar affinities, and acts as a full agonist on both CB_1 and CB_2 receptors (Ki = 58-472 nM and 145-1400 nM respectively) (Pertwee, 2005). AEA and 2-AG are synthesised by membrane components (arachidonic acid) and released 'on demand' (Di Marzo et al., 1994). AEA and 2-AG are broken down by the enzymes fatty acid amidohydrolase (FAAH) and monoglyceride lipase (MAGL) respectively (Cravatt et al., 1996; Dinh et al., 2002).

Endocannabinoids regulate a variety of cellular effects via inter- (paracrine) and intracellular (autocrine) communication. The endogenous ligands bind to the v or CB_2 receptor, which affects ion channels or second messenger signalling pathways (Bosier et al., 2008; Prather et al., 2000; Su and Vo, 2007; Mackie et al., 1995; Twitchell et al., 1997). The exact pathway depends on the receptor subtype that is activated (Figure 3). CB_1 receptors in the nervous system are located on the pre-synapse. In this way, endocannabinoids act as retrograde synaptic messengers (Figure 4). The receptors are able to regulate activation and inhibition of the post-synaptic cell by stimulating the release of neurotransmitters like GABA and glutamate (Twitchell et al., 1997; Guo and Ikeda, 2004; Binzen et al., 2006).

PATHOLOGY OF THE ENDOCANNABINOID SYSTEM

Because of its essential basic physiological functions and its widespread presence throughout the body, the endocannabinoid system might be involved with many different pathological conditions. Although many findings are still controversial, studies in animal models and patients demonstrated changes in the endocannabinoid system activity in certain diseases or disease models, such as increased AEA levels in the CSF of schizophrenic patients (for example, see Richardson et al. (2008)). However, whether a deregulated system is a cause or a result of the disorder remains to be investigated and only little is known about the pathophysiology of the cannabinoid system.

PSYCHIATRY AND NEUROLOGY – Due to the clear psychotomimetic effects of cannabis consumption, the pathophysiology of the endocannabinoid system in psychiatric and neurologic disorders is relatively well studied. Many studies have led to the theory that chronic cannabis consumption can contribute to schizophrenia (for a review, see Ferretjans, Moreira, Teixeira, & Salgado (2012)). Several labs studied the endocannabinoid system in schizophrenia pathology, however, no consistency could be found regarding CB_1 expression in the brain, or blood and tissue concentrations of the major endocannabinoids as outlined in a review

by Ferretjans, Moreira, Teixeira, & Salgado (2012). It has been suggested, however, that schizophrenia is associated with polymorphisms of the CNR1 gene, which is responsible for encoding the CB_1 receptor (Ujike et al., 2002), although many other genes have also been implicated. Variations of the CNR1 gene are also associated with major depression and with the mediation of antidepressant drug effects (Mitjans et al., 2013).

Other evidence regarding the pathophysiology of the endocannabinoid system in minor and major depression and suicide is contradictory (as outlined in a review by Micale, Di Marzo, Sulcova, Wotjak & Drago (2013)). It is more certain, however, that the endocannabinoid system plays a gate-keeper role with regard to activation of the hormonal hypothalamic-pituitary-adrenal (HPA) axis, which has a major role in controlling reactions to stress. Stress has a large influence on cognition, anxiety and mood, and chronic stress can lead to depression-like symptoms. Endocannabinoids regulate the neurotransmitter release leading to hormonal release by retrograde messaging, which is mostly related to downregulation of excitatory, glutamatergic transmission.

Also for other brain regions, there are possible relationships between the endocannabinoid system and pathology. However, the exact role in pathological states remains still unclear. For example, in a rat model of autism the endocannabinoid system showed downregulation of 2-AG degrading enzymes in certain brain areas and higher tissue concentrations of endocannabinoids following social exposure (Kerr et al., 2013), but no conclusions can be drawn regarding the pathophysiology of the endocannabinoid system in autism. Also, due to the expression of CB_1 receptors on inhibitory GABAergic as well as excitatory glutamatergic neurons, cannabinoids can be both pro- and anti-convulsive, but their role in epilepsy is not well studied. A study by Sagredo et al. (2007) found that the CB_1 receptor is downregulated in early stages of Parkinson's disease, and the cannabinoid system becomes overactive in advanced stages of the disease (Sagredo et al., 2007). Although previous studies reported beneficial effects of cannabinoids on symptoms of Alzheimer's disease, including mood, sleep and cognitive decline, and on neuroprotection, the exact role of the endocannabinoid system in Alzheimer's disease is unknown (for review, see Orgado, Fernandez-Ruiz, & Romero (2009)). The endocannabinoid system is involved in the majority of the processes that occur before, during and after ischemia, and result in vasodilatation, neuroprotection, immunomodulation and antioxidation (Orgado et al., 2009; Martinez-Org et al., 2007). Also, the endocannabinoid system is involved in nociception, chronic inflammatory and neuropathic pain (Zogopoulos et al., 2013). Recently, it has come to light that some metabolites of AEA and 2-AG can either exacerbate or inhibit nociceptive signalling (Rani et al., 2012). The exact role of the endocannabinoid system in ischemia and pain modulation is still under investigation at several labs.

The major function of the endocannabinoid system is believed to be the regulation of the feeding system (De Petrocellis et al., 1999). This applies to both the feeling of hunger and the direct involvement in energy regulation. An obvious example includes getting 'the munchies' or a craving for high caloric food after cannabis use. Also, endocannabinoid activity directs towards energy storage, for example by stimulating adipogenesis and gluconeogenesis (for review, see Silvestri & Di Marzo (Silvestri and Di Marzo, 2013) and Osei-Hyiaman et al. (2008)). This inspired academy and industry to investigate the possibilities of the endocannabinoid system in the light of eating disorders such as obesity and anorexia. However, studies on the potential therapeutic validity of cannabinoids in eating disorders are scarce and inconclusive. The same counts for substance abuse, in which no conclusions can be drawn on the exact mechanisms. However, it has been found that CB_1 contributes to the motivational and reinforcing properties of ethanol, and chronic consumption alters endocannabinoid transmitter levels and CB_1 expression in brain addiction pathways (Pava and Woodward, 2012). Also, several studies associated polymorphisms in the CNR1 and FAAH genes with drug-related behaviours (Lopez-Moreno et al., 2012).

IMMUNOLOGY – Immunologic disorders for which the endocannabinoid system has been investigated include multiple sclerosis, arthritis, sepsis, inflammatory bowel disease, pancreatitis, uveitis and peridontitis. Studies performed *in vitro*, preclinically and in humans showed an upregulation of the endocannabinoid system in inflammation (Richardson et al., 2008) (for an overview of the studies in multiple sclerosis, see the review by Pertwee (2007)). For example, AEA and 2-AG have been found in synovial fluid of arthritic patients, whereas in the synovial fluid of healthy volunteers, no cannabinoids were detected (Richardson et al., 2008). In post-mortem lesioned brain tissue from patients with chronic multiple sclerosis, the concentration of anandamide was significantly elevated compared to brain tissue from healthy controls (Eljaschewitsch et al., 2006). These examples suggest a protective role of the endocannabinoid system in inflammation.

ENDOCRINOLOGY – Several studies demonstrated that the upregulation of endocannabinoids and CB_1 and CB_2 stimulation increases food intake, obesity-related inflammation and adipogenesis (Gamage and Lichtman, 2012) (for an overview, see review by Cluny, Reimer, & Sharkey (2012) and Faurholt Bennetzen (2010)). Clinical studies found that obese subjects have a decreased subcutaneous CB_1 expression compared to lean subjects, and that the endocannabinoid system reduction is normalised with weight loss (Faurholt Bennetzen, 2010). This could imply a reactive compensation in obese patients.

In line with these observations, mice lacking the CB_1 receptor in hepatocytes, although still susceptible to diet-induced obesity, are protected against liver steatosis, hyperglycemia, dyslipidemia, and insulin resistance (Osei-Hyiaman et al., 2008). Blocking CB_1 function is associated with alleviation of hyperglycemia and dyslipidemia. In line with these findings, several studies indicate that endocannabinoids have negative effects on glucose tolerance and insulin secretion (for review, see Doyle (2011)). Studies in patients with advanced diabetic nephropathy and in mice, suggested that CB_2 signalling was impaired (Barutta et al., 2011). The exact role of the endocannabinoid system in the pathophysiology of diabetes, however, still needs to be investigated.

CARDIOVASCULAR – The endocannabinoid system affects heart and arterial performance in pathological conditions, including regulation of vessel contractility and atherogenesis. This happens directly or indirectly via alteration of cardiometabolic risk factors and CB_1 and CB_2 receptors often seem to act in opposing ways (for a review, see Montecucco & Di Marzo (2012)).

GLAUCOMA – A study in patients showed lower COX-2 expression and lower PGE2 concentration in aqueous humor compared to healthy individuals (Maihofner et al., 2001). As COX-2 and PGE2 can be increased by cannabinoids and glaucoma can be treated by cannabinoids, it has been suggested that the endocannabinoid system might contribute to the control of processes leading to glaucoma (for review, see Nucci et al. (2008)).

ONCOLOGY – Endocannabinoids might represent one of the many adaptive responses aimed at counteracting tumour cell growth. Several studies demonstrated that cannabinoids exert anti-proliferative and apoptotic effects (for review, see Hermanson & Marnett (2011)). Also, increased endocannabinoid signalling is found in some human malignancies compared with the corresponding healthy tissues, as well as in human cancer cells with a high degree of invasiveness (see review by Di Marzo, Bifulco, & De Petrocellis (2004)). However, over-expression of CB_2 receptors on haematopoietic precursor cells has been suggested to be associated with, and possibly a causative factor of, human acute myeloid leukaemia.

In summary, we can conclude that endocannabinoid changes accompany a wide variety of disorders, although many changes are still controversial. This is largely due to the physiological complexity of the endo-

cannabinoid system, which often involves feedback mechanisms at a local tissue level, or indirect influences on processes that are also regulated by other systems. Changes in signalling sometimes represent an attempt to counteract a pathological process, and in other instances could be one of the causative factors underlying the disease or its symptoms. Although it is premature to view endocannabinoids as markers of pathological states, a general conclusion from previous studies is that, endocannabinoids seem to have a protective or ameliorating role in many cases.

Complexities of cannabinoid drug development

Endocannabinoids are involved in complex physiological systems that play an important role in a huge number of diseases in almost all areas of medicine. In principle, this makes them appealing targets for drug development. However, because of this complexity and the relatively recent discovery of the endocannabinoid system, endocannabinoid research is still in a premature stage. Cannabinoid research and drug development is complicated further by a number of factors which are summarised in the following paragraphs.

LIMITED SUBTYPE SPECIFICITY

The limited number of receptor subtypes and the limited number of endogenous ligands and their ubiquitous presence makes it difficult to identify the exact local steering processes. For example, in contrast, the GABA-A receptor has at least half a dozen subtypes and the serotonin-system has over a dozen of 5-HT receptor subtypes. This creates ample opportunities for the development of highly selective compounds as research tools or potential drugs, or to develop genetic knock-in or knock-out models to study the functional role of a specific receptor subtype. In the case of the endocannabinoid system, such models and interventions generally affect many systems at the same time. The number of enzymes

that are involved in endocannabinoid synthesis and degradation is also limited. Consequently, there is a shortage of good pharmacological interventions to manipulate the endogenous cannabinoids, such as inhibitors of degradation but also of reuptake or transport.

WIDESPREAD DISTRIBUTION

The endocannabinoid system is one of the most widely distributed pharmacological systems in the body (for review, see Pertwee (1997)). This complicates systemic or organ-specific targeting. By trying to target a specific location, the ubiquitous presence of the system easily causes unnecessary or undesirable effects elsewhere, which limits the development of therapeutically specific drugs.

HIGH LIPOPHILICITY

To optimise specific targeting of cannabinoids to those parts of the body that are involved in a disease, pharmacokinetics of endogenous and exogenous cannabinoids can be modified, for example by changing administration routes, dosing quantity and time intervals, or by differentiating peripheral and central drug distribution. However, pharmacokinetic optimisation is limited by the strong lipophilic character of exogenous and endogenous cannabinoids (e.g. Log P values for anandamide and 2-AG are 6.31 and 8.01 respectively (Stanton et al., 2005). Although lipophilic compounds are generally well absorbed gastro-intestinally, they carry the risk not to be optimally distributed systemically, due to the rapid diffusion from the blood to fatty organs, such as adipose tissue, liver and brain. As a consequence, a relatively large concentration is located at specific sites, whereas other sites are much less exposed to the compound. Also, very lipophilic compounds are often only slowly redistributed from fatty organs back into the blood, as a result of which the compounds accumulate and remain detectable in the blood for long time periods after

dosing. Furthermore, lipophilic compounds can be rapidly metabolised, resulting in fast metabolite exposure. They generally have a high protein binding, resulting in a low free drug fraction and thereby more variable drug exposure. Also lipophilic compounds generally have a somewhat limited specificity, i.e. 'pharmacological promiscuity'. Consequently, the lipophilicity of cannabinoids creates a large complexity for specific dosing in terms of target and time frame.

COMPLEX PHYSIOLOGICAL INTEGRATION

Due to the ancient phylogeny of the endocannabinoid system and its involvement in primitive systems, it is deeply embedded in basic functions and complex physiological systems. Locally, these systems can have very diverse signalling pathways, cellular messaging and functions (Figure 3). Most systems in which endocannabinoids are involved, such as the central nervous system or immune system) form highly integrated networks, with many layers of feedback and regulation. This makes it enormously difficult for pharmacology to precisely interfere with one specific signalling pathway. For the same reasons there are also many uncertainties regarding the exact role of endocannabinoids in pathophysiology, which in most diseases has not been unequivocally demonstrated. For the few diseases in which consistent involvement of the endocannabinoid system has been found, it is still unclear to what extent a deregulation is part of the cause or merely a consequence or sign of dysfunction.

COMPLICATED EFFECT MEASUREMENTS

The integration of the endocannabinoid system at subcellular levels of complex multicascadic physiological mechanisms and the wide range of effects create a major challenge for measurement of changes in their activity, which is essential in drug development. The methodology currently used in clinical research is unable to track all drug- or disease-induced changes. Therefore, it is easy to miss relevant effects. This can be the case in acute single dosing studies, where the, very often subtle, changes in homeostasis can be easily overlooked.

Optimisation of early cannabinoid development

In spite of the complexity of endocannabinoids, many efforts have been made to develop drugs that are targeted on this system. In general, several options are available to overcome the pharmacologic limitations and the problems with effect measurements that are described in the previous sections. This section deals with these options, and how they were approached in this thesis.

DRUG DESIGN OPTIMISATION

PHARMACOLOGICAL OPTIMISATION – To act on pathological conditions, which are often very local or limited to a single physiological system, receptor subtypes should be targeted as specifically as possible. thc is the most well-known cannabinoid and is generally used as an experimental compound in CB_1 agonist studies and THC-challenge studies and is a major compound in various registered and experimental medical formulations, including medicinal cannabis, Sativex® and Marinol®. However, THC lacks cannabinoid receptor specificity and exhibits its effects as a partial agonist on both the CB_1 and the CB_2 receptor (for a review, see Pertwee (2008)). Also, THC is very lipophilic (log P = 6.97) and accordingly, after administration, THC is very quickly distributed to the peripheral fatty tissues including lungs, adipose tissue and the brain (Thomas et al., 1990; Lemberger et al., 1970; Ryrfeldt et al., 1973; Brunet et al., 2010). Besides the option of exogenous targeting of the cannabinoid receptors, the endogenous cannabinoid levels could be manipulated.

Options for manipulation of the endogenous cannabinoid levels include influencing synthesis, transport, release, and degradation. The

most well-known example of current investigation of this type of manipulation is the development of FAAH-inhibitors, which inhibit the metabolism of AEA. Clinical studies with FAAH-inhibitors for the indications of several pain and inflammation states are still ongoing. Studies with inhibitors of monoacylglycerol lipase (MAGL) and transporters are still in pre-clinical *in vitro* phase (ICRS, 2012). Despite these attempts, the options are very limited due to the small number of available compounds influencing endogenous cannabinoids and are unlikely to allow enough pharmacological selectivity for a wide array of disease-specific cannabinoid-targeted treatments.

Another way to improve the therapeutic window of drugs with limited pharmacological selectivity is to control their action site penetration. This can be achieved by pharmacokinetic optimisation of drug levels and tissue penetration.

PHARMACOKINETIC OPTIMISATION

REDUCING SYSTEMIC VARIABILITY: ADMINISTRATION ROUTE – The administration route can influence pharmacokinetic aspects such as time of drug absorption or peak concentration and distribution, and thereby time of effect onset, and the number and magnitude of concentration-related therapeutic and undesirable effects. The most common administration route is the oral route. Oral administration is generally very easy and convenient, however, pharmacokinetically, there are some risks with this administration route. Oral administration could result in variable plasma concentrations, as absorption to the blood is dependent on GI tract activity, pH variations and food interactions. Also, the compounds reach the liver before they reach the systemic blood circulation, resulting in metabolism and possible modification of the activity of compounds that are metabolised by CYP450 enzymes. These enzymes are also situated in the gut wall, and their activity can vary due to genetic variations and interactions with foods and drugs.

In order to avoid gut- and liver metabolism, drugs can be administered intravenously, directly into the blood stream (i.e. 100% bioavailability). This administration route is limited by its invasiveness. Non-invasive ways of avoiding hepatic metabolism are for example intrapulmonal, sublingual and transdermal administrations. These routes are not suitable for each compound. They may give less variable pharmacokinetics compared to oral administration, but the administration routes are less practical. Another way to enhance the bioavailability of a compound is by galenic manipulation. Changing the formulation can improve the resorption of a compound and affect the exposure profile.

In this thesis, we investigated the pharmacokinetics and pharmacodynamics of several different administration routes of THC. In previous studies by Zuurman et al. we have optimized the intrapulmonary administration of THC, using a vaporizer and pure THC rather than the more usual method of smoking cannabis extracts (Zuurman et al., 2008). Although inhalation of vaporized pure THC produces reliable pharmacokinetic profiles, it is a less convenient mode of administration, which gives little control over the exposure profile. This can be improved by the so called repeated paced puffing protocol, which uses predetermined dosages and times to achieve a desired exposure profile, however with relatively variable results (Chapter 3, 4, 5 and 6).

In Chapter 2, we investigate different oral and sublingual administrations of Namisol, a new tablet containing THC. Namisol is manufactured with Alitra™, a novel lipophilic compound delivery technology that has an improved absorption of poor water soluble compounds in the human blood, thereby improving bioavailability with reduced variability. Th most favourable administration route was chosen for further development of Namisol for the indication of pain and spasms in multiple sclerosis.

PHARMACOKINETIC CONTROL OF THE THERAPEUTIC WINDOW: DOSAGE AND TIME INTERVAL – Besides the route of administration, the actual

dose given and the time interval between multiple doses are essential factors to maintain drug concentrations within the therapeutic window for the required time periods. Overdosing could lead to unwanted (side) effect profiles and even to toxicological effects. It is essential therefore to accurately predict the optimal therapeutic window for a new drug. This may be problematic if the effects are difficult to measure; either because they are part of an integrated system with many homeostatic mechanisms; or because specific tests are lacking; or because the beneficial effects are chronic (e.g. weight reduction or cardiovascular risk) or infrequent (*e.g.* epileptic seizures or exacerbations of multiple sclerosis). This was a problem with the first cannabinoid antagonists like rimonabant, where traditional methods like preclinical dose predictions and maximum tolerability levels in healthy subjects were used to determine the doses for clinical trials (Cohen, 2010). Although this approach led to the registration of rimonabant for obesity, the drug was withdrawn soon after launch because of unacceptable psychiatric side effects in a minority of patients. It is important therefore to determine the concentration range that has an optimal effect on the right pharmacological target: not too much or too little inhibition or stimulation; and not at action sites that are not involved in the disease. The determination of dose- or concentration-effect relationships for different mechanisms of action of cannabinoids is an important part of this thesis. In Chapter 2 a first in human study with the novel THC tablet Namisol aimed to find the optimal dosage for single dose administration by evaluating pharmacokinetics and pharmacodynamic effects. In Chapters 4-6 we try to establish the concentration-effect relationships for different cannabinoid agonists and antagonists, with the aim of establishing a dosing regimen with an optimal pharmacological effect.

TARGET SPECIFICITY – Options for improving specificity in drug development are limited due to the limited number of cannabinoid receptor subtypes (i.e. two) and their presence all throughout the body. Another option to improve specificity for a specific target or location would be to improve the delivery of the compound to a specific location. For example, manipulation of the compound's permeability for the blood brain barrier could keep a compound outside of the central nervous system. In Chapter 5, we actually tested TM38837, a compound that showed peripheral restriction in preclinical studies with the aim to demonstrate peripheral activity without central activity. In this study, we compared TM38837 with the centrally and peripherally active antagonist rimonabant, using biomarkers of peripheral and central CB_1-activity that have been previously identified (i.e. feeling high and heart rate) (Zuurman et al., 2009). TM38837 is under development for treatment of peripherally associated disorders (including hepatic disorders and obesity) with reduced central side-effects.

METHODOLOGICAL OPTIMISATION

General challenges in drug development are to precisely and accurately detect and measure relevant (side) effects, and to ensure translatability of drug responses from preclinical animals and healthy volunteers to patients and vice versa. These challenges particularly apply to the development of cannabinoid drugs.

ACCURATE EFFECT MEASUREMENTS – The endocannabinoid system is deeply embedded within a variety of physiological networks. When endocannabinoid changes are induced in the network, these changes can be quickly modified by other homeostatic processes. The complex interactions between the networks and their eventual results are not always immediately measurable. Consequently, results from acute dosing studies cannot always be extrapolated to multiple dose studies in which more chronic effects are studied.

Cannabinoids can induce a wide palette of effects which makes measuring the relevant effects related to various physiological networks

quite challenging. It should be tested whether the compound reaches the target site and other sites and to what magnitude and in what time frame the effects take place. With the availability of a broad range of tests, especially of the central nervous system (CNS), an adequate set of tests should be chosen in order not to miss any relevant effects.

TEST SELECTION – Zuurman et al. performed a systematic literature review on biomarkers for the effects of cannabis and THC in healthy volunteers (Zuurman et al., 2009). Consistently, she found increases of heart rate frequency and feeling high, and decreases of motor control. This trio of consistent effect measurements are considered as the basic cannabinoid responses that were measurable in a consistent way, and were therefore applied in all studies in this thesis.

For the detection of these effects in healthy volunteers, the Centre for Human Drug Research (CHDR) developed the NeuroCart test battery. This test battery includes all of the functions mentioned, using non-invasive tests with a short duration and limited learning effect, which allows for repeated measures. The NeuroCart battery include visual analogue scales of mood, alertness and calmness by Bond & Lader (1974), psychedelic effects by Bowdle et al. (1998), and body sway measurement, and was applied to the studies reported in Chapters 2, 4, 5 and 6 and partially in Chapter 3.

Even with a battery of tests, effects of the endocannabinoid system can be easily missed. There is a need therefore, for methods that provide integrated representations of functional activities, which are highly sensitive and specific to pharmacological effects. For the immune system and metabolism, biochemical analyses have been developed to examine specific functional arrays (immunoarrays and metabolic arrays), and the more general 'omic' approaches (proteomics, metabolomics) can provide broad screens of functional changes on an individual level (Powanda and Beisel, 2003; Ahmed et al., 2013). Biochemical effects are more difficult to determine in the central nervous system, but network analysis could provide sensitive indications for a wide range of CNS effects. In Chapter 3, we examined the novel technique of pharmacological resting state functional magnetic resonance imaging (RS-FMRI). This technique seems very valuable for clinical phases of drug development; however, it has not yet been applied for this purpose. Besides better understanding the pharmacodynamics of THC, we aimed to bring RS-FMRI one step closer towards application in drug development.

OPTIMAL STUDY DESIGNS

Pharmacological therapies try to achieve a correction of homeostasis (i.e. healthy state) by artificially interfering with the disturbed elements in a disordered biological system (e.g. stress or pathology). Since early phase clinical research investigates cannabinoids in healthy humans, one should find possibilities for translating the effects seen in healthy volunteers to clinically relevant outcomes in patients. The latter is a specific challenge if the acute effects of a pharmacological manipulation are not measurable in healthy subjects. It is difficult for instance to show the effects of a pharmacological stimulus (e.g. a receptor agonist), if the target system is already maximally active. Such ceiling effects are well-known for cognitive enhancers in healthy students. It is also challenging to show effects of pharmacological inhibition (e.g. a receptor antagonist) in case of 'floor effects', when the endogenous system is dormant under physiological conditions. The low basic activity of the endocannabinoid system may be the reason why cannabinoid antagonists do not show any effect in healthy volunteers at doses that are clearly effective in various disease states (Rodriguez de Fonseca et al., 1999). In such cases, pharmacological or functional challenge tests can be used to perturb the target system in such a way, that it is possible to show correction by the drug. For example, a scopolamine-challenge causes cognitive deterioration, which can be improved by procognitive drugs (Snyder et al., 2005). To enable detection and quantification of

effects of cannabinoid antagonists, CHDR developed the THC challenge test (Zuurman et al., 2008; Zuurman et al., 2010). This test allows indirect quantification of agonistic effects by measuring the antagonistic inhibition of THC-induced effects. In practice, this means that on one study occasion an agonist is used to induce acute effects (e.g. feeling high), whereas on another occasion the agonist is dosed together with an antagonist, which can now be shown to reduce the agonistic effects. This provides unequivocal proof that the antagonist has reached pharmacologically active concentrations in relevant parts of the body, which is an essential prerequisite for therapeutic activity. Obviously, these relevant body parts need to be represented by the measurements that are used in the study.

The THC challenge test has been developed as a standard method that has been applied in over ten studies, including both antagonist studies and studies investigating THC effects only (Zuurman et al., 2008; Zuurman et al., 2010) and it was applied for Chapters 4, 5 and 6.

CONCENTRATION-EFFECT MODELLING

Modelling is a very powerful tool to simulate and predict pharmacokinetics (PK) and pharmacodynamic effects (PD). These models allow the optimisation of a study design by predicting effective dosages and concentrations and relevant effects, but they can also be applied to 'translate' data from experimental animals and healthy volunteers to patient groups.

The mathematical models that relate PK and PD are referred to as PK-PD models. These models are data driven mathematical models that best describe the relationship between the plasma concentration and a particular pharmacodynamic effect, based on a relatively simple underlying function (usually an E_{MAX}-model). During recent years, the field of modelling underwent major improvements with the development of new theoretical concepts, including the receptor theory and dynamical systems analysis, which takes into account the specific physiological characteristics of a body system (such as blood flow and lipophilicity). Also, statistical and technical improvements led to the more widespread application of visual predictive checks and objective assessments of model complexity (minimal value of objective function), thereby improving the quality of model predictions. Previously, CHDR developed PK-PD models for THC effects (Strougo et al., 2008). These models were used for the design of all THC challenge studies described in this thesis. In this thesis, we tried to expand these models with new PK-PD models for the CB_1 antagonists drinabant (AVE1625), surinabant (SR147778) and TM38837 and CB_1 inverse agonist rimonabant (SR141716) based on inhibition of THC-induced effects (Chapter 6).

In summary, the aim of this thesis is to improve cannabinoid drug development in early phase clinical research, by investigating new cannabinoid compounds and new formulations to improve pharmacological effects, experimenting with new methodologies to optimise effect measurement, and applying new concentration-effect models to improve the simulation and prediction of optimal dosing regimens of cannabinoid agents for future studies.

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BOX – THC PHARMACOKINETICS

As the most well known constituent of cannabis, THC is often used as a cannabinoid (partial) agonist in pharmacological studies (Pijlman et al., 2005). Also, THC is only one of the few cannabinoid agonists available for clinical use. Therefore, we chose THC as the agonist of preference in this thesis. THC is very lipophilic and its pharmacokinetics are complex. In this box, we give an overview of the most important aspects of THC pharmacokinetics in clinical trials.

ADMINISTRATION

INTRAVENOUS – Intravenous THC administration is a very uncommon and merely experimental administration route that was only applied in a limited number of clinical experiments (Bhattacharyya et al., 2010; Carbuto et al., 2011; Lemberger et al., 1973). Intravenous administration has a bioavailability of 100%, and it thereby allows assessment of absolute bioavailablity when compared to other formulations.

INHALATION – One of the most common administration routes of THC is via smoking cannabis. This has several methodological disadvantages in addition to the problems of smoking. Cannabis is usually smoked in combination with tobacco, resulting in the inhalation of a varied mixture of (noxious) compounds which could influence the THC-induced effects. When smoked as pure cannabis, the mixture of cannabinoids could as well influence the THC-induced effects. For example, cannabidiol (CBD) abolishes the well-known THC-induced 'feeling high' effect (Dalton et al., 1976). Moreover, the lack of dosage control makes smoking less suitable for clinical research; the exact amount of THC that is inhaled cannot be controlled due to partial combustion of the THC at times when the cigarette smoke is not inhaled. Moreover, efficiency of smoking is dependent on the experience of cannabis users.

To avoid these disadvantages, a THC inhalation method using a vaporiser was developed at the Centre for Human Drug Research (CHDR, Leiden). Pure THC diluted in 100% ethanol is applied on the Volcano® device. Hot air from the vaporiser vaporises the THC dilution into a balloon that is attached to the vaporiser. The balloon is closed with a valve that opens when the content is inhaled. Using a paced puffing protocol, volunteers inhale an exactly known amount of THC from vapour in the balloon (Hazekamp et al., 2006; Zuurman et al., 2008). This inhalation method is used in several chapters of this thesis. An overview of the average loss of THC during the THC administration using the vaporiser, and the quantity of THC that is inhaled is given in Figure 5.

ORAL – Oral THC administration is another very commonly used method for both recreational and clinical usage. Cannabis could be processed into baked products, such as biscuits and cakes, or decocted and served as 'tea'. The disadvantages of oral administration are the variation of cannabinoid composition and the late onset and unpredictable magnitude of effects. The cannabinoid composition is dependent on way the cannabis is processed. For example, due to the lipophilic character of some cannabinoids such as THC, the composition of cannabinoids in tea shifts to relatively lower concentrations of THC and higher concentrations of THC-acid (THCA) (Hazekamp et al., 2007). Also, the temperature during processing is of relevance for cannabinoid composition due to conversion of cannabinoid acids (Hazekamp et al., 2007).

To avoid the problems of variable cannabinoid composition, several oral formulations of cannabis derived medicines (CDM) and THC have been developed containing predefined amounts of cannabinoids. These formulations include Marinol®, a capsule with a synthetic form of THC dissolved in sesame oil, and Cesamet®, a capsule containing THC analogue nabilone.

OROMUCOSAL AND SUBLINGUAL – Sativex® is a CDM that, besides its major compounds THC and cannabidiol (CBD), contains a mixture of several other cannabinoids (presented during the 20th and 21st Symposiums of the International Cannabinoid Research Society, 2010 and 2011). At present, Sativex® is the only registered CDM that is applied for oromucosal and sublingual administration. Sativex® is administered by spraying into the oral cavity.

OTHER – Other THC and CDM administration routes such as dermal and rectal have been applied as well in clinical trials (Mattes et al., 1994; Callaway et al., 2005). These administration routes are not applied for currently registered cannabinoid medicines, and are beyond the scope of this thesis.

ABSORPTION

Plasma concentration profiles of THC for different administration routes are given in Figure 6. THC profile after inhalation of pure THC is comparable to the profile after intravenous administration, with an instant time to peak plasma concentration (T_{MAX}) within 3 min and a steep decline of plasma concentration (Ohlsson et al., 1980). Although the oral administration route is more practical, THC absorption is less favourable compared to intravenous or intrapulmonary administration routes. The oral T_{MAX} lies between 60 to 90 min after eating of a 20 mg THC-containing chocolate cookie (Ohlsson et al., 1980) and between 2.8 to 3 h for 5-20 mg Marinol® (Schwilke et al., 2009; Karschner et al., 2011). An oromucosal THC-CBD dosage, administered as a spray, gives a relatively late THC T_{MAX} of 4 h (Karschner et al., 2011).

Previous pharmacokinetic studies reported that bioavailability of THC inhalation was between 10 and 28.7% on average. Frequent cannabis users had higher THC plasma levels compared to infrequent users after smoking (Ohlsson et al., 1982; Lindgren et al., 1981). However, a study by Ohlsson et al. (1982) found that intravenous THC administration resulted in only small plasma concentration differences between infrequent and frequent users. This indicates that a substantial amount of THC from cigarettes is not absorbed and that the amount of THC intake is relatively variable. However, the intrapulmonary administration route has advantages over intravenous administration with regards to familiarity and its non-invasive character. Therefore, the Centre for Human Drug Research developed a standardised THC inhalation protocol that was reported by Zuurman et al. This protocol was applied for studies with repeated measurements for the assessment of concentration-effect relationship modelling, and in challenge tests (Zuurman et al., 2008; Strougo et al., 2008; Zuurman et al., 2008).

Oral bioavailability is relatively small, varying on average from 6 to 20% (Ohlsson et al., 1980; Wall et al., 1983). The relative bioavailability of oral THC was 87.2% when compared to sublingual THC+CBD, and 93.9% when compared to buccal THC+CBD administration (Guy and Robson, 2003). A study with oromucosal THC+CBD administration (both sublingual and buccal) found a 92.6 to 98.8% bioavailability of oral THC (Karschner et al., 2011).

DISTRIBUTION

Although extensive data are available from studies in animals, only little is reported on the distribution of cannabinoids in humans. Due to its lipophilic nature, THC is distributed to peripheral tissues, such as lungs, adipose tissue and kidneys. This happens very quickly after central absorption, as can be seen by the steep concentration decline in Figure 6 (Lemberger et al., 1970; Ryrfeldt et al., 1973; Brunet et al., 2010). Gronewold and Skopp (2011) investigated distribution of THC and its metabolites in five human post mortem cases (Gronewold and Skopp, 2011). Bile contained high concentrations of THC and metabolites and muscle tissue also contained high concentrations of THC, although metabolites could hardly be detected. In the liver, THC had low concentrations or was even undetectable, while 11-NOR-9-CARBOXY-THC glucuronide (THC-

COOglu) had appreciably concentrations in both liver and kidney. Furthermore, THC was present in lung specimens. Metabolites were largely absent in brain tissue, with 11-HYDROXY-THC (11-OH-THC) being completely absent. Gronewold and Skopp (2011) suggested that muscle tissue serves as a matrix for detection of cannabis use, and that retention from muscle tissue, in addition to retention in fat, could be a source of the prolonged elimination period of cannabinoids (Gronewold and Skopp, 2011). Findings from bile supported extensive enterohepatic recirculation of THC-COOglu (Gronewold and Skopp, 2011). The role of enterohepatic circulation in the distribution pattern of THC has also been described in animal studies (Garrett and Hunt, 1977; Klausner and Dingell, 1971). In daily cannabis users, a previous study on cannabinoids in oral fluid described the abundant presence of THC-COOH in 98.2% of the samples (Milman et al., 2010). Conversely, 11-OH-THC was not detected in any sample, whereas THC was present in only 20.7% of plasma samples. Previous studies also described the distribution and determination of THC in detail in vitreous humour, oral fluid, breast milk and foetuses (Jenkins and Oblock, 2008; Milman et al., 2011; Perez-Reyes and Wall, 1982). These aspects are beyond the scope of this thesis, and are therefore not described.

METABOLISM

In humans, THC is predominantly metabolised by hydroxylation and oxidation via cytochrome P450 (CYP) enzymes (Yamamoto et al., 1995). CYP2C9 and to a lesser extend CYP2C19 play the major roles in humans (Watanabe et al., 2007). Metabolism mainly takes place in the liver, and to lesser extend in the heart and lungs, as reported from animal studies (Nakazawa and Costa, 1971; Widman et al., 1975). Many pre-clinical studies reported on metabolic rates, but extrapolation of the results is limited by interspecies differences that could be explained by differences in CYP profiles (Harvey and Brown, 1991). The major metabolism pathway is visualised in Figure 2. The ratios at which the metabolites occur after human administration, is largely dependent on the administration route.

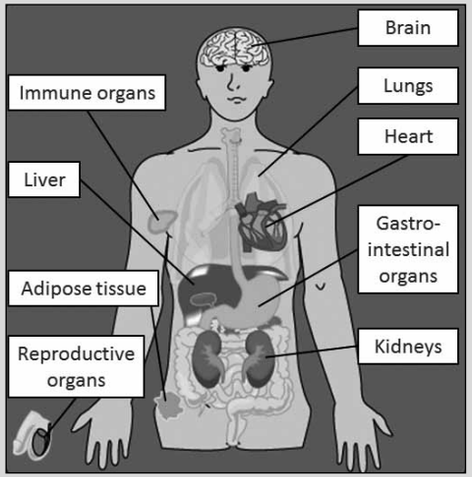
ELIMINATION

After reaching the maximum concentration (C_{MAX}) for THC inhalation and right after intravenous administration, THC plasma concentration has a steep decline until the concentration reaches a second phase, resembling an equilibrium (Figure 6). This equilibrium occurs between approximately 20 minutes and 6 hours after THC administration. After 6 hours a third phase is reached in which the plasma concentration has a flatter slope compared to the second phase. The exact course of elimination phases in humans is unknown, but preclinical studies reported up to 6 phases (Leuschner et al., 1986).

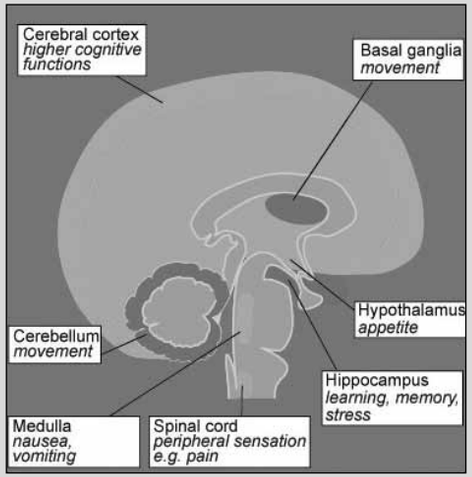
The steep decline in the first phase, which could be attributed by a combination of rapid distribution and metabolism, has a half-life (initial half life or $t_{1/2}^{INIT}$) of 30 min (Lemberger et al., 1970). In the second and third phase, equilibriums between plasma and tissue are reached (Chiang and Rapaka, 1987; Lemberger et al., 1970). The terminal plasma $t_{1/2}^{TERM}$ was calculated up to 57 hours (Lemberger et al., 1971). It should be noted that the actual $t_{1/2}$ calculation is difficult and is limited by difficulties in the quantitative analysis of very low plasma concentrations that are found in phase 3. The clearance of THC in the third phase is between 0.0033 and 0.06 L/h, while the maximum clearance at t = 100 min was reported to be 1.2 L/h (Ohlsson et al., 1982; Wall et al., 1983; Hunt and Jones, 1980). The slow elimination of THC from the plasma could be explained by redistribution from peripheral tissues, such as the adipose tissue, into the blood compartment.

About 15-30% of THC is excreted in urine, mainly as acid metabolites with less than 0.05% of unchanged THC. About 30-65% is excreted in faeces, less than 5% of an oral dose as unchanged drug (Lemberger et al., 1970; Hunt and Jones, 1980; Wall et al., 1983). Most of the THC metabolites in urine were excreted as polar acidic metabolites during day 1.

FIGURE 1 Distribution of cannabinoid receptors in the body and brain. CB_1 receptors are widely distributed in areas related to metabolism and energy storage, such as the adipose tissue, digestive tract, liver, brain, but also in the kidney, lungs and reproductive organs (1a). CB_2 receptors are mainly present in the immune system, such as tissues of the spleen, tonsils, and thymus gland, and immune cells including glia cells in the brain, monocytes, macrophages, B-cells and T-cells and hematopoetic stem cells (1a). **FIGURE 1B** shows the location of CB_1 receptors in the brain in more detail. The CB_1 receptor is predominantly found in the hypothalamus (associated with appetite regulation), hippocampus (associated with memory and stress), amygdala (associated with emotion), basal ganglia and cerebellum (associated with coordination and movement), medulla (associated with basal functions, including vomiting), spinal cord (associated with sensations, including pain) and in the cortex (associated with higher cognitive functions).

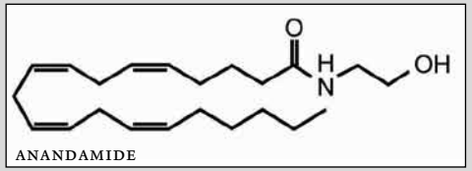


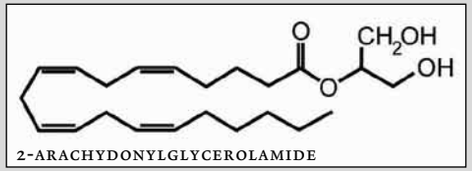
A *(see inside cover for this figure in colour)*



B *(see inside cover for this figure in colour)*

FIGURE 2 Structural formulas of the endocannabinoids anandamide and 2-arachydonylglycerolamide (2a), and the metabolic pathway of the exogenous cannabinoid Δ^9-tetrahydrocannabinol that is metabolised to 11-nor-9-carboxy-THC glucuronide, a water soluble congener which can be more easily excreted by the body (2b).





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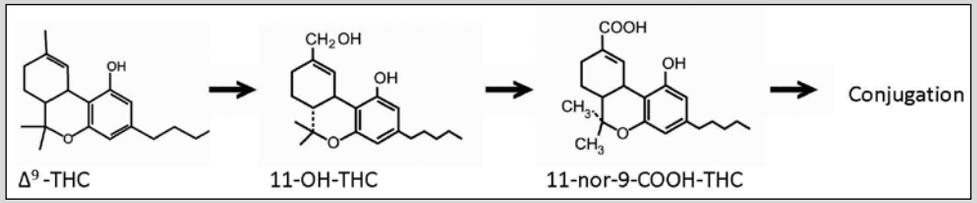


B

FIGURE 3 Complexity of intracellular CB_1 receptor signalling. As for G protein coupled receptors in general, the CB_1 receptor has the ability to activate multiple G proteins. Consequently, different functions are regulated by a variety of pathways. For example, cell survival and cell death are regulated by the MAPK cascades, whereas ion currents are directly involved in the process of neurotransmitter release. The triggering of the variety of intracellular pathways and thereby functional responses elicited by cannabinoid receptors is dependent on several factors, including the type of cells or tissue targeted, the type of ligand and the duration of receptor activation (Sanchez et al., 2001; Galve-Roperh et al., 2000). For example, successive activation might lead to a biphasic concentration-response profile or to tolerance by a molecular switch between G proteins (Asimaki and Mangoura, 2011; Sulcova et al., 1998; Paquette et al., 2007).

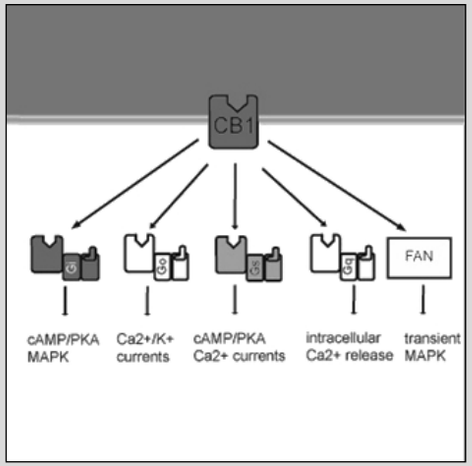


FIGURE 4 Signalling of endocannabinoids on CB_1 receptors located at the axon terminals is via a retrograde pathway. Endocannabinoids, such as anandamide and 2-arachidonoyl glycerol are released post-synaptically. Via the synapse, the molecules migrate to the pre-synaptic cell, where they give feedback via stimulation of the CB_1 receptor. Upon stimulation, a second messenger pathway influences ion channels (e.g. inhibition of calcium) thereby regulating the release of neurotransmitters including glutamate and GABA *(see inside cover for this figure in colour)*.

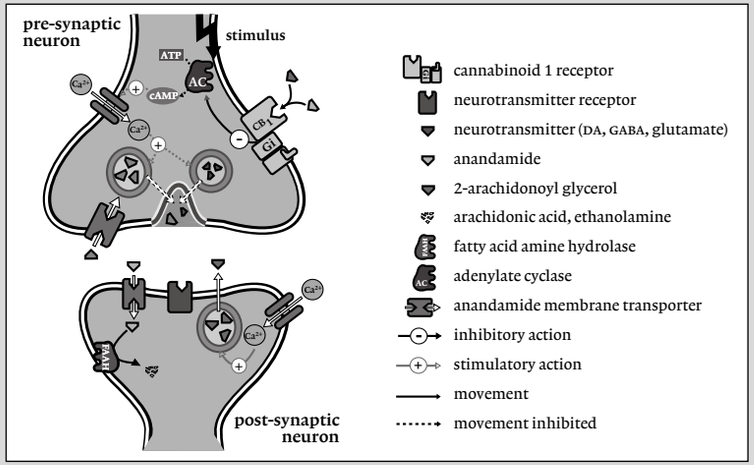


FIGURE 5 Overview of the different steps of the THC administration process where THC loss occurs. The given percentages are mean values. Eventually, 25.8% of the THC dose stays in the lungs. The exact percentage of the THC that actually reaches the blood stream is unknown, since THC metabolising enzymes are present in the lungs. The data are derived from work by Hazekamp et al. (2006).

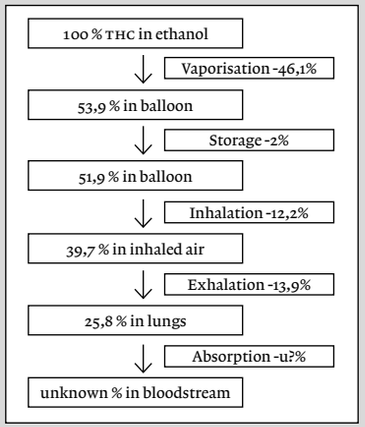
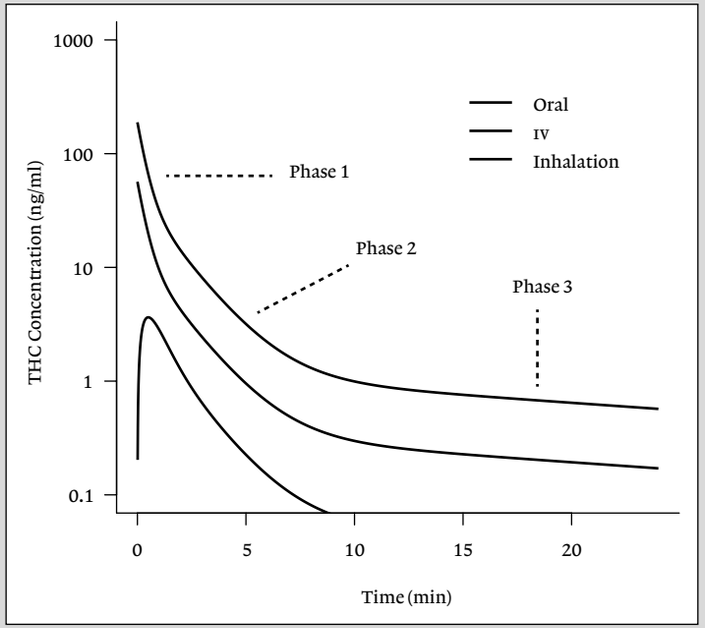


FIGURE 6 Plasma concentration profiles of 10 mg THC after inhalation, intravenous and oral absorption as simulated from a CHDR THC model in 2012 (data on file). With this model, we are able to distinguish three elimination phases: a steep decline of plasma concentration (phase 1) occurs in all administrations and lasts for a few minutes. Subsequently, a less steep decline occurs (phase 2), which changes into a flat phase that could last for over an hour (phase 3).



CHAPTER II

Novel Δ^9-tetrahydrocannabinol formulation Namisol has beneficial pharmacokinetics and promising pharmacodynamic effects

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ABSTRACT

AIM Among the main disadvantages of currently available Δ^9-tetrahydrocannabinol (THC) formulations are dosing difficulties due to poor pharmacokinetic characteristics. Namisol® is a novel THC formulation, designed to improve THC absorption. The study objectives were to investigate the optimal administration route, pharmacokinetics (PK), pharmacodynamics (PD), and tolerability of Namisol®.

METHODS This first in human study consisted of two parts. Panel I included healthy males and females (n = 6/6) in a double-blind, double-dummy, randomised, cross-over study with sublingual (crushed tablet) and oral administration of Namisol® (5 mg THC). Based on these results, male and female (n = 4/5) participants from panel I received oral THC 6.5-, 8.0 mg or matching placebo in a randomised, cross-over, rising dose study during panel II. PD measurements were: body sway; visual analogue scales (VAS) mood, psychedelic; heart rate. THC and 11-OH-THC population PK analysis was performed.

RESULTS Sublingual administration showed a flat concentration profile compared to oral. Oral THC apparent $t_{1/2}$ was 72-80 min, T_{MAX} was 39-56 min, and C_{MAX} 2.92-4.69 ng ml^{-1}. THC affected body sway (60.8%; 95%CI 29.5-99.8), external perception (0.078 log mm; 95%CI 0.019-0.137), alertness (-2.7 mm; 95%CI -4.5-/-0.9) feeling high (0.256 log mm; 95%CI 0.093-0.418), and heart rate (5.6 BPM; 95%CI 2.7-6.5). Namisol® was well tolerated.

CONCLUSIONS Oral Namisol® showed promising PK and PD characteristics. Variability and T_{MAX} of THC plasma concentrations were smaller for Namisol® than reported for studies using oral dronabinol and nabilone. This study was performed in a limited number of healthy volunteers. Therefore, future research on Namisol® should study clinical effects in patient populations.

INTRODUCTION

Components of the *Cannabis sativa* L. plant, or cannabis, have been used for medical purposes for thousands of years. Nowadays, cannabis derived compounds, or cannabinoids, are registered in several countries for a variety of indications, including antinociception and muscle relaxation in patients suffering from multiple sclerosis (Ungerleider et al., 1987; Zajicek et al., 2003; Zajicek et al., 2005), and anti-nausea and anti-emetic effects in cancer patients (Chang et al., 1979; Orr et al., 1980; Sallan et al., 1975). Cannabis consists of several cannabinoid compounds, some of which are still subject of clinical research. For the registered products, Δ^9-tetrahydrocannabinol (THC) is generally considered to be the active compound responsible for the clinical effects (Buccellato et al., 2010; Baker et al., 2000).

THC induces its effects via activation of cannabinoid receptor types 1 and 2 (CB_1 and CB_2) (Alexander et al., 2008). CB_1 are mainly located in the central nervous system, as well as in peripheral tissues such as the heart, adipose tissue and sympathetic ganglions, while CB_2 are mainly present in immune cells (Engeli et al., 2005; Herkenham, 1992; Ishac et al., 1996). The major metabolite of THC is 11-OH-THC (Grotenhermen, 2003). This metabolite induces effects via CB_1 and has been described to be equally or up to seven times as potent as THC (Wilson and May, 1975; Karler and Turkanis, 1987). This could mean that the clinical effects of THC are related to the combined activities of THC and 11-OH-THC.

The common medicinal cannabis administration routes are via smoking, after vaporising, and orally as tea or in baked goods. After smoking, THC plasma levels increase quickly (Huestis et al., 1992). However, smoking is not a very practical route and it can lead to stigmatisation, which may be limiting factors particularly for non-smokers. Also, cannabis, especially when co-administered with tobacco, contains a mixture of other compounds, some of which interact with the effects of THC, and some of which are noxious. Moreover, part of the active substances is not inhaled and will be lost. Also, depth and

frequency of inhalations vary considerably between individuals. This lack of controlled dosing may reduce clinical efficacy or induce side effects and may also occur after vaporisation of cannabis or THC. With regards to oral administration of THC using cannabis tea, a previous study found tea to have a different cannabinoid composition compared to non-decocted cannabis (Hazekamp et al., 2007), affecting the clinical effects. To bypass these problems, methods have been developed to purify THC from cannabis and to formulate it in a stable dosage form.

Marinol® and Cesamet® are two oral THC formulations registered for anorexia in AIDS patients, and nausea and vomiting in cancer patients. Marinol® contains synthetic THC, or dronabinol, and is registered in Germany and the USA. Cesamet® contains nabilone, a THC analogue, and is registered in Canada and the USA. An oromucosal spray containing mainly THC and cannabidiol, a non-psychoactive cannabinoid, is registered in Canada and in some European countries as Sativex® against pain and spasms in MS. Disadvantages of the current administration forms are the long TMAX-values for these formulations, ranging from 1 to 4 hours for Marinol® and Cesamet® (Davis, 2008; Schwilke et al., 2009), and 3.3 to 4.0 hours for Sativex® (Karschner et al., 2011). Long times to reach a maximal concentration can be a disadvantage for on demand symptomatic treatment. Oral dronabinol formulations, such as Marinol®, have variable pharmacokinetics, as peak plasma concentration variations from 150% to 200% were observed in previous studies (Naef et al., 2003; Wall and Perez-Reyes, 1981). This is unfavourable for accurate dose regulation.

In the current study, Namisol® was examined, a novel tablet formulation of pure THC that was produced using Alitra™ (Echo Pharmaceuticals b.v., Nijmegen), an emulsifying drug delivery technology. This technology was designed to improve the uptake of poorly soluble lipophilic compounds, using less surfactant (less than 10% w/w). This is a first in human trial investigating the optimal administration route of Namisol®, the safety, pharmacokinetics, pharmacodynamics and tolerability. The first objective was to compare the sublingual and oral dosing routes of Namisol® tablets with respect to pharmacodynamic effects and pharmacokinetics of THC and its active metabolite 11-OH-THC and to choose the most favourable administration route. This was decided on factors such as a short time to maximal THC concentration, and a high maximal concentration. The second objective was to use the most favourable administration route in a subsequent dose-ranging study, in order to evaluate the pharmacokinetics and pharmacodynamic effects of different doses. With these objectives, which intend to explore the pharmacokinetic and pharmacodynamic properties of Namisol®, no registered cannabis based medicines were taken as an additional treatment arm in at this early stage of development.

METHODS

Design

The study consisted of two parts. In the first part of the study, the pharmacokinetic differences between oral and sublingual administration, and the most favourable administration route were determined, referred to as 'Panel I'. Panel I had a double blind, double dummy, two-way cross-over design. Panel II referred to the dose-ranging part of the study, which was a randomized, double blind, placebo-controlled, 3-way dose-escalation trial. For both panels, the wash-out period between two treatments was at least two weeks. Subjects were medically screened within 3 weeks before dosing. Subjects had a follow-up visit after the 24-hour PK sample of the last visit of Panel II.

Sample size

This was an explorative study for which no sample size calculation was performed. For Panel I, 12 healthy subjects (6 male, 6 female) were included, and for Panel II, 9 subjects (mixed gender) were included. These numbers are usually sufficient to demonstrate significant dose-related pharmacodynamic effects of THC after inhalation (Zuurman et al., 2008; Bossong et al., 2009). Participants from Panel I were allowed to continue in Panel II.

Inclusion and exclusion criteria

After signing the informed consent form, subjects were medically screened. Subjects were between 18 and 55 years old and had a body mass index between 18.0 and 28.5 kg m^{-2} (extremes included). They had to be cannabis users for at least one year, to minimise the risk of oversensitivity to THC in naive subjects. To prevent pharmacokinetic and pharmacodynamic tolerance, the maximal use was limited to once per week, and subjects were not allowed to have used cannabis from at least two weeks prior to the first treatment period to the end of the last study day. Subjects were not allowed to smoke more than ten cigarettes per day and had to refrain from smoking during study days. Subjects using more than six units of (methyl)xanthine products (e.g. coffee, tea, cola, chocolate) were not included, and subjects had to stop using xanthine containing products from 12 hours prior to dosing until discharge. An irregular diurnal rhythm and consumption of grapefruit (juice) were not allowed from two weeks prior to the first dose until the last study day. Quinine and alcohol use were not allowed from two days prior to dosing until discharge. Use of medication was not allowed from one week prior to dosing until the last study day. Use of illicit drugs were not allowed during the study, and each study day prior to dosing, illicit drug (including cannabis) use was tested using drug screening urine tests. In order to keep a consistent level of sex hormones, female subjects were only included if they used the Nuvaring® or one of the monophasic oral contraceptives, and were able and willing to skip the pill or ring-free week from screening until the end of the study. Pregnant and/or breastfeeding women were excluded, and urinary pregnancy tests were performed prior to study drug administration. The study was approved by the Ethical Review Board of Leiden University Medical Center.

Treatments

Namisol® and matching placebo (Echo Pharmaceuticals b.v., Nijmegen) were administered as 1.5 mg and 5 mg tablets. In Panel I, one tablet (5.0 mg THC), and in Panel II, three tablets (one 5.0 mg and two 1.5 mg tablets active or matching placebos) were used for the administration of 6.5 mg or 8.0 mg THC or placebo respectively. Oral administrations were done

with 200 ml mineral water. Namisol® tablets were not designed for sublingual use. Due to a relatively long in vitro disintegration time of up to 15 minutes of this experimental formulation, tablets were crushed before sublingual administration using Pillmaster (Sell-Plan, Weesp) to increase the surface area of the tablet, and, as a result, improve sublingual absorption. The crushed tablet was then placed under the tongue using cigarette rolling paper.

In Panel I, the following treatments were administered within one minute of t = 0: (1) oral Namisol® 5 mg + sublingual matching placebo, (2) sublingual Namisol® 5 mg + oral matching placebo. After Panel I, an interim analysis of safety, pharmacokinetic and pharmacodynamic data was performed. Based on this analysis, the most favourable administration route of Namisol® was selected for Panel II. The dose levels for Panel II were also based on the interim results of Panel I, leading to an oral dose selection of 6.5 mg, 8.0 mg or matching placebo.

Pharmacokinetics

For determination of the plasma concentration of THC and its active metabolite 11-OH-THC, venous blood was collected in EDTA tubes of 4 ml at the following time points: pre dose, 0h11m, 0h30m, 0h45m, 1h00m, 1h30m, and at 2, 3, 4, 6, 8, 12 and 24 hours. The 24-hour blood sample was only drawn in Panel I. After blood collection the tubes were put in ice water in light-shielded containers and were centrifuged within one hour (10 min, 2000g, 4^{0}C). The handling of THC samples was done at low ambient lighting. Plasma samples were stored at a temperature of at least –70 ^{0}C and analysed by Analytisch Biochemisch Laboratorium b.v., (Assen) using liquid chromatography/mass spectrometry/mass spectrometry (LC-MS/MS) according to good laboratory practice procedures. The lower limit of quantification for both THC and 11-OH-THC was 0.100 ng/ml.

Pharmacodynamics

Pharmacodynamic measurements were performed in 'test-blocks', in a quiet room with subdued lighting, with only one subject in the same room per session. Test-blocks were performed at the following time points: twice pre-dose, 0h15m, 0h32m, 0h47, 1h02m, 1h32m, and at 2 minutes past 2, 3, 4, 6, and 8 hours. Within three weeks before the first occasion, subjects had a training session in order to get acquainted with the pharmacodynamic tests and to minimise learning effects during the study.

BODY SWAY METHODOLOGY

Two-minute measurements of postural stability were performed using a body sway meter as described previously (Zuurman et al., 2008).

VISUAL ANALOGUE SCALES

The Bond and Lader visual analogue scales (VAS) were used to measure subjective alertness, mood, and calmness (Bond and Lader, 1974). The Bowdle VAS of psychedelic effects were performed in order to measure subjective feeling high, and clustered scales that quantify effects on internal and external perception (Bowdle et al., 1998; Zuurman et al., 2008). Internal perception reflects inner feelings that do not correspond with reality, including mistrustful feelings, whereas external perception reflects a misperception of external stimuli or changes in the awareness of the subject's surroundings. The data were clustered and log transformed, and are expressed as units as described previously (Zuurman et al., 2008).

HEART RATE

Electrocardiogram (ECG) measurements (Cardiofax V equipped with ECAPS12 analysis program, Nihon Kohden) were taken in triplicate after

having been in a supine position for at least 5 min at the following time points: pre-dose, 1h15m and 24h08m (Panel II only). The QT-intervals were corrected for heart rate according to Bazett and Fridericia's QT correction. Blood pressure and heart rate measurements were performed using Nihon-Kohden (BSM-1101K) or Colin (Pressmate BP 8800) automated device after sitting for at least 5 min. Safety heart rate and blood pressure measurements were performed at the following time points: pre-dose, 1h03m and 23h58m (Panel II only). Heart rate measurements that were also recorded as pharmacodynamic endpoints, at time points described in that pertaining section.

Data analysis

As the first part of the study was not placebo-controlled, statistical analysis of safety and pharmacodynamics was performed for both study panels separately. For the pharmacokinetic parameters, all treatments were analysed together. After Panel I, an interim analysis was performed for adverse events, pharmacokinetics and pharmacodynamics, to adapt the design of Panel II.

Non-compartmental pharmacokinetic analysis

Descriptive statistics were calculated for the plasma concentrations of THC, 11-OH-THC, and unbound active moiety (THC + 11-OH-THC) at each time point and for peak plasma concentration (C_{MAX}), time to peak plasma concentration (T_{MAX}), apparent terminal half-life ($t_{1/2}$), and area under the curve from t = 0 to infinity ($AUC_{0-\infty}$). Dose-proportionality was assessed for C_{MAX} and $AUC_{0-\infty}$. Pharmacokinetic parameters were compared with a mixed model analysis of variance and reported with 95% confidence intervals around the estimated differences. All effects were considered significant at the 5% level.

Compartmental pharmacokinetic analysis

A population pharmacokinetic model was developed for the most favourable Namisol® formulation, in order to make predictions of pharmacokinetic profiles for further clinical development. Pharmacokinetic modelling was conducted using NONMEM (version 7.1.2). Pharmacokinetics of THC and 11-OH-THC were described using a sequential compartmental modelling approach, which was used previously (Strougo et al., 2008; Zhang et al., 2003). The model part of 11-OH-THC was linked to the individual empirical Bayes estimates determined for the THC pharmacokinetic parameters. Different absorption models were tested, including first-order absorption and transit models, as well as different elimination models, including linear elimination and Michaelis-Menten elimination, which was used in a previous model (Strougo et al., 2008). Model discrimination was performed using the likelihood ratio test, using a difference in objective function values of 6.64 as significance criterion (chi-square test, $\alpha = 0.01$, df = 1). All models were also graphically evaluated using goodness of fit plots, depicting individual and population predicted versus observed. Potential model misspecification was assessed using plots of residuals versus time and the dependent variable. Predictive performance of the final models for internal validation was evaluated using a visual predictive check depicting the model simulated distribution together with the observed values versus time.

Pharmacodynamic analyses

Average baseline values per subject and visit for each variable were obtained by calculation of the mean of two baseline assessments. Body sway was log transformed to correct for the log normal distribution. All pharmacodynamic parameters were analysed by mixed model analyses of variance (using SAS PROC MIXED) with subject, subject by treatment

and subject by time as random effects, with gender, treatment, occasion, time, treatment by gender and treatment by time as fixed effects, and the average baseline value was included as covariate. For panel I the contrast oral THC 5 mg versus sublingual THC 5 mg was calculated. For panel II the calculated contrasts were: placebo versus oral 6.5 mg, placebo versus oral 8.0 mg, oral 6.5 mg versus oral 8.0 mg. All effects were considered significant at the 5% level.

RESULTS

Subjects

For Panel I, 14 subjects (7 males and 7 females) were included in order to get 12 complete data sets. Data sets from 13 subjects were used for pharmacodynamic and pharmacokinetic analysis. One subject dropped out after a vasovagal collapse, and one subject for personal circumstances. Four males and five females from Panel I continued the study in Panel II. On average, the subjects were 21.4 years old, and had a body mass index of 21.7 kg m^{-2}. Demographic details per panel can be found in Table 1.

Adverse effects

All adverse events were of mild to moderate intensity and transitory in nature. A vasovagal syncope occurred during the first occasion, 32 minutes after administration of Namisol® oral 5 mg + Placebo Namisol® sublingual, which was considered to be possibly related to treatment and led to the subject's withdrawal. In Panel I, the frequencies and types of adverse events were similarly distributed over sublingual and oral administration. In Panel II, compared to placebo, more subjects in THC treatment groups had adverse events that were classified as nervous system disorders, especially in the 8.0 mg THC treatment group (9/9 subjects; 6.5 mg THC, 7/9 subjects; placebo, 4/9 subjects), with dizziness as the most frequent adverse event. The same trend was found for the psychiatric disorder class (8.0 mg THC, 5/9; 6.5 mg THC, 3/9; placebo, 0/9), which mainly concerned self reported euphoric mood ('feeling high').

No clinically relevant changes in blood pressure, body temperature, haematology, biochemistry, urinalysis or any of the ECG intervals were found. Heart rate increase after treatment was analysed as a pharmacodynamic parameter.

Noncompartmental pharmacokinetic analysis

Noncompartmental pharmacokinetic parameters of sublingual and oral THC are summarised in Table 2 and the concentration profiles of THC and 11-OH-THC are given in Figure 1. Based on the interim PK analysis, the oral administration route was chosen above the sublingual route. A shorter T_{MAX} and a higher C_{MAX} of oral THC indicated a possibly larger effect with a faster onset compared to sublingual administration. These differences in T_{MAX} and C_{MAX} between oral and sublingual administration were not statistically significant. Sublingual administration showed a significant longer apparent $t_{1/2}$ compared to oral administration (+122 min; 95% CI 64 / 181; p = 0.0002). $AUC_{0-\infty}$ and C_{MAX} of oral THC were dose proportional and T_{MAX} and $t_{1/2}$ were similar for all doses.

The difference between pharmacokinetic parameters for oral and sublingual THC 5 mg administration were not significantly different for 11-OH-THC, except for the dose corrected peak concentration (0.30 ng/ml/mg; 95% CI 0.10/0.49; p = 0.0047). Pharmacokinetic profiles for oral 5.0-, 6.5-, and 8.0 mg THC were also not different, except for $t_{1/2}$, where 5 mg was shorter than both 6.5- and 8.0 mg (115 min; 95% CI 8 / 222; p = 0.0366; and 110 min; 95% CI 3 / 217; p = 0.0441 respectively).

Compartmental pharmacokinetic analysis

The two-compartment model for THC pharmacokinetics had first-order absorption, linear elimination and a lag time. A proportional model was used for the residual error. The estimates for clearance and volumes are apparent values, i.e. CL F-1 and V F-1, since this study had no intravenous administration and therefore absolute bioavailability (F) could not be determined. Peripheral volume of distribution of THC was approximately two times larger than central volume (1780 L vs. 889 L), while the peripheral volume of 11-OH-THC was approximately 19 times larger than the central volume of distribution (1010 L vs. 52.6 L). Inter-individual variability was estimated for clearance and central volume. THC clearance had a variability of 28.4%. 11-OH-THC had a large variability of clearance of 70.4%. Inter-individual variability of the central volume of distribution was large for THC with 56.3%, and was especially large for 11-OH-THC with 413%. Almost all parameters showed a relative standard error (RSE) that was smaller than 30%. An overview of the pharmacokinetic parameters after oral administration of Namisol® is given in Table 3. Visual predictive checks demonstrated that the predictive performance of the THC and 11-OH-THC models slightly overestimated the variability during wash-out. The visual predictive checks can be found in Figure 2.

The pharmacokinetic model of THC was used for a stochastic simulation of THC and 11-OH-THC concentrations during a multiple dose design of two daily 5 mg THC doses. The graphical representation of this simulation can be found in Figure 3. In this simulation the plasma concentration of THC and 11-OH-THC will not drop below the lower limit of quantification (0.100 ng/ml for both THC and 11-OH-THC) in steady state before the next dose is administered. The accumulation factor of the plasma concentration is 1.02 for THC, and 1.11 for the active metabolite as based on this single dose study.

Pharmacodynamics

Contrasts of pharmacodynamic parameters are summarised in Table 4. As an example of the graphical representation of the pharmacodynamic parameters, the effect of Namisol® on body sway is given in Figure 4. In panel I, oral THC administration gave a statistically significant increase in VAS calmness, compared to sublingual administration. This difference was not considered clinically relevant, as the absolute peak difference was 3 mm on a 100 mm scale. Between oral and sublingual administration, no clinically relevant differences in PD parameters were observed. In panel II, significant increases were found between THC 6.5 mg and placebo on VAS external perception, VAS feeling high, and heart rate. THC 8.0

mg produced a decrease on VAS alertness, and increases on body sway, VAS external perception, VAS feeling high, and heart rate compared to placebo. The THC-effects changed in a dose-dependent way, which was significant for body sway when comparing THC 6.5 mg and 8.0 mg.

DISCUSSION

Available oral THC formulations and cannabis based medicines generally show disadvantageous pharmacokinetics that cause difficulties in dose regulation. Namisol® is a new THC formulation that was developed to achieve a more favourable pharmacokinetic profile. Since pharmacokinetic characteristics of THC ultimately determine its pharmacodynamic features, a fast onset of action and less variable response, as found in this study, are expected to lead to a more rapid and consistent clinical response. This study was designed to investigate two administration routes of Namisol® and three different oral doses of Namisol® in healthy volunteers.

Route of administration

The pharmacokinetic differences after oral and sublingual administration were small. Sublingual administration showed more flat concentration profiles of THC and 11-OH-THC, compared to oral administration, a late and small maximal concentration and a long apparent terminal half-life. This could be explained by a relatively small absorption constant of THC from the oral mucosa into the blood, with an absorption that could be slower than the elimination or distribution. The slow absorption from the oral mucosa after sublingual administration could be caused by the lipophilic character of THC. Furthermore, no in-vitro data are available that support a slow absorption. The more favourable pharmacokinetic profile of the oral tablet compared to the sublingual route implies beneficial pharmacodynamic properties of oral Namisol®, such as an improvement of speed and accuracy of the onset and of the extent of the effects. Therefore, combined with the practical convenience of the administration procedure, the oral administration route was found to be most optimal.

Pharmacokinetics

Oral Namisol® showed a short time to reach maximal THC concentration (39-56 min) compared to reported values in previous studies using oral THC (60-240 min), nabilone (120-240 min), or oral-mucosal THC+CBD (Sativex®, 198-240 min) (Valeant Pharmaceuticals International, 2006; Davis, 2008; Schwilke et al., 2009; Karschner et al., 2011; Naef et al., 2003). Namisol® also had a shorter time to maximal concentration of the active metabolite 11-OH-THC (46-84 min) compared to what has been published for dronabinol (120-204 min) and Sativex® (216-234 min) (Naef et al., 2003; Karschner et al., 2011). Although direct comparative studies are needed to corroborate these findings, the differences seem large enough to be realistic, and to be clinically relevant if the therapeutic effects follow the plasma concentrations reasonably directly. If so, Namisol® could give faster clinical effects compared to other oral formulations with THC or cannabis based medicines that are currently in clinical use. The short time to reach maximal THC and 11-OH-THC concentrations could be explained by a fast absorption of Namisol®. Inter-individual variability of Namisol® parameters was relatively large when compared to THC inhalation, as shown by compartmental analysis on THC pharmacokinetic parameters (Strougo et al., 2008). However, variability of THC maximal concentration was two to five times smaller than reported previously for dronabinol, which was based on non-compartmental analysis (Naef et al., 2003; Wall et al., 1983). This first in human study was primarily intended to explore the pharmacokinetic and pharmacodynamic properties of Namisol®. At this early stage of development therefore, no registered cannabis based medicines were taken as an additional treatment arm. Although there are clear limitations to comparisons with literature data, in summary, the pharmacokinetic properties suggest that THC from Namisol® might have a faster absorption and a less variable maximal concentration. Therefore, pharmacokinetics of Namisol® could be more favourable than currently registered oral dronabinol formulations and cannabis based medicines.

The pharmacokinetic model that was developed for THC and 11-OH-THC can be used to predict concentration-time profiles of alternative dosing scenarios. Hence, 'what-if' questions that are related to pharmacokinetics can be answered in further clinical development of this compound. Compartmental pharmacokinetic analysis assessed that the apparent terminal half-life of 11-OH-THC was shorter for oral 5.0 mg compared to 6.5 and 8.0 mg. This could be explained by the fact that the concentration after 5.0 mg drops below the lower limit of quantification more rapidly than for higher doses, and this does not necessarily imply that the actual half-life is different for oral than for sublingual administrations. A previous study administering 5 mg of labelled THC intravenously found that THC was still detectable in plasma 72 h after administration (Ohlsson et al., 1982), while in the current study no THC or 11-OH-THC was detected in plasma at 24 h after administration. This confirms our implication that the limitations of the limit of quantification and the time frame of sampling in the current study thwarted an accurate estimation of the half-life of oral and sublingual Namisol®.

Compared with intravenous administration and inhalation, the concentration of the 11-OH metabolite after oral THC administrations from Namisol® was relatively high (Committee for medicinal products for human use, 2010; Valeant Pharmaceuticals International, 2006; Wilson and May, 1975). The ratio of 11-OH-THC:THC (based on peak plasma concentrations) was 1:30 for intravenous administration and 1:7 for inhalation, while this ratio was 1:0.6-0.8 for Namisol® (Naef et al., 2004; Grotenhermen, 2003; Strougo et al., 2008). Previous studies with oral dronabinol and Sativex® also gave a lower metabolite concentration compared to Namisol® (11-OH-THC:THC was 1:1.2-2.0) (Schwilke et al., 2009; Karschner et al., 2011). The relatively high levels of 11-OH-THC compared to the parent compound THC could be explained by several concomitant or alternative factors that could not be identified in this

study. High concentrations of the metabolite suggest that considerable first-pass metabolism is taking place. Considering the absorption rate constant of 0.04 per minute suggested by the PK-model, it is possible that THC stays in the gastro-intestinal tract for a relatively long time where much of it is locally metabolised to 11-OH-THC. The metabolite is then absorbed from the gastrointestinal tract to the blood, where it is not as rapidly distributed to fatty tissues as THC, due to the metabolite's less lipophilic character. At the same time, THC could rapidly disappear from blood into more fatty tissues, leading to low plasma concentrations. Long blood sampling schedules and very low detection thresholds for THC and its metabolites in plasma or mass balance studies would be needed to resolve the complex pharmacokinetics of THC in more detail.

Pharmacodynamics

Although the THC plasma concentrations after oral Namisol® administration were relatively low after completion of Panel I, the pharmacodynamic effects were larger than we had expected, and comparable to those observed in a THC inhalation study in which high peak THC plasma concentrations were found (Zuurman et al., 2008). This could reflect a large pharmacological effect of the 11-OH-metabolite. Preclinical studies have found 11-OH-THC to be a highly potent CB_1-agonist (Karler and Turkanis, 1987; Wilson and May, 1975), and clinical studies also reported more rapid and larger effects after 11-OH-THC administration compared to THC (Lemberger et al., 1972; Lemberger, 1973; Lemberger et al., 1973). In itself, this would have allowed us to predict the pharmacodynamic effects of higher doses in Panel II, by reference to the results of other oral THC formulations in the literature which also produce high concentrations of 11-OH-THC. However, quantitative comparisons were quite difficult to make because of differences in methodology and study designs (Curran et al., 2002; Zuurman et al., 2009). Moreover, it was impossible to exclude the alternative (or additional) explanation that the large pharmacodynamic effects are due to a more efficient absorption of THC from the Namisol® formulation, with rapid redistribution to the CNS during the absorption phase. Since after Panel I we could not be certain about the dose proportionality of Namisol® at higher doses, we decided to continue the study in Panel II with two conservatively small dose increases (to 6.5 and 8 mg) for reasons of safety and tolerability, and to increase the dose further if necessary and possible.

The first pharmacodynamic effects of Namisol® 6.5 and 8.0 mg were already observed during the first assessments, 15 min after dosing. Namisol® had a faster onset of action than reported in a previous study with oral dronabinol (Marinol®), which had an onset of action between 0.5 and 1 hour, and peak effects that were reached between 2 and 4 hours (Solvay Pharmaceuticals, 2004). The time profile of the pharmacodynamic effects was more similar to the concentration curve of 11-OH-THC than that of THC. A previous study reported that 11-OH-THC induces a quicker onset of the pharmacodynamic effects compared to THC (Lemberger et al., 1972; Lemberger, 1973; Lemberger et al., 1973). These results in this study are quite promising for a fast onset of the clinical effects in a patient population, although future studies should carefully investigate the relation between pharmacodynamic effects in healthy volunteers and clinical effects in patients. Also, a more detailed analysis of the CNS-effects of THC and 11-OH-THC should be done in humans to separate the contributions of both compounds to the effects. A future study where the effects of THC are compared with those of 11-OH-THC alone could provide meaningful information about the relative contributions of 11-OH-THC to the CNS-effects of THC and cannabis.

In conclusion, Namisol® is a novel formulation of THC that is well-tolerated and absorbed quickly after ingestion, and reaches peak plasma concentrations within one hour and maximal effects between 1 to 2 hours after Namisol® administration. Compared to the literature on registered dronabinol formulations and cannabis based medicines, these

results imply that Namisol® may also have favourable pharmacokinetic and pharmacodynamic characteristics in patients. Further clinical studies are needed to show that these apparent advantages are also therapeutically relevant.

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TABLE 1 Summary of subject demographics of Panel I and Panel II

	Variable	N	Mean	Std	Min	Max
PANEL I	Gender (M:F)	7:7				
	Age (yrs)	14	21.4	3.3	18	27
	BMI (kg m^{-2})	14	21.71	1.52	18.4	24.5
	Height (m)	14	1.783	0.103	1.62	1.96
	Weight (kg)	14	69.09	10.13	55.3	90.1
PANEL II	Gender (M:F)	4:5				
	Age (yrs)	9	21.9	3.8	18	27
	BMI (kg m^{-2})	9	22.31	0.97	21.1	24.5
	Height (m)	9	1.766	0.099	1.62	1.91
	Weight (kg)	9	69.70	8.91	55.3	80.6

TABLE 2 Pharmacokinetic parameters of THC and 11-OH-THC after sublingual and oral administration of Namisol®. All data are presented as means with coefficient of variation (%).

Panel	**I (n=13)**	**I (n=13)**	**II (n=9)**	**II (n=9)**
Parameter	**5.0 mg sublingual**	**5.0 mg oral**	**6.5 mg oral**	**8.0 mg oral**
THC				
C_{max} (ng ml^{-1})	2.30 (44)	2.92 (51)	4.43 (42)	4.69 (62)
T_{max} (min)	74.5 (52)	56.0 (73)	39.3 (20)	43.6 (26)
$AUC_{0-\infty}$ (ng.min ml^{-1})	235.8 (47)	188.7 (40)	286.6 (36)	377.2 (46)
$t_{1/2}$ (min)	252.9 (98)	71.9 (24)	80.0 (22)	78.8 (21)
11-OH-THC				
C_{max} (ng ml^{-1})	3.08 (42)	4.68 (42)	5.94 (44)	6.10 (53)
T_{max} (min)	83.6 (63)	74.1 (68)	46.1 (28)	78.4 (63)
$AUC_{0-\infty}$ (ng.min ml^{-1})	522.9 (50)	648.1 (49)	848.7 (42)	1087.3 (50)
$t_{1/2}$ (min)	279.0 (51)	196.0 (33)	318.7 (54)	314.1 (58)

* C_{max} and $AUC_{0-\infty}$ were dose-corrected for treatment p-value calculation
C_{max}=peak plasma concentration; T_{max}=time to peak plasma concentration;
$AUC_{0-\infty}$=area under the curve from t=0 to infinity; $t_{1/2}$= apparent terminal half-life.

TABLE 3 THC population pharmacokinetic parameters after oral Namisol®.

	THC		11-OH-THC	
Parameter	**Estimate (RSE)**	**IIV**	**Estimate (RSE)**	**IIV**
Clearance/F (L min^{-1})*	26.5 (10.6)	28.4	9.53 (25)	70.4
Central volume of distribution/F (L)*	889 (22.5)	56.3	52.6 (47.9)	413
Peripheral volume of distribution/F (L)*	1790 (21.9)	-	1010 (15.3)	21.1
Intercompartmental clearance/F (L min^{-1})*	13.3 (17)	-	4.46 (34.5)	50.7
Absorption rate constant (min^{-1})	0.0401 (22)	-	-	-
Proportional residual error (SD mean^{-1})	0.509 (8)	-	0.461 (6.2)	-
Absorption lag time (min)	11.5 (0.9)	-	-	-

* This parameter is an apparent parameter as bioavailability could not be calculated.
RSE = relative standard error (%); IIV=inter-individual variability (coefficient of variation, %).

TABLE 4 Pharmacodynamic effects after Namisol® dosing. Treatment differences are given in estimated differences of least square means with 95% confidence intervals and p-values. Log transformed VAS (scores in mm + 2) are given in units (U).

Panel	**I (n=13)**	**II (n=9)**	**II (n=9)**	**II (n=9)**
Parameter	**5.0 mg oral vs 5.0 mg sublingual**	**6.5 mg oral vs placebo**	**8.0 mg oral vs placebo**	**8.0 mg vs 6.5 mg oral**
Body sway (%)	7.66 (-4.62, 21.53) p=0.2037	22.06 (-1.05, 50.57) p=0.0610	60.82 (29.46, 99.79) **p=0.0003***	31.76 (6.53, 62.96) **p=0.0145***
VAS Alertness (mm)	-0.3 (-2.0, 1.5) p=0.7124	-1.4 (-3.2, 0.4) p=0.1161	-2.7 (-4.5, -0.9) **p=0.0057***	-1.3 (-3.1, 0.5) p=0.1390
VAS Mood (mm)	0.8 (-0.1, 1.6) p=0.0653	0.1 (-0.3, 0.5) p=0.5357	0.2 (-0.2, 0.6) p=0.3686	0.1 (-0.4, 0.5) p=0.7815
VAS Calmness (mm)	1.8 (0.1, 3.5) **p=0.0443***	0.7 (-0.1, 1.4) p=0.0665	0.5 (-0.2, 1.2) p=0.1246	-0.1 (-0.9, 0.6) p=0.7080
VAS Feeling high (U)	0.111 (-.042, 0.265) p=0.1347	0.229 (0.073, 0.384) **p=0.0071***	0.256 (0.093, 0.418) **p=0.0044***	0.027 (-.129, 0.183) p=0.7145
VAS External perception (U)	0.037 (-.017, 0.090) p=0.1482	0.061 (0.002, 0.121) **p=0.0446***	0.078 (0.019, 0.137) **p=0.0141***	0.017 (-.042, 0.076) p=0.5507
VAS Internal perception (U)	0.006 (-.014, 0.026) p=0.5247	0.013 (-.003, 0.029) p=0.1057	0.002 (-.015, 0.019) p=0.8312	-0.011 (-.028, 0.005) p=0.1632
Heart rate (BPM)	0.2 (-3.6, 4.0) p=0.9261	5.3 (2.4-8.2) p=0.0019*	5.6 (2.7-8.5) **p=0.0014***	0.3 (-2.7, 3.2) p=0.8524

* Statistically significant values

FIGURE 1 THC (A) en 11-OH-THC (B) concentrations after sublingual 5.0 mg and oral 5.0-, 6.5- and 8.0 mg Namisol® administration as estimated with a mixed model. Closed circles are sublingual THC 5 mg, open circles are oral THC 5.0 mg, triangles are oral THC 6.5 mg, and squares are oral THC 8.0 mg. Error bars represent standard deviations.

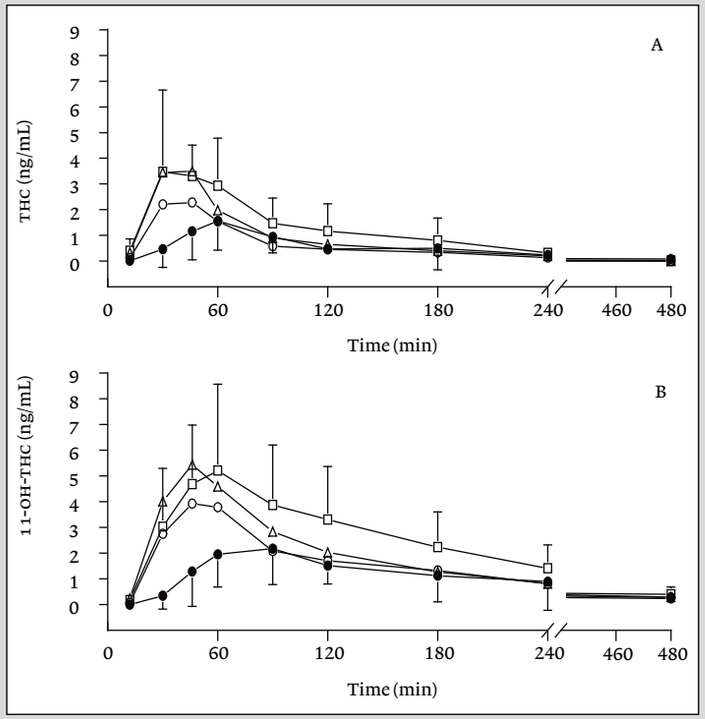


FIGURE 2 Visual predictive checks of THC concentrations after THC 5.0-, 6.5-, and 8.0 mg (figures A, B and C), and of 11-OH-THC concentrations (figures D, E, and F). Lower limit of quantification for THC and 11-OH-THC is 0.1 ng/ml.

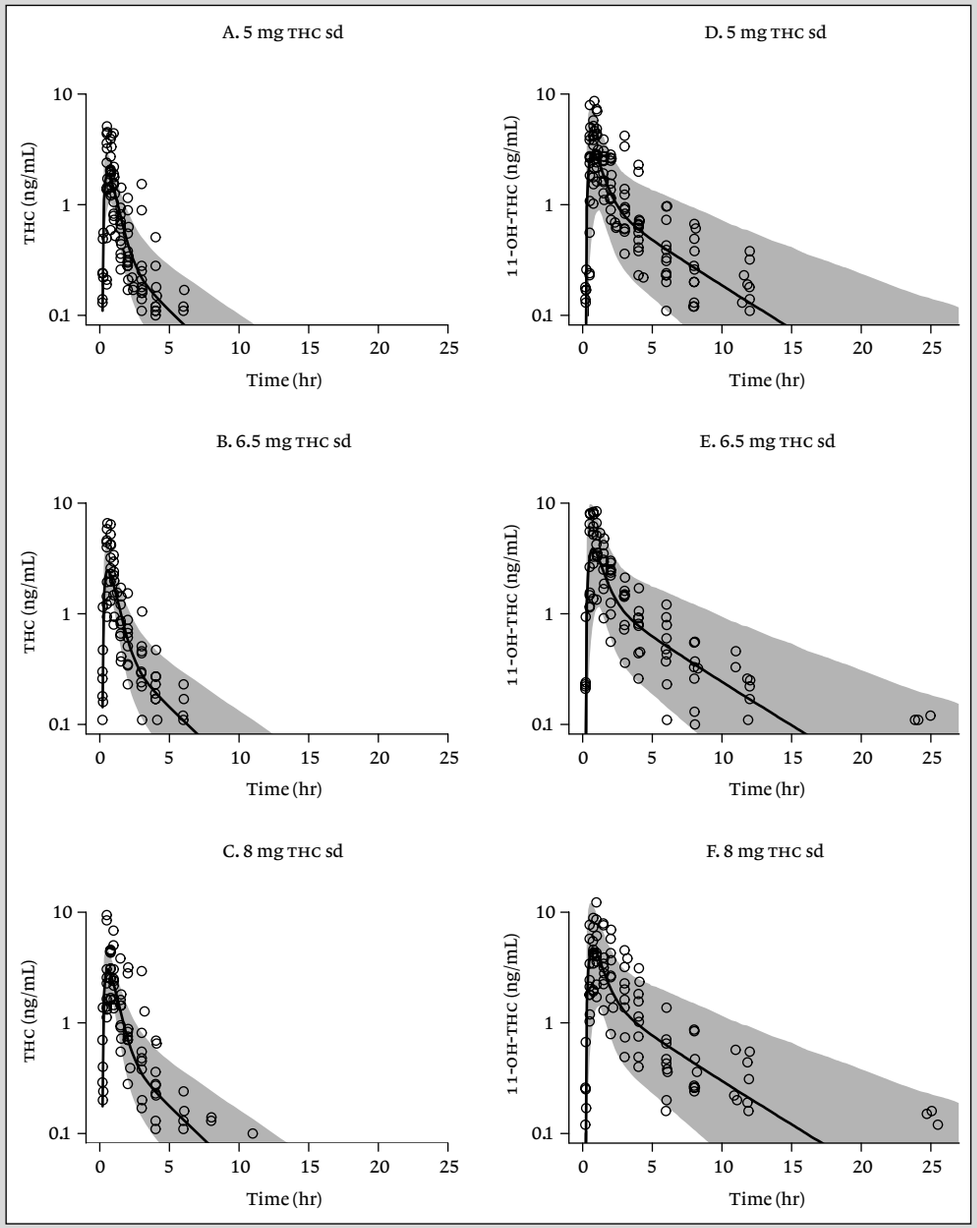


FIGURE 3 Stochastic simulations (n=2000) of concentrations of THC after a single 5 mg dose (A), and after 21 dosages, 5 mg two times per day (B) and simulations of 11-OH-THC concentrations after a single 5 mg dose (C), and after 21 dosages, 5 mg two times per day (D). Sd = single dose; bid = two doses per day.

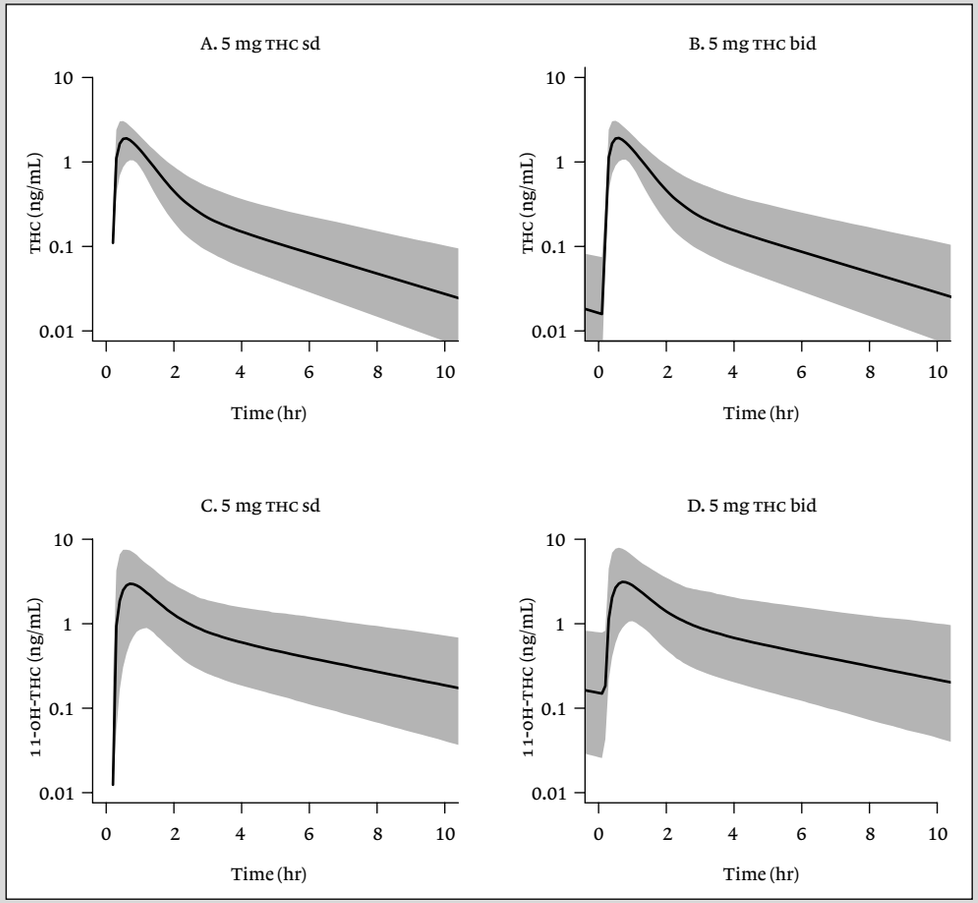
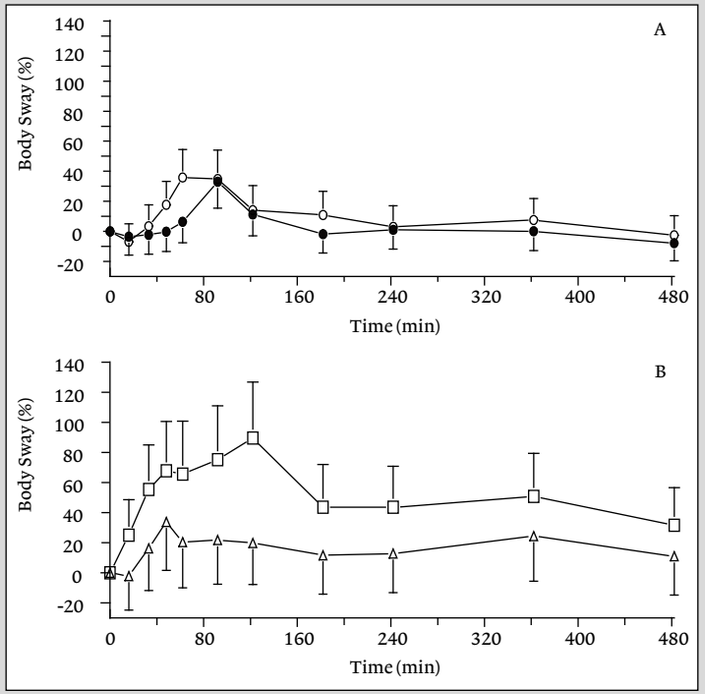


FIGURE 4 Effect-time profiles of baseline corrected body sway least square means in %, with 95% confidence interval error bars. Figure A shows the results from Panel I of the study, including sublingual THC 5.0 mg as closed circles, and oral THC 5.0 mg as open circles. Figure B has the results of Panel II, with oral THC 6.5 mg as triangles, and oral THC 8.0 mg as squares.



CHAPTER III

Manipulating brain connectivity with Δ^9-tetrahydrocannabinol: a pharmacological resting state fMRI study

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ABSTRACT

AIM Resting state-functional magnetic resonance imaging (RS-FMRI) is a neuroimaging technique that allows repeated assessments of functional connectivity in resting state. While task-related FMRI is limited to indirectly measured drug effects in areas affected by the task, resting state can show direct CNS effects across all brain networks. Hence, RS-FMRI could be an objective measure for compounds affecting the CNS. Several studies on the effects of cannabinoid receptor type 1 (CB_1)-receptor agonist Δ^9-tetrahydrocannabinol (THC) on task-dependent FMRI have been performed. However, no studies on the effects of cannabinoids on resting state networks using RS-FMRI have been published. Therefore, we investigated the effects of THC on functional brain connectivity using RS-FMRI.

METHODS Twelve healthy volunteers (9 male, 3 female) inhaled 2, 6 and 6 mg THC or placebo with 90-minute intervals in a randomized, double blind, cross-over trial. Eight RS-FMRI scans of 8 minutes were obtained per occasion. Subjects rated subjective psychedelic effects on a visual analogue scale after each scan, as pharmacodynamic effect measures. Drug-induced effects on functional connectivity were examined using dual regression with FSL software (FMRIB Analysis Group, Oxford). Eight maps of voxel-wise connectivity throughout the entire brain were provided per RS-FMRI series with eight predefined resting-state networks of interest. These maps were used in a mixed effects model group analysis to determine brain regions with a statistically significant drug-by-time interaction. Statistical images were cluster-corrected, and results were Bonferroni-corrected across multiple contrasts.

RESULTS THC administration increased functional connectivity in the sensorimotor network, and was associated with dissociable lateralised connectivity changes in the right and left dorsal visual stream networks. The brain regions showing connectivity changes included the cerebellum and dorsal frontal cortical regions. Clear increases were found for feeling high, external perception, heart rate and cortisol, whereas prolactin decreased.

CONCLUSIONS This study shows that THC induces both increases and (to a lesser extent) decreases in functional brain connectivity, mainly in brain regions with high densities of CB_1-receptors. Some of the involved regions could be functionally related to robust THC-induced CNS-effects that have been found in previous studies (Zuurman et al, 2008), such as postural stability, feeling high and altered time perception.

INTRODUCTION

Ideally, early clinical phase drug development for neurological and psychiatric indications should use tests that measure effects in an objective way and repeatedly over time across different species. These tests should also be able to distinguish unique effect profiles for different classes of drugs. Traditionally, measurements of drug effects on the central nervous system (CNS) in healthy volunteers include cognitive tasks, various questionnaires, neurophysiological measurements, and increasingly also neuroimaging. The wide diversity of these tests and their numerous variations limits their applicability for decision making in clinical practice or drug development. In addition, pharmacological studies can only include a limited number of pre-defined pharmacodynamic tests, which can easily miss drug effects in CNS domains that are not tested. Moreover, most CNS effects are influenced by various functions like attention and motor coordination, and therefore do not provide direct information on an exact site of drug action.

Imaging techniques have the advantage of objectively assessing direct effects in the body. However, positron emission tomography (PET) studies have radiation dose restrictions that limit repeated measurements within subjects, and the targeted pharmacological or functional system is restricted by the availability of an appropriate imaging agent. Functional magnetic resonance imaging (FMRI) on the other hand is a non-invasive imaging technique based on blood-oxygen-level-dependent (BOLD) measurements that represent brain activity. Until recently, FMRI was applicable in task-related designs only, in which pharmacologically induced changes in BOLD signals were measured in response to a specific task. The application of FMRI in drug development has several restrictions, imposed by the need for a pre-defined hypothesis about how the drug affects the task, and by limitations related to the scanning environment and to repetitive testing.

Resting state (RS) FMRI is a recently developed imaging technique that measures spontaneous BOLD changes of subjects who are in a resting state, without the interference of any task or specific stimulus. This means that RS-FMRI can be applied in studies without *a priori* hypotheses on action site. The fact that RS-FMRI is non-invasive and not affected by variability or limitations of task performance and that it can be frequently and rapidly repeated, could make it a highly valuable technique in CNS drug development. Although experience is still limited, RS-FMRI could be applied in pre-clinical animal studies, healthy volunteers and patients, which could make it a suitable translational instrument in drug development.

Previous studies found that coherent resting state BOLD fluctuations form spatially correlated brain maps, or resting-state networks (RSNS) (Beckmann et al., 2005; Biswal et al., 2010). RSNS have shown to be consistently present across human subjects, and could represent brain regions that are anatomically and functionally connected, and related to behavioural outcomes and clinical conditions (De Luca et al., 2006; Greicius et al., 2004; Fox et al., 2007; Quigley et al., 2003; Smith et al., 2009; Damoiseaux et al., 2006). A previous study by Mennes et al. suggested that inter-individual differences in RS-FMRI could predict the response to task-induced BOLD activity (Mennes et al., 2010). Only a few studies investigated the effects of pharmacologically active CNS compounds on the functional topography of RSNS. We recently conducted a study where RS-FMRI was repeated while plasma levels of morphine and alcohol were kept stable (Khalili-Mahani et al., 2011). In order to develop a broad basis for this technique by investigating reliability and reproducibility, more studies using different drug classes should be performed. This would provide important methodological information and reference data for the use of RS-FMRI as a biomarker for CNS drug research (Wise and Preston, 2010).

In the current study we investigated the effects of Δ^9-tetrahydrocannabinol (THC) on the brain using RS-FMRI. THC is a major pharmacologically active constituent of the plant *Cannabis sativa* L. In the body, THC

binds to two cannabinoid receptors (CB_1 and CB_2) of which CB_1 receptors are predominantly present in various brain areas (Herkenham, 1992). The action of THC on the CB_1 receptors is generally considered responsible for the commonly known pharmacodynamic effects, such as feeling high and postural instability (Zuurman et al., 2008).

Previous PET and FMRI studies with THC that investigated regional cerebral blood flow and BOLD signal fluctuation found THC-induced effects on the limbic system (thalamus, amygdala, hippocampus, parahippocampal gyrus, cingulate cortex) and connected areas (basal ganglia, frontal cortex), which are involved in reward, emotion, memory, awareness, pain, and executive functions (Bhattacharyya et al., 2009; Mathew et al., 1998; Mathew et al., 1999; Mathew et al., 2002; Stokes et al., 2010; van Hell et al., 2011). THC also affects areas of sensory (insula, postcentral gyrus, superior temporal gyrus), and motor coordination systems (cerebellum). The functions associated with these regions are related to the behavioural effects after THC or cannabis use (Zuurman et al., 2009).

The primary aim of this study was to investigate the effects of THC on task-independent RS-FMRI functional connectivity patterns using repetitive measures in healthy volunteers. Based on previous studies using other psychopharmacological manipulations (Khalili-Mahani et al., 2011) we hypothesised that THC would induce changes in brain connectivity compared to placebo. In addition, we measured the plasma concentrations of THC and its active metabolite 11-HYDROXY-THC (11-OH-THC) as well as a number of well-known THC-related CNS effects. Based on our previous studies, we expected to measure clear THC and metabolite plasma concentration profiles, and prominent pharmacodynamic effects, other than RS-FMRI (Strougo et al., 2008; Zuurman et al., 2008).

METHODS

Design

This was a double-blind, randomized, placebo-controlled, two-way cross-over study with a wash-out period of at least 2 weeks.

Subjects

Healthy, right-handed male and female volunteers aged 18 to 45 years with a body mass index of 18.0 to 28.5 kg/m^2 were included in the study. Subjects with a history of psychiatric or neurological illness, or with a history of hereditary psychiatric illness in first degree relatives or neurological illness in first- or second degree relatives were excluded from participation. Subjects had to be cannabis users for at least 1 year with use frequency of no more than once a week, and had to be able to refrain from using cannabinoids from at least 2 weeks prior to the first treatment period up to the end of the study. They had to refrain from nicotine and caffeinated products on study days. Subjects were excluded if they used medication other than contraceptives, and if they were pregnant (as assessed by HCG urine test). They were not allowed to have a positive alcohol breath test or drug urine test at the screening visit or at the start of a study day, neither a history of alcohol or drug dependence. Subjects could not participate if they had metal body implants or claustrophobia.

As this was an explorative study, no sample size calculation could be performed. We planned a sample size of 12 volunteers (6 male and 6 female) who completed two occasions, since in all drug studies that we have performed so far, numbers of 12 subjects were found to be sufficient (Strougo et al., 2008; Desmond and Glover, 2002), and a similar number was also mentioned in a study about the power of FMRI and RS-FMRI. Subjects who were not able to complete two occasions would be replaced.

Procedure

Subjects gave written informed consent before any study-specific procedure was performed. Eligible subjects were enrolled in the study after a general health screen within three weeks before the first study day. Subjects were acquainted with the visual analogue scales questionnaire and the inhalation procedure using THC vehicle. At each study day, THC or placebo was administered at 0m, 1h30m and 3h00m. Pharmacodynamic (PD) and pharmacokinetic (PK) measurements were frequently performed on all study days at fixed time points, as chronologically indicated in Figure 1. At the beginning of each study day a venflon cannula was inserted intravenously for all blood samples that were drawn on both study days. Subjects were fasted for at least 4 hours at arrival, and standardized meals were provided pre-dose, and at 3h40m and after the last study day activity at 6h47m. The wash-out period between study days was at least two weeks. The study protocol was approved by the Medical Ethics Review Board of Leiden University Medical Center and complied with the principles of ICH-GCP, the Helsinki declaration and Dutch laws and regulations.

Treatments

Each study day, subjects received three doses of THC (2-, 6-, and 6 mg) or placebo via inhalation with 1.5 h intervals. Two mg purified THC was dissolved in 200 µl 100% ethanol. The THC dosages were selected to reach and maintain clear central nervous system effects as predicted by PK-PD models that were based on a previous study (Strougo et al., 2008). Procedures for vaporizing the solution and inhalation of the vapour were done according to a method as previously described (Zuurman et al., 2008). In addition to this procedure, the current study used a nose clip during the THC or placebo administrations to prevent nasal exhalation, in order to reduce pharmacokinetic variability by minimizing undetected loss of THC vapour.

Outcome measures

A schematic representation of the time points of the study day activities is given in Figure 1. The precision of all activities is imposed by the tight time schedule.

PHARMACOKINETIC MEASUREMENTS AND BIO-ANALYSIS

To determine THC plasma concentration, venous blood samples were collected in 4 ml EDTA tubes at 5, 20 and 88 min after each administration and at 178 min after the third administration only. After collection, the tubes were kept on ice water in aluminium foiled containers and centrifuged within one hour for 10 minutes at 2000G at 4 ^{0}C. THC samples were handled sheltered from light. Plasma samples were stored at –20 ^{0}C and sent to Analytisch Biochemisch Laboratorium (ABL, Assen) for analysis. Plasma THC as well as metabolite concentrations (11-HYDROXY-THC and 11-NOR-9 CARBOXY-THC) were determined using tandem mass spectrometry with a lower limit of quantification of 1.00 ng/ml.

PHARMACODYNAMIC ASSESSMENTS

IMAGING – Resting state functional magnetic resonance imaging (RS-FMRI) scans were made pre-dose and at 10 and 70 min after the first and second THC administrations, and 10, 100 and 190 min after the third administration. The differences in time points of measurements performed after the first and second administration versus after the third THC administration were chosen to investigate a more extended time course of THC and metabolite plasma concentrations, and pharmacodynamics. As the interval of the THC dosing schedule was 90 minutes, the time frame in which measurements could be performed that were related to the previous THC administration was limited

to 90 minutes. Subjects were asked not to move or talk and to look at a fixation cross during scanning to improve the subject's comfort in THC conditions, and to minimize the risk of falling asleep during scanning. Four chest electrodes and the scanner's flexible pressure belt were used to record heart rate and respiration rate during scanning. A 3T Achieva scanner (Philips Medical System, Best) was used for image acquisition. RS-FMRI scans were T2*-weighted and consisted of 220 gradient echo 'echo planar imaging' (EPI) volumes (repetition time interval = 2180 ms; echo time interval = 30 ms; flip angle = 80^0; 38 axial slices; 64x64x38 isotropic resolution 3.44 mm; scan time = 8.1 min). For anatomical registration, a T1-weighted scan was obtained for each subject at the end of each study day.

VISUAL ANALOGUE SCALES (VAS) – VAS by Bond and Lader is a 16-item subjective assessment of subjective effect on alertness (composition of items alert/drowsy, strong/feeble, muzzy/clear-handed, well coordinated/clumsy, lethargic/energetic, mentally slow/quick-witted, attentive/dreamy, incompetent/proficient, and interested/bored), on mood (composition of items contended/discontented, troubled/tranquil, happy/sad, antagonistic/amicable, and withdrawn/gregarious), and calmness (composition of items calm/excited, and tense/relaxed) (Bond and Lader, 1974). The adapted version of VAS by Bowdle et al. (1998) is a 13-item assessment of subjective effects on item 'feeling high' and on factors 'internal perception' and 'external perception', which are both compositions of items that are affected differently by THC as previously described (Zuurman et al., 2008). VAS were included in this study to provide a positive control for THC-induced pharmacodynamic effects, as previous studies showed clear effects on the VAS (Zuurman et al., 2008; Zuurman et al., 2008; Zuurman et al., 2009). The measurements were taken twice pre-dose, and at time points: 29 and 59 min, 1h23min, 1h59min, 2h29min, 2h53min, 3h35min, 4h29min, 4h57min, 5h59min, and 6h42min.

HEART RATE AND BLOOD PRESSURE – Heart rate and blood pressure were taken as safety measurements using a Nihon-Koden (Lifescope EC, Tokyo, Japan) blood pressure apparatus. Heart rate measurements were used as an objective measure for treatment effects, as previous studies showed clear heart rate effects (Zuurman et al., 2008; Zuurman et al., 2008; Zuurman et al., 2009). Heart rate measurements were taken 3 minutes after each time point of VAS measurements as mentioned in the previous paragraph. Blood pressure was measured pre-dose, and at 6h45min.

HORMONES – Prolactin levels (µgr/l) were measured as a biomarker for dopaminergic activity (de Visser et al., 2001). Cortisol (µmol/ml), luteinizing hormone (LH, ng/ml) and follicle-stimulating hormone (FSH, U/l) were measured as exploratory biomarkers of hypothalamic-pituitary activity (Chen et al., 2010). Due to the diurnal rhythm of cortisol, the two study days of each subject were consistently scheduled at the same time of the day. Blood samples for LH, FSH, prolactin and cortisol were collected twice pre-dose, at 20 and 1h28min after each THC administration and an additional sample was taken at 5h58min. Serum was separated by centrifugation (2000g at 4^{0}C for 10 min) within 1 h of collection. The samples were analyzed by the Central Clinical Chemistry Laboratory (Leiden University Medical Center, Leiden) using an electrochemoluminiscence-immunoassay for prolactin and cortisol, and a fluoro-immunoassay for LH and FSH.

METABOLIC BLOOD MEASURES – The study was also used to perform an exploratory analysis of several metabolic effects of THC. Glucose (mmol/l), high-density lipoprotein (HDL) cholesterol (mmol/l), leptin (µg/l) and triglycerides (mmol/l) serum samples were analyzed by the Central Clinical Chemistry Laboratory (Leiden University Medical Center, Leiden). For description of serum collection and time points, see 'hormones' section.

Statistical analyses

CLINICAL EFFECTS

For vital signs [heart rate (HR) in beats per minute (bpm) and blood pressure (mmHg)], raw data and changes from baseline were analyzed by type of measurement and parameter and treatment using descriptive statistics. HR and PR-, QRS-, and QT-intervals, corrected QT (QTC) (all in ms) from automatic reading were analyzed as raw parameter value and change from baseline (for HR and QTC only). Adverse events were coded according to the Medical Dictionary for Regulatory Activities (MedDRA version 13.0).

PHARMACOKINETICS

All concentrations and maximal concentration (C_{MAX}), time of maximal concentration (T_{MAX}), area under the curve from zero to infinity ($AUC_{0-\infty}$), and terminal half-life ($t_{1/2}$) of THC and its metabolites 11-OH-THC, and THC-COOH were summarized by mean, standard deviation (SD), standard error of the mean (SEM), coefficient of variation (CV%), and number of available observations. Also, a population pharmacokinetic analysis was performed based on a previously described two-compartmental model (Strougo et al., 2008), with the addition of the active metabolite 11-OH-THC in a separate compartment. A post hoc analysis on gender differences was performed using a linear mixed effect model with treatment, period, time and treatment by time as fixed effects, subjects and subject by treatment as random effects and with baseline value as covariate (SAS for windows V9.1.2 ; SAS Institute, Inc., Cary, NC, USA).

PHARMACODYNAMICS

Resting State FMRI data processing was carried out using the Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (FSL 4.1, Oxford, UK), using the same analysis techniques for pharmacological RS-FMRI as reported previously (Khalili-Mahani et al., 2011).

For preprocessing the following standard procedure was performed: head motion correction, brain extraction, Gaussian smoothing with a 5 mm FWHM kernel, grand-mean scaling of each BOLD FMRI dataset by a single multiplicative factor; high-pass temporal filtering (FWHM = 100s). After preprocessing, the EPI data were affine-registered to the anatomical T1-weighted scan, and the anatomical scan was subsequently affine-registered to the MNI 152 standard space (Montreal Neurological Institute, Montreal, Canada). FMRI images in MNI space were interpolated to 2x2x2 mm voxels.

RSN functional connectivity was determined as similarity of the BOLD fluctuations in each brain voxel in relation to characteristic fluctuation in eight predefined networks of interest (NOIS). These networks were obtained from a published model-free analysis of the spatio-temporal structure of the resting-state BOLD fluctuations (Beckmann et al., 2005). The template NOIS include over 80% of the total brain volume and comprise the following networks: medial and lateral visual (NOIS 1 and 2, respectively, including primary visual areas), auditory and somatosensory (NOI 3, including areas involved with hearing), sensorimotor (NOI 4), default mode [NOI 5, of which is hypothesized that these regions are associated with the representation of the world around us and spatial attention (Miller and Cohen, 2001)], executive control (NOI 6, these areas have been hypothesized to provide bias signals to other areas of the brain in order to implement cognitive control), and right and left-lateralized fronto-parietal dorsal visual [NOIS 7 and 8, probably representing information relevant for (visual) attention, but related to visuospatial and verbal attention respectively] (Beckmann et al., 2005; Laird et al., 2011). The predefined networks, as determined by the weighted template NOIS, were calculated for the study sample.

Connectivity to each of the 8 NOIS, for each voxel, was measured using dual-regression (Filippini et al., 2009; Beckmann et al., 2005) followed by

a mixed effects model group analysis. Dual-regression analysis generated whole-brain statistical maps of z-scores representing voxel-wise functional connectivity across all regions with the characteristic activity in each of the NOIs (12 subjects x 8 scans x 2 occasions x 8 NOIs). The higher the absolute value of the z-score, the stronger the connectivity to a given NOI.

Variations in heart rate and respiratory rate could be induced pharmacologically by THC administrations (Zuurman et al., 2008; Zuurman et al., 2008), and these fluctuations could induce variance in the resting-state BOLD signal unrelated to functional CNS-effects (Beckmann and Smith, 2005; Birn et al., 2008; Chang et al., 2009). It has been shown that BOLD signal fluctuations measured in the white matter (WM) and cerebrospinal fluid (CSF) are reliable representations of non-neuronal physiological noise in RS-FMRI data (Birn, 2012). Therefore, we included separate WM and CSF confounds, as well as six motion parameters, as nuisance variables in the second stage of the dual regression analysis for each scan. These separate WM and CSF confounds were measured for each scan by calculating tissue-specific segmentations of each subject's high-resolution T1 structural scan (segmented using FSL FAST) (Zhang et al., 2001), transforming the resulting WM and CSF maps to the corresponding subject's EPI space and subsequently extracting mean time series from that functional scan within the space of each of these tissue-specific maps.

For group analyses, treatment and time were used as fixed factors and subject was used as a random factor. Average respiration and heart rates per RS-FMRI scan were also added as nuisance covariates (Khalili-Mahani et al., 2011). Within-subject average z-maps were modelled with separate fixed factors, to allow the model to estimate the correlation between z-maps. Permutation-based statistical inference was used (5000 repeated permutations) on the treatment by time interactions (Nichols and Holmes, 2002). Higher-level analyses were restricted to study population-specific grey matter regions by registration of the grey matter volumes resulting from FAST segmentation to MNI space and subsequent summing across subjects. Significant THC effects on functional connectivity were defined using threshold-free cluster enhancement ($p < 0.05$, family-wise error-corrected) (Smith and Nichols, 2009). Correction for 16 multiple comparisons was done using Bonferroni correction. The multiple comparisons consisted of 2 comparisons (either connectivity increase or decrease after THC administration) for 8 NOIs.

VAS and heart rate were analyzed using a linear mixed effect model with treatment, period, time and treatment by time as fixed effects, subjects and subject by treatment as random effects and with baseline value as covariate (SAS for windows V9.1.2; SAS Institute, Inc., Cary, NC, USA). From this model, pair wise differences and corresponding 95% confidence intervals were estimated to verify the effects of THC. Measurements from VAS Bowdle (e.g. feeling high, external and internal perception) were log (VAS score+2) transformed for statistical analysis and reported in 'units' (U).

RESULTS

Subject characteristics

Twenty-two healthy volunteers (eleven male, eleven female) were randomized and treated, twelve of whom completed two occasions and were included in the pharmacodynamic and pharmacokinetic analysis. One of these subjects missed the last two scans on the placebo occasion due to nausea and vomiting. For safety analysis, all treated subjects were included. Eight female subjects and one male subject dropped out from the study due to adverse events during THC occasions. Details on the nature of the adverse events can be found in section 0. One male subject discontinued the study after the first occasion with THC treatment, for personal reasons. Also, this subject had an incomplete first THC administration (2 mg) due to leakage of the vaporizer. Details on subject demographics can be found in Table 1.

Adverse effects

Nine subjects dropped out due to adverse effects during THC occasions only. Two subjects dropped out due to a vasovagal collapse, one female 22 min after the first THC inhalation, and one male 27 min after the second THC inhalation. One female subject discontinued due to nausea that started 4 min after the second THC inhalation, and another female became nauseous after the third THC inhalation. One female dropped out because of nausea and anxiety that started 12 min after the second THC inhalation. Four other females discontinued due to anxiety: two at 12 and 21 minutes after the first THC administration, and two at 10 and 12 minutes after the second. Most adverse events that were observed in this study were typically related to THC use. The most occurring treatment related adverse effects were feeling high (7/22 subjects), nausea (7/22), and anxiety (6/22).

Pharmacokinetics

Five minutes after each THC administration, a peak plasma concentration was observed (Figure 2). Mean peak plasma concentrations were: 29.5 ng/ml (SD 11.6) after the first administration (2 mg THC), 139.9 ng/ml (SD 42.1) after the second administration (6 mg THC) and 109.1 ng/ml (SD 55.3) after the third administration (6mg THC). After each peak, a rapid decline in plasma concentration was observed. An overview of the pharmacokinetic parameters of THC and 11-OH-THC is given in Table 2.

GENDER

The unexpectedly large number of THC-related adverse events in females raised questions about potential sex-related pharmacokinetic differences. Therefore, a post hoc analysis of THC and metabolite plasma concentrations was performed in males and females. In Figure 3 the THC concentration curves of males and females are given. When compared graphically, the average plasma concentration for females was higher compared to males. A reliable statistical analysis could not be performed for subjects who completed the entire study, since only three females received all treatments. However, THC concentrations were significantly higher in the eleven females who inhaled the first dose of THC 2 mg (42.3 ng/ml), than in the nine males (26.3 ng/ml; difference 61.0%, 95% CI 13.3-128.7, $p = 0.0087$).

Resting State Connectivity

Each of the 8 NOIs showed treatment effects on connections with several brain regions (Table 3). After Bonferroni correction, treatment-related connectivity differences were observed within the sensorimotor and right and left dorsal visual stream networks (NOI 4, 7, 8), which are depicted in Figure 4. Most changes occurred in connectivity patterns of the right dorsal visual stream network (NOI 7). After THC administration,

connectivity of this network increased with the left and bilateral frontal pole and dorsomedial prefrontal cortex, and with the left superior pre-frontal cortex (t = 5.69 with 149 voxels; t = 4.97 with 130 voxels respectively, Bonferroni corrected), with an extension into the left superior frontal gyrus. Also, a connectivity decrease (t = 5.44; 53 voxels) was found in the right and dorsal visual stream network (NOI 7). This decrease was observed in the area covering the superior frontal pole, middle and inferior frontal gyrus, and dorsolateral prefrontal cortex, with all regions being lateralized to the right hemisphere. An increase of connectivity was found between the cerebellum and the sensorimotor network (NOI 4) after THC administration (t = 6.36; 6101 voxels, Bonferroni corrected). The area including the occipital pole and lateral occipital cortex showed an increased connectivity (t = 5.01; 52 voxels) with the left dorsal visual stream network (NOI 8).

Other pharmacodynamic parameters

Graphs of feeling high and heart rate plotted against time are given in Figure 5. Treatment comparison of the pharmacodynamic effects other than FMRI measurements demonstrated significant increases after THC administration on VAS external perception (0.225 (U); 95%CV 0.054 - 0.396; p = 0.0149), feeling high (0.768 (U); 95%CV 0.578 - 0.957; p = <.0001), and heart rate (10.3 bpm; 95%CV 4.4 - 16.2; p = 0.0026). The centrally mediated external perception and feeling high scores increased after the first and second THC administration, but not after the third THC inhalation. The decrease of these effects was relatively slow. Heart rate remained stable during placebo treatment; whereas THC induced acute heart rate elevations that declined relatively rapidly after each dose (Figure 5B). Stress-hormone cortisol showed a 32.2% increase (95%CV 11.9 - 56.3; p = 0.0051) after THC, whereas prolactin decreased with 21.0% (95%CV -33.0 -/-7.0; p = 0.0100). The THC effect on cortisol was maximal around the third THC administration. The first prolactin measurement after the first THC administration showed no significant differences between THC and placebo treatment, however, as time progressed, concentration differences increased by a continuously decreasing prolactin concentration after THC compared to placebo. The mean glucose concentration over time increased by 7.2% after THC treatment (95%CV 0.1 – 14.8; p = 0.0468). Visual inspection indicated that this difference was solely caused by a larger glucose increase in the THC arm after a standardized meal (at t = 4.28 h, 48 min after lunch and 1h28m after the third THC administration) (6.19 mmol/l in the placebo group and 8.34 mmol/l in the THC treated group). No significant changes were seen for HDL cholesterol, leptin and triglycerides. An overview of the pharmacodynamic parameters can be found in Table 4.

DISCUSSION

This study demonstrated that THC induced changes in RSN functional connectivity. As predicted by the PK-PD models that were based on a previous study (Strougo et al., 2008) the THC and 11-OH-THC concentrations were within the effective range, inducing significant effects on external perception and feeling high from VAS Bowdle and on heart rate measures.

Connectivity and function

THC induced significant effects on functional connectivity between various brain areas and the sensorimotor and right and left dorsal visual stream networks. In general, an increase of network connectivity was found after Bonferroni-correction, with one area showing decreased connectivity in the left dorsal visual stream cortex. The areas that were found to be most affected by THC in this study were comparable to findings from previous THC studies using PET, in which changes in resting-state blood flow or [11C]-raclopride binding potential were found, including the cerebellum, frontal pole, left superior frontal gyrus, right middle frontal gyrus (Mathew et al., 1998; Stokes et al., 2010).

NOI 4

The areas displaying THC-induced connectivity changes in the sensorimotor network could be associated with the functional changes that are observed after THC administration, such as the increase of external perception as seen in this study. The cerebellum, which showed connectivity increase, is associated with motor coordination and time perception (which is one aspect of external perception) and has high CB_1 receptor density (Nyberg et al., 2010; Stoodley and Schmahmann, 2009; Romero et al., 2002). A previous study with THC reported a correlation between altered time perception and cerebellar blood flow (Mathew et al., 1998). Also, the cerebellum is likely to be involved in THC-induced postural stability changes as previously observed (Zuurman et al., 2008; Zuurman et al., 2008; Zuurman et al., 2009). A possible correlation between the increase of external perception (a composite scale including the item 'altered time perception') and cerebellar connectivity changes should be further explored in a future study using PK-PD modelling.

NOI 7

The bilateral and left DMPFC, and the left frontal pole and left superior frontal gyrus (SFG) had an increased connectivity to the right dorsal visual stream network, whereas the right superior frontal pole, right dorsolateral PFC (DLPFC), and the right inferior and middle frontal gyri had a decreased connectivity. The DMPFC and frontal pole are functionally associated with decision making and cognitive control, such as subserving the monitoring of action outcomes and cognitive branching, the ability to put on hold an alternative course of action during the concurrent performance of the ongoing one (Venkatraman et al., 2009; Daw et al., 2006; Koechlin et al., 1999). We have not studied these functions in our study, but the literature includes a few studies of the effects of THC/cannabis on complex problem solving and planning tasks (Tinklenberg et al., 1972; Crockett et al., 1976), which could be attributed to fronto-polar PFC changes.

The SFG is involved in higher cognitive functions, such as the executive functions of working memory processing, and is suggested to be associated with the excitatory and inhibitory influences on craving, as found in a lesion study and a study with tobacco cigarettes (du Boisgueheneuc et al., 2006; Rose et al., 2011). In human brain tissue, CB_1 receptors are present in the SFG (Eggan and Lewis, 2007), suggesting effects of cannabinoids on the higher cognitive functions. However, in healthy subjects, THC demonstrated no effects on cognitive functions such as plan-

ning and reasoning in the very limited available literature (Ramaekers et al., 2009; Morrison et al., 2009). The right inferior and middle frontal gyri are involved in risk attitudes and contingency awareness (Carter et al., 2006). These behaviours are affected by THC (Foltin et al., 1990), but are much dependent on the exact type of behaviour that is tested (McDonald et al., 2003; Zuurman et al., 2009). One FMRI-study, for example, showed that THC attenuated activity in the right inferior frontal and anterior cingulate gyri when performing the Go/No-Go task for response inhibition, but no difference was seen on the task performance itself (Borgwardt et al., 2008). The DLPFC is involved with organization of working memory (Jha et al., 2006), which can also be affected by THC use (Bocker et al., 2010).

NOI 8

This study showed that the right posterior pole and lateral occipital cortex had an increased connectivity with the left visual dorsal stream network. The occipital regions are functional visual areas (Hine, 1918; Kolmel, 1988). Previous studies found that THC influences several aspects of vision that could be attributed to visual cortex changes (Koethe et al., 2006; Emrich et al., 1991; Winton-Brown et al., 2011). In this study, THC had clear effects on VAS external perception, which includes several scales of changes of colours and shapes.

In summary, the different NOIs show significant connectivity effects on brain areas that are functionally related and that have been found to be significantly affected by THC administration in previous studies. This suggests that connectivity changes that are found with RS-FMRI could be related to functional alterations. Since similar conclusions were previously reached with morphine and ethanol (Khalili-Mahani et al., 2011), RS-FMRI can possibly be a useful technique for prediction of drug effects, although more studies are needed to understand the potential role of this technique in drug development.

Other pharmacodynamic parameters and gender effects

CORTISOL

The cortisol increase, or reduced decrease (which occurs during daytime due to the diurnal rhythm), is consistent with previous findings (Goodwin et al., 2011; Ranganathan et al., 2009). Pre-clinical studies found that the cannabinoid-induced hypothalamic-pituitary axis activity increase is caused by cannabinoid action in the paraventricular nuclei in the hypothalamus and the pituitary gland, where CB_1 and corticotrophin releasing hormone receptors are co-expressed (Corchero et al., 1999; Dewey et al., 1970; Wenger et al., 1999). No clear connectivity changes were found in hypothalamic regions. The question whether connectivity changes could be expected between, for example, the hypothalamus and limbic regions after a THC-induced cortisol increase remains unanswered, as the relationship between connectivity changes and functional changes is unknown and should be further investigated. Possibly, the THC-induced enhancement of postprandial glucose elevations was due to a THC-induced cortisol increase, which may have induced gluconeogenesis. No comparable studies to our study have been reported, but similar findings have been reported in pre-clinical studies and a clinical study in fasting conditions (Kim et al., 2011; Benowitz et al., 1976). As the subjects were served a standardized meal, glucose elevation due to larger carbohydrate intake is unlikely. Future studies may reveal interesting information about the circuitry involved in adaptive regulation of the brain-body function.

FEELING HIGH

Most subjects experienced the familiar feelings of subjective ‘high’ after administration of THC. This raises the question of which networks

could be associated with these psychomimetic effects. The answer to this question cannot be given easily, since THC causes many different effects with very similar time profiles (Strougo et al., 2008). Consequently, it is difficult if not impossible to distinguish the network activities that are uniquely associated with feeling high, from those related to other THC-effects like upright postural instability or sedation. As our database of similar pharmacological studies with other psychomimetic drugs expands, we may be able to address the question of neural correlates of 'feeling high' by integration and, for example, factor analyses of data from different drugs in the future.

PHYSIOLOGICAL VARIATIONS

We have found a significant effect of THC on heart rate. Because physiological pulsations may cause movement of large vessels, various retrospective processing techniques are proposed to correct for correlated physiological noise. As recently recommended in (Birn, 2012) we have used the BOLD fluctuations within individual's CSF and WM masks as an indirect measure of physiological noise. Furthermore, we have used average physiological variables as covariates at the higher-level group analysis. Previously, it has been shown that the functional connectivity of the default mode network (NOI 5) *in particular* is susceptible to heart pulsations (Chang et al., 2009). However, the impact of such corrections is likely to vary depending on the method used for estimating functional connectivity (e.g. ICA, dual-regression or seed-based) without any significant impact at the group level analysis (Starck et al., 2010). Because the aim of our study is to localize drug effects in the brain, we have refrained from performing any physiological correction that assumes a hemodynamic response function for the respiration and heart rate variations. Therefore, our results should be interpreted with the caveat that some of the regional drug effects might be confounded with signal change due to vascular motion.

Gender

The post-hoc analysis on pharmacokinetic gender differences showed a higher THC plasma concentration in females compared to males after the 2 mg dose. This study did not anticipate gender differences, which have rarely been examined in the literature. We administered a fixed dose using a nose clip to prevent surreptitious exhalation, whereas during recreational use (as cannabis), individual titration to the subjective effect could easily obscure most gender differences. Possible explanations for the pharmacokinetic gender differences found in this study are differences in height, weight, body composition, metabolism, hormones, or frequency of habitual cannabis use. This could not be explored further in this study, which was not designed to examine pharmacokinetic or pharmacodynamic gender differences. This would require future studies with a larger sample size and adequate considerations of other sex-associated confounders.

Concluding remarks and future directions

In line with previous findings, this study confirms that RS-FMRI seems a promising technique for clinical pharmacological studies and drug development (Khalili-Mahani et al., 2011; Cole et al., 2010). The possible THC concentration-effect relationship including the active metabolite 11-OH-THC needs to be further studied using PK-PD modelling. This would allow the quantitative examination of THC-induced effects on connectivity, including changes at low concentrations that might be observed without pronounced behavioural effects.

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TABLE 1 Demographics of the subjects that were included for pharmacodynamic and pharmacokinetic analyses. SD = standard deviation

Gender	Variable	N	Mean	SD
All	Age (year)	12	22.17	2.95
	BMI (kg/m²)	12	22.36	2.55
	Height (m)	12	1.82	0.09
	Weight (kg)	12	74.33	13.17
Female	Age (year)	3	23.33	2.52
	BMI (kg/m²)	3	22.07	0.32
	Height (m)	3	1.70	0.06
	Weight (kg)	3	63.83	4.93
Male	Age (year)	9	21.78	3.11
	BMI (kg/m²)	9	22.46	2.97
	Height (m)	9	1.86	0.05
	Weight (kg)	9	77.82	13.32

TABLE 2 Pharmacokinetic parameters of THC as assessed by non-compartmental pharmacokinetic analysis. CV = coefficient of variation, IIV = interindividual variability, Cl = clearance, F = bioavailability, V = distribution volume, Q= intercompartmental clearance, $t_{1/2}$ = initial half-life, NA = not applicable.

Parameter Units	Median	Uncertainty (%CV)	IIV (%CV)
CL/F (L/hr)	145	11.2	37.4
V/F (L)	20.1	12.5	37.4
V peripheral (L)	78.6	15.8	37.4
Q (L/hr)	95.6	15.1	37.4
$t_{1/2}$ (hr)	0.986	4.91	NA

TABLE 3 Overview of the significant decreases and increases of connectivity (p<0.05, threshold-free cluster enhancement corrected). The areas in grey are significant regions after Bonferroni correction.

Networks		Region (Harvard-Oxford)	t-value[a]	X	Y	Z	Voxel number	THC effect[b]
NO11: Medial visual	L	Superior and medial frontal gyrus (premotor cortex)	5.32	-24	-2	46	26	-
	B	Dorsal ACC	5.02	2	12	34	20	-
	L	Temporal occipital fusiform cortex	4.9	-30	-48	-18	17	-
NO12: Lateral visual	L	Temporal occipital fusiform cortex (extending into para-hippocampal gyrus & hippo-campus)	4.91	-30	-48	-12	163	+
	L	Ventromedial cerebellum	5.03	-22	-60	-44	36	+
	R	Temporal occipital fusiform cortex (extending into parahip-pocampal gyrus)	4.16	26	-38	-18	22	+
	R	Middle frontal gyrus, dlPFC	5.34	28	20	44	14	+
	B	Posterior precuneous cortex	4.8	2	-74	42	203	-
	R	occipital pole, lateral occipital cortex	4.3	34	-92	-10	27	-
	R	Precentral gyrus	5.54	46	-10	58	12	-
NO13: Auditory	R	Parahippocampal gyrus (extending into hippocampus)	4.66	40	-34	-10	132	+
	B	PCC, retrosplenial cortex	5.04	0	-46	2	20	+
	R	Caudate	5.86	20	32	2	16	+
	B	Supramarginal gyrus, superior/ medial/inferior temporal gyri, temporal pole, parahippocampal gyrus, lateral OFC	6.07	62	-30	28	25075	-
	B	Superior frontal gyrus, dmPFC	4.83	0	50	28	262	-
	R	Frontal pole, dmPFC	4.13	10	60	22	104	-
	L	Precentral gyrus, superior parietal cortex	3.85	-10	-16	64	52	-
	B	Mid-cingulate cortex	4.26	-4	-8	34	38	-
	L	Precentral gyrus, superior mid-cingulate cortex	4.11	-4	-20	50	30	-
	R	vmPFC	4.12	16	54	6	28	-
	R	Middle frontal gyrus	3.36	46	10	40	19	-
	R	Precentral gyrus, superior mid-cingulate	4.12	6	-26	54	17	-

(Table continues on next page)

Networks		Region (Harvard-Oxford)	t-value[a]	X	Y	Z	Voxel number	THC effect[b]
NO14: Sensorimotor	L	Midbrain	3.92	-8	-28	-18	22	+
	B	**Cerebellum (more extensive in right hemisphere)**	**6.36**	**14**	**-70**	**-50**	**6101**	+
	L	**Cerebellum (antero-ventral)**	**5.53**	**-20**	**-48**	**-54**	**47**	+
	L	**Cerebellum (ventromedial)**	**5.15**	**-10**	**-64**	**-50**	**5.15**	+
	R	Postcentral gyrus	5.09	40	-32	62	184	-
	R	Precuneous cortex	4.28	14	-46	44	169	-
	R	Superior posterior parietal cortex	4.39	20	-56	62	142	-
	L	Superior posterior parietal cortex	3.73	-22	-54	50	99	-
	L	Postcentral gyrus, superior parietal cortex	4.39	-18	-40	64	71	-
	R	Superior posterior parietal cortex (mid-superior)	4.24	30	-42	64	57	-
NO15: Default mode	L	Frontal pole, dorsal PFC	5.17	-28	46	16	35	+
	L	Intracalcarine (visual) cortex	5.63	-18	-80	10	17	+
NO16: Executive/ salience	L	Precuneous Cortex.	5.19	-8	-58	36	17	-
NO17: Right dorsal visual stream	**B**	**Frontal pole, dmPFC**	**5.69**	**-12**	**66**	**10**	**149**	+
	L	**Frontal pole, dmPFC**	**4.97**	**-12**	**54**	**30**	**130**	+
	L	**dmPFC, frontal pole, superior frontal gyrus**	**4.53**	**-2**	**52**	**30**	**15**	+
	R	**Superior frontal pole, middle frontal gyrus, dlPFC, inferior frontal gyrus**	**5.44**	**38**	**38**	**24**	**53**	-
	R	Superior frontal pole, inferior and medial frontal gyrus, dlPFC	5.44	38	38	24	324	-
	R	Superior frontal pole, inferior frontal gyrus (partial)	4.16	32	50	14	138	-
	R	Frontal pole (inferior), ventrolateral PFC	4.61	44	52	-6	109	-
NO18: Left dorsal visual stream	R	Occipital pole, lateral occipital cortex	5.01	42	-92	2	770	+
	L	Pre and post-central gyrus, central sulcus	4.74	-44	-16	40	29	+
	R	**Occipital pole, lateral occipital cortex**	**5.01**	**42**	**-92**	**2**	**52**	+
	B	PCC	4.7	-2	-38	24	105	-

a Uncorrected peak t-value
b The minus (-) indicates a connectivity decrease after THC, and the plus (+) an increase.
Abbreviations: L - left, R - right, B - bilateral, ACC/PCC - anterior/posterior cingulated cortex, PFC - prefrontal cortex, dlPFC - dorso-lateral PFC, dmPFC - dorso-medial PFC, vmPFC - ventro-medial PFC, OFC - orbito-frontal cortex

TABLE 4 Overview of the non-FMRI pharmacodynamic parameters

	LSM Treatment			Contrasts	LSM change from baseline	
Parameter	Placebo	THC	P-value	THC vs Placebo	Placebo	THC
VAS Alertness (mm)	52.5	45.9	0.0646	-6.6 (-13.7, 0.5)	1.3	-5.3
VAS Calmness (mm)	53.9	55	0.2248	1.1 (-0.8, 3.0)	0.3	1.4
VAS Mood (mm)	55	55	0.9787	0.1 (-4.6, 4.8)	-0.6	-0.5
VAS External log (mm)	0.32	0.545	0.0149*	0.225 (0.054, 0.396)	0.008	0.233
VAS Internal log (mm)	0.308	0.346	0.0718	0.037 (-.004, 0.079)	0.004	0.041
VAS feeling high log (mm)	0.285	1.053	<.0001*	0.768 (0.578, 0.957)	-0.02	0.748
Heart rate (BPM)	66.7	77	0.0026*	10.3 (4.4, 16.2)	-1.8	8.4
FSH (U/L)	2.327	2.291	0.5601	-1.56% (-7.11%, 4.33%)	-3.87	-5.37
LH (ng/ml)	4.16	3.15	0.0935	-24.20% (-45.9%, 6.12%)	2.02	-22.71
Cortisol (µmol/ml)	0.36	0.47	0.0051*	32.21% (11.87%, 56.25%)	-29.24	-6.45
Prolactin (µgr/l)	8.41	6.64	0.0100*	-21.00% (-33.0%, -7.01%)	-28.68	-43.69
Glucose (mmol/l)	4.7	5.1	0.0468*	7.2% (0.1%, 14.8%)	-4.64	2.25
HDL cholesterol (mmol/l)	1.12	1.12	0.8933	0.63% (-9.21%, 11.53%)	0.93	1.57
Leptin (µg/l)	3.6	3.7	0.8328	1.95% (-16.6%, 24.62%)	-2.94	-1.04
Triglycerides (mmol/l)	0.98	0.97	0.9086	-0.70% (-13.2%, 13.64%)	3.47	2.74

* Statistically significant values

FIGURE 1 Visual representation of the chronological study day activities after each THC inhalation. The horizontal axis represents the time line and should be read from left to right. The vertical lines connected to the dots represent the relative time points for the activities indicated in the boxes. The grey lines represent measurements that were only performed after the third THC inhalation. The time points are given in hours and minutes relative to the THC administration, and refer to THC administration and RS-MRI measurements. At the first blood sample (1) for each cycle, only a PK sample was taken.

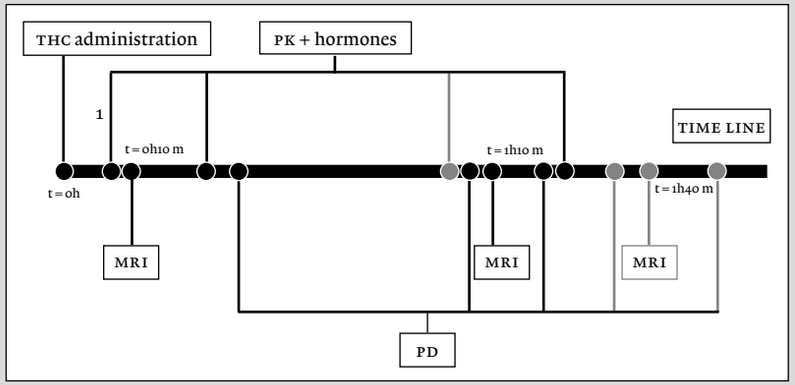


FIGURE 2 Mean THC plasma concentration (+ standard deviation) graph. THC was administered at 0 min (2 mg), 90 min (6 mg), and 180 min (6 mg).

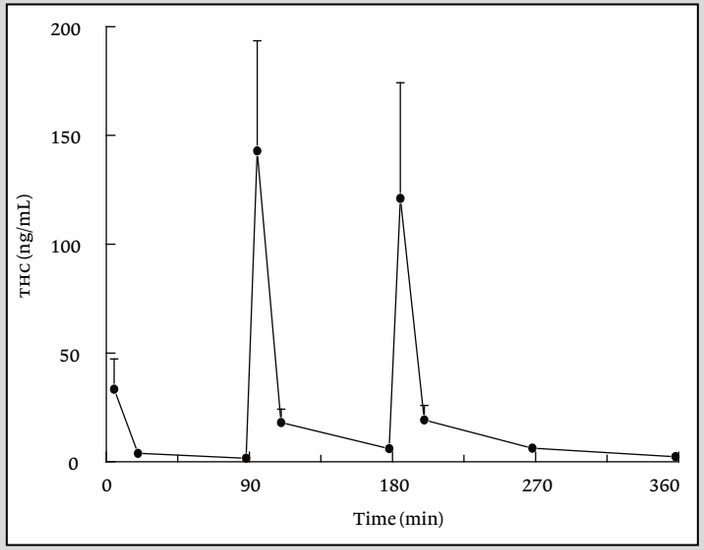


FIGURE 3 Mean THC plasma concentration (+ standard deviation) graph by gender. Dots = males; Circles = females.

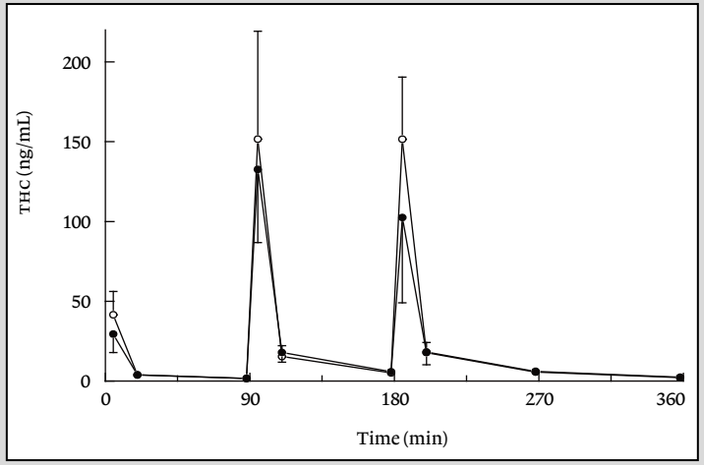
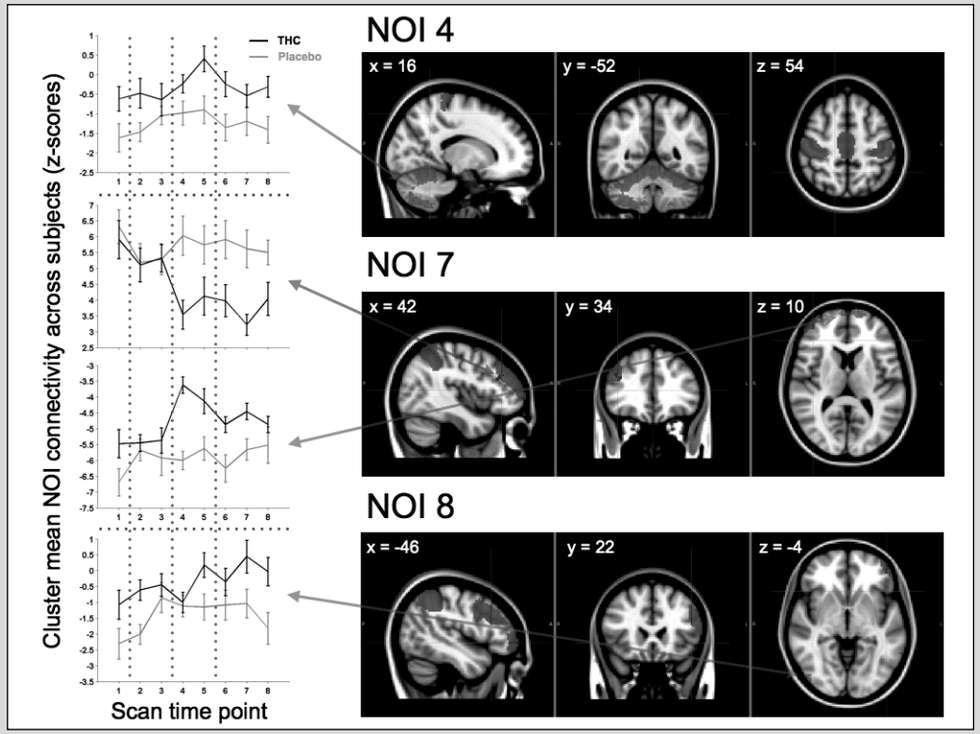
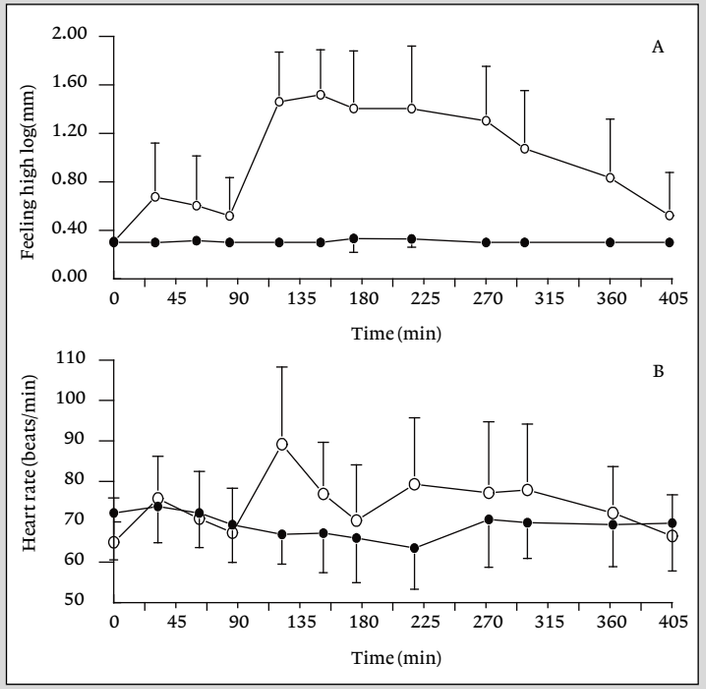


FIGURE 4 Brain regions showing clusters of significant differences (Bonferroni corrected) in NOI functional connectivity following THC relative to placebo. Spatial maps (right): Axial and coronal slices are displayed in radiological convention such that left=right. Green = NOI, Red = Connectivity increase after THC relative to placebo, Blue = Connectivity decrease after THC relative to placebo; crosshairs indicate position of displayed slices. Connectivity changes across time (left): plots visualise z-scores resulting from each significant contrast (Bonferroni corrected) only, split by scan time point and averaged across clusters and subjects, separately for placebo (grey) and THC (black) conditions. Error bars represent the standard error of the mean. Vertical green dotted lines indicate the three points at which a dose was inhaled. Red and blue arrows link the associated spatial and temporal information.



(see inside cover for this figure in colour)

FIGURE 5 Graphs of pharmacodynamic effects, with VAS feeling high (figure A) average scores of log (mm) + standard deviations (SD), and mean heart rate + SD (figure B). Open circle: THC, closed circle: placebo. THC inhalations were given at time points 0, 90, and 180 min.



CHAPTER IV

Surinabant, a selective CB1 antagonist, inhibits THC-induced central nervous system and heart rate effects in humans

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ABSTRACT

AIM Cannabinoid receptor type 1 (CB_1) antagonists are developed for the treatment of obesity and associated risk factors. Surinabant is a high affinity CB_1 antagonist in vitro. The aim of this study was to assess the magnitude of inhibition by surinabant of CNS effects and heart rate induced by Δ^9-tetrahydrocannabinol in humans.

METHODS This was a double blind, placebo-controlled, randomized, four-period six-sequence cross-over study. Thirty healthy young male occasional cannabis users (<1/week) were included. A single oral dose of surinabant (5, 20 or 60 mg) or placebo was administered followed 1.5 hours later by four intrapulmonary THC doses (2, 4, 6 and 6 mg) or vehicle, administered at 1h intervals. The wash-out period was 14-21 days. Subjective and objective pharmacodynamic (PD) measurements were performed. A population PK-PD model for THC and surinabant quantified PK and PD effects.

RESULTS Surinabant 20 and 60 mg inhibited all THC-induced PD effects in a similar range for both doses with inhibition ratios ranging from 68.3% (95%CI = 32.5, 104.2; heart rate) to 91.1% (95%CI = 30.3, 151.8; body sway). IC_{50} ranged from 22.0 ng/ml (relative standard error = 45.2%; body sway) to 58.8 ng/ml (RSE = 44.2%; internal perception). Surinabant 5 mg demonstrated no significant effects.

CONCLUSIONS The dose-related inhibition by surinabant, without any effect of its own, suggests that this compound behaves as a CB_1 receptor antagonist in humans at these concentrations. A single surinabant dose between 5 to 20 mg and above was able to antagonize THC-induced effects in humans.

INTRODUCTION

Research on the cannabinoid system started several decades ago with the isolation of Δ^9-tetrahydrocannabidiol (THC) from the plant Cannabis Sativa (Mechoulam and Gaoni, 1965). Since the 1990s, when cannabinoid receptors type 1 (CB_1) and type 2 (CB_2) (Alexander et al., 2008) were cloned, the number of studies on the cannabinoid system and its application to the medical practice increased rapidly (Munro et al., 1993; Matsuda et al., 1990b). Modulators of CB_1 receptors became of special interest for medical indications. CB_1 receptors are mainly located in brain areas such as the cortex, basal ganglia, hippocampus, hypothalamus, and cerebellum, in the spinal cord, and in peripheral tissues such as adipose tissue, the heart, and intestines (Bermudez-Silva et al., 2010). THC is the most well-known agonist of the CB_1 receptor and induces a wide range of effects corresponding to the widespread location of CB_1 CB_1 receptors. These effects include involvement in feeding behaviour and pain (Bermudez-Silva et al., 2010; Ravinet et al., 2003; Van Gaal et al., 2005; Pertwee, 2009).

In the 1990s, the alimentary effects led to the theory that if appetite enhancement is regulated by CB_1 receptors, then antagonism of these receptors would suppress appetite, resulting in weight loss. With the increasing global problem of obesity and related factors, this topic became of special interest for pharmaceutical companies. From 1994, the first CB_1 inverse agonist rimonabant (at that time believed to be an antagonist) was discovered and developed by Sanofi (Rinaldi-Carmona et al., 1994). Besides efficacy in obesity and associated risk factors (Ravinet et al., 2003; Van Gaal et al., 2005), results from pre-clinical and clinical research also showed the beneficial effects of CB_1 antagonists on alcohol and nicotine abuse (Rodriguez de Fonseca et al., 1999; Centre for Reviews and Dissemination, 2004; Cohen et al., 2005; Cohen et al., 2002). In

2006, the European Commission granted a marketing authorisation for rimonabant as an adjunct to diet and exercise for the treatment of obese patients, or overweight patients with associated risk factors such as dyslipidaema, diabetes mellitus type 2, or cardiovascular risk factors (Wathion, 2009).

However, after a recommendation of suspension of rimonabant's marketing authorisation by the European Medicines Agency (EMEA) in 2008, rimonabant was withdrawn from the market (The European Medicines Agency (EMEA), 2008). The EMEA had drawn the conclusions that the weight loss did not outweigh the psychiatric side effects, especially depression (The European Medicines Agency (EMEA), 2008). At around the same time, Merck announced the withdrawal of their CB_1-antagonist taranabant from phase II and III studies for the indications of smoking cessation and obesity, also due to psychiatric side effects including depression, irritability, anxiety, and suicidality (Merck & Co., 2008; Aronne et al., 2010; Kipnes et al., 2010; Proietto et al., 2010; Morrison et al., 2010). The results of clinical studies on rimonabant and taranabant showed that both the desired and undesired effects were dose related, with greater efficacy and more adverse events in the highest doses (Aronne et al., 2010; Kipnes et al., 2010; Merck & Co., 2008; Van Gaal et al., 2005). While the significant weight loss effects can only be measured after a few weeks, Morrison et al. reported that with taranabant, the largest percentage of psychiatric adverse events occurred within the first four days of treatment (Morrison et al., 2010).

These clinical findings with CB_1 antagonists do not invalidate attempts to address obesity treatment or smoking cessation via antagonism of the CB_1 receptor. However, careful attention should be paid to potentially harmful effects as clearly explained by Kirilly, Gonda & Bagdy (2012). The clinically effective level might be found in a lower dose range of the CB_1 antagonist compared to doses that cause psychiatric side effects (Cohen, 2010). In the available literature on CB_1 antagonists, there is a lack of information on different dose or plasma concentration ranges, and the relation between the various pharmacodynamic parameters, i.e. efficacy parameters and safety profile. Therefore, for future CB_1 antagonist studies a possible safety window between clinically effective dose levels and doses with undesirable effects should be examined carefully.

For example, rimonabant 20 mg demonstrated a reduction of both weight gain and smoking cessation in humans, whereas Tonstad and Aubin found, that CB_1 antagonist surinabant 5 mg did not improve smoking cessation, but had a small effect on reducing weight gain (Tonstad and Aubin, 2012; Cahill and Ussher, 2011).

Acute administration of CB_1 antagonists does not give measurable effects in healthy volunteers, which hampers the accurate determination of dose-response relationships and prediction of minimal pharmacological effect levels in early drug development. Therefore, we previously developed the THC-challenge test (Zuurman et al., 2008; Zuurman et al., 2008). This test is used to quantify the displacement of the concentration effect curve of CB_1 agonist THC, by different doses of a CB_1 antagonist for various pharmacodynamic parameters. The THC-challenge test showed clear dose-related effects of CB_1 antagonist drinabant (AVE1625) in a previous study, after single doses that did not cause any detectable effect of their own, and which were lower than predicted from preclinical experiments (Zuurman et al., 2008). As a consequence, the dose range for subsequent phase II-trials was reduced. A very recent study on smoking cessation found that another CB_1 antagonist surinabant had a small effect on weight gain, whereas it had no effect on smoking cessation (Tonstad and Aubin, 2012).

After repeated-dose oral administration for 14 days in young subjects, surinabant was rapidly absorbed with a median T_{MAX} of 2 h. After a single-dose administration of 20 to 80 mg, C_{MAX} and AUC increased less than dose-proportionally (2.0-and 2.9-fold respectively). The 4-fold dose increase in a repeated dosing study had a 2.1- and 2.1-fold increase of C_{MAX} and $AUC_{0\text{-}24}$. The terminal half-life was not dose-proportional and ranged between 161 and 183 hours for 14-day multiple doses (20 to 80

mg/day). Steady state was achieved by Day 13 and the mean accumulation ratios were 1.3 (C_{MAX}) and <2.6 (AUC_{0-24}) (sanofi-aventis, 2006). A previous pharmacokinetic trial in humans found that surinabant elimination took place primarily through the faeces (sanofi-aventis, 2006c). An in vitro study identified CYP3A4 as the major CYP isoform involved in the metabolism of surinabant (sanofi-aventis, 2006).

The aim of this study was to investigate the pharmacodynamic/pharmacokinetic relationships of surinabant using the THC-challenge test in healthy volunteers.

METHODS

Study design

This was a single-centre double-blind, placebo-controlled, randomized, 6-treatment, 4-period, 6-sequence incomplete balanced cross-over study with a wash-out period of at least 2 weeks.

Subjects and power calculation

Healthy male volunteers aged 18 to 45 years were included in the study. Subjects had to be cannabis users for at least 1 year with a frequency of use of no more than once a week to minimise the risk on adverse effects from naive subjects, as well as to avoid tolerance. Subjects had to be able to refrain from using cannabinoids from at least 3 weeks prior to the first treatment period up to the end of the study.

Thirty-six subjects were planned to be randomised and treated in order to obtain at least 24 subjects completing the 4 periods (4 subjects per sequence, each treatment given to a total of 16 subjects). A sample size of 16 subjects per treatment group was to provide a power of at least 90% to demonstrate a 50% inhibition of THC-induced effect on body sway, alertness and feeling high, using a two-sided paired t-test at 5% alpha level. These parameters gave consistent and robust THC effects in previous studies, and were therefore chosen for power calculation (Strougo et al., 2008; Zuurman et al., 2008; Zuurman et al., 2008). Calculations were based on CB_1 antagonist placebo + THC effects and within-subject standard deviations as demonstrated in a previous study (Zuurman et al., 2008).

Procedure

Subjects gave written informed consent after full explanation of what was involved, and before any study-specific procedure was performed.

Eligible subjects were enrolled in the study after a general health screen within three weeks before the first study day. Subjects were acquainted with the experimental methods and conditions in a training session including the inhalation procedure using THC vehicle. Alcohol breath test and urine drug screen had to be negative on each study day. Pharmacodynamic (PD) and pharmacokinetic (PK) measurements were frequently performed on all study days. A follow-up visit was scheduled between 12 and 18 days after the last study day. The study protocol was approved by the Medical Ethics Review Board of Leiden University Medical Center and complied with the principles of ICH-GCP, the Helsinki declaration and Dutch laws and regulations.

Treatments

Subjects received randomised administration of 4 out of the following 6 treatments: surinabant 5 mg or 20 mg or 60 mg + THC, surinabant 60 mg + placebo THC, placebo surinabant + THC, and placebo surinabant + placebo THC. Starting from the expected T_{MAX} of surinabant at 1.5 hours, 4 doses of inhaled THC (2, 4, 6 and 6 mg) or placebo were administered at 1-hour intervals.

Surinabant was administered as oral capsules (Sanofi-Synthélabo Recherche, Toulouse, France). The soft gelatine capsules contained 5 mg, 10 mg, or 20 mg surinabant or placebo and the following excipients: polyoxyl 40 hydrogenated castor oil, propylene glycol monolaurate type II, triglycerides medium-chain (caprylic-capric acid 60-40), caprylocaproyl macrogolglycerides type 400, gelatine, glycerol, titanium dioxide, and purified water.

THC 2, 4, and 6 mg was diluted in 200 µl 100% ethanol (Farmalyse b.v., Zaandam) or THC vehicle, which consisted of ethanol only. This amount of ethanol was considered too small to cause any effects that would interfere with THC effects. The THC was vaporised into a balloon using a Volcano vaporizer® (Storz & Bickel GmbH & co. KG, Tuttlingen, Germany). Subjects inhaled the full contents of the balloon within 2 minutes using a standard paced puffing protocol as previously described by Zuurman et al (Zuurman et al., 2008).

Surinabant dosages were selected in order to obtain sub-effective and effective plasma concentrations, based on phase 2 efficacy results in obesity, and on PK data from a phase 1 study (study numbers DR15029 and TDR 5736, data on file). THC dosages were selected in order to reach and maintain clear, sub-maximal central nervous system effects, based on PK-PD model simulations that were based on a previous study (Strougo et al., 2008). Procedures to evaporate the solution and inhalation of the vapour were done according to a method previously described by Zuurman et al. (2008).

Outcome measures

PHARMACOKINETIC MEASUREMENTS

For surinabant and THC PK analysis, venous blood samples were taken via a cannula that was inserted at the start of the study day thirty minutes after arrival, before any measurements were performed. Surinabant samples were drawn pre-dose and at fixed time points after dosing from t = oh45m up to t = 24h. THC samples were taken pre-dose and three times after each of the first three THC administrations, and four times after the fourth THC administration.

PHARMACODYNAMIC ASSESSMENTS

The choice of the PD endpoints was based on a previous review and previous studies by Zuurman et al. (2008; 2008; 2009). The PD measurements were performed twice pre-dose, twice after surinabant administration before the first THC inhalation, three times after each of the first three THC inhalations and nine times after the fourth THC

inhalation up to t = 9h16m. Vital signs (heart rate and blood pressure) were measured ten times per study day of which twice pre-dose.

BODY SWAY – The body sway meter (André Ibelings, TNO/ICT, Delft) is an objective assessment of antero-postural sway in mm per two minutes. The antero-postural sway is regulated by different factors, such as attention and motor coordination, involving the central and peripheral nervous system and vestibular processes. Visual feedback was eliminated by closing the eyes. Measurements were performed according to a procedure previously described (Zuurman et al., 2008).

VISUAL ANALOGUE SCALES (VAS) – VAS by Bond and Lader is a 16-item assessment of subjective effect on alertness (composition of items alert/drowsy, strong/feeble, muzzy/clear-handed, well coordinated/clumsy, lethargic/energetic, mentally slow/quick-witted, attentive/dreamy, incompetent/proficient, and interested/bored), on mood (composition of items contended/discontended, troubled/tranquil, happy/sad, antagonistic/amicable, and withdrawn/gregarious), and calmness (composition of items calm/excited, and tense/relaxed) (Bond and Lader, 1974). The adapted version of VAS by Bowdle (1998) is a 13-item assessment of subjective effects on feeling high and on factors of internal and external perception, which are both compositions of items that are affected differently by THC as previously described (Zuurman et al., 2008).

HEART RATE AND BLOOD PRESSURE – Heart rate and blood pressure were measured using Nihon-Koden (Lifescope EC, Tokyo, Japan) blood pressure apparatus. All heart rate measurements were used for PD analysis.

Adverse events and concomitant medication were continuously recorded from screening until follow-up period.

Bioanalyses

SURINABANT SAMPLES

Venous blood was collected in 4.5 ml EDTA tubes. The blood samples were kept on ice and centrifuged within 30 min of collection at 2000xg at 4^{0}C for 10 minutes. The plasma was transferred into 2 ml Sarstedt polypropylene tubes and stored at –20^{0}C. Samples were analysed by the Global Metabolism and Pharmacokinetics department of Sanofi (Malvern, PA, USA) using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) method with a lower limit of quantification (LLOQ) of 1.0 ng/ml.

THC SAMPLES

For determination of the concentration of plasma THC and its metabolites 11-HYDROXY-THC (11-OH-THC) and 11-NOR-9-CARBOXY-THC (THC-COOH) venous blood was collected in 4 ml EDTA tubes. As cannabinoids are photosensitive compounds, samples were protected from light at all times. The tubes were kept on ice and centrifuged for 10 minutes at 2000xg at 4^{0}C. The plasma was transferred into 2 ml brown Sarstedt polypropylene tubes and stored at –20^{0}C. Plasma samples were analysed by PRA International (Zuidlaren). Plasma THC as well as metabolite concentrations (11-OH-THC and THC-COOH) were determined using LC-MS/MS method with a LLOQ of 0.5 ng/ml.

Statistical Analyses

ADVERSE EFFECTS

Evaluation of the safety data was based on the review of individual values and descriptive statistics. Vital signs (heart rate and blood pressure were analysed using descriptive statistics. Adverse events were coded according to the Medical Dictionary for Regulatory Activities (MedDRA version 9.0).

NONCOMPARTMENTAL PHARMACOKINETICS

PK parameters of surinabant, THC, 11-OH-THC, and THC-COOH were determined for each period by noncompartmental analysis of plasma concentrations and real time values using PKDMS Version 1.3 with WinNonlin Professional Version 4.01.

PHARMACODYNAMICS

PD parameters were analyzed using a linear mixed effect model with treatment, period, time and treatment by time as fixed effects, subject and subject by treatment as random effects and with baseline value as covariate. The baseline value was defined as the calculated mean of pre-dose assessments for each occasion. From this model, pairwise differences and corresponding 95% confidence intervals were estimated to verify the effects of THC and to assess the intrinsic and inhibitory activity of surinabant. This analysis was conducted on data measured from the third THC inhalation up to three hours after the fourth inhalation to measure at maximum THC effects. The model was fitted by estimated generalized least squares (GLS) using SAS PROC MIXED.

Inhibition ratios as defined in percentages were estimated (with 95% confidence interval) within the mixed model framework for each surinabant dose separately using the following formula below. Each parameter in the formula represents the effect that was measured at a certain time point for the indicated treatment:

$$\frac{\textit{Surinabant dose} + \textit{THC challenge versus placebo} + \textit{THC challenge}}{\textit{Placebo surinabant} + \textit{thc vehicle versus placebo} + \textit{THC challenge}}$$

Body sway data and item 'feeling high' on the VAS Bowdle were analyzed after log (VAS score+2) transformation.

Population PK-PD modelling

Population PK and PK-PD modelling was performed using the nonlinear mixed effect modelling package NONMEM (version 5, ICON Development Solutions, Ellicott City, Maryland, USA) (NONMEM project group, 1992) running on a Linux cluster (Speth, 2004). Model development was guided by visual (goodness-of-fit plots) and statistical criteria based on minimisation of the objective function value, uncertainty of parameter estimates, and biologically plausible values. The first order conditional estimation method with interaction (FOCE-I) was used throughout the analysis.

Population PK-models were developed to describe the time course of surinabant- and THC concentrations. Subsequently, PK-PD models were developed for the separate PD measures that quantify the relationship between the plasma concentrations of surinabant and THC and the observed effects, using an agonist-antagonist interaction model, as shown in Equation 1:

$$(1) \quad Effect_{THC+SR} = \frac{E_{max} \times \frac{THC_{conc}}{EC_{50,THC}}}{1 + \frac{SR_{conc}}{IC_{50,SR}} + \frac{THC_{conc}}{EC_{50,THC}}}$$

$$(2) \quad Effect_t = E_{0,occasion} + Effect_{THC+SR,t}$$

Equation 2 models the effect at a specific time point and occasion. The empirical Bayes estimates of the individual PK parameters were used to develop separate PK-PD models for the evaluated PD parameters.

The PK-PD relationship for THC was described using an effect compartment model in which the effect compartment rate constant (K_{e0}) accounts for the delay between PK and PD (i.e. hysteresis).

This parameter can also be expressed as the effect compartment equilibrium half-life (T_{50}), which was calculated by the following equation:

(3) $$T_{50} = \frac{\ln(2)}{K_{eo}}$$

The relation between the effect compartment concentration and the observed effect was initially modelled using a maximal effect model, in terms of baseline, EC_{50} and E_{MAX}. When the data showed no maximal effect relationship, a linear slope function was estimated.

As all subjects had PK sampling on more than one occasion for THC, interoccasion variability (IOV) was evaluated for the relative bioavailability. A THC dose was defined as an occasion. Interindividual variability (IIV) and IOV in a PK parameter, P, were included in the model and assumed to be log-normally distributed, according to Equation 4:

(4) Pjk = TVP · e (ηj+τk)

where Pjk is an individual PK parameter for the jth individual and the kth occasion, TVP is the typical value of the PK parameter, and j and k are the independent and normally distributed between- and within-subject random variability with mean of zero and variance P and ϖP, respectively. Different combinations of correlation (-block) and fixed at zero were evaluated. The selection of an -block, if any, was made on the basis of the decrease of the objective function value (OFV). The residual variability was evaluated using a proportional error model for the population PK analysis and using an additive error model for the population PK-PD analysis according to Equations 5 and 6, respectively:

(5) Cobs = Cpred · (1+ε)

(6) Cobs = Cpred + ε

where Cobs was the observed concentration or effect; Cpred was the corresponding model predicted concentration or effect; and was the departure of the observed from the predicted concentration or effect, which was assumed to follow a random normal distribution with a mean of o and variance.

RESULTS

Subject demographics

Thirty healthy young males were randomised and treated, and 28 subjects completed 4 occasions. One subject discontinued from the study after the first study occasion (surinabant 5 mg + THC) due to personal reasons, and one subject discontinued due to an adverse event during the second visit (placebo surinabant + THC). Thirty subjects were evaluated for pharmacodynamic and pharmacokinetic analysis. Subject demographics were balanced for all treatment arms (mean age = 23.2 years, SD = 5.3; weight = 78.94 kg, SD = 8.23; height = 187.7 cm, SD = 6.7; BMI = 22.39 kg/m², SD = 1.94). All subjects were of Caucasian ethnicity (one subject was of half Asian, half Caucasian origin).

Adverse effects

Adverse events were of mild to moderate intensity and transitory in nature, and no serious adverse events were reported during the study. One subject discontinued his second occasion with placebo + THC challenge treatment due to vasovagal syncope, which occurred 8 minutes after the second THC inhalation (4 mg). The safety profile of adverse events was similar in the surinabant 60 mg group (10 out of 18; 56% of the subjects had adverse events) compared with the placebo group (8 out of 19; 42%). The most frequent adverse events in the surinabant 60 mg + THC vehicle group were headache (28%), somnolence (17%), and nausea (17%). A higher incidence of psychiatric, nervous system and gastrointestinal disorders was observed during THC treatment (95%), which were dose dependently decreased by surinabant co-treatment (90% in the surinabant 5 mg group; 82% in the surinabant 20 mg group, and 63% in surinabant 60 mg + THC treatment group). These adverse events include

euphoric mood (feeling high, collected after spontaneous reporting independent from the VAS feeling high scores, 45%), dizziness (50%), somnolence (45%), headache (30%), dry mouth (20%), and nausea (15%).

No clinically relevant changes were found for blood pressure, haematology, biochemistry, urinalysis or any of the ECG intervals. Heart rate changes were analysed as PD parameters.

PK analysis

SURINABANT

Mean surinabant plasma concentration-time profiles are shown in Figure 1 and an overview of surinabant PK parameters is given in Table 1. Mean surinabant exposure was generally similar with or without THC challenge after surinabant 60 mg (Figure 1). Median T_{MAX} was 1.58 hours for all surinabant dosages, corresponding to the start time of the THC challenge. Surinabant exposure increased in a less than dose proportional manner. A twelve-fold increase in surinabant dose (from 5 mg to 60 mg) gave a 6.91-fold increase of C_{MAX} ($p<0.0001$) and an 8.08-fold increase of AUC_{0-24} ($p<0.0001$).

Population PK analysis showed that surinabant PK was best described with a two-compartment model with first-order elimination and first-order absorption with a lag time. Population PK parameters were estimated with good precision (relative standard errors <22.0). Population PK parameters estimates are given in Table 2.

THC

Mean THC plasma concentration-time profiles are shown in Figure 2. THC peak plasma concentration increased for the fourth inhalation, as co-administration of surinabant increased (Figure 2, $p = 0.0006$). A similar increase was observed for 11-OH-THC and to a lesser extent for THC-COOH (data not shown).

A two-compartmental model with linear elimination best described the THC PK data. A model with Michaelis-Menten elimination, as was used in a previous study (Strougo et al., 2008), did not significantly improve the model (data not shown). PK parameter estimations were relatively good, with a relative standard error up to 14.6%. Relative bioavailability fractions were implemented for each dose within an individual allowing the estimation of intra-individual variability in absorption. Inter-occasion variability of bioavailability was shown to significantly improve the model, and was estimated to be 55.8%. Inter-individual variability was estimated for central clearance and central volume of distribution. An overview of population pharmacokinetic parameters is given in Table 2.

Pharmacodynamics

THC-induced significant effects on all pharmacodynamic measurements, except for VAS calmness, compared with the placebo surinabant + placebo THC condition. Surinabant 20 and 60 mg were able to significantly reduce all THC-induced effects on the central nervous system and heart rate compared to surinabant placebo + THC challenge. The inhibition ratios for surinabant 20 mg and 60 mg did not differ significantly. Surinabant completely or almost completely (>80% inhibition) inhibited THC-induced effects, except on heart rate and feeling high where submaximal inhibition was observed (Table 3). Surinabant 5 mg was not able to inhibit any of the THC-induced effects significantly. By itself, 60 mg surinabant did not induce any significant effect on the central nervous system parameters nor on heart rate, compared with surinabant placebo+THC placebo treatment. A graph with the observed effects of feeling high can be found in Figure 3.

Population PK-PD

A schematic representation of the basic structure of the PK-PD model is visualised in Figure 4. The effect of THC on body sway and feeling high

were best described by maximum effect models, relating the effect to the concentration in the effect compartment (Ferron et al., 2008). These models included inter-individual variability on the baseline value, E_{MAX} and Keo (Table 4). The effect by surinabant on THC-induced feeling high was best described using a partial antagonism model. Internal and external perception and alertness were best described by a linear response model, relating the effect to the concentration in the effect compartment. These models included variability on the baseline value along with inter-individual variability on baseline, slope and Keo (Table 4). As some subjects appeared not to show any changes in internal perception following the THC challenge, a model excluding non-responders was evaluated, but no improvement was seen. The effect compartment equilibrium half-lives for alertness (120 min) and body sway (89 min) were larger compared to feeling high (40 min), internal (44 min) and external perception (48 min). This means that THC-induced effects on alertness and body sway have a later onset than effects on feeling high, internal, and external perception and that they last longer. For heart rate, no PK-PD model was developed. In the placebo group, the sampling scheme during the postprandial period in which heart rate increase was observed was too sparse for accurate PK-PD modelling.

The EC_{50} of THC for body sway was similar to that of feeling high (7.24 ng/ml and 6.98 ng/ml respectively). No EC_{50} could be calculated for the other PD parameters, as a linear model best described these parameters. The IC_{50} of surinabant for body sway was approximately half of the IC_{50} value for internal perception (22.0 ng/ml vs. 58.8 ng/ml). This means that 50% inhibition of THC-induced body sway increase is established with a surinabant concentration that is approximately half of the concentration needed to reduce the effects on internal perception by half. IC_{50} values for feeling high, alertness, and external perception were similar (30.5 ng/ml, 33.6 ng/ml and 37.1 ng/ml respectively). A summary of population PK-PD model parameters can be found in Table 4.

DISCUSSION

The objective of this study was to investigate the interaction of oral surinabant and inhaled THC on central nervous system effects and heart rate in healthy subjects. We have demonstrated that doses of 20- and 60 mg surinabant are able to inhibit THC-induced effects on central nervous system parameters and heart rate by 68.0% to 91.6%, whereas surinabant 5 mg was unable to antagonize any THC-effect. Surinabant 60 mg alone had no acute effects, particularly not on mood.

Pharmacokinetics

With increasing doses of surinabant, maximum plasma concentration (C_{MAX}) and area under the plasma concentration curve from time 0 to 24 hours (AUC_{0-24}) increased in a less than dose-proportional manner. This was also found in the population PK model; a negative dose effect on the absorption rate constant improved the model. Physiologically, this could be explained by saturation of absorption of surinabant, poor dissolution, or an increase of transit time from the blood. The exact mechanism is unknown.

THC peak plasma concentration increased as co-administration of surinabant increased, which was represented in the population PK model by a relatively high inter-occasion variability on bioavailability of 55.8%. Rather than representing a PK interaction, this could be due to a pharmacodynamic compensation in this group of experienced cannabis users. Subjects who received surinabant in combination with THC experienced less of their familiar subjective effects while inhaling THC. Consequently, they may have tried to inhale maximally THC during concomitant surinabant treatment. On the other hand, less THC was required to induce the desired high feelings, while on surinabant placebo. The standardized paced puffing inhalation

protocol should have prevented this type of variability. However, it is possible that some subjects were able to regulate the amount of THC by breathing out through the nose. Therefore, the inhalation protocol has since been adapted by adding the use of a nose clamp during future studies.

Pharmacodynamics

In contrast to a paced puffing protocol, complete self-regulation of THC administration would allow subjects to titrate for the expected or desired PD effects. This would lead to inaccurate estimations of the antagonistic effects, which could explain the differences in the effect size between our study and a previous study by Huestis et al. In the latter study in which a cannabis challenge was applied, rimonabant doses up to 90 mg gave 43% inhibition on subjective feeling high of and 59% inhibition on heart rate increase (Huestis et al., 2001; Huestis et al., 2007), whereas for surinabant, reductions were 70% and 75% respectively. The rimonabant doses produced plasma concentrations in the upper range of the therapeutic window, suggesting that the levels of inhibition that were found in the current study could be over the therapeutic range. Although this cannot be excluded without a comparison with the results of clinical studies, it is perhaps more likely that the disparate estimates are related to differences in inhalation methodology. In Huestis' study, subjects inhaled THC from cannabis cigarettes, which allows a certain freedom to self-regulate the amount of inhaled THC by the deepness and the number of the inhalations. THC C_{MAX} was 130 ng/ml in the study by Huestis et al., and 83.48 ng/ml in the current study. With self-regulated titration for PD effects, subjects compensate for a certain amount of effect inhibition, leading to an underestimation of rimonabant's antagonistic potency. This is more difficult if THC is administered with an evaporation device and subjects are instructed to inhale the full contents of the balloon. In view of these differences, it seems more likely that the suppression caused by surinabant is in the same range as the effects of rimonabant. Furthermore, the variety of active compounds from cannabis could interfere with the THC and antagonist effects. The time period from which the inhibition ratios were calculated was different for both studies (1 hour vs. 4.5 hours).

Another study using the CB_1 antagonist drinabant (AVE1625) had a similar design as the current study (Zuurman et al., 2008). Drinabant 20 mg and 60 mg induced maximal inhibition on heart rate, VAS feeling high, internal and external perception, but not on body sway and VAS alertness. Surinabant caused suppression of all these THC-responses, including near-complete inhibition of body sway and VAS alertness, but it had sub-maximal effects on heart rate and high feeling. This indicates possible differences in clinical efficacy between surinabant and drinabant. We have argued that THC-induced tachycardia is (primarily) mediated peripherally, based on a previous PK-PD study in which the equilibration half-life of heart rate was significantly shorter compared to the other centrally mediated effect parameters (Strougo et al., 2008). In line with this conjecture, pre-clinical studies also suggest that surinabant and drinabant have different central and peripheral mediated effects. Conversely, effects on food intake, which could be peripherally mediated (Gomez et al., 2002), are found at 0.3 mg/kg oral drinabant, while the effective dose of oral surinabant was 3.0 kg/mg (unpublished data). No plasma concentrations or PK-PD-relations were determined in these preclinical experiments. These findings could be explained by a larger or faster brain penetration for surinabant compared to drinabant, whereas drinabant appears to have a relatively larger peripheral effect. If so, the effect of surinabant on feeling high seems small (around 70%) compared to drinabant (up to 101%), but the reliability of this inhibition ratio may have been diminished by a fairly large intra-individual variability (124%).

Surinabant 5 mg was unable to significantly inhibit any of the THC-induced central nervous system effects and heart rate, which were

suppressed by surinabant 20 mg and 60 mg. This implies that surinabant effects are dose-dependent. Inhibition ratios of surinabant 20 mg were similar to 60 mg, indicating that 20 mg is able to induce maximal effects.

PK-PD

The PK-PD models adequately described the time-course of PK and PD effects of THC and the antagonism of these PD effects by surinabant. The THC models of body sway, feeling high and alertness are generally comparable with the THC model that was constructed in a previous study by Strougo et al. (2008). The maximal effect of THC on feeling high was smaller in the current study compared to the previous study by Strougo et al. (0.713 log mm vs. 1.68 log mm). A linear response model best fit the external perception data in this study, while Strougo et al. found a maximal effect model to best describe their data. The difference observed in this study might be explained by the THC dose range, which could have been insufficient for detecting a maximal effect.

For surinabant, various IC_{50} values were found for central nervous system parameters, with a smaller IC_{50} value for body sway, which may be regulated by central as well as peripheral processes, compared to the purely centrally mediated measures. This variability of PK-PD parameters implies that surinabant has a variety of effect compartments, even within the central nervous system, which could be functional or kinetic. Also, the effect compartment equilibrium rate constant, or K_{eo}, showed differences among the various pharmacodynamic measures, which means that some effects have a later onset and longer duration than other effects. This could be caused by several factors that could not be determined in this study, such as a difference in penetration rate between the different effect compartments. These findings also support the hypothesis that the clinically effective level of surinabant might be found at different concentrations compared to the levels that are needed to induce adverse side effects.

This agonist-antagonist PK-PD interaction model can be used for prediction of surinabant concentration-effect profiles in future studies, even if these studies have a different design or dosing regimen. As surinabant and rimonabant are very similar in structure and action, the population PK-PD model of surinabant could also be used to estimate concentration-effect profiles of rimonabant to a certain extent. Conversely, as rimonabant has been used extensively in patient studies, a patient population PK-PD model could theoretically be used to predict concentration-effect profiles for surinabant in patients, with the aim of finding an optimal therapeutic window, ranging between the dose-dependent desired and undesired effects. Currently however, such quantitative predictions are hampered by the as yet unknown relationships between the pharmacodynamic (central and peripheral) biomarkers and the clinical (metabolic and psychiatric) endpoints. At any rate, surinabant was found to be a potent CB_1-antagonist, at single doses that did not cause any adverse systemic or central nervous system effects in healthy subjects. However, this information is insufficient to draw conclusions on the effects after a multiple dose regimen. Therefore, future studies should investigate the optimal surinabant dose and its effects after long term use, with a particular focus on the occurrence of psychiatric side effects.

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TABLE 1 Non-compartmental PK parameters for surinabant (5, 20 and 60 mg), Mean (CV%) ± SD of surinabant PK parameters

	Surinabant 5 mg + THC challenge (n=20)	Surinabant 20 mg + THC challenge (n=19)	Surinabant 60 mg + THC challenge (n=19)	Surinabant 60 mg + THC vehicle (n=18)
C_{max} (ng/ml)	104 (31) ± 32.6	334 (24) ± 79.0	719 (26) ± 190	749 (21) ± 157
T_{max}* (h)	1.58 (0.750,1.58)	1.58 (0.750, 2.58)	1.58 (0.750, 2.58)	1.58 (0.750, 2.58)
AUC_{0-24} (ng.h/ml)	543 (50) ± 271	1860 (30) ± 557	4390 (32) ± 1420	4870 (28) ± 1380

* median (minimum, maximum)

TABLE 2 Population PK parameters for surinabant and THC. F=Bioavailability; CV=Coefficient of variation (%); RSE=Relative Standard Error (%); IIV=inter-individual variability (%).

Parameter	Surinabant		THC	
	Estimate (RSE)	IIV (RSE)	Estimate (RSE)	IIV (RSE)
Clearance/F (L/h)	4.69 (13.0)	72.1 (27.7)	293 (7.58)	11.8 (25.0)
Central volume of distribution/F (L)	3.74 (22.0)	74.8 (34.9)	43.6 (8.03)	15.2 (36.0)
Peripheral volume of distribution/F (L)	491 (6.27)	30.6 (23.9)	136 (8.97)	-
Intercompartmental clearance/F (L/h)	15.3 (3.70)	16.3 (30.8)	166 (8.01)	-
Absorption rate constant (ka; h^{-1})	0.406 (3.18)	6.40 (115)	-	-
Lag time (h)	0.591 (5.91)	-	-	-
Dose effect on ka*	-0.00164 (16.4)	-	-	-
Interoccasion variability on relative bioavailability (CV%)	-	-	55.8 (12.6)	-
Proportional residual error (CV%)	18.2 (10.0)	-	15.9 (14.6)	-

* Dose effect on k^1 (α): k_a (dose) = k_a (5 mg) + $\alpha \cdot$ (dose-5)

TABLE 3 Ratios and 95% confidence intervals of inhibition by surinabant (5, 20 and 60 mg) on THC-induced effects, measured from the third THC inhalation until three hours after the fourth inhalation.

PD assessment	Surinabant dose (mg)	% Inhibition (estimate)	95% CI
Body Sway	5	13.6	(-32.6, 59.7)
	20	93.1	(31.9, 154.3)
	60	91.1	(30.3, 151.8)
VAS alertness	5	-8.9	(-54.9, 37.0)
	20	72.5	(18.3, 126.7)
	60	82.5	(25.7, 139.4)
VAS feeling high	5	10.0	(-20.9, 40.9)
	20	68.0	(31.6, 104.4)
	60	70.0	(33.2, 106.9)
VAS External Perception	5	17.1	(-18.3, 52.6)
	20	88.7	(43.2, 134.3)
	60	89.0	(43.3, 134.7)
VAS Internal Perception	5	37.9	(-5.1, 80.9)
	20	89.9	(37.0, 142.8)
	60	91.6	(38.3, 145.0)
Heart rate	5	17.6	(-13.0, 48.1)
	20	75.4	(38.4, 112.3)
	60	68.3	(32.5, 104.2)

TABLE 4 Population PK-PD parameter estimates for body sway, VAS feeling high, alertness, external perception, and internal perception

PD parameter	Population parameter estimate (RSE%)	Inter-individual variability CV% (RSE%)	Inter-occasion variability CV% (RSE%)
Body sway			
Baseline (ln mm)	5.46 (1.26)	6.66 (24.3)	3.00 (32.2)
E_{max} (log mm)	0.829 (24.5)	68.8 (40.2)	-
EC_{50} (ng/ml)	7.24 (42.8)	-	-
K_{e0} (h^{-1})	0.466 (17.9)	73.4 (33.6)	-
IC_{50} (ng/ml)	22.0 (45.2)	-	-
Residual variability (SD of additive error)	0.212 (10.5)	-	-
Feeling high			
Baseline (log mm)	0.321 (3.96)	21.6 (38.5)	-
E_{max} (log mm)	0.713 (31.6)	124 (39.6)	-
EC_{50} (ng/ml)	6.98 (33.5)	-	-
K_{e0} (h^{-1})	1.04 (17.4)	71.6 (32.4)	-
IC_{50} (ng/ml)	30.5 (61.6)	-	-
Maximum inhibition	0.751 (20.6)	-	-
Residual variability (SD of additive error)	0.254 (19.1)	-	-
Alertness			
Baseline (mm)	49.4 (1.10)	5.13 (47.9)	180 (37.0)
Slope (/ng/ml)	0.547 (45.2)	98.1 (53.5)	-
K_{e0} (h^{-1})	0.347 (33.7)	4.64 (26.0)	-
IC_{50} (ng/ml)	33.6 (45.8)	-	-
Residual variability (SD of additive error)	3.30 (18.3)	-	-
External perception			
Baseline (log mm)	0.367 (0.529)	-	3.86 (46.1)
Slope (/ng/ml)	0.00258 (41.9)	154 (29.4)	-
K_{e0} (h^{-1})	0.868 (16.9)	69.9 (30.1)	-
IC_{50} (ng/ml)	37.1 (59.6)	-	-
Residual variability (SD of additive error)	0.0182 (19.1)	-	-
Internal perception			
Baseline (log mm)	0.366 (0.508)	2.68 (68.2)	1.46 (36.9)
Slope (/ng/ml)	0.000869 (38.2)	151 (35.1)	-
K_{e0} (h^{-1})	0.955 (20.1)	71.4 (45.5)	-
IC_{50}	58.8 (44.2)	-	-
Residual variability (SD of additive error)	0.0123 (22.8)	-	-

* EC_{50} of THC effect. RSE = Relative Standard Error (%); CV = Coefficient of variation (%); E_{max} = Maximal effect; EC_{50} = Concentration producing 50% of E_{max}; K_{e0} = effect compartment equilibration rate constant; IC_{50} = Concentration producing 50% of inhibition of THC E_{max}; SD = standard deviation.

FIGURE 1 Mean and predicted plasma concentration-time curve of surinabant with standard deviations. Surinabant was administered at time point zero, and the first blood sample for bio-analysis was taken pre-dose. The open circles are surinabant concentrations after surinabant 5 mg + THC, the open triangles are surinabant 20 mg + THC, the open squares are surinabant 60 mg + THC treatment and the closed squares are after surinabant 60 mg + placebo THC treatment. The dotted lines with plus signs represent the predicted surinabant plasma concentration-time curves.

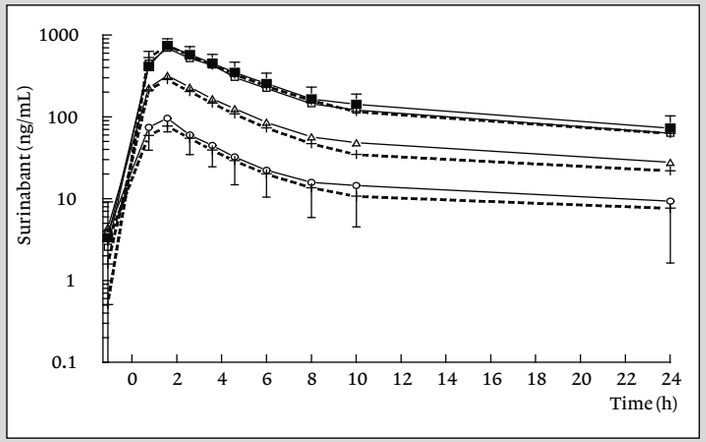


FIGURE 2 Mean plasma concentration-time curve of THC with standard deviations. The arrows indicate the time points of THC administration. The closed circles are the THC concentrations after placebo surinabant + THC treatment, the open circles are surinabant 5 mg + THC, the triangles are surinabant 20 mg + THC, and the squares are surinabant 60 mg + THC treatment. The graph shows a rather repetitive pattern after each THC administration: the blood samples were taken at 5, 30 and 57 minutes after the 1st, 2nd and 3rd inhalation, and at 5, 20, 89 and 130 minutes after the 4th THC inhalation.

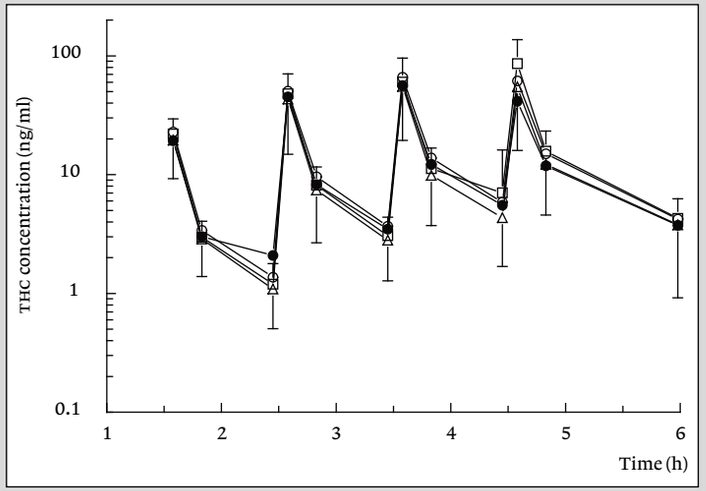


FIGURE 3 Graph with observed feeling high effects and standard deviations. Two baseline measurements were recorded before surinabant administration. The closed triangles are feeling high scores after placebo surinabant + placebo THC administration, the closed circles are after placebo surinabant + THC treatment, the open circles are surinabant 5 mg + THC, the open triangles are surinabant 20 mg + THC, the open squares are surinabant 60 mg + THC treatment and the closed squares are after surinabant 60 mg + placebo THC treatment.

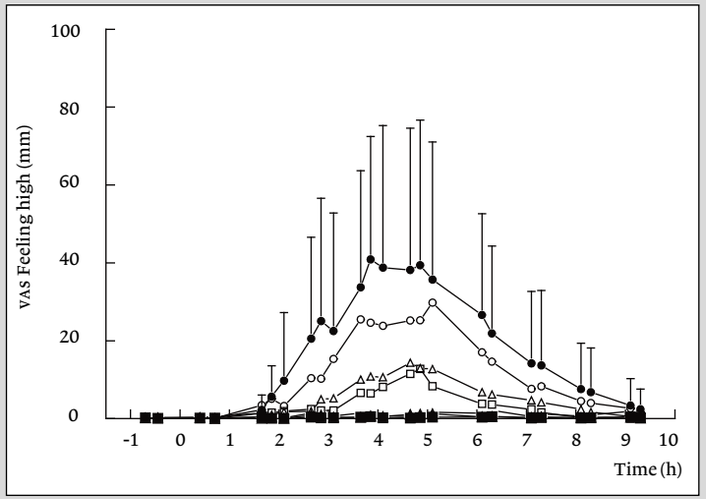
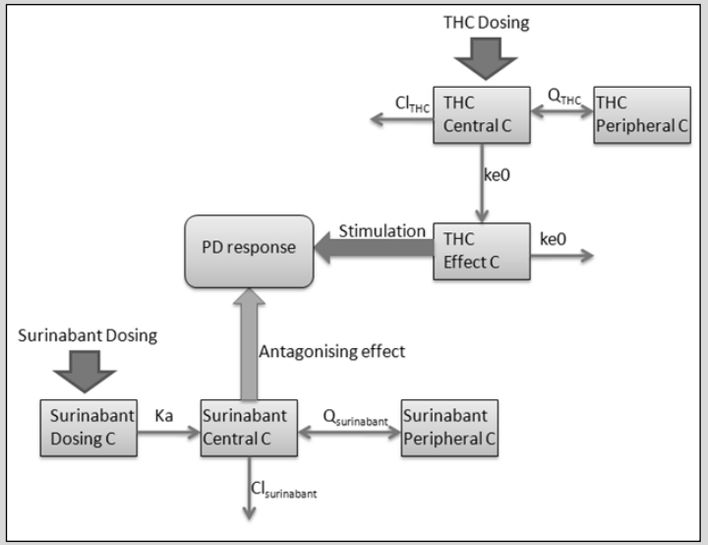


FIGURE 4 Schematic overview of PK-PD model (for detailed background information, see ref. (Mager, Wyska, & Jusko, 2003)). The central compartment refers to the central circulation. C = compartment, k_a = absorption rate constant, K_{e0} = effect compartment equilibration rate constant, Q = intercompartmental clearance, Cl = clearance



CHAPTER V

Peripheral selectivity of the novel cannabinoid receptor antagonist TM38837 in healthy subjects

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ABSTRACT

AIM Cannabinoid receptor type 1 (CB_1) antagonists show central side effects, whereas beneficial effects are most likely peripherally mediated. In this study, peripherally selective CB_1 antagonist TM38837 was studied in humans.

METHODS This was a double-blind, randomized, placebo-controlled, cross-over study. In occasion 1-4, 24 healthy subjects received 5x4mg THC with TM38837 100 mg, 500 mg, or placebo, or placebos only. During occasion 5, subjects received placebo TM38837+THC with rimonabant 60 mg or placebo in parallel groups. Blood collections and pharmacodynamic effects (PD) were assessed frequently. Pharmacokinetics (PK) and PD were quantified using population PK-PD modelling.

RESULTS TM38837 plasma concentration profile was relatively flat compared to rimonabant. TM38837 showed an estimated terminal half-life of 771 hours. THC induced effects on VAS feeling high, body sway, and heart rate were partly antagonized by rimonabant 60 mg [-26.70% (95%CI -40.9-/-12.6%); -7.10%, (95%CI -18.1-5.3%); -7.30%, (95%CI -11.5%-/-3.0%) respectively] and TM38837 500 mg [-22.10% (95%CI -34.9-/-9.4%); -12.20% (95%CI -21.6%-/-1.7%); -8.90% (95%CI -12.8%-/-5.1%) respectively]. TM38837 100 mg had no measurable feeling high or body sway effects, and limited heart rate effects.

CONCLUSIONS Rimonabant showed larger effects than TM38837, however, heart rate effects were similar. TM38837 100 mg had no impact on CNS-effects, suggesting that this dose does not penetrate the brain. This TM38837 dose is predicted to be at least equipotent to rimonabant with regard to metabolic disorders in rodent models. These results provide support for further development of TM38837 as a peripherally selective CB_1 antagonist for indications such as metabolic disorders, with a reduced propensity for psychiatric side effects.

INTRODUCTION

Research on the cannabinoid system has largely increased in the last decades, since the discovery of cannabinoid receptors type 1 and 2 (CB_1 and CB_2) and endogenous cannabinoids from 1988 onwards (Devane et al., 1988; Matsuda et al., 1990; Munro et al., 1993; Sugiura et al., 1995; Alexander et al., 2008). The endogenous cannabinoid system, or endocannabinoid system, is located throughout the body. CB_1 receptors are present in the central nervous system and at peripheral sites such as the heart, liver, pancreas and adipose tissue (Bermudez-Silva et al., 2010; Bermudez-Silva et al., 2008), whereas CB_2 receptors are mainly present in immune cells (Munro et al., 1993; Galiegue et al., 1995; Schatz et al., 1997).

Although the exact functions of the endocannabinoid system are unknown, the widespread presence suggests that the system could have a variety of functions, which could be studied for various clinical indications. Obesity and associated diseases are among the major medical conditions for which involvement of the endocannabinoid system is currently studied. Obesity, or severe overweight, is a condition that affects approximately 500 million adults worldwide, and the World Health Organization estimates this number to increase to 700 million adults in 2015 (World Health Organization, 2011).

Rimonabant was the first CB_1 receptor antagonist that was registered in 2006 as an adjunct to diet and exercise for the treatment of obese patients, or overweight patients with associated risk factors such as dyslipidaemia, diabetes mellitus type 2, or cardiovascular risk factors (Wathion, 2009). However, two years later, rimonabant was withdrawn from the market due to adverse psychiatric effects such as depression (The European Medicines Agency (EMEA), 2008; The European Medicines Agency (EMEA), 2008). The beneficial effects of rimonabant in patients included decrease of appetite, weight loss, and weight loss independent improvement of metabolic parameters such as HDL cholesterol, triglycerides, fasting glucose and insulin levels (Van Gaal et al., 2008; Pan et al., 2011; Van Gaal et al., 2005).

CB_1-receptors are widely distributed throughout the brain, including central nervous system areas that are involved in the regulation of food intake and metabolism (for review, see Pertwee (1997)). Nevertheless, there is considerable evidence to suggest that the beneficial metabolic effects of CB_1 antagonists are mediated by CB_1 receptors that are present at locations which are specifically associated with metabolic regulation, such as the liver, the pancreas, and fat cells (Bermudez-Silva et al., 2010; Bermudez-Silva et al., 2008). A study in rats demonstrated that centrally administered rimonabant did not affect feeding behaviour, whereas peripheral rimonabant inhibited food intake (Gomez et al., 2002). Other studies found that peripheral, but not central, CB_1 antagonism induced beneficial effects on metabolism and feeding behaviour (Nogueiras et al., 2008; Cluny et al., 2010). A recent study by Tam et al. suggests that peripheral CB_1 inverse agonism reduces obesity by reversing obesity-related leptin resistance (Tam et al., 2012). This suggests that the beneficial metabolic effects of rimonabant might be regulated by peripheral CB_1 receptors, whereas the psychiatric side effects could be regulated by centrally located CB_1 receptors.

TM38837 is a new peripheral-acting CB_1 antagonist that demonstrated efficacy in pre-clinical studies (7TM Pharma A/S, 2009). TM38837 showed 30 times less potency on centrally induced body temperature effects compared to rimonabant, whereas TM38837 was only 3 to 10 times less potent than rimonabant on gastro-intestinal effects (7TM Pharma A/S, 2009). In a first in human trial, dosages up to 900 mg were well tolerated in healthy subjects, obese patients, and liver fibrotic patients (7TM Pharma A/S, 2009).

In the current study the central and peripheral effects of TM38837 and rimonabant in healthy subjects were investigated. Since acute administration of CB_1 antagonists does not have measurable effects in healthy volunteers, the Δ^9-tetrahydrocannabinol (THC)-challenge test was used in this study (Zuurman et al., 2008; Zuurman et al., 2008). The THC-challenge test is able to quantify the displacement of the concentration effect curve of the CB_1 agonist THC by different doses of a CB_1 antagonist for various pharmacodynamic parameters. These parameters include measures that are mediated via the central nervous system, such as the subjective effect 'feeling high', measures that could be affected by processes at multiple locations, such as postural stability, and heart rate, which is likely to be peripherally mediated (Strougo et al., 2008). In this way, the central and peripheral characteristics of the effect profile in healthy subjects can be assessed. Rimonabant was used as a positive control for both central and peripheral effects. Quantification of modulation of the concentration-effect curve of THC by CB_1 antagonists was done by building a population PK-PD model for THC, TM38837, and rimonabant.

Our hypothesis was that TM38837 would show no effects or small effects on central nervous system parameters, while showing clear effects on biomarkers that are more likely to be peripherally mediated, such as heart rate.

METHODS

Study design

This was a double-blind, double dummy, partially randomized, placebo controlled, cross-over, partial parallel study with a washout period of at least 12 days.

Subjects

Healthy male volunteers aged 18 to 45 years were included in the study. Subjects had to be cannabis users for at least 1 year with using frequency of no more than once a week, and had to be able to refrain from using cannabinoids from at least 3 weeks prior to the first treatment period up to the end of the study. Previous studies reported that black subjects have different rimonabant pharmacokinetics compared to subjects from other races (sanofi, 2008; Martinez et al., 2007). Therefore, black people were excluded from the study.

Twenty-four healthy male volunteers were planned to complete five periods. The study was powered as a bio-equivalence study (Committee for medicinal products for human use (CHMP), 2010). This was based on the hypothesis that there is no or small difference in central nervous system response between THC alone and THC + TM38837, which could be defined as a lack of effects when comparing TM38837 with THC alone treatment, or *bio-equivalent* effects according to the bio-equivalence guideline (Committee for medicinal products for human use (CHMP), 2010). At the time of study performance, these guidelines included the criteria that the 90% confidence intervals of the rate ratios for the main effects of the two treatments would lie within the range 0.80-1.25.

Procedure

Subjects gave written informed consent before any study-specific procedure was performed. Eligible subjects were enrolled in the study after a general health screen within three weeks before the first study day. Subjects were acquainted with the experimental methods and conditions in a training session including the inhalation procedure using THC vehicle. At all treatment visits, subjects stayed at the clinic for 2 days. Alcohol breath test and urine drug screen had to be negative on each treatment visit. Pharmacodynamic (PD) and pharmacokinetic (PK) measurements were frequently performed on all study days (indicated in Table 1). A follow-up visit was scheduled approximately 14 days after the last study day. The study protocol was approved by the Medical Ethics Review Board of Leiden University Medical Center and complied with the principles of ICH-GCP, the Helsinki declaration and Dutch laws and regulations.

Treatments

The treatments that were administrated can be found in Table 2. Each CB_1 antagonist or placebo administration was followed by 5 inhaled doses of vaporised THC 4 mg diluted in 400 µl 100% ethanol or THC vehicle, which consisted only of vaporised ethanol. THC was vaporised using a Volcano vaporizer® (Storz & Bickel GmbH & co. KG, Tuttlingen, Germany). Procedures for vaporising the solution and inhalation of the vapour were done according to a method previously described by Zuurman et al. (2008). The T_{MAX} of TM38837 was expected at approximately 4 hours after administration, whereas rimonabant had a T_{MAX} of 2 hours (sanofi, 2008; 7TM Pharma A/S, 2009). Therefore, oral TM38837 was dosed at time point 0h, oral rimonabant was dosed 2 hours later to account for expected differences in T_{MAX}, and three subsequent intrapulmonary THC doses were given from t = 4 hours with 2.5-hour

intervals. In this way, the first THC inhalation would be administered at the expected T_{MAX} of TM38837 and rimonabant. Twenty-four hours after TM38837 administration, two THC doses were administered with 2.5-hour intervals. A schematic overview of the administrations and other study day procedures can be found in Table 1.

Rimonabant has a terminal half-life of 6-9 days after multiple ascending doses in healthy volunteers (Turpault et al., 2006). To minimize the risk of long-lasting carry-over effects that could complicate the interpretation of the effects of TM38837, each rimonabant treatment arm was always scheduled at the fifth occasion, thereby splitting the study design into a 4-way cross-over part and a parallel part (Table 2).

TM38837 dosages were based on preclinical and clinical studies (7TM Pharma A/S, 2009). The 100 mg dose was selected in order to explore exposure of the anticipated therapeutic level. A 500 mg dose in fed state was expected to give similar exposure to that seen after the highest dose (900 mg) explored in the fasted state, as examined in the first in man single ascending dose study (7TM data on file). This exposure was well tolerated by all subjects. Rimonabant 60 mg dosage was selected in order to obtain plasma concentrations in the clinically effective range. The recommended therapeutic dose of rimonabant was 20 mg; however, as steady state exposures are 3.3-fold higher than those observed after a single dose (sanofi, 2008), a single dose of 60 mg rimonabant per subject was administered in this study in order to achieve a maximum plasma concentration that was comparable to the steady state concentration with therapeutic dosages. Based on previous cannabinoid challenge studies with CB_1 antagonists such as rimonabant, we expected that a dose of 60 mg rimonabant would be sufficient to suppress THC-induced effects (Huestis et al., 2001; Huestis et al., 2007; Zuurman et al., 2010). THC dosages and dosing schedules were selected in order to obtain and maintain clear, sub-maximal central nervous system effects as predicted by PK-PD models that were based on previous studies (Zuurman et al., 2008; Zuurman et al., 2010).

Outcome measures

PHARMACOKINETIC ASSESSMENTS AND BIO-ANALYSES

Time points of venous blood sampling for pharmacokinetic analyses of TM38837, rimonabant and THC can be found in Table 1.

TM38837 and rimonabant – Venous blood was collected in 4 ml Li-Hep tubes. The blood samples were kept on ice and centrifuged within 30 min of collection at 2000G at 4^0C for 10 minutes. The supernatant plasma was divided into three or four 2 polypropylene tubes. Samples were stored at -80^0C and sent to Quotient Bioresearch (Fordham, UK) for analysis. Measurements of TM38837 and rimonabant concentrations in human plasma samples were performed according to bioanalytical methods that were validated. Concentrations of TM38837 and rimonabant were measured by liquid chromatography with tandem mass spectrometry method with a lower limit of quantification of 0.1 ng/ml for TM38837, and 1.0 ng/ml for rimonabant. For TM38837 analysis precision was 4.3% accuracy was -1.3% and for rimonabant precision was 4.5% and accuracy -1.3%.

THC – For determination of the concentration of plasma THC and its metabolites 11-OH-THC and 11-NOR-9-CARBOXY-THC venous blood was collected in 2 ml EDTA tubes. As cannabinoids are photosensitive, samples were protected from light at all times. After blood collection the tubes were put in ice water in aluminium foiled containers, and were centrifuged within one hour for 10 minutes at 2000G at 4^0C. The supernatant plasma was divided into two 2 ml brown polypropylene tubes. Plasma samples were stored at a temperature of -20^0C and sent to ABL (Assen) for PK analysis. Plasma THC as well as metabolite concentrations (11-HYDROXY-THC and 11-nor-9 carboxy-THC) were determined using tandem mass spectrometry with a lower limit of quantification of 0.1 ng/ml.

PHARMACODYNAMIC ASSESSMENTS

The choice of the pharmacodynamic (PD) endpoints was based on a previous review and prior studies by Zuurman et al. (2008; 2010). Pharmacodynamic measurements were performed at time points indicated in Table 1.

BODY SWAY – The body sway meter (André Ibelings, TNO/ICT, Delft) is an objective assessment of antero-postural sway in mm per two minutes. The antero-postural sway is regulated by different factors, such as attention and motor coordination, involving the central and peripheral nervous system and vestibular processes. Visual feedback was eliminated by closing the eyes. Measurements were performed according to a procedure previously described (Zuurman et al., 2008).

VISUAL ANALOGUE SCALES (VAS) – VAS by Bond and Lader is a 16-item subjective assessment of subjective effect on alertness (composition of items alert/drowsy, strong/feeble, muzzy/clear-headed, well coordinated/clumsy, lethargic/energetic, mentally slow/quick-witted, attentive/dreamy, incompetent/proficient, and interested/bored), on mood (composition of items contended/discontended, troubled/tranquil, happy/sad, antagonistic/amicable, and withdrawn/gregarious), and calmness (composition of items calm/excited, and tense/relaxed) (Bond and Lader, 1974). The adapted version of VAS by Bowdle (1998) is a 13-item assessment of subjective effect on item feeling high and on factors internal perception and external perception, both compositions of items that are affected differently by THC as previously described (Zuurman et al., 2008).

BECK'S DEPRESSION INVENTORY II (BDI) – The BDI is a 21-item self-report questionnaire for measuring the severity of depression with a four-point Likert scale for each question (Beck et al., 1996). The questionnaire was included in the study to check for possible mood changes, since previous multiple dose studies with rimonabant reported a larger incidence of subjects suffering from depression (Van Gaal et al., 2005; Van Gaal et al., 2008). The BDI was performed one time per occasion at 9h30m after TM38837 or placebo TM38837 administration.

HEART RATE AND BLOOD PRESSURE – Heart rate and blood pressure were measured using Nihon-Koden BSM-1101K monitor (Lifescope EC, Tokyo, Japan) blood pressure apparatus. All heart rate measurements were used for PD analysis. Adverse events and concomitant medication were recorded from screening until follow-up period.

Data analysis

For the direct clinical effect, PK and PD comparisons of TM38837 and rimonabant, data were used only from subjects who received rimonabant 60 mg + THC treatment during the fifth study occasion.

CLINICAL EFFECTS

Evaluation of the safety data were based on the review of individual values and descriptive statistics. Analysis of laboratory parameters was performed using screening and end-of-study assessments. For vital signs (heart rate and blood pressure), raw data and changes from baseline were analyzed by type of measurement and parameter and treatment using descriptive statistics. Heart rate, PR-, QRS-, and QT-intervals, corrected QT (QTC) from automatic reading were analyzed as raw parameter value and change from baseline (for HR and QTC only). Adverse events were coded according to the Medical Dictionary for Regulatory Activities (MedDRA version 13.0).

PHARMACOKINETICS

All concentrations and maximal concentration (C_{MAX}), time of maximal concentration (T_{MAX}), area under the curve from zero to infinity ($AUC_{0-\infty}$), and terminal half-life ($t_{1/2}$) of TM38837, rimonabant, THC, and its metabolites 11-OH-THC, and THC-COOH were analysed using noncompartmental analysis (SAS PROC MIXED 9.1.3).

Pharmacodynamics

To study the effect of repeated doses of THC on the pharmacodynamic measures or other carry-over effects, the fifth occasion of the twelve subjects receiving only THC was compared graphically and statistically with the previous occasion in which subjects only received THC. If no significant period effect could be established, the fifth occasion would be used for a 5-way cross-over analysis. For this 5-way partial cross-over subanalysis, and for the 4-way cross-over part of the study, the pharmacodynamic variables were analysed with a mixed model analysis of variance (using SAS PROC MIXED 9.1.3) with treatment, time, and treatment by time as fixed effects, with subject, subject by treatment and subject by time as random effects, and the average baseline value was included as covariate. The parallel part was analysed with subject as random effect, with treatment, time, and treatment by time as fixed effects, and the average baseline value as covariate. A 90% confidence interval around the ratio was used for statistical comparison between TM38837 + THC and placeboTM38837 + THC treatment with $\alpha = 0.05$ two-sided (Committee for medicinal products for human use (CHMP), 2010). Graphs of the Least Squares Means estimates over time by treatment were presented with 95% confidence intervals as error bars. Body sway was log transformed before analysis to correct for the log-normal distribution. All pharmacodynamic effects were statistically compared with heart rate effects. We assumed that heart rate primarily represents a peripheral CB_1-effect and that beneficial effects mediated by CB_1-antagonists are peripherally mediated.

Population PK and PK-PD modelling

Population PK and PK-PD modelling was performed using nonlinear mixed effect modelling (NONMEM version 7.1.0, GloboMax LLC, Ellicot City, MD). The pre-dose samples that were taken at an occasion following a study day where TM38837 was administered were also used for the pharmacokinetic model of TM38837. The compartmental population PK analysis was based on the results of previous CHDR studies with multiple THC inhalations, which used a two-compartment model with bolus administration (Strougo et al., 2008). The empirical Bayes estimates from the THC pharmacokinetic analysis were used to describe the THC profile. Parameter estimation for population PK modelling of THC, Rimonabant and TM38837 was performed under ADVAN 5, and the PK-PD modelling of all PD parameters was performed under ADVAN6 TOL 5. First order conditional estimation with interaction was the standard method of estimation, with exception for VAS feeling high, for which LAPLACE was used. Within each model, additive- and proportional residual error models were compared.

For PK-PD modelling, an effect compartment was incorporated to account for delay in response, in which the concentration-effect was modelled as a linear- and a maximal effect relationship. The drug-effect relations were assumed to cause a horizontal shift on the concentration-effect profile, therefore the drug effects relationships were only applied to the parameter describing the concentration at which half the maximum effect is reached (EC_{50}). Internal model selection and validation was performed using minimum objective function value, goodness of fit plots and visual predictive checks (VPC). For the VPC, 1,000 replications of the model were simulated and the median, 5th and 95th percentiles were calculated for each simulated time-point and compared visually with the actual data (Post et al., 2008).

The inhibition ratios are defined in percentages and quantify the maximum inhibition of the THC-induced effect (defined as 100%) by

either rimonabant, TM38837 100 mg or 500 mg. The median of the inhibition ratios was calculated with their 90% confidence intervals (90%CI) by using the PK-PD models, simulated for 1000 individuals.

To minimize the effect of over- and under-dispersion due to the subjectivity of the VAS scale, and to include non-response in the model, the VAS feeling high scale was translated to a binary scale, to accommodate the possibility to construct a probability model for feeling high. The anchor point for this translation was the median of all scores higher than 0.

RESULTS

Subject demographics

Thirty-six healthy young males were randomised and treated, and 24 subjects completed five occasions. Concerning the parallel part of the study, 10 subjects received rimonabant + THC treatment, and 14 subjects received placebo rimonabant + THC treatment. Four subjects dropped out for personal reasons (i.e. time schedule conflict and not liking the study days), two after the first and two after the second occasion. Three subjects dropped because of adverse events: two during the first occasion (occasion with THC alone treatment) and one during the second (occasion 1 was placebo, occasion 2 THC alone). The adverse events were THC related (i.e. derealisation, pre-syncope and anxious feeling). One subject was not compliant and dropped out after the third occasion. Four subjects suffered study schedule delays and could eventually not complete five occasions because of irremediable expiry dates of rimonabant and THC: one subject after occasion three and three subjects after occasion four. All 36 subjects' completed occasions were included in the analyses. Subject demographics were balanced for all dose arms; the average age was 21.2 years (SD 3.8 years), the average BMI was 22.9 kg/m^2 (SD 2.1 kg/m^2), height was 183.42 cm (SD 6.99 cm) and the average weight was 77.25 kg (SD 10.18 kg).

Adverse effects

Adverse events were of mild to moderate intensity and transitory in nature. No serious adverse events were reported during the study. One subject discontinued during his first occasion with placebo TM38837 + THC treatment due to a pre-syncope and anxiety, which occurred 11 minutes after the first THC inhalation. Another subject discontinued

the first occasion with placebo TM38837 + THC due to anxiety that started 1h29m after placebo TM38837 administration. During the second occasion with placebo TM38837 + THC, one subject decided to discontinue due to derealisation that started 14 minutes after the first THC inhalation.

The total number of subjects that had an adverse event was similar for all treatment groups (90.0%-96.9%), except for the placebo TM38837 + placebo group (63.3%). Most of the adverse events were classified as psychiatric and nervous system disorders, and mainly the psychiatric disorders were considered to be probably THC-related. Euphoric mood ('feeling high') was by far the most frequently reported psychiatric effect, especially in the treatment groups that received THC in combination with the 100 mg dose of TM38837, placebo TM38837, or placebo rimonabant (23/32 or 71.9%, 25/34 or 73.5%, and 13/14 or 92.9% respectively). Euphoria was less common when THC followed administration of TM38837 500 mg or rimonabant (16/31 or 51.6%, and 5/10 or 50% respectively). In the nervous system disorder class, the most frequent adverse event was somnolence, which occurred in a similar frequency for TM38837 100 mg (20/32 or 62.5%), 500 mg (20/31 or 64.5%), rimonabant (5/10 or 50.0%), and placebo TM38837 + THC (19/34 or 55.9%) treatment groups, to a larger extent in the placebo rimonabant + THC treatment group (12/14 or 85.7%) and to a lesser extent in the placebo group (10/30 or 33.3%).

Other frequently occurring adverse events in all treatment groups, including placebo, were: fatigue (14.3% to 41.2%), dizziness (3.3% to 35.7%), headache (6.7% to 18.8%), and hypersomnia (6.7% to 28.6%). These adverse events were less frequent in the placebo group, and of similar frequency in the active treatment groups.

No clinically relevant changes were found for blood pressure, haematology, biochemistry, urinalysis or any of the ECG intervals. Heart rate changes were analysed as pharmacodynamic parameters.

Pharmacokinetics and population pharmacokinetic models

The pharmacokinetics of THC, TM38837 and rimonabant was described by a two compartmental pharmacokinetic model with first-order elimination. The oral absorption of rimonabant and TM38837 followed a first-order process, and the pulmonary absorption of THC was considered as a bolus administration. The increase of the concentration after administration of rimonabant was insufficiently detailed to estimate the first-order absorption rate constant with sufficient precision. Therefore this parameter was fixed to the value for the absorption rate constant reported by Martinez et al. (2007) at Ka = 1.17 h^{-1}. For all models, the residual error model was proportional and individual empirical Bayes' estimates were employed to describe the concentration profile used in the population pharmacokinetic and PK-PD analyses.

An overview of the pharmacokinetic parameters can be found in Table 3 (non-compartmental analysis) and in Table 4 (compartmental analysis). TM38837 and rimonabant show different concentration-time profiles (Figure 1). TM38837 had a relatively flat pharmacokinetic profile compared with rimonabant, which was related to the low absorption rate constant and the low clearance. This caused similar exposure of TM38837 during all five THC challenge tests within a study occasion, whereas for rimonabant during the first 3 THC challenges the exposure levels to the CB_1 antagonist were distinctly higher than for the 2 THC challenges that were given on the second day of a study occasion. TMAX of TM38837 (12.55 h to 13.01 h) was larger compared with rimonabant (4.11 h). TM38837 had a long half-life of 771 h, whereas the half-life of rimonabant was 12.7 h as estimated using compartmental analysis.

Pharmacodynamics

THC showed significant increases on body sway, heart rate, feeling high and external perception. For internal perception, almost half of the subjects showed no response (12 non-responders vs. 16 responders) and the average effect was limited. No THC effect was found on VAS mood, and only limited effects were found on VAS alertness and calmness (estimate of difference: -5.5% and 4.1% respectively). Therefore VAS internal perception, alertness, mood, and calmness were not considered relevant efficacy parameters for evaluating inhibition of THC induced effects, and were therefore not further described.

THC period effects were found for VAS feeling high (0.101 log mm, p = 0.0004) and heart rate (1.4 beats per minute, p = 0.0338). As these changes were very small compared to the treatment effects, the period effects of VAS feeling high and heart rate parameters were not considered to have had a significant impact on the study results.

A graphical representation of TM38837 and rimonabant effect profiles on THC-induced feeling high can be found in Figure 2. TM38837 antagonizing effects started on day 1 and reached their maximum on day 2, whereas for rimonabant the effects were maximal on the first measurement of day 1 (4 hours post dosing) and diminished during the second day. Overall this seemed to be consistent with the plasma concentration time profiles and the shorter T_{MAX} (≈4 h) and $t_{\frac{1}{2}}$ (13 h) of rimonabant as compared to TM38837 using non-compartmental pharmacokinetic analysis.

Because of the different time frames of TM38837 and rimonabant time-effect profiles, no proper comparison of peak effects could be made. Instead, the complete effect profiles were compared from the data of the 5-way subanalysis. The results of these comparisons are given in Table 5. The results of the 4-way cross-over analysis including all subjects were very similar to the results from the 5-way cross-over subanalysis and are therefore not shown. TM38837 100 mg did not significantly inhibit THC effects, except for a small reduction of VAS external perception, which failed to reach significance with the higher dose of TM38837. Both rimonabant 60 mg and TM38837 500 mg inhibited all other THC effects.

Another graphical analysis of effects was performed to avoid the differences in time frames between TM38837 and rimonabant. Figure 3 represents a visualisation of heart rate effects plotted against body sway and P (feeling high›12) expressed as inhibition ratios that were estimated using the PK-PD models. Each point estimate represents the effects that were measured after one of the five THC dosages. The estimated values and 90% confidence intervals (90%CI) are given in Table 6. When comparing the effects on heart rate to those on P (feeling high›12) (expressed in inhibition of the THC effect), rimonabant has similar effect magnitudes for both heart rate and P (feeling high›12) (around 80% inhibition of the THC-effect). TM38837 500 mg maximally inhibits THC-induced heart rate increase by 87.8% (90% confidence interval 80.4, 92.5%) and feeling high by 30.4% (90%CI 24.0, 38.6%). TM38837 100 mg shows a 59.3% (90%CI 45.6, 71.3%) heart rate inhibition against a 7.47% (90%CI 5.5, 10.0%) inhibition of feeling high. The relationship between heart rate and body sway is comparable with the association between heart rate and P (feeling high›12) although slightly less pronounced (see Figure 3 for the relationship between the effects, and Table 6 for the estimated values and 90%CI).

PK-PD modelling

In Figure 4, schematic overviews of the population PK-PD models of TM38837 and rimonabant are given. All PK-PD models included a baseline level, effect compartments that equilibrated with the plasma concentration, and a model to relate the effect compartment concentration to the pharmacodynamic response. The period effects of VAS feeling high and heart rate were included in the THC PK-PD model. Heart rate and body sway were best described by a maximum effect model. For feeling

high a probability model was used to quantify the probability for a VAS score > 12 at the study population level. The VAS value of 12 was the central value in the distribution of positive VAS scores in the study, which served as a reference point. All models included the THC challenge effect and the antagonizing effect of rimonabant and TM38837, either by shifting the EC_{50} (maximum effect model), or decreasing the P (feeling high>12). An overview of the PK-PD parameters can be found in Table 7. The results of the VPCs can be found in the article's supplement.

The population PK-PD models confirmed the expected THC-induced increase of heart rate, body sway, and P (feeling high>12). The equilibration time of THC with the effect compartment was relatively small (0.217 hr for heart rate, 1.94 hr for body sway, and 1.31 hr for P (feeling high>12), indicating a fast onset of the effects. The equilibration half-life of TM38837 was long compared with rimonabant (Heart rate: 85.5 versus 1.27 hr, body sway: 89.4 versus 1.21 hr, feeling high: 4.63 versus 1.27 hr). This caused a larger delay in the onset of TM38837 effects, compared with rimonabant. For heart rate the half maximal inhibitory concentrations (IC_{50}) were similar for TM38837 (65.2 ng/ml) and rimonabant (95.3 ng/ml), whereas for body sway and feeling high, the IC_{50} of rimonabant was 4 times and 56 times larger respectively than for TM38837 (Body sway; TM38837: 49.9 ng/ml, rimonabant: 206 ng/ml. Feeling high; TM38837: 347 ng/ml, rimonabant: 19,500 ng/ml).

Similarly to the graphical differences in time-effect profiles in the previous sections, the inhibition ratios of heart rate, body sway and P (feeling high>12) that were estimated using the PK-PD models suggested a maximal inhibition of TM38837 on day 2, whereas rimonabant's inhibition was maximal at the first measurement on day 1, as can be seen in Table 6. These effect profiles are comparable with the pharmacokinetic profiles of the compounds in Figure 1.

DISCUSSION

This study aimed to investigate the central and peripheral effectivity of TM38837 and rimonabant in healthy subjects. We hypothesised that TM38837 would show no effects or small effects on central nervous system parameters, whereas rimonabant would show large effects on all pharmacodynamic tests. This study suggested that TM38837 is 56 times less potent than rimonabant in antagonizing the THC effect on feeling high when comparing the IC_{50} values, and 4 times less potent on body sway in healthy male volunteers. However, the antagonizing effect on heart rate increase had a similar potency for TM38837 and rimonabant. TM38837 100 mg, which is an anticipated effective human therapeutic dose, did not clearly antagonize THC effects when complete time-effect curves were compared, but between 24 to 27 hours after administration this dose caused close to 60% inhibition of THC-induced tachycardia. In contrast, a therapeutic concentration of rimonabant showed pronounced maximal inhibition ratios that were about 80% for heart rate, body sway as well as feeling high. With heart rate increase being suggested to represent a primarily peripheral effect of THC (Strougo et al., 2008), this altogether implies that TM38837 is able to induce clear peripheral effects with much less central activity, whereas rimonabant shows relatively large central effects. These acute outcomes suggest that TM38837 could be effective for peripherally associated clinical indications such as metabolic disorders, with a lower propensity for centrally mediated side effects than rimonabant (Van Gaal et al., 2005; Van Gaal et al., 2008), although this clearly still needs to be demonstrated in prolonged studies with more relevant metabolic endpoints.

Population PK and PK-PD analysis

The current population PK and PK-PD models could be used for simulations of new study designs. However, the possibilities of TM38837 multiple dose designs could not be explored accurately. Due to the low clearance and the long terminal half-life, an unknown accumulation of the TM38837 plasma concentration and effects could occur after multiple dosing. The low clearance and long terminal half-life could be caused by factors like a slow inter-compartmental clearance, but this could not be examined any further with the current study design. A future multiple dose study, or a study using labelled TM38837 could investigate the influence of the pharmacokinetic parameters on the accumulation of TM38837 in a multiple dose design.

The population pharmacokinetic model of TM38837 calculated a terminal half-life of 771 h, whereas the non-compartmental analysis found a terminal half-life of approximately 12 to 13 hours. The reason for this apparent discrepancy is that the population pharmacokinetic model included the pre-dose 'baseline' samples, which often contained measurable TM38837 concentrations of previous occasions despite long washout periods. This provided an improved accuracy of terminal half-life estimation. The non-compartmental analysis was not able to include the pre-dose 'baseline' because of the sparse and relatively short sampling scheme during the terminal elimination. The terminal half-life that was estimated using the non-compartmental analysis is therefore not a reliable estimation of the terminal half-life, but rather an estimation of a pre-terminal half-life.

The current study design did not include measurements between 11 and 24 hours after drug administration, which would have been difficult to perform and interpret during the night. This could have influenced the estimation of some of the pharmacodynamic and pharmacokinetic parameters, such as the (time of) maximal concentration and effect, which for TM38837 may well have fallen within this period. In a future study, the PK-PD model should be optimised by the integration of data from another sampling scheme that would compensate for the time points between 11 and 24 hours after TM38837 administration. This could result in more accurate estimations of the pharmacokinetic and pharmacodynamic effects. However, the current study found a large variability on the bioavailability of TM38837 that could not be explained completely by the time gap in the sampling scheme. This large inter-individual variability in TM38837 pharmacokinetics could be caused by several factors, such as inter-individual differences in absorption, which could not be explored in the current study.

In conclusion, TM38837 induces relatively strong effects on heart rate compared to the central nervous system effects. At the anticipated therapeutic dose, no clear central nervous system effects were found, in contrast to pronounced heart rate effects. Compared to rimonabant, TM38837 exhibits relatively strong heart rate effects compared to central nervous system effects, which might be an indication of peripheral selectivity. TM38837 doses up to 100 mg can possibly induce beneficial effects in patients suffering from metabolic disorders, without centrally mediated side effects.

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TABLE 1 Overview of study day procedures

Time (hours)	Procedures study day
-1h30m – 0h00m	Arrival, breakfast, vital signs, drug screen, alcohol breath test, PD-block* (twice), blood sampling TM38837 or rimonabant
0h00m	**TM38837 administration**
1h30m	Snack
2h00m	**Rimonabant administration**
2h48m – 4h00m	PD-block* (twice), vital signs, lunch
4h00m	**1st THC administration**
04h05m – 06:30h	Blood sampling TM38837 or rimonabant (04h06m) and THC (04h05m, 4h19m, 5h50m), PD-block* (thrice), snack
6h30m	**2nd THC administration**
6h35m-9h00m	Blood sampling TM38837 or rimonabant (6h36m) and THC (6h35m, 6h49m, 8h20m), PD-block* (thrice), dinner
9h00m	**3rd THC administration**
9h05m – 1d00h00m	Blood sampling TM38837 or rimonabant (9h06m) and THC (9h05m, 09h19m, 10h50m), PD-block* (four times), vital signs (twice), breakfast
1d00h00m	**4th THC administration**
1d00h05m – 1d02h30m	Blood sampling TM38837 or rimonabant (1d00h06m) and THC (1d00h05m, 1d00h19m, 1d00h50m), PD-block* (thrice), vital signs, lunch
1d02h30m	**5th THC administration**
1d02h30m – 1d06h00m	Blood sampling TM38837 or rimonabant (1d04h21m) and THC (1d02h35m, 1d02h49m, 1d04h20m), PD-block* (thrice), vital signs, snack

* Pharmacodynamic block consists of body sway measurement, VAS B&L and Bowdle, heart rate measurement. AEs and concomitant medication were recorded continuously.

TABLE 2 The study consisted of a 4-way cross-over part and a parallel part. Rimonabant or placebo rimonabant were always randomly administered at the fifth occasion. All subjects received all treatments from occasion 1 to 4 and the subjects were split up in two groups for occasion 5 with half of the subjects receiving rimonabant 60 mg and the other half received placebo rimonabant 60 mg.

Occasion Study design	Study sample	TM38837*	Rimonabant	THC**
Occasion 1-4 Cross-over	100%	100 mg	Placebo	Placebo
		500 mg	Placebo	Placebo
		Placebo	Placebo	5·4mg
		Placebo	Placebo	Placebo
Occasion 5 Parallel	50%	Placebo	Placebo	5·4mg
	50%	Placebo	60 mg	Placebo

* Penn Pharma, Gwent, United Kingdom
** Farmalyse b.v., Zaandam

TABLE 3 Pharmacokinetic parameters from non-compartmental analysis with means (standard deviation). Median $t_{1/2term}$ was 12.08 h for TM38837 100 mg and 12.98 h for TM38837 500 mg.

	TM38837 100 mg	TM38837 500 mg	Rimonabant
C_{max} (ng/ml)	2,860 (2,377)	12,449 (1,620)	620 (113)
T_{max} (h)	13.01 (8.28)	12.55 (8.53)	4.11 (0.03)
$AUC_{0-\infty}$ (ng.h/ml)	86,088 (49,862)	327,907 (190,569)	6,952 (1534)

TABLE 4 Population PK parameter estimates for TM38837, rimonabant and THC with relative standard error (RSE, %) inter-individual variability as %CV. F=Bioavailability; IIV=inter-individual variability (%) IOV=inter-occasion variability (%)

	THC			TM38837		Rimonabant	
Parameter estimate	Estimate (RSE)	IIV	IOV	Estimate (RSE)	IIV	Estimate (RSE)	IIV
Clearance/F (L/h)	200 (5.9)	31.2	-	2.20 (9.29)	66.2	9.30 (6.87)	25.6
Central volume/F (L)	28.5 (8.91)	40.8	25.1	18.7 (16.3)	132.0	39.3 (15.5)	20.6
Peripheral volume of distribution/F (L)	107 (14.3)	-	-	10.8 (42.4)	-	93.0 (12.8)	-
Intercompartmental clearance/F (L/h)	106 (6.9)	-	-	0.00975 (22.0)	-	17.9 (17.2)	-
Absorption rate constant (Ka; h^{-1})	-	-	-	0.0789 (9.72)	-	1.17**	-
Terminal half-life (h)*	1.11 (11.0)	4.98	-	771 (21.8)	-	12.7 (11.5)	-

* Parameter derived from the model
** Fixed parameter

TABLE 5 Statistical analysis of pharmacodynamic parameters. Estimated difference (90% confidence interval) and p-value. Bold numbers are the significant differences (P<0.05). Data were analysed for the complete time profile.

	VAS feeling high *	VAS external *	Body sway	Heart rate
Rimonabant vs Placebo	-	-	29.60% (14.3%, 47.1%) **p=0.0012**	4.60% (-0.2%, 9.3%) p=0.1177
TM38837 100 mg vs THC	0.20% (-12.5%, 12.9%) p=0.9753	-10.20% (-18.1%, -2.2%) **p=0.0379**	-1.20% (-11.6%, 10.5%) p=0.8605	-3.70% (-7.5%, 0.2%) p=0.1137
TM38837 500 mg vs THC	-22.10% (-34.9%, -9.4%) p=0.0059	-7.80% (-15.7%, 0.0%) p=0.1005	-12.20% (-21.6%, -1.7%) p=0.0588	-8.90% (-12.8%, -5.1%) **p=0.0003**
Rimonabant vs THC	-26.70% (-40.9%, -12.6%) p=0.0030	-17.80% (-26.5%, -9.1%) **p=0.0016**	-7.10% (-18.1%, 5.3%) p=0.3287	-7.30% (-11.5%, -3.0%) **p=0.0063**
TM38837 100 mg vs Rimonabant	-26.90% (-40.9%, -12.9%) **p=0.0025**	-8.50% (-18.1%, 1.1%) p=0.1440	-6.00% (-17.0%, 6.3%) p=0.4017	-3.70% (-8.2%, 0.7%) p=0.1650
TM38837 500 mg vs Rimonabant	-5.90% (-23.9%, 12.1%) p=0.5815	-10.80% (-20.2%, -1.5%) p=0.0586	5.70% (-6.6%, 19.7%) p=0.4547	1.80% (-2.8%, 6.5%) p=0.5089

* Tests were analysed without placebo results, as these showed no variance.

TABLE 6 Simulated inhibition ratios (%) with 90% confidence intervals (90%CI) of THC-induced effects by TM38837 100 mg and 500 mg, and rimonabant 60 mg calculated per THC administration.

Parameter	THC dose	100 mg TM38837		500 mg TM38837		Rimonabant	
		Estimate	90%CI	Estimate	90%CI	Estimate	90%CI
Body Sway	1 (1st day)	3.24	(1.34, 7.85)	14.1	(6.33,29.1)	77.4	(58.2, 88.4)
	2 (1st day)	4.89	(1.94, 11.8)	20.3	(8.98,39.7)	71.3	(46.2, 87.9)
	3 (1st day)	6.42	(2.47, 15.3)	25.4	(11.2,47.0)	61.2	(34.3,82.7)
	4 (2nd day)	18.8	(8.84, 35.9)	53.2	(32.2, 73.5)	44.4	(22.7, 69.7)
	5 (2nd day)	16.3	(7.19, 33.3)	49.2	(27.9, 71.1)	35.7	(16, 62.2)
Heart Rate	1 (1st day)	16.4	(10.2,25.5)	47.7	(35.2, 60.4)	75.6	(60.6, 85.2)
	2 (1st day)	27	(17.6, 39.0)	64	(51.1, 75.5)	74	(54.8, 86.3)
	3 (1st day)	35.3	(24.1, 48.5)	72.6	(61, 82.1)	67.2	(46.0, 82.5)
	4 (2nd day)	58.4	(44.8, 70.7)	87.4	(79.9, 92.2)	43.8	(25.0, 63.6)
	5 (2nd day)	59.3	(45.6, 71.3)	87.8	(80.4, 92.5)	39.9	(22.1, 60.1)
Feeling high	1 (1st day)	3.52	(2.11, 5.77)	16	(9.91, 24.5)	84.6	(75.7, 89.8)
	2 (1st day)	5.75	(3.57, 8.73)	24.6	(16.1, 34.4)	83.4	(66.1, 89.8)
	3 (1st day)	7.13	(4.76, 10.6)	29.7	(21.0, 40.3)	78.9	(56.9, 86.1)
	4 (2nd day)	7.47	(5.5, 10.0)	30.4	(24.0, 38.6)	52.2	(33.4, 63.1)
	5 (2nd day)	6.85	(5.09, 9.31)	28.3	(22.3, 36.2)	47.8	(30.5 59.1)

TABLE 7 Population PK-PD parameter estimates for VAS feeling high, body sway and heart rate with relative standard error (RSE, %). T_{50} = equilibration half-life; E_{max} = maximal effect; EC_{50} = concentration at 50% of maximal effect; IC_{50} = concentration of antagonist at 50% of maximal inhibition; C_{THC} = concentration of THC

Parameter	VAS feeling high P (feeling high >12) (RSE)	Body sway (mm per 2 min) (RSE)	Heart rate (BPM) (RSE)
T_{50} THC (hr)	1.31 (7.9)	1.94 (92.6)	0.217 (19.2)
T_{50} Rimonabant (hr)	1.27 (77.5)	1.21 (26.2)	1.27 (32.4)
T_{50} TM38837 (hr)	4.63 (56.4)	89.4 (24.7)	85.1 (14.3)
Baseline	-	230 (5.25)	63.2 (1.8)
E_{max}	-	134 (33.5)	44.0 (39.0)
EC_{50} THC (ng/ml)	-	3.49 (89.2)	33.1 (54.1)
IC_{50} Rimonabant (ng/ml)	-	49.9 (71.2)	95.6 (55.2)
IC_{50} TM38837 (ng/ml)	-	206 (56.3)	65.4 (33.4)
Baseline P (feeling high >12)	0.00656 (30.0)	-	-
C_{THC} at P=0.5 (ng/ml)	9.75 (4.4)	-	-
C_{THC} at P=0.5 during 1st dose (ng/ml)	6.21 (7.3)	-	-
IC_{50} Rimonabant (ng/ml)	347 (31.9)	-	-
IC_{50} TM38837 (ng/ml)	19500 (27.9)	-	-

FIGURE 1 Mean concentration-time profile of TM38837 100 mg (black dots) and 500 mg (blank dots) in the left graph, and the profile of rimonabant in the right graph. TM38837 treatments were administered at t=0 h, and rimonabant was administered at t=2 h. Error bars represent the standard deviation.

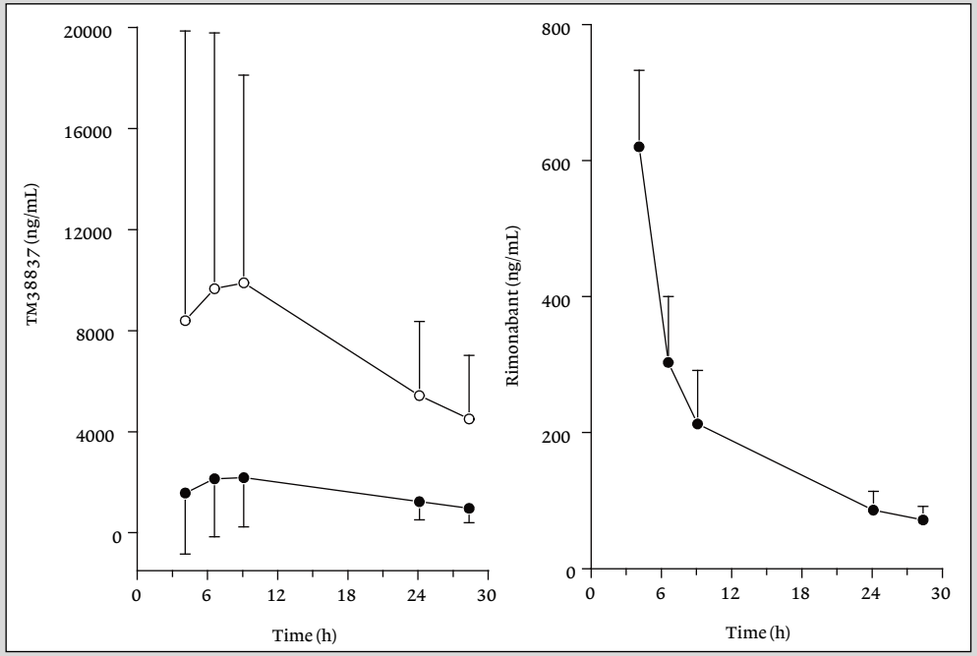


FIGURE 2 Effect-time profiles of feeling high after TM38837 100 mg (triangles), 500 mg (open dots), rimonabant (squares) or placebo antagonist (black dots) administration.

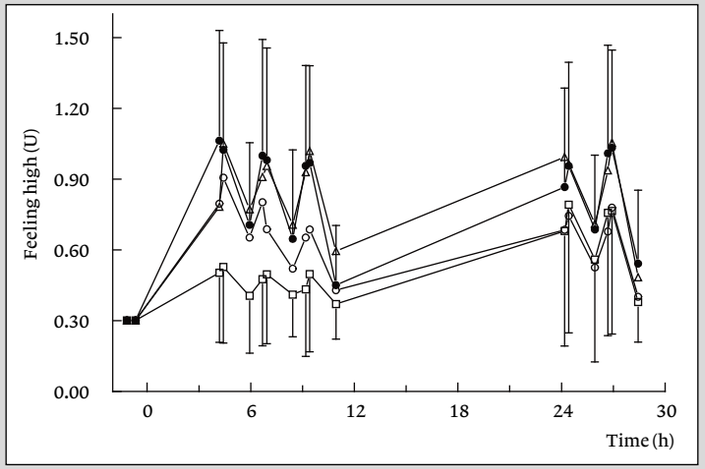


FIGURE 3 Simulated inhibition ratios (%) of THC-induced effects by TM38837 100 mg (triangles) and 500 mg (squares), and rimonabant 60 mg (dots) calculated per THC administration. Figure A gives the relationship between heart rate (assumed to primarily represent a peripheral CB_1 effect) and body sway, and figure B between heart rate and P (feeling high<12).

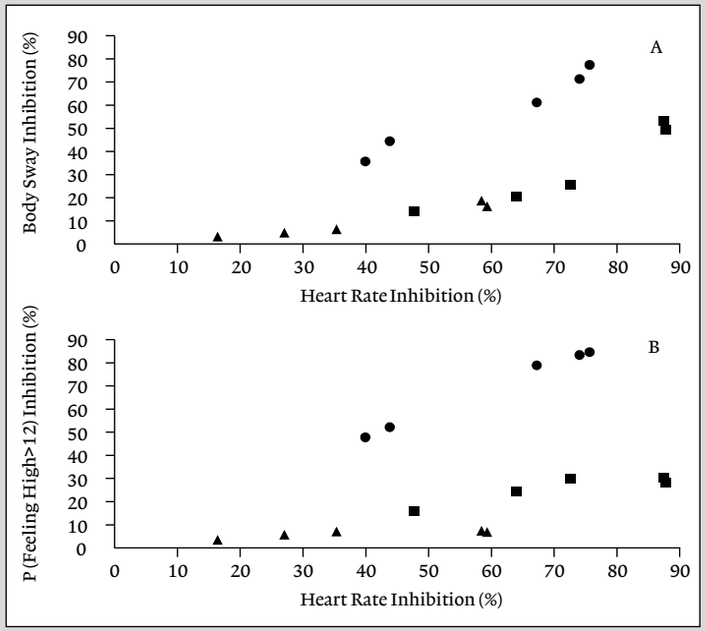
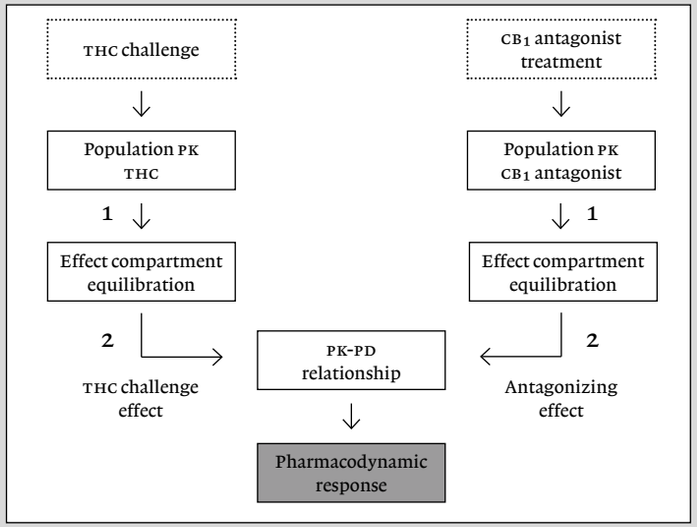


FIGURE 4 Schematic representation of the PK-PD models of TM38837 and rimonabant. 1: influence of plasma concentration, 2: influence of concentration in effect compartment.



CHAPTER VI

Pharmacokinetic / pharmacodynamic modelling and simulation of the effects of different CB1 antagonists on Δ9-tetrahydrocannabinol challenge tests in healthy volunteers

Submitted for publication

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ABSTRACT

AIM Although CB_1 antagonists are less widely studied due to market withdrawal of rimonabant, this drug class is still very interesting due to a the therapeutic potential. The severe psychiatric side effects might be overcome due to careful management of drug development, including improved studies in healthy volunteers by using CB_1 agonist challenge tests and thorough pk/pd analyses. We aimed to build pk/pd models suitable for direct comparisons of pharmacological compounds in complex clinical setting using a pharmacological challenge test. Secondly, we wanted to apply the model to make a direct comparison between four CB_1 antagonists.

METHODS The pharmacokinetic models of multiple thc administrations and the four antagonists drinabant, surinabant, rimonabant and tm38837 were built separately. Next, the thc-induced effects in healthy volunteers, including changes on heart rate and the visual analogue scale of feeling high were modelled by a PK/PD model linked to the thc pk model. Then, the inhibition of the thc-induced effects by the antagonists was quantified by incorporating components representing the inhibitory effect. The delay between drug concentration and drug effect was described using a biophase compartment. A benchmark simulation was then used based on a constructed model to evaluate the reduction rate of each antagonist on the reversal of the thc-induced effect in a unified simulation scenario.

RESULTS The final PK model of THC and antagonists was a two-compartment model with first order absorption and first order elimination. An E_{MAX} model and logistic regression model were used as effect measures and the antagonist effect was added in these models in a competitive binding manner. $T_{1/2ke0}$ ranged from 0.00462 to 63.7 hours for heart rate and from 0.964 to 150 hours for VAS. IC_{50} ranged from 6.42 to 202 ng/ml for heart rate and from 12.1 to 376 ng/ml for VAS. RSEs were <100% for heart rate, and <65% for feeling high, except for a 193% RSE on T_{50} after rimonabant administration. After the benchmark simulation, drinabant and TM38837 showed relatively larger effects on heart rate than feeling high compared to surinabant and rimonabant.

CONCLUSIONS Our PK/PD modelling and simulation approach was suitable for modelling and simulation of heart rate and feeling high for four CB_1 antagonists in a THC challenge test. We were able to directly compare four antagonists and we found differences in efficacy profiles that might be translated to differences in therapeutic efficacy in future studies.

INTRODUCTION

Obesity is one of the world wide, emerging, serious, life threatening diseases (World Health Organization, 2011). The lack of efficient and well-tolerated drugs to treat obesity has led to an increased interest in new targets for the development of new drugs (Patel and Pathak, 2007; Barth, 2005). A specifically interesting target is the CB_1 receptor, which is located in the central nervous system (CNS) and at peripheral sites such as the heart, liver, pancreas and adipose tissue (Bermudez-Silva et al., 2008; Bermudez-Silva et al., 2010). At these sites, the CB_1 receptor has a modulatory role in the regulation of a variety of complex physiological systems, such as the nervous system, and the digestive and endocrine system in metabolism (for a review, see Melamede (2005)). Activation of the CB_1 receptor leads to effects including feeling high and altered time perception, increased body sway and getting hungry ('the munchies') (for a review, see (Zuurman et al., 2009)).

This widespread involvement of the CB_1 receptor and its ligands provides numerous opportunities for the development of new medicines for neuronal and metabolic disorders including movement disorders, diabetes mellitus, and dyslipidemia. In the late 1990's the pharmaceutical industry became particularly interested in the metabolism effects of CB_1 receptors and focused on new chemical entities that could decrease appetite by CB_1 receptor antagonism. It was found that CB_1 antagonists were indeed able to block feeding behaviour and they also showed other characteristics (including decreased gastric emptying and increased insulin sensitivity (Patel and Pathak, 2007; Xie et al., 2007)) that underlined the potential of CB_1 antagonist in obesity treatment.

In 2006, the first CB_1 antagonist rimonabant (formerly known as SR141716) was registered for the treatment of obesity and overweight with obesity-associated disorders (Wathion, 2009). Besides rimonabant, Sanofi developed more CB_1 antagonists, such as drinabant (formerly known as AVE1625 with possible inverse agonism properties) and surinabant (SR147778). However, in 2008, rimonabant was withdrawn from the market due to unacceptable psychiatric adverse effects. Almost all pharmaceutical companies, including Sanofi, terminated all studies involving CB_1 receptor antagonists (such as rimonabant, otenabant and taranabant).

Nevertheless, there are studies suggesting that the beneficial metabolic effects of rimonabant might be regulated predominantly by peripheral CB_1 receptors, whereas the psychiatric side effects could be regulated by centrally located CB_1 receptors (Cluny et al., 2010; Nogueiras et al., 2008). There is considerable evidence to suggest that the beneficial metabolic effects of CB_1 antagonists are mediated by CB_1 receptors that are present at locations which are specifically associated with metabolic regulation, such as the liver, the pancreas, and fat cells (Bermudez-Silva et al., 2008; Bermudez-Silva et al., 2010; Gomez et al., 2002). If the therapeutic effects of CB_1 antagonists have their target site in peripheral tissues and the (serious) side effects originate in certain regions of the CNS, it is crucial to understand how the specific antagonist could affect the several central and the peripheral target sites.

One of the problems with the investigation of the different sites and effects of CB_1 antagonism is that there are now validated measurements of these effects after acute administration of CB_1 antagonists or in healthy subjects. To partly overcome this problem, challenge tests with the $CB_1/_2$ partial antagonist Δ^9-tetrahydrocannabinol (THC) were developed (Klumpers et al., 2013; Strougo et al., 2008; Zuurman et al., 2008). With this challenge test, the endocannabinoid system is stimulated using THC, which induces a range of dose- and concentration-related responses. Several of these measures, such as the characteristic euphoric 'high' feeling, are clearly indicative of central nervous system effects. Other parameters like heart rate are more likely to be peripherally mediated (Strougo et al., 2008; Zuurman et al., 2008; Zuurman et al.,

2008). The THC-challenge has been found to be an effective tool to demonstrate the pharmacological effects of a CB_1 antagonist, since co-administration of a selective CB_1 antagonist causes a near-complete block of the acute THC-induced effects. The use of the variety of measures such as feeling high, body sway and heart rate allow us to create individual effect profiles for the different CB_1 antagonists.

Previously, our clinical research centre separately investigated the concentration-effect relationships of four different CB_1 antagonists: rimonabant, surinabant, AVE1625 (drinabant) and TM38837 (Zuurman et al., 2010; Klumpers et al., 2013). This was performed in three separate studies by using THC-challenge tests, all with different THC dosages and dosing time intervals. This approach allowed us to analyse the pharmacological characterisation of the individual antagonists. However, a thorough comparison among the antagonists was hampered by the different dose regimes of the THC challenge tests. In the current study, we built an integrated PK/PD model for all antagonists that would compensate for these differences between the THC challenge tests, allowing a direct comparison of the different CB_1 antagonists with regards to PK and PD characteristics.

PK/PD modelling is an approach to characterize the concentration–time profile and the relationship between concentrations and effects using a mathematical model. Model estimation can be based on both individuals and populations. The assumption that all individual concentration–effect relationships can be described with the same structural model is based on the notion that the drug activates the same pharmacological system in all subjects (or systems for different responses). PK/PD modelling is performed by using a non-linear mixed effect modelling approach which provides estimates of the population average parameters (assuming that each individual can be described using the same structural model) and their associated inter-individual variability, which allows individuals to differ from each other. Residual error describing the variability of the difference between predicted values and the observations is also estimated (Beal, 2013; Holford and Sheiner, 1981). Simulation is a subsequent step, following the modelling. It can be used to predict model outcomes using an existing model structure given different scenarios (model input), for instance with different dosages, sampling times and other covariates.

Our first aim was to build an integrated PK/PD model that would be suitable for direct comparisons of pharmacological compounds in complex clinical setting using a pharmacological challenge test. We would do this for four different CB_1 antagonists (drinabant, surinabant, rimonabant and TM38837) and a THC-challenge test for efficacy parameters feeling high and heart rate. Our second aim was to apply the model for direct comparisons of the different pharmacokinetic profiles and efficacy of the four different CB_1 antagonists to better understand the behaviour of CB_1 antagonists in healthy humans.

METHODS

Study designs

From 2003 until 2009, three THC challenge studies were performed at CHDR in healthy male volunteers, in which four CB_1 antagonists were administered: a study with drinabant (AVE1625), one with surinabant (SR147778), and another study that investigated both rimonabant (SR141716) and TM38837 (referred to as 'the rimonabant-TM38837 study') (Tonstad and Aubin, 2012; Zuurman et al., 2008). The three studies were all performed in a double-blind, randomized, placebo-controlled, (partial) cross-over manner. The complete design and clinical results of these studies were published separately (Zuurman et al., 2010b; Klumpers et al., 2013c; Klumpers et al., 2013). The treatments per study and subject demographics are summarized in Table 1 and Table 2, respectively. In short, each CB_1 antagonist or placebo administration was followed by a series of inhaled doses of a vaporized solution of THC in ethanol or THC vehicle, which consisted only of vaporized ethanol. THC was vaporized using a Volcano vaporizer® (Storz & Bickel GmbH & co. KG, Tuttlingen, Germany). In each study, the first THC dose was administered around the expected T_{MAX} of the CB_1 antagonist. Blood sampling for PK and selected PD responses were taken accordingly after multiple THC challenge and/or antagonist administration and the last sampling time points were shortly after the last challenge dose of THC.

Pharmacokinetic and pharmacodynamic measurements

Blood samples of THC and four antagonists were analyzed as published before (Zuurman et al., 2010b; Klumpers et al., 2013c; Klumpers et al., 2013). In short, THC samples were measured using tandem mass spectrometry with a lower limit of quantification of 0.1 ng/ml. Concentration of AVE1625 was measured using Flow Chromatography – Mass Spectrometry/Mass Spectrometry (TFC-MS/MS) and the limit of quantification was 0.2 ng/ml. Concentration of surinabant was measured using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) method with a lower limit of quantification (LLOQ) of 1.0 ng/ml. Concentrations of TM38837 and rimonabant were measured by liquid chromatography with tandem mass spectrometry method with a lower limit of quantification of 0.1 ng/ml for TM38837, and 1.0 ng/ml for rimonabant.

In all studies, Visual analogue scales (VAS) according to Bowdle (psychedelic effects) and heart rate were assessed frequently (Bowdle et al., 1998; Zuurman et al., 2008). Heart rate was measured using Nihon-Koden BSM-1101K monitor (Lifescope EC, Tokyo, Japan) blood pressure apparatus. The adapted version of the Bowdle scales consists of 100 mm visual analogue lines, to indicate subjective feeling high, and on a range of other subjective effects that cluster as factors internal perception and external perception, both composite scores that are affected differently by THC as previously described (Zuurman et al., 2008).

Modelling and simulation

PK and PK/PD modelling was performed using population approach nonlinear mixed effect modelling program NONMEM 7.1.0 (Beal, 2013). Nonlinear mixed effect modelling considers the repeated observations as a function of time in a population of individuals. The model to describe these observations adopts a common structural model and distribution of residuals, while allowing the parameters in the model to vary between individuals. The location (typical value or fixed effect) and spread between individuals (variability or random effect) of the model parameters are estimated by fitting the parameters to the data by minimizing an objective function based on the log likelihood (–2 x LL). Using the popu-

lation values (both location and spread), individual specific empirical Bayes' estimates (post hoc estimates of individual deviates (ETAS) from the random effects distributions) are determined that allow description of individual time profiles.

Different models are compared with increasing complexity in the structural model and the number of random effects. The objective is to find the simplest model that describes the data adequately. Competing models are compared using the likelihood ratio test, which compares the difference between log-likelihoods for the models (difference in objective function value, Δ OFV) to a Chi-square distribution with degrees of freedom corresponding to the difference in number of parameters between the two models (p-value used was less than 0.01: Δ OFV = -6.63). Models were qualified by visual inspection for goodness of fit and check of weighted residuals.

A general overview of the two-step modelling approach is displayed in Figure 1. First, PK models for THC and four antagonists were built separately for every compound to obtain estimated PK parameters based on OFV and goodness of fit. The PK model was only built to optimally describe the PK profile. Therefore, a separate THC model (if possible with a similar structure) was built for each of the three studies. Secondly, the PK/PD model was built. The integrated models only regard the PD models to enable direct comparison of the different CB_1 antagonists. Individual empirical Bayes' estimates were determined to describe the concentration profile and used in the subsequent PK/PD analyses. Parameter estimation for population PK modelling of THC and antagonists was performed under ADVAN 5 and the PK/PD modelling of all PD parameters was performed under ADVAN6 TOL 5. The RSE (relative standard error) was calculated for all parameters. Inter-individual variability (IIV) and inter-occasion variability expressed as coefficient of Variation (%CV) using:

(1) $$\%\mathrm{CV} = 100 \times \sqrt{\exp(\omega^2) - 1}$$

First order conditional estimation (FOCE) with interaction was the standard method of estimation, with the exception of VAS feeling high PD model, for which LAPLACE was used. Within each model, additive and/or proportional residual error models were compared.

Pharmacokinetic and pharmacodynamic analyses

The population PK model of THC was based on the results of previous CHDR studies with multiple THC inhalations, using a two-compartment model with bolus administration (Strougo et al., 2008) and first order elimination. PK analyses of four antagonists were performed in a similar way with compartmental model, including first order absorption and first order elimination.

A biophase compartment is used when drug action is delayed by distribution from plasma to the site of action. The rate of equilibration of drug in the plasma with the site of action is denoted k_{eo}, the rate constant for exit of drug from the biophase compartment (Groenendaal et al., 2008; Hull et al., 1978; Sheiner et al., 1979).

A biophase compartment was first used to account for the delayed response of VAS. To minimize the effect of over- and under-dispersion due to the subjectivity of the VAS scale, and to include non-response in the model, the VAS feeling high scale was translated into a binary scale, to accommodate the possibility to construct a probability model for feeling high (Klumpers et al., 2013). The anchor point for this translation was the median of all scores higher than 0 (on a 100 point scale) for the treatment arms where only THC was dosed. Inverse logit transformation is used for binary data:

(2) $$P(VAS > CUT) = \frac{\exp(-kd \times TAD) \times \exp(x)}{1 + \exp(x)}$$

With

(3) $$x = \frac{\beta_1 \cdot C_{THC}}{1 + \beta \cdot C_{Antgonist}}$$

In which CUT was the anchor point that changed depending on the study; b_1 is the coefficient of THC effect; b is the coefficient of the shift of the THC effect caused by the antagonist. The effect of antagonists in the above equation reverses the THC induced increase in probability of scoring a VAS>CUT. Every subject receives multiple THC inhalations, causing a tolerability that affected the scores of the VAS. To cope with this, *kd*, the elimination rate of tolerance, was included to decrease the possibility of feeling high caused by time factor TAD, the time after the first dosing time point.

A biophase compartment was used to account for the delayed response of heart rate as well. Because the all antagonists bind with the CB_1 receptor in competition with THC, the biophase compartment concentration of THC and respective antagonist was used for the PD analyses by using a maximum effect equation (Eq. 4). In this equation, the antagonist could cause a shift to the right of the apparent EC_{50} depending on the impact of the THC challenge effect and the effect is described as:

(4) $$E = E_0 + \frac{E_{\max} \times C_{THC}}{EC_{50} + C_{THC} + \beta \times C_{Antagonist}}$$

Where E^0 is the baseline of the effect; E_{MAX} is the maximal achievable effect; C_{THC} is THC concentration in biophase compartment; EC_{50} is the concentration that causes 50% of the E_{MAX}. *b* is the coefficient that describes the antagonist shift by the THC effect; Cantagonist is the CB_1 antagonist concentration in the biophase compartment.

Based on PD model parameters, IC_{50} and $t_{1/2ke0}$ can be then be derived from parameter estimation by using equations 3 and 4. These two parameters describe the inhibition potential of CB_1 antagonists. IC_{50} is a measure of the effectiveness of a compound in inhibiting biological function. It indicates how much of a particular antagonist is needed to inhibit a given effect of THC by half. $t_{1/2e0}$ is the apparent half life of a drug effect. It is derived from k_{e0}, which indicates the rate constant of the elimination of a drug effect:

(5) $$IC_{50} = \frac{EC_{50}}{\beta}$$

(6) $$T_{1/2ke0} = \frac{\log 2}{k_{e0}}$$

Visual predictive checks

Visual predictive checks (VPC) were performed for all PK and PD models using R version 2.12.0 (R: A Language and Environment for Statistical Computing, R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria, 2010) with the lsoda (deSolve Package 1.8.1) and mvrnorm functions (MASS Package v7.3-8). The visual predictive check encompassed a projection of the simulated dependent variable as a function of time using the final model on the observations. The simulations were performed considering the estimated population parameters (Q vector) as well as the covariance matrix describing IIV (W matrix). The residual variability (S matrix) was not included in the simulations. The simulations and the data were grouped by antagonists' dose. Summary statistics of the simulations (median and the 95% prediction interval of the simulated IIV) enabled a comparison of the predicted and the observed variability. For each dose group 1000 individuals were simulated.

Simulation

We selected a benchmark scenario to try to maximally cover the major part of the original study designs. Due to differences in T_{MAX}, the time points of the first THC administration relative to the antagonist administration were different among the different study designs. As original study designs, the time between administration of CB_1 antagonists and first THC was the same as the T_{MAX} of antagonist. In this way, the first THC inhalation would be administered at the expected T_{MAX} of CB_1 antagonists. We kept this the same in the benchmark scenario and we compensated for these differences by simulating the

THC challenge profile rather than using the actual challenges. During this simulated challenge, individuals received 4 doses (2, 4, 6 and 6 mg) of THC inhalation at an hourly interval. Drinabant, surinabant, rimonabant and TM38837 were simulated as single dose administered at 3, 1.5, 2 and 4 hours before THC challenge, respectively, similar to the dose regimens in the actual studies. A wide dose range of the antagonists was simulated with dosages from 2mg to 1000mg to optimise the dose response curve. The reduction rate was used as drug response in the dose response curve and was calculated as the difference between the AUC (area under the curve) of the PD response of the THC challenge only and the THC challenge + antagonist. For AUC calculation observations were used from the first administration of THC until one hour after the 4th THC administration. The reduction rate was calculated as:

$$(7) \quad RR = \frac{AUC_{THC} - AUC_{Antagonist+THC}}{AUC_{THC}} * 100$$

Where RR is the reduction ratio, AUC_{THC} is the area under the curve of THC alone; $AUC_{antagonist+THC}$ is the area under the curve of co-administration of THC and the antagonist.

Simulations were performed in a similar way as for VPC by implementing the identified models and the estimated parameters in R using the function lsoda from the deSolve library (version 1.8.1) and the function mvrnorm from the MASS library (version 7.3-8). The results of the simulations were used to plot the population-typical dose-response curves.

RESULTS

THC pharmacokinetic modelling

In all three studies, a two-compartmental structure model with first-order elimination was the best model to describe THC concentration-time curve. The pulmonary administration was implemented as a bolus input in the central compartment.

The PK parameters of THC of the three separate studies are presented in Table 3. No significant differences were found among the studies for the model parameter estimations and they were also similar to the parameters from the models by Strougo et al. (2008). All RSEs of the estimations were smaller than 30% (from 5.19% to 14.3%). Inter-individual variability (IIV) was identified on the apparent central distribution volume, ranging from 10.3% to 40.8%. IIV on apparent clearance was 18.8% and 31.2% for the drinabant study and the rimonabant-TM38837 study, separately. For the surinabant study the IIV on the apparent clearance could not be identified. Additionally, inter-occasion variability on the apparent central distribution volume was included to account for differences in bioavailability between individual dosing occasions in the surinabant study and the rimonabant-TM38837 study and was 78.0% and 25.1% respectively. The residual error model was only proportional to concentration.

Antagonist pharmacokinetic modelling

The PK models of the four antagonists were built separately. All of them could be described using a two-compartmental model with first-order elimination and first-order absorption. Surinabant was found to have a lag time of 0.550 hours (RSE = 5.7%) and its ka was dose-proportional with a dose effect of 0.00486 (RSE = 14.4%) as defined by the following equation:

(8) $ka(dose) = 0.448 \times (1 - \alpha \times dose)$

In wich α is the dose effect to ka. For each compound, the RSEs of the parameter estimations varied between 3.91% and 42.4% (Table 4). IIV and IOV were incorporated in the model if it improved goodness of fit. IIV for the clearance of surinabant, rimonabant and TM38837 ranged from 25.6% to 66.2%. For apparent central distribution volume, the IIV varied from 20.6% to 132%. The goodness of fit plot was improved by adding an IOV of 24% for the central distribution volume of drinabant. Inspection of the data showed that the upswing of the concentration after administration of rimonabant was insufficiently detailed to estimate the first-order absorption rate constant. Therefore, this parameter was fixed to the value for the absorption rate constant as reported by Martinez (Martinez et al., 2007). The PK parameter estimations of the antagonists were presented separately in Table 4, including the RSE, inter-individual variability and inter-occasion variability. VPCs and diagnostic plots were also performed for all four antagonists PK model for model validation.

The THC-induced effects were modelled using data from treatment arms with THC dosages only. To enable a direct comparison of the antagonists, an integrated THC PD model was applied on the three trials for the same set of PD parameters: heart rate and feeling high. An E_{MAX} model gave the best fit for heart rate. The baseline was estimated at 64.2 bpm with a RSE of 1.14%. Within the study, the highest heart rate observed was around 120 bpm. Although physiologically, higher heart rates are possible for higher THC dosages, we chose to fix the E_{MAX} of heart rate to two times the baseline, resulting in proper diagnostic plots and VPCs. IIV and IOV were both incorporated at the baseline at 7.98% and 5.91%. RSEs of all heart rate model parameters were below 30%.

A logistic regression model was used for modelling the VAS feeling high, the parameters of which had a relatively low RSE (smaller than 20%). The estimated parameters of VAS feeling high are shown in Table 5.

Antagonist pharmacodynamic modelling

An effect compartment was built for THC and the antagonists to describe the time delay between the concentration effect profiles. An equilibration half-life ($t_{1/2ke0}$) was defined, which ranged from 0.00462 (0.502%) to 63.7 (35.4%) hours for heart rate with all RSEs smaller than 100%; and 0.964 (193%) to 150 (16.8%) hours for VAS. These wide CV ranges suggested a large variability in drug distribution rates to the target locations for the different antagonists. Rimonabant presented a relatively high RSE, which was the only one that was bigger than 100%. This suggested a low uncertainty of the parameter estimation.

The range of IC_{50} also ranged widely, from 6.42 (36.9%) to 202 (38.6%) ng /ml for heart rate, and from 12.1 (25.9%) to 376 (15.3%) ng /ml for VAS feeling high with all RSE smaller than 100%.

All PD parameter estimates of the four different antagonists are presented in Table 6. Both diagnostic plot and VPC were performed, which confirmed that the proposed model fit the data properly with acceptable predictive ability.

Dose-response curve simulations

The simulations of two dose-response curves (in this case dose-reduction rate curves) of the antagonists are graphically displayed in Figure 2. The dose range for the simulation ranged from 2 to 1000 mg. All antagonists caused a maximal reduction of THC-induced effects of 70% to 85%. The order and shape of the curves that depict the relations between dosages and reduction rates varied considerably among the different CB_1 antagonists and effects. For example, the reduction rates for heart rate were larger than for VAS high in the case of drinabant and TM38837, whereas for surinabant and rimonabant, VAS feeling high had a higher reduction rate than heart rate. This suggests that different antagonists can show different selectivity for various target sites.

DISCUSSION

Our aims were to build integrated PK/PD models for THC and four CB_1 antagonists and to apply them for direct comparison of the different antagonists to improve our understanding on the behaviour of CB_1 antagonists in healthy volunteers.

We found that our PK/PD modelling and simulation approach was suitable for direct comparisons of pharmacological compounds in complex clinical settings using a THC challenge test, even when the data came from different studies with different THC dosing regimens. Our integrated PK/PD models have a few advantages and disadvantages compared to the individual PK/PD models that we built in previous studies (Strougo et al., 2008; Klumpers et al., 2013a; Klumpers et al., 2013b). Integration on the PD level enabled us to compare the different antagonists directly; however this approach resulted in enlarged inaccuracy of parameter estimation. The method of calculating the inhibition ratios of the antagonists as performed in the surinabant study and the rimonabant-TM38837 study was highly dependent on sampling time points and did not consider the whole effect-time profile (Strougo et al., 2008; Klumpers et al., 2013a; Klumpers et al., 2013b), while our study presented an improved method to calculate inhibition ratios based on the AUC of PD responses. In this way, we were able to make estimations along the whole time-effect curve.

We have found that surinabant and rimonabant induced larger effects on inhibition of THC-induced VAS feeling high than on inhibition of THC-induced heart rate rising effect, whereas drinabant and TM38837 showed an opposing behaviour. This was consistent when (graphically) comparing the findings from previous studies (Zuurman et al., 2010; Klumpers et al., 2013a; Klumpers et al., 2013b). The different effect profiles in healthy humans of drinabant and TM38837 compared to surinabant and rimonabant suggest differences in clinical efficacy in patient groups. Considering the previously suggested associations of heart rate effects and peripheral effectivity, it would be tempting to imply that drinabant and TM38837 have a larger preference for peripheral target sites, resulting in larger peripheral effects compared to centrally induced effects. This would be a more desired effect profile, considering the severe unwanted psychiatric side effects as previously observed in clinical rimonabant dosages. However, patient studies are needed to investigate the efficacy of compounds with increased peripheral selectivity and their translation to efficacy parameters in healthy volunteers.

Despite the market withdrawal of rimonabant, it would still be very interesting to investigate the efficacy and tolerability of rimonabant as well as surinabant in more detail. From our previous research (Klumpers et al., 2013a) we analysed that the clinically used CB_1 antagonist dosages and steady state plasma concentrations were well above the dosage and concentration that maximally blocked THC-induced effects. The analyses were performed over specific time periods during which the antagonist concentrations where at maximum reaching maximum inhibition of THC-induced effects. This implies that the clinically applied rimonabant dosage might have been higher than needed to induce favourable therapeutic effects and high enough to induce severe unwanted side effects. We hypothesise that a lower dose and concentration of rimonabant (and the right dose for surinabant) might result in an acceptable balance between efficacy and side effects, which could be different for different patient groups. To confirm this, future research should perform additional patient studies and carefully translate our model (i.e. the results from studies in healthy subjects) to patient groups.

In conclusion, we were able to build suitable PK/PD models in which CB_1 antagonists drinabant, surinabant, rimonabant and TM38837, and the agonist THC were integrated. We found that the effects of the antagonists showed different profiles, with drinabant and TM38837 showing relatively larger heart rate effects than effects on VAS feeling

high compared with surinabant and rimonabant. We suggest that drinabant and TM38837 might have a larger therapeutic potential than rimonabant and surinabant, due to the potential higher risk of severe psychiatric side effects for the latter two compounds, which is based on their relatively large central effects (i.e. feeling high).

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TABLE 1 Subject demographics. Mean with standard deviation (SD). BMI: Body Mass Index.

Name of study	Subject number	Age	Weight (kg)	Height (cm)	BMI (kg/m²)
Drinabant	36	22 (3)	76 (11)	183 (6)	23 (3)
Surinabant	30	23.2 (5.3)	78.94 (8.23)	187.7 (6.7)	22.39 (1.94)
Rimonabant-TM38837	36	21.2 (3.8)	77.25 (10.18)	183.42 (6.99)	22.9 (2.1)

TABLE 2 Treatments per study.

Name of study	Treatment	Time of THC administration after antagonist administration (hr)	THC challenge administration dosage (mg)
Drinabant	Placebo drinabant+ THC vehicle	3, 4, 5, 6	2, 4, 6, 6
	Placebo drinabant + THC challenge		
	20 mg drinabant + THC challenge		
	60 mg drinabant + THC challenge		
	120 mg drinabant + THC challenge		
	120 mg drinabant + THC vehicle		
Surinabant	Placebo surinabant + THC vehicle	1.5, 2.5, 3.5, 4.5	2, 4, 6, 6
	Placebo surinabant + THC challenge		
	5 mg surinabant + THC challenge		
	20 mg surinabant + THC challenge		
	60 mg surinabant + THC challenge		
	60 mg surinabant + THC vehicle		
Rimonabant-TM38837	Placebo TM38837 + Placebo rimonabant+ THC vehicle	2, 4.5, 7, 22, 24.5*	4, 4, 4, 4, 4
	Placebo TM38837 + Placebo rimonabant+ THC challenge		
	100 mg TM38837 + Placebo rimonabant+ THC challenge		
	500 mg TM38837 + Placebo rimonabant+ THC challenge	4, 6.5, 9, 24, 26.5**	4, 4, 4, 4, 4
	Placebo TM38837 + 60 mg rimonabant+ THC challenge		
	Placebo TM38837 + Placebo rimonabant+ THC challenge		

* Time of THC administration after rimonabant administration
** Time of THC administration after TM38837 administration

TABLE 3 PK parameters of THC in the different studies, with the relative standard error (RSE, %) and the inter-individual variability (IIV) as %CV. F=Bioavailability; IOV=inter-occasion variability (%)

	Drinabant			Surinabant			Rimonabant-TM38837		
Parameter	Estimate (%RSE)	IIV	IOV	Estimate (%RSE)	IIV	IOV	Estimate (%RSE)	IIV	IOV
Clearance/F (L/h)	228 (5.19)	18.8	-	228 (7.39)	-	-	200 (5.9)	31.2	-
Central volume/F (L)	35.5 (6.95)	10.3	-	35.2 (8.88)	38.9	78	28.5 (8.91)	40.8	25.1
Peripheral volume of distribution/F (L)	145 (6.45)	-	-	103 (6.79)	-	-	107 (14.3)	-	-
Intercompartmental clearance/F (L/h)	134 (6.08)	-	-	128 (7.16)	-	-	106 (6.9)	-	-

TABLE 4 PK parameters of drinabant, surinabant, rimonabant and TM38837 with the relative standard error (RSE, %) and the inter-individual variability (IIV) as %CV. F=Bioavailability; IOV=inter-occasion variability (%)

	Drinabant			Surinabant			Rimonabant			TM38837		
Parameter	Estimate (%RSE)	IIV	IOV	Estimate (%RSE)	IIV	IOV	Estimate (%RSE)	IIV	IOV	Estimate (%RSE)	IIV	IOV
Clearance/F (L/h)	32.5 (14.8)	-	-	4.4 (12.7)	62.5	-	9.30 (6.87)	25.6	-	2.20 (9.29)	66.2	-
Central volume/F (L)	213 (9.57)	36.3	24	4.99 (16.3)	66.4	-	39.3 (15.5)	20.6	-	18.7 (16.3)	132	-
Peripheral volume of distribution/F (L)	2170 (30)	-	-	515 (12.5)	102.	-	93.0 (12.8)	-	-	10.8 (42.4)	-	-
Intercompartmental clearance/F (L/h)	32.5 (11.4)	-	-	15.9 (6.5)	91.2	-	17.9 (17.2)	-	-	0.00975 (22.0)	-	-
Absorption rate constant (ka; h^{-1})	1.09 (8.22)	39.8	-	c0.448 (3.91)	7.83	-	1.17 (fixed)	-	-	0.0789 (9.72)	-	

TABLE 5 PK/PD parameter estimates of THC alone for heart rate and VAS feeling high with percentage coefficient of variation (CV). T_{50} = equilibration half-life of the elimination from the biophase compartment; E_{max} = maximal effect; EC_{50} = concentration at 50% of maximal effect; IIV = inter individual variability; IOV = inter occasion variability; BetaTHC = coefficient of the antagonist-induced shift of the THC effect; Kd = elimination rate of tolerance

	Parameter	Units	Estimate (%RSE)	IIV	IOV
Heart rate	$t_{1/2}$	hr	0.33 (28.2)	-	-
	E_0	BPM	64.2 (1.14)	7.98	5.91
	E_{max}	BPM	64.2 (-)	-	-
	EC_{50}	ng/ml	73.7 (18.4)	-	-
Feeling high	$t_{1/2}$	hr	2.26 (16.3)	-	-
	CUT1		2.78 (2.98)	-	-
	BetaTHC		-0.519 (16.7)	-	-
	Kd		0.131 (18.6)	-	-

TABLE 6 PK-PD parameter estimates of antagonists for VAS feeling high, body sway and heart rate with percentage coefficient of variation (CV). $T_{1/2e0}$ = equilibration half-life; IC_{50} = concentration of antagonist at 50% of maximal inhibition

	Parameter	Units	Estimate (%RSE)	Estimate (%RSE)	Estimate (%RSE)	Estimate (%RSE)
			Drinabant	**Surinabant**	**TM38837**	**Rimonabant**
Heart rate	$t_{1/2e0}$	hr	6.25 (34.6)	0.00462 (0.502)	63.7 (35.4)	1.12 (26.3)
	IC_{50}	ng/ml	6.42 (36.9)	107 (34.4)	175 (36.6)	202 (38.6)
Feeling high	$t_{1/2e0}$	hr	1.75 (34.7)	6.7 (62.9)	150 (16.8)	0.964 (193)
	IC_{50}	ng/ml	12.1 (25.9)	61.6 (44.9)	376 (15.3)	92.8 (65)

FIGURE 1 Schematic representation of the PK-PD models.

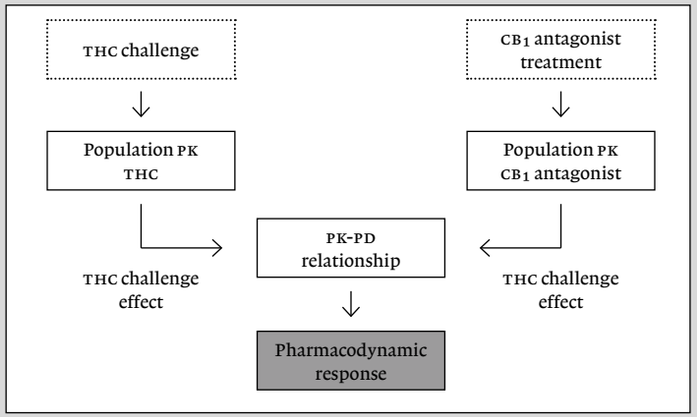
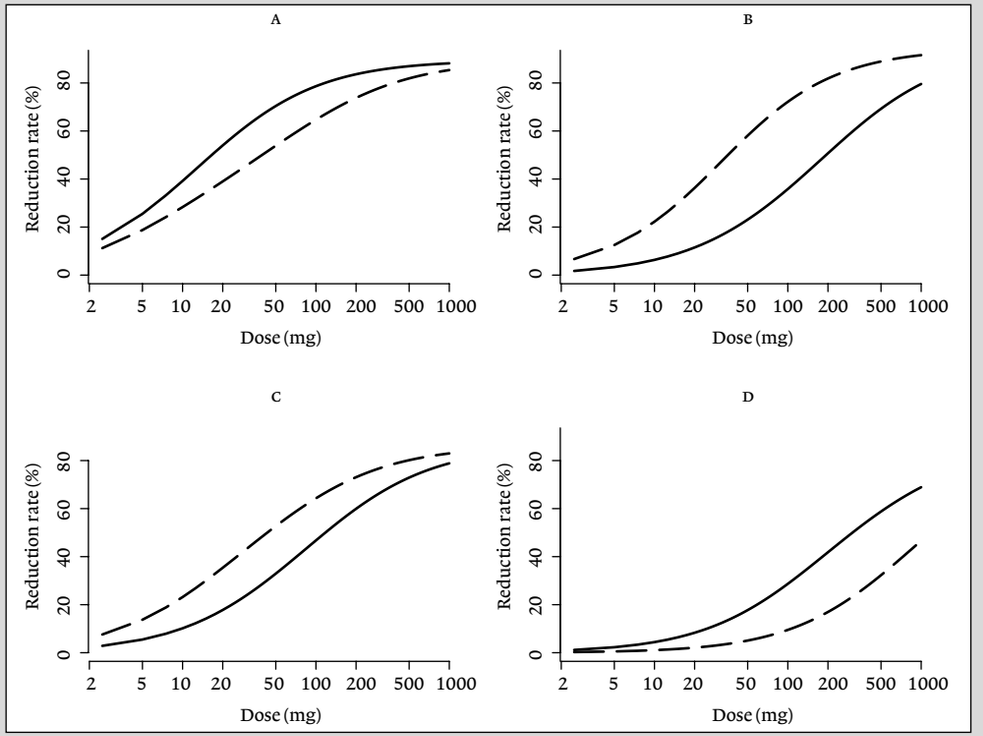


FIGURE 2 Simulated dose-effect relationship and the estimated reduction rate (i.e. antagonism of THC-induced effects) of heart rate (solid line) and VAS feeling high (dashed line) of: (A) drinabant; (B) surinabant; (C) rimonabant; (D) TM38837. From the curves we observed that drinabant and TM38837 induce relatively larger heart rate effects than VAS feeling high effects, whereas this is opposite for surinabant and rimonabant.



CHAPTER VII

Discussion

For already thousands of years, cannabis has been the most widely used illicit drug for recreational and medicinal purposes. The receptors on which cannabinoids act are part of one of the most phylogenically ancient and widely preserved pharmacological systems in biology. Nonetheless, this endocannabinoid system has only been discovered during the last few decades, and scientific progress in understanding the relevance of this system in health and disease has been limited and slow. As a result, only a few drugs that act on the endocannabinoid system have been registered, most of which are components of *Cannabis sativa*. At the end of 2006, just when cannabis research was flourishing, the industry suddenly lost its interest due to concerns of the FDA about the safety of CB_1 antagonist rimonabant, an anti-obesity drug with inverse agonistic properties. The registration of rimonabant, which was hailed as a breakthrough in drug research and in treatment of the metabolic syndrome, was quickly followed by a market withdrawal in 2008. This resulted in an almost complete and unanimous ban on CB_1 antagonist research, which is still felt today.

The demise of rimonabant reflects many of the difficulties of cannabinoid drug development. Some of these are related to public perceptions. Research on cannabinoid agents is often regarded suspiciously because of concerns of abuse. Many of the most promising potential indications for cannabinoid drugs are disputed: obesity is not considered a disease by the American Medical Association (ama Council, 2013), addiction is often viewed as an individual failure of character (Gartner et al., 2012), and many pharmaceutical industries regard psychosis as too risky for commercial drug development (van Gerven and Cohen, 2011).

The unfortunate fate of rimonabant is also related to some fundamental scientific complexities of endocannabinoid research. Some of these are mentioned in the introduction: widespread distribution, limited subtype specificity, local production of highly lipophilic and rapidly degraded transmitters, complex physiological integration, lack of good effect measures, all leading to unclear pathophysiological involvement. But the focus of endocannabinoid research on cannabis can also be misleading. Many of the putative indications for cannabinoid agonists or antagonists are inspired by the well-known effects of recreational cannabis consumption. Although feeling pleasant and 'high', getting the 'munchies', or suffering from panic attacks after using cannabis, are undoubtedly founded in the functional pharmacology of endocannabinoid systems, these effects reflect limited aspects of gross overstimulation. They hardly represent the local functions of endocannabinoids in the subtle regulatory modulation of normal physiological processes, or their involvement in intercellular or systemic derangements of complex multicascadic functional networks.

The failure of rimonabant also reflects some weaknesses of current drug development. Rimonabant was considered a 'miracle drug' for the treatment of obesity and smoking (Boekholdt and Peters, 2010), with 'blockbuster' potential. Its development was based on the 'logical' notion that blocking hunger or reward (and associated physiological processes) will reduce weight and craving (i.e. induce the opposite of cannabis-associated munchies and abuse). But it was disregarded that along the same reasoning, inhibition of pleasant feelings (i.e. inverse of cannabis-induced euphoria) would be expected to have a negative impact on mood. Nonetheless, to the best of our knowledge, emotional or cognitive effects have never been studied systematically in clinical trials. There seems to have been no systematic evaluation of the balance of the inferred beneficial and detrimental effects of rimonabant, which would be essential for the determination of a therapeutic window.

In this thesis, we explore some improvements in the early development of cannabinoids, by systematically investigating new cannabinoid compounds and formulations to enhance their pharmacological activities, experimenting with new methodology to optimise effect measurement, and applying new concentration-effect models to improve the simulation and prediction of future studies.

IMPROVING PHARMACOLOGY

In Chapter 2 we investigated the pharmacology of different administration methods of Namisol® THC tablets, which are based on an improved emulsifying technology to enhance absorption. Somewhat unexpectedly, we have found that oral administration resulted in a quicker THC absorption into the blood compartment compared to sublingual administration of a crushed tablet and we suggested that the absorption via oromucosal tissue is relatively slow compared to gastrointestinal absorption. If we compare our results to the findings from THC inhalation studies (e.g. from Chapter 3, 4 and 5), the proportion of active metabolite (11-OH-THC) to THC is larger for the oral administration, meaning that relatively more active metabolite is formed than after inhalation. Compared with the literature on other oral THC formulations, Namisol® seems to have a shorter absorption time (or T_{MAX}) with reduced variability, probably contributing to a faster and more predictable onset of effects. We concluded that Namisol® seems to have benefits over common oral cannabis and THC treatments and we suggested that Namisol® offers potential improvements over currently registered cannabinoid-treatments including registered oral THC formulations. However, this would require a direct comparison of the pk and pd of the oral thc tablet Namisol® with the current registered oral, oromucosal and sublingual formulations. Also, the absolute bioavailability of the various formulations should be studied, although this would be limited by the lack of a standardised intravenous dosage.

Because the endocannabinoid system is relative inactive under physiological circumstance, cannabinoid antagonists show no acute effects under normal resting conditions. We therefore used a THC-challenge test in Chapter 4 and 5 to examine the pharmacology of new CB_1 antagonists in healthy subjects. The effects of a thc challenge test are clearly measurable in healthy subjects and we have previously demonstrated that these effects can be inhibited by CB_1 antagonists (Zuurman et al., 2010).

In Chapter 4 we investigated the pharmacokinetics of surinabant and its pharmacodynamic effects on those of THC. As a consequence of the recent rimonabant incident, we aimed to characterize the dose-effect relationships for surinabant, to support the prediction of optimal effects and minimal risk for unwanted (central) side effects in patient studies. Although surinabant exhibited no effects of its own on a wide range of different CNS-function tests, we concluded that the dose-related inhibition of THC, demonstrates unequivocal CB_1 receptor antagonism in humans. A single surinabant dose between 5 to 20 mg was able to completely antagonize THC-induced effects in humans. Higher doses were well tolerated, but did no show additional pharmacological activity. During the time of study performance we hoped that our results would allow the determination of clinically effective doses with minimised central side effects. However, shortly after our study surinabant development was ceased due to adverse psychiatric effects in phase 2. The relevant doses were determined prior to the start of our study, based on different grounds than those of our study. The plasma concentration range at which the adverse events prominently occurred were relatively high compared to our study (Sanofi, personal communication).

To improve the therapeutic window between metabolic improvements and mental disturbance, TM38837 was developed as a peripherally selective CB_1 antagonist. In rodents, this antagonist only hardly penetrates through the blood-brain barrier in dosages that show beneficial effects on metabolism. Chapter 5 describes the first study of this compound in humans. It was also the first direct comparison of two cannabinoid antagonists (TM38837 and the formerly registered rimonabant) in a clinical study, which is an efficient way to characterize new compounds. This study gave us insight into the PK and effect profiles of a peripherally acting antagonist and the differences compared to rimonabant by statistical analyses and PK and PK-PD modelling. When directly compared to rimonabant, TM38837 had a relatively large inhibiting effect on THC-induced heart rate (which had previously been argued to be a mainly pe-

ripheral effect (Zuurman et al., 2009; Strougo et al., 2008)) with relatively small effects on subjective scores associated with CNS activity (e.g. feeling high) and body sway. The lowest TM38837 dose of 100 mg was predicted to be at least equipotent to rimonabant with regard to metabolic disorders in rodent models and had no significant impact on CNS-effects in our study. These results provide support for further development of TM38837 as a peripherally selective CB_1 antagonist for indications such as metabolic disorders, with a reduced propensity for psychiatric side effects. The PK-PD analyses were put in a larger perspective in Chapter 6, in which the results from Chapter 4 and 5 and the results from a previous study with drinabant (ave1625) (Zuurman et al., 2010) were all used for building a general antagonist model. These analyses confirmed our graphical interpretation that tm38837 has a relatively larger peripheral effect than central effects when compared to rimonabant, surinabant and drinabant. We concluded that the relatively low central activity and the large effects on heart rate suggest a potential for therapeutic treatment development of tm38837, with minimal risks of the central side effects attributed to rimonabant.

OPTIMISING MEASUREMENTS

Besides the limited knowledge on the endocannabinoid system, and the pharmacology that limits the possibilities of pharmacotherapy, optimisation of drug development is limited by the lack of validated effect measurements. New techniques can be important tools to better understand the physiology of the cannabinoid system, to optimize dose selection and effect profiling, and to improve our understanding of the involvement of cannabinoid systems in general.

Since all central nervous system (CNS) functions ultimately depend on the activity of neuronal networks, connectivity analyses of neurophysiological (electroencephalography (EEG), magnetoencephalography (MEG)) or neuroimaging technologies (positron emission tomography (PET), functional magnetic resonance imaging (FMRI)) may provide useful tools for a more direct assessment of drug- or disease-induced functional CNS-changes. In Chapter 3 we measured brain connectivity changes after THC administration by using resting-state functional MRI (RS-FMRI.) We found that THC induced increases and decreases of brain connectivity for various networks of interest. These clear effects (which are also found with other medications by other members of our research group) suggested that RS-FMRI is a suitable method to apply in early clinical stages of drug development. The brain regions in which the connectivity changes were found were comparable with the functional regions that are associated with the behavioural effects after THC or cannabis use such as postural stability and altered time perception. RS-FMRI has some unique features, compared with other CNS measures that we used in this thesis. As opposed to more commonly applied neurophysiological, functional and subjective methods, RS-FMRI is able to detect a wide range of direct and indirect (acute) effects and to 'objectively' measure effect profiles, with less concealed interference from compensatory mechanisms and motivational aspects or other factors that can affect responses and performance. Moreover, this might enable early phase clinical research on compounds at low concentrations with a functional impact that can be easily compensated, or which is too limited to noticeably affect performance. RS-FMRI may also show effects of antagonists that do not induce acute measurable effects in the commonly used neurophysiologic tests, although this remains to be established.

Overall, we concluded that THC induces connectivity changes in brain regions that are comparable with the functional regions that are associated with the behavioural effects after THC or cannabis use, and that RS-FMRI is a suitable technique for clinical drug development, including development of cannabinoid pharmacotherapies. Future research could maturate the applicability of the RS-FMRI methodology by investigating dose-effect relationships, for example by developing a PK-PD model for RS-FMRI. Also, it would be interesting to understand

the implications of the methodology in a wider perspective, for example by exploring the relationships between the connectivity changes and functions. This will allow us to optimise the usability of the technique, but also to improve our understanding of the biological systems of the brain in general.

IMPROVING ANALYSES

For the analysis of Chapter 5 which describes a study with the peripherally specific CB_1 antagonist TM38837 and rimonabant in a THC-challenge test, PK-PD models were developed for heart rate, postural stability and feeling high. All PK-PD models included a baseline level, effect compartments that equilibrated with the plasma concentration, and a model to relate the effect compartment concentration to the pharmacodynamic response. Heart rate and body sway were best described by a maximum effect model. For feeling high a probability model was used to quantify the probability for a VAS score > 12 at the study population level. All models included the THC challenge effect and the antagonizing effect of rimonabant and TM38837. The equilibration half-life of TM38837 was long compared with rimonabant, causing a larger delay in the onset of TM38837 effects. For heart rate the half maximal inhibitory concentrations (IC_{50}) were similar for TM38837 and rimonabant, whereas for body sway and feeling high the IC_{50} of rimonabant was 4 times and 56 times larger respectively than for TM38837. This suggests that TM38837 induces relatively smaller central effects than peripheral effects when compared with rimonabant. The time profiles of the effects were comparable with the pharmacokinetic profiles of the compounds. Unfortunately, no therapeutic trials have been performed with TM38837 so far, to verify these predictions.

In Chapter 6 we built PK-PD models for four different CB_1 antagonists: drinabant (AVE1625), surinabant (SR147778), rimonabant (SR141716) and TM38837. This approach gave us insight in the differences of the PK and PD between the four antagonists and increased our knowledge on the behaviour of CB_1 antagonists in general. Compared to TM38837, surinabant and rimonabant effect profiles induced relatively larger centrally regulated PD effects than heart rate effects. The models can be applied for optimization as well as development of future clinical studies by simulation and prediction of the PK and PD of cannabinoid antagonists. As of today, research continues developing the mechanism-based PK-PD modelling with for example more emphasis on disease system analysis. Mechanism-based PK-PD modelling is an important field that should continue in the future. Besides the desire to develop a translational tool from healthy subjects to patients, also in other phases of drug development tools for translation, simulation and prediction of pharmacokinetics and effects could be applied (e.g. from preclinical to clinical studies).

GENERAL CONCLUSION

The aim of this thesis was to explore some ways to advance cannabinoid drug design, by improvements of study designs, measurements and analyses in early phase clinical studies. Such improvement seem to be needed to increase our understanding of the pharmacology of cannabinoids in healthy people, and enable a more effective control of the cannabinoid system in pathology.

In this thesis, we have introduced a new oral THC formulation and a new CB_1 antagonist, which we tested in healthy subjects. We concluded that the new formulations showed more beneficial pharmacological effects compared to current treatments. We have also optimized and applied new methodologies. We have provided indications that resting state FMRI is a suitable technology for early phase clinical drug development. We have also demonstrated that the THC-challenge test can be applied for pharmacological characterisation and dose optimisation of antagonists. For this, we developed PK-PD models for THC and the

CB1 antagonists drinabant, surinabant, TM38837 and inverse agonist rimonabant. These models can be applied for simulation and prediction of PK and PD, for example to optimise future study designs. These methods provide more information than the 'traditional' approaches in early development, where dose selection is essentially based on extrapolation of preclinical results, pharmacokinetic optimisation of dosing regimens, and estimation of maximum tolerated doses – at best supplemented with some indications of pharmacodynamic effects. This approach can easily fail if the investigative compound has a novel mechanism of action, and particularly when it has no effects under physiologically stable conditions. This seems to have been the case for rimonabant, which had to be withdrawn shortly after registration, because of adverse psychiatric events that perhaps in hindsight were not unexpected. We used the PK-PD-approach that is described in this thesis to determine a pharmacologically optimised dose for rimonabant as well as for other novel CB1-antagonists. This analysis suggested that rimonabant may have been overdosed, possibly because the compound is so well tolerated in healthy subjects where it has no 'spontaneous' effects. Clearly, this remains speculative as long as confirmatory studies have not been performed.

At present, there are still questions about the predictive value of the pharmacological biomarkers, for clinically relevant therapeutic or inadvertent effects of CB1-antagonists. It remains to be established, therefore, whether functional challenge studies and pharmacological PK-PD-analyses would actually allow the determination of a therapeutic window that is large enough for a safe and effective use of CB1-antagonists. Nonetheless, a pharmacological approach gives hope that drug development in the field of endocannabinoids is feasible and potentially useful, despite the many problems that are inherent to this complex system. The hope for cannabinoid research and drug development may also be fuelled by the break-down of taboos on cannabis use. The recreational use of cannabis gradually gained more acceptance since the 1970's, and an increasing number of countries and states in the USA have decriminalised cannabis [(Robison, 2013) for visualisation, see Reeve (2013)]. The spread of cannabis use, particularly for medical purposes also increases general social acceptance and stimulation of further research of cannabis-related compounds.

OVERALL CONCLUSIONS

Our results lead to the conclusion that there is room for improvement in cannabinoid research – enough to give confidence that the cannabinoid system still has potential as a target for pharmacological therapies, despite the setback after the market withdrawal of the first registered cannabinoid antagonist shortly after launch. Although the current amount of cannabinoid research is relatively low, and clinical research is particularly limited, the social acceptance of cannabis, also as a medicine, could facilitate a revival of research on the cannabinoid system. Our research shows that this requires novel approaches to the administration of cannabinoids, to the measurements and the study designs, and to the analyses of the effects. This reflects the complexity of the highly integrated endocannabinoid system, but also sets the stage for other innovative drug development programs.

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NEDERLANDSE SAMENVATTING

Al duizenden jaren is cannabis wereldwijd een van de meest populaire drugs. Cannabis wordt vooral gebruikt voor recreatieve en medische doeleinden. De CB_1- en CB_2-receptoren waaraan cannabinoïden (cannabisachtige stoffen) zich binden, zijn onderdeel van het endocannabinoidesysteem, een van de oudste farmacologische systemen in de biologie die wijd verspreid in verschillende organismen voorkomen.

Ondanks haar ouderdom is het cannabinoïdesysteem pas enkele decennia geleden ontdekt en is de algemene kennis over de relevantie van het systeem in ziekte en gezondheid nog steeds erg beperkt. Bovendien levert nieuw wetenschappelijk onderzoek slechts langzaam nieuwe informatie op. Dit heeft tot gevolg dat er maar enkele geneesmiddelen geregistreerd zijn die op het endocannabinoïdesysteem werken. De meeste stoffen die hierbij gebruikt worden zijn direct afkomstig uit de plant *Cannabis sativa*. Eind 2006, toen het cannabinoïdenonderzoek op een bloeiend hoogtepunt was, verloor de industrie plotseling haar interesse. Dit werd veroorzaakt doordat de FDA zorgen uitte over de veiligheid van CB_1-antagonist rimonabant, een medicijn tegen obesitas met een werking die tegengesteld is aan de werking van cannabinoïden. De registratie van rimonabant, die werd gezien als een grote doorbraak in het geneesmiddelenonderzoek voor onder meer de behandeling van het metaboolsyndroom, werd bijna direct, in 2008, weer van de markt gehaald. Dit leidde ertoe dat de farmaceutische industrie indertijd vrijwel volledig stopte met het onderzoek naar CB_1-antagonisten. De consequenties daarvan zijn nog steeds voelbaar.

Buiten de grote nalatenschap van rimonabant zijn er verschillende andere moeilijkheden waarmee het cannabinoïdenonderzoek te maken heeft. Zo is er bijvoorbeeld de maatschappelijke kwestie waarbij onderzoek naar cannabinoïde stoffen vaak in verband wordt gebracht met cannabismisbruik. Bovendien zijn veel van de potentiële indicaties voor cannabinoïde middelen mikpunt van discussie: vetzucht wordt niet als een ziekte gezien door bijvoorbeeld de *American Medical Association* (AMA Council, 2013) en verslaving wordt vaak gezien als een gedragsstoornis in plaats van een ziekte (Gartner, Carter, & Partridge, 2012). Bovendien is binnen de farmaceutische industrie de mening ontstaan dat de ontwikkeling van middelen voor psychiatrische aandoeningen in het algemeen te risicovol is (van Gerven & Cohen, 2011).

Het lot van rimonabant is ook gerelateerd aan de wetenschappelijk fundamentele complexiteiten van het endocannabinoïdenonderzoek, zoals: wijdverspreide (receptor)distributie, beperkte receptorsubtypespecificiteit, locale productie van zeer lipofiele en snel degraderende transmitters, complexe fysiologische integratie en gebrek aan goede effectmaten. Dit alles tezamen leidt tot onduidelijke betrokkenheid binnen de pathofysiologie. Aan de andere kant kan de focus van het endocannabinoïdenonderzoek misleidend zijn. Veel van de vermeende indicaties voor cannabinoïde agonisten en antagonisten worden gekoppeld aan de bekende effecten van recreationeel cannabisgebruik. Alhoewel een prettig en 'high' gevoel, de 'munchies' (hongeraanvallen) en de paniekaanvallen na cannabisgebruik ongetwijfeld diep geworteld zijn in de functionele farmacologie van het endocannabinoïdesysteem, zijn dit slechts enkele gevolgen van een excessieve overstimulatie. Door juist op deze grote effecten te focussen raken de lokale functies van cannabinoïden, die vooral een rol spelen bij subtiele regulatoire modulatie van normale fysiologische processen, en hun betrokkenheid bij intercellulaire of systemische balansverstoringen van complexe multicascadische functionele netwerken, ondergesneeuwd.

Het falen van rimonabant legt ook een teken van zwakte bloot van het huidige geneesmiddelenonderzoek. Rimonabant werd indertijd gezien als een wondermiddel voor de behandeling van obesitas en roken (Boekholdt & Peters, 2010) met zogenaamd 'blockbuster'-potentieel. Rimonabants ontwikkeling was gebaseerd op het gegeven dat het blokkeren van gevoelens van honger of beloning (en de daarmee geassocieerde fysiologische processen) leidt tot afname van gewicht en verslaafbaarheid; of met andere woorden: het stopt de cannabisgeassocieerde 'munchies' en het misbruik. Opvallend genoeg werd de mogelijkheid genegeerd, dat

hiermee plezierige gevoelens kunnen worden geremd (als potentieel gevolg van het tegengaan van cannabisgeïnduceerde euforie). Voor zover ons bekend, zijn de emotionele of cognitieve effecten van rimonabant indertijd niet specifiek bestudeerd in klinisch onderzoek. Het lijkt erop alsof er nooit een systematische evaluatie van de balans tussen de gunstige en de nadelige effecten van rimonabant heeft plaatsgevonden, wat juist essentieel is voor het bepalen van een therapeutisch venster.

In dit proefschrift onderzochten we verbeteringen in vroege klinische ontwikkeling van cannabinoïden, waarbij we systematisch te werk probeerden te gaan. We onderzochten nieuwe cannabinoïdeliganden en formuleringen om de farmacologische activiteit te vergroten, we experimenteerden met een nieuwe methodologie om effectmetingen te optimaliseren en we pasten nieuwe concentratie-effectmodellen toe om simulaties en voorspellingen van toekomstige studies te verbeteren.

FARMACOLOGISCHE VERBETERINGEN

In hoofdstuk 2 onderzochten we de farmacologie van verschillende toedieningsmethoden van de THC-tablet Namisol®. Deze tablet is geproduceerd met een verbeterde emulsietechnologie om de absorptie te verhogen. Tegen onze verwachting in vonden we dat de orale toedieningsvorm een snellere THC-absorptie naar de bloedbaan gaf dan sublinguale toediening van een verkruimelde tablet. Hieruit leidden we af dat de absorptie via oromucosaal weefsel relatief langzaam is vergeleken met gastrointestinale absorptie. Toen we onze resultaten vergeleken met de bevindingen uit inhalatiestudies met THC (zoals bijvoorbeeld in hoofdstuk 3, 4 en 5), zagen we dat de verhouding van actieve metaboliet (11-OH-THC) tot THC groter is voor de orale toedieningen, wat betekent dat er relatief meer actieve metaboliet wordt gevormd dan bij inhalatie. Toen we Namisol® vergeleken met andere THC-formuleringen in de literatuur, bleek dat Namisol® een kortere absorptietijd (ofwel T_{MAX}) en een geringere variabiliteit heeft, wat waarschijnlijk bijdraagt aan snellere en beter voorspelbare effecten. We concludeerden dat Namisol® waarschijnlijk farmacologische voordelen heeft boven de bekende orale cannabis- en THC-middelen, die zich mogelijk vertalen in therapeutische voordelen voor patiënten. Om deze hypothese te kunnen bevestigen, is een vervolgstudie nodig waarbij de farmacokinetiek (PK) [en eventueel de farmacodynamiek (PD)] van Namisol® direct wordt vergeleken met de huidige geregistreerde orale, oromucosale en sublinguale formuleringen. Er zou hierbij ook naar de absolute biologische beschikbaarheid van de verschillende middelen gekeken kunnen worden, alhoewel dit beperkt wordt door het ontbreken van een gestandaardiseerde intraveneuze toedieningsvorm.

Het endocannabinoïdesysteem is onder normale omstandigheden weinig actief, waardoor cannabinoïde-antagonisten bij gezonde mensen geen directe effecten laten zien. Om in hoofdstuk 4 en 5 toch de farmacologie van nieuwe CB_1-antagonisten te kunnen testen, hebben we een THC-challengetest toegepast bij gezonde vrijwilligers. De effecten van een THC-challenge zijn ook bij gezonden duidelijk meetbaar. Al eerder hadden we aangetoond dat deze effecten door de CB_1-antagonisten sterk kunnen worden onderdrukt (Zuurman et al., 2010).

In hoofdstuk 4 onderzochten we de farmacokinetiek van surinabant en haar farmacodynamische effecten op THC-geïnduceerde effecten. Door het recente gebeuren rond rimonabant wilden we de dosis-respons-relatie voor surinabant onderzoeken waarmee we een dosisvoorspelling konden doen met enerzijds optimale effecten en anderzijds een minimaal risico op ongewenste (centrale) bijwerkingen tijdens patiëntenstudies. Alhoewel surinabant zelf geen effecten liet zien in de verschillende centraal zenuwstelsel (CZS)-testen, concludeerden we dat de dosisgerelateerde remming van THC-effecten wijst op CB_1-receptorantagonisme in mensen. Een enkele dosis surinabant tussen 5 en 20 mg kon de effecten van THC compleet antagoneren. Hogere enkelvoudige doseringen werden goed verdragen, maar lieten geen extra farmacologische remming zien. Ten tijde van de studie-uitvoer hoopten we dat onze resultaten zouden leiden tot

het bepalen van een klinisch effectieve dosering met minimale centrale bijwerkingen. Echter, kort na de uitvoer van onze studie werd de verdere ontwikkeling van surinabant gestaakt door psychiatrische bijwerkingen in een fase II-studie. Deze studie was al eerder in gang gezet met doses die op andere gronden waren gekozen. De plasmaconcentratie waarbij deze bijwerkingen duidelijk optraden was relatief hoog vergeleken met de concentraties in onze studie (Sanofi, persoonlijke communicatie).

Om het therapeutische venster te vergroten tussen metabole verbeteringen en psychiatrische bijwerkingen, is de perifeer selectieve CB_1-antagonist TM38837 ontwikkeld. Dit middel dringt bij proefdieren nauwelijks door de bloedhersenbarrière heen, in doseringen die wel gunstige metabole effecten hebben. Hoofdstuk 5 beschrijft de eerste studie met dit middel in mensen. Dit was ook de eerste keer dat twee cannabinoïde-antagonisten (namelijk TM38837 en rimonabant) direct werden vergeleken binnen dezelfde klinische studie, wat een efficiënte manier is om nieuwe middelen te karakteriseren. Deze studie verschafte inzicht in de PK en de effectprofielen van een perifeer werkende antagonist en de verschillen met rimonabant, zoals bepaald door statistische analyses en PK- en PK-PD-modellering. Vergeleken met rimonabant veroorzaakt TM38837 een relatief grote remming op THC-geïnduceerde hartslagversnelling, een effect waarvan voorheen beredeneerd is dat het vooral samenhangt met perifere cannabinoïde werking (Zuurman, Ippel, Moin, & van Gerven, 2009; Strougo et al., 2008). Er waren relatief geringe effecten op subjectieve effecten in het CZS, zoals high gevoel en op de 'body sway', een maat van houdingsstabiliteit. De metabole effecten van de laagste dosis TM38837 van 100 mg zouden (op grond van voorspellingen vanuit knaagdiermodellen) tenminste equipotent zijn aan die van de onderzochte dosering van rimonabant. TM38837 had in deze lage dosis in onze studie geen significante CZS-effecten, in tegenstelling tot rimonabant. De resultaten bevestigen dat verdere ontwikkeling van TM38837 als een perifeer selectieve CB_1-antagonist interessant is voor indicaties zoals metabole ziekten, met daarbij een lagere kans op psychiatrische bijwerkingen.

In hoofdstuk 6 worden de PK-PD-analyses in een groter perspectief geplaatst. In dit hoofdstuk worden de resultaten van hoofdstukken 4 en 5 en de resultaten van een eerdere studie met drinabant (AVE1625) (Zuurman et al., 2010) gebruikt voor het bouwen van een algemeen antagonistenmodel. Deze analyses laten zien dat TM38837 relatief grotere perifere effecten dan centrale effecten veroorzaakt vergeleken met rimonabant, surinabant en drinabant. Hieruit concludeerden we dat TM38837 potentie heeft om doorontwikkeld te worden voor perifere indicaties, zoals het metaboolsyndroom, met beperkte risico's op de centrale bijwerkingen die tot de terugtrekking van rimonabant hadden geleid.

METINGEN OPTIMALISEREN

Naast de beperkte kennis over het cannabinoïdesysteem en de beperkte mogelijkheden die de farmacologische eigenschappen van dit systeem bieden, wordt geneesmiddelenontwikkeling verder beperkt door het gebrek aan gevalideerde effectmetingen. Nieuwe meetmethoden kunnen belangrijk zijn om de fysiologie van het cannabinoïdesysteem beter te begrijpen, om de juiste dosering te selecteren, om de effecten van stoffen beter te vergelijken, en om ons algehele begrip van het cannabinoïdesysteem te verbeteren.

Effecten in het CZS manifesteren zich als activiteit van neurale netwerken. Deze activiteiten kunnen direct worden gemeten, door middel van connectiviteitsanalyses. Er zijn verschillende technieken die hier gebruik van maken, met name neurofysiologische methoden ([electroencefalografie (EEG), magnetoencefalografie (MEG)] en neurovisualisatietechnologieën [*positron emissie tomografie* (PET), functionele magnetische resonantiebeeldvorming (fMRI)]. Met behulp van deze netwerkanalyses kunnen verandering in het CZS die door ziekten of medicijnen worden veroorzaak, directer worden gemeten dan met testen van CZS-functies zoals gedragsmaten of subjectieve veranderingen.

In hoofdstuk 3 hebben we veranderingen in hersenconnectiviteit gemeten na THC-toediening, met behulp van de zogenaamde resting-state functionele MRI (RS-FMRI). We vonden dat THC in sommige 'networks of interest' een significante toename in hersenconnectiviteit veroorzaakt en in andere netwerken juist een significante afname. Deze duidelijke effecten, welke ook werden gevonden in studies van onze groep bij andere middelen, wekken de suggestie dat RS-FMRI een geschikte methode is voor vroege fase klinisch geneesmiddelenonderzoek. De hersengebieden waarin de connectiviteitsveranderingen werden gevonden waren vergelijkbaar met de functionele hersengebieden die worden geassocieerd met de bekende gedragseffecten na THC- of cannabisgebruik, zoals houdingsinstabiliteit en een veranderde tijdsperceptie (beide in het cerebellum). RS-FMRI heeft als methodologie unieke eigenschappen vergeleken met andere (gangbaardere) CZS-metingen die we in dit proefschrift hebben toegepast. In contrast met de algemeen toegepaste neurofysiologische, functionele en vooral subjectieve methoden is RS-FMRI in staat om een zeer wijd spectrum van zowel directe als indirecte (acute) effectprofielen te meten. Daarbij is deze manier van meten 'objectief'; dat wil zeggen: er is minder verborgen interferentie van compensatoire mechanismen of motivatie-aspecten of andere factoren die de respons en de uitvoering van testen kunnen beïnvloeden. Bovendien biedt deze methodologie de ruimte om in vroege fase klinisch onderzoek met zeer lage geneesmiddelenconcentraties te werken, waarvan de effecten eenvoudig gecompenseerd worden door compensatoire mechanismen, of in andersoortige testen gewoonweg niet kunnen worden waargenomen. Ook kan RS-FMRI geschikt zijn om functioneel 'stille' geneesmiddel effecten waar te nemen, zoals van CB_1-antagonisten die bij gezonde vrijwilligers geen acuut meetbare veranderingen laten zien van de gangbare neurofysiologische testen. Dit moet echter nog wel worden onderzocht.
Samengevat concluderen we dat THC connectiviteitsveranderingen teweeg brengt in hersengebieden die geassocieerd zijn met gedragseffecten na THC- of cannabisgebruik. RS-FMRI lijkt een geschikte techniek voor klinisch geneesmiddelenonderzoek, waaronder de ontwikkeling van cannabinoïde farmacotherapieën. Met behulp van verder onderzoek naar dosis-effect-relaties, bijvoorbeeld door PK-PD-modellen voor RS-FMRI te ontwikkelen, kan de toepasbaarheid van RS-FMRI verder worden uitgebouwd. Ook is het interessant om de implicaties van de methodologie in een breder perspectief te begrijpen, bijvoorbeeld door de relatie tussen connectiviteitsveranderingen en hersenfuncties verder te onderzoeken. Op deze manier kunnen we de toepassing van de techniek optimaal benutten en tegelijkertijd onze nog beperkte kennis over endocannabinoïde systemen in de hersenen vergroten.

ANALYSES VERBETEREN

In hoofdstuk 5, waarin een studie wordt beschreven met de perifeer selectieve CB_1-antagonist TM38837 en met rimonabant, ontwikkelden we PK-PD-modellen voor hartslagfrequentie, houdingsstabiliteit (body sway) en het high gevoel. Alle PK-PD-modellen bevatten een basisniveau (baseline), effectcompartimenten die equilibreren met de plasmaconcentratie en een model om de effectcompartimentconcentratie te relateren aan de farmacodynamische respons. De modellen voor hartslag en body sway werden het best omschreven door een maximaal-effect-model. Voor het high gevoel werd een waarschijnlijkheidsmodel ('probability model') gebruikt, waarbij de kans werd bepaald dat de VAS-score boven of onder de mediaan van de studiepopulatie zou liggen. Alle modellen bevatten zowel de effecten van de THC-challenge-effecten als de remmende werking van rimonabant en TM38837. De equilibratie-halfwaardetijd van TM38837 was lang vergeleken met rimonabant. Dit veroorzaakte een grotere vertraging in de aanvang van de effecten van TM38837. Hartslag liet half-maximale inhibitoire concentraties (IC_{50}) zien die voor TM38837 en rimonabant overeenkomstig waren, terwijl rimonabant bij body sway en high gevoel zelfs een IC_{50} had van respectievelijk 4 en 56 keer groter dan voor TM38837. Dit doet vermoeden dat TM38837 relatief

kleinere centrale dan perifere effecten heeft dan rimonabant. De tijdprofielen van de effecten zijn vergelijkbaar met de PK-profielen van beide stoffen. Helaas zijn er op dit moment nog geen studies uitgevoerd die de therapeutische mogelijkheden van TM38837 nader onderzoeken en onze hypotheses over het grotere therapeutische venster van TM38837 kunnen verifiëren.

Voor vier verschillende CB_1-antagonisten, namelijk drinabant (AVE1625), surinabant (SR147778), rimonabant (SR141716) en TM38837, hebben we in hoofdstuk 6 PK-PD-modellen gebouwd, waarbij de verschillende antagonisten per PD-parameter werden geïntegreerd in één model. Deze aanpak verschafte ons inzicht in de PK- en PD-verschillen tussen de vier antagonisten en verbeterde onze kennis over het gedrag van CB_1-antagonisten in het algemeen. Vergeleken met TM38837 lieten surinabant en rimonabant effectprofielen zien met relatief grotere centraal gereguleerde PD-effecten dan effecten op de hartslag. Drinabant leek meer op TM38837 dan op de andere CB_1-antagonisten. Deze modellen kunnen onder meer worden toegepast voor de ontwikkeling en optimalisatie van toekomstige klinische studies door simulatie en voorspellingen van de PK en PD van cannabinoïde-antagonisten te genereren. Onderzoek op het gebied van 'mechanism-based' PK-PD-modelleren blijft zich ook vandaag de dag nog ontwikkelen met bijvoorbeeld meer nadruk op systeemanalyse van ziekten. Het is een onderzoeksveld van grote betekenis en verdere ontwikkeling in de toekomst is belangrijk; niet alleen voor de ontwikkeling van translationele modellen van gezonde personen naar patiënten, maar ook in andere fases van geneesmiddelenontwikkeling zijn modellen voor translatie, simulatie en voorspelling van PK en PD van groot belang (bijvoorbeeld in de overgang van preklinisch naar klinisch onderzoek).

ALGEMENE CONCLUSIES

Het doel van dit proefschrift was om verschillende manieren te onderzoeken die kunnen bijdragen aan de verbetering van cannabinoïdegeneesmiddelen, door te kijken naar verbeteringen in het ontwerp van vroege fase klinische studies, en door toepassing van nieuwe meetmethoden en analyses. Deze verbeteringen zijn hard nodig voor ons nog beperkte begrip van de farmacologie van cannabinoïden in gezonde personen en om cannabinoïden effectief te kunnen gebruiken voor de behandeling van ziekten.

In dit proefschrift hebben we een nieuwe orale THC-formulering en een nieuwe CB_1-antagonist geïntroduceerd in studies met gezonde personen. Hieruit concludeerden we dat de nieuwe formuleringen betere farmacologische effecten lieten zien vergeleken met de huidige behandelingen. Ook hebben we in dit proefschrift nieuwe methodologieën geoptimaliseerd en toegepast. Zo lieten we onder meer zien dat resting state-fMRI een geschikte technologie is voor vroege fase klinisch geneesmiddelenonderzoek en dat de THC-challengetest toegepast kan worden voor farmacologische karakterisering en dosisoptimalisatie van antagonisten. Om dit verder uit te breiden hebben we PK-PD-modellen ontwikkeld voor THC en voor de CB_1-antagonisten drinabant, surinabant, TM38837 en de inverse agonist rimonabant. De toepassing van deze modellen is van belang voor simulatie en voorspelling van PK en PD, bijvoorbeeld om toekomstige studie-ontwerpen te kunnen optimaliseren. In vroege fasen van geneesmiddelenontwikkeling levert deze methodologie meer informatie op dan de meer traditionele aanpak waarbij de selectie van doseringen in feite voornamelijk gebaseerd wordt op extrapolatie en allometrische schalen van preklinische resultaten, PK-optimalisatie van doseringsschema's en de schatting van de maximaal getolereerde dosering – welke in het beste geval wordt aangevuld met enkele indicaties van farmacodynamische effecten. De traditionele aanpak geeft vooral gemakkelijk verkeerde informatie wanneer bijvoorbeeld het te onderzoeken geneesmiddel een nieuw werkingsmechanisme heeft, en ook wanneer er geen meetbare effecten zijn onder fysiologisch stabiele condities. Het lijkt er sterk op dat dit van toepassing was op rimonabant, dat kort na registratie van de markt af werd gehaald vanwege psychiatrische

bijwerkingen die achteraf gezien misschien verwacht hadden kunnen worden. In dit proefschrift onderzochten we op gestructureerde manier de PK-PD-relaties om zowel voor rimonabant als voor de andere nieuwe CB_1-antagonisten de farmacologisch optimale dosering te bepalen. Onze analyse geven aanwijzingen dat rimonabant in de praktijk mogelijk werd overgedoseerd, wat vermoedelijk werd veroorzaakt door de goede verdraagbaarheid van het middel in gezonde personen, die immers geen 'spontane' effecten lieten zien. Dit blijft speculatief zolang er geen vervolgstudie is uitgevoerd die onze hypothese bevestigt.

Het is nog steeds een grote vraag wat de voorspellende waarde van de toegepaste farmacologische biomarkers is voor klinische relevante therapeutische of juist ongewenste effecten van CB_1-antagonisten. Verder onderzoek is dan ook nodig naar de vraag in welke mate functionele challengestudies en PK-PD-analyses in staat zijn om een therapeutisch venster te bepalen dat groot genoeg is voor een veilig en effectief gebruik van CB_1-antagonisten. Toch vergroot een farmacologische aanpak de hoop dat geneesmiddelenonderzoek naar (endo)cannabinoïden realiseerbaar is en potentie heeft, ondanks de serieuze problemen die inherent zijn aan onderzoek naar een dergelijk complex systeem. Deze hoop voor cannabinoïdenonderzoek en geneesmiddelenontwikkeling wordt mede gevoed door de afname van taboes op cannabisgebruik. Het recreationele gebruik wordt sinds de jaren 1970 steeds meer geaccepteerd en een toenemend aantal landen en staten in de VS decriminaliseren cannabis [(Robison, 2013), voor een illustratie, zie Reeve (2013)]. Deze verspreiding, met name bij gebruik voor medische toepassingen, vergroot de algemeen maatschappelijke acceptatie van cannabis, wat een stimulans kan bieden aan verder onderzoek naar cannabisgerelateerde middelen.

ALGEHELE CONCLUSIE

Onze resultaten leidden tot de conclusie dat er veel ruimte is voor verbeteringen in het cannabinoïdenonderzoek – voldoende om het vertrouwen te geven dat het cannabinoïdesysteem nog steeds potentie heeft in het kader van farmacologische therapieën, ondanks dat de eerste geregistreerde cannabinoïde-antagonist kort na registratie weer van de markt gehaald werd. Alhoewel er momenteel weinig cannabinoïdenonderzoek met vooral erg weinig klinisch werk plaatsvindt, kan de toenemende maatschappelijke acceptatie van cannabis, ook als een geneesmiddel, bijdragen aan een herleving van onderzoek aan het endocannabinoïdensysteem. Ons onderzoek laat zien dat hiervoor nieuwe manieren nodig zijn om cannabinoïden toe te dienen, de studies te ontwerpen en om hun effecten te meten en te analyseren. Dit reflecteert de complexiteit van het diep geïntegreerde endocannabinoïdesysteem en effent ook de weg voor andere innovatieve geneesmiddelenontwikkelingsprogramma's.

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CURRICULUM VITAE

In 1980 I was born in Rotterdam. After finishing grammar school in 1999 at the Gymnasium Camphusianum in Gorinchem, I studied medical biology with a focus on neurobiology at the University of Amsterdam. After graduation (M.Sc.) in January 2004, travelling and working as a full-time musician, I worked as a project leader at the Centre for Human Drug Research from 2006 to 2011. The research described in this thesis was performed during that period. In 2010, I was awarded the Predoctoral student award at the International Cannabinoid Research Society conference in Lund, Sweden for my presentation of the results of 'Peripheral selectivity of the novel cannabinoid receptor antagonist TM38837 in healthy subjects'. For publication of this research, I was awarded the BJCP Prize in London in 2013. During my work at the CHDR, I was trained as a clinical pharmacologist. From 2012 to 2013, I worked as a senior business analyst at A.T. Kearney. In October 2013, I started as a business development manager at SkylineDx, a medical diagnostics company.

PUBLICATION LIST

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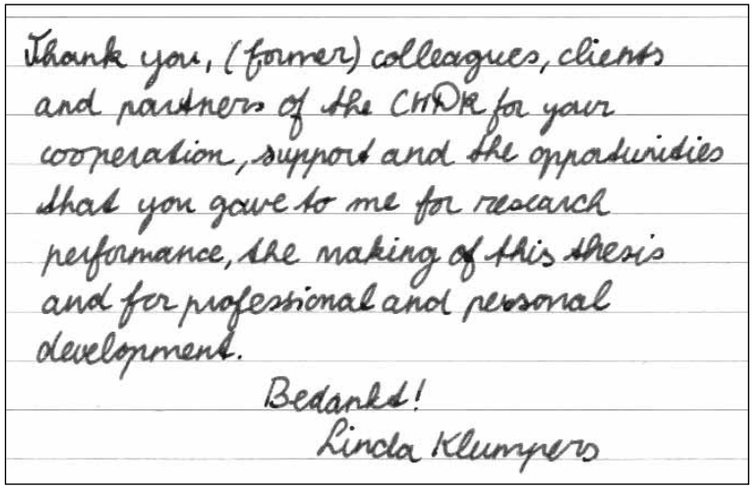
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