Novel approaches in clinical development of cannabinoid drugs

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NOVEL APPROACHES IN CLINICAL DEVELOPMENT OF CANNABINOIDS DRUGS
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ACKNOWLEDGEMENTS
The publication of this thesis was financially supported by the foundation Centre for Human Drug Research, Leiden

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NEDERLANDSE SAMENVATTING / SUMMARY IN DUTCH
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Chapter I

Introduction to the endocannabinoid system as a target for drug development
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**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 hydrids, 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 monocular 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). Δ9-
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: CB1 and CB2 receptors, which have different functions and localisation patterns. CB1 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, CB2 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 CB2 receptor is less widely expressed than the CB1 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)). CB1 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 CB1 and CB2 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 (CB₁ and CB₂) 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 CB₁ receptor (Ki = 61-543 nM) compared to CB₂ (Ki = 279-1940 nM) (Pertwee, 2005). 2-AG has shown higher efficacy with similar affinities, and acts as a full agonist on both CB₁ and CB₂ 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 CB₂ receptor, which affects ion channels or second messenger signalling pathways (Bozier 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). CB₁ 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 postsynaptic 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 CB₁ expression in the brain, or blood and tissue concentrations of the major endocannabinoids as outlined in a review
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 CB1 contributes to the motivational and reinforcing properties of ethanol, and chronic consumption alters endocannabinoid transmitter levels and CB1 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).
I. Introduction to the Endocannabinoid System

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 CB1 and CB2 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 countering 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 CB2 receptors on hematopoietic 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-

Immunology – Immunologic disorders for which the endocannabinoid system has been investigated include multiple sclerosis, arthritis, sepsis, inflammatory bowel disease, pancreatitis, uveitis and peritonitis. 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 CB1 and CB2 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 CB1 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 CB1 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 CB1 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 CB2 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.

Gastroenterology – Immunologic disorders for which the endocannabinoid system has been investigated include multiple sclerosis, arthritis, sepsis, inflammatory bowel disease, pancreatitis, uveitis and peritonitis. 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.

Endocannabinoids have been implicated in the pathogenesis of inflammatory bowel disease (IBD). The endocannabinoid system is involved in the regulation of immune function, including the production of pro-inflammatory cytokines and the modulation of immune cells. For example, CB1 receptor antagonists have been shown to reduce inflammatory responses in animal models of IBD. Additionally, endocannabinoids have been shown to modulate the gut microbiome, which is important for maintaining gut health and preventing inflammation.

Endocannabinoids also play a role in the regulation of gastrointestinal motility. CB1 receptors are expressed in the gut and are involved in the control of gut motility. CB1 agonists and antagonists have been shown to affect gastrointestinal motility, with CB1 agonists slowing motility and CB1 antagonists accelerating motility.

In summary, endocannabinoids play a role in the pathogenesis and regulation of inflammatory bowel disease. Further research is needed to fully understand the role of the endocannabinoid system in IBD and to develop therapeutic strategies targeting this system for the treatment of IBD.

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the endocannabinoid 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 \textit{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 CB1 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 CB1 and the CB2 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
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. The most favourable administration route was chosen for further development of Namisol for the indication of pain and spasms in multiple sclerosis.

**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.
Another option to improve specificity for a specific target or location would be to improve the delivery of the compound to a specific location. 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 CB1-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
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 EMAX-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 CB1 antagonists drinabant (AVE1625), surinabant (SR147778) and TM38837 and CB1 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.
REFERENCES

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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 bioavailability 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 cannabinoïds (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 cannabinoïds 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 $t_{1/2INIT}$) 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 $t_{1/2TERM}$ 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.
Chapter I – Introduction to the Endocannabinoid System

Novel approaches in clinical development of cannabinoid drugs

Figure 3

Complexity of intracellular CB1 receptor signalling. As for G protein coupled receptors in general, the CB1 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 CB1 receptors located at the axon terminals is via a retrograde pathway. Endocannabinoids, such as anandamide and 2-arachidonoylglycerol are released post-synaptically. Via the synapse, the molecules migrate to the pre-synaptic cell, where they give feedback via stimulation of the CB1 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 bloodstream 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 CMBR 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 $\Delta^9$-tetrahydro-cannabinol formulation
Namisol has beneficial pharmacokinetics and promising pharmacodynamic effects


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ABSTRACT

AIM Among the main disadvantages of currently available Δ9-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 t1/2 was 72-80 min, TMAX was 39-56 min, and CMAX 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 TMAX 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, Δ9-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 (CB1 and CB2) (Alexander et al., 2008). CB1 are mainly located in the central nervous system, as well as in peripheral tissues such as the heart, adipose tissue and sympathetic ganglia, while CB2 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 CB1 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°C). The handling of THC samples was done at low ambient lighting. Plasma samples were stored at a temperature of at least –70°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 (Cmax), time to peak plasma concentration (Tmax), apparent terminal half-life (t1/2), and area under the curve from t = 0 to infinity (AUC0-∞). Dose-proportionality was assessed for Cmax and AUC0-∞. 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, α = 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 $T_{\text{MAX}}$ and a higher $C_{\text{MAX}}$ of oral THC indicated a possibly larger effect with a faster onset compared to sublingual administration. These differences in $T_{\text{MAX}}$ and $C_{\text{MAX}}$ between oral and sublingual administration were not statistically significant. Sublingual administration showed a significant longer apparent $t_{\text{CL/1}}$ compared to oral administration (+122 min; 95% CI 64/181; $p = 0.0002$). $AUC_{0-\infty}$ and $C_{\text{MAX}}$ of oral THC were dose proportional and $T_{\text{MAX}}$ and $t_{\text{CL/1}}$ 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 $t_{\text{CL/1}}$, 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. $C_{\text{L}}$ and $V_{\text{F}}$, 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 gastrointestinal 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 CB1-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.

REFERENCES


Chapter II – PK and PD of Namisol®

Table 1: Summary of subject demographics of Panel I and Panel II

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Mean</th>
<th>Std</th>
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<td></td>
<td></td>
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<td>Age (yrs)</td>
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<td>21.9</td>
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<td>BMI (kg m^-2)</td>
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<td>22.31</td>
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<td>1.91</td>
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<td>Weight (kg)</td>
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<td>69.70</td>
<td>8.91</td>
<td>55.3</td>
<td>80.6</td>
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</table>

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 (%).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Panel</th>
<th>Panel</th>
<th>Panel</th>
</tr>
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<tr>
<td></td>
<td>I (n=13)</td>
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<td>I (n=13)</td>
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<tr>
<td>THC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax (ng ml^-1)</td>
<td>2.30 (44)</td>
<td>2.92 (51)</td>
<td>4.43 (42)</td>
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<tr>
<td>Tmax (min)</td>
<td>74.5 (52)</td>
<td>56.0 (73)</td>
<td>39.3 (20)</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-&lt;/sub&gt;t&lt;sub&gt;∞&lt;/sub&gt; (ng.min ml^-1)</td>
<td>235.8 (47)</td>
<td>188.7 (40)</td>
<td>286.6 (36)</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (min)</td>
<td>252.9 (98)</td>
<td>71.9 (24)</td>
<td>80.0 (22)</td>
</tr>
<tr>
<td>11-OH-THC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax (ng ml^-1)</td>
<td>3.08 (42)</td>
<td>4.68 (42)</td>
<td>5.94 (44)</td>
</tr>
<tr>
<td>Tmax (min)</td>
<td>83.6 (63)</td>
<td>74.1 (68)</td>
<td>46.1 (28)</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-&lt;/sub&gt;t&lt;sub&gt;∞&lt;/sub&gt; (ng.min ml^-1)</td>
<td>522.9 (50)</td>
<td>648.1 (49)</td>
<td>848.7 (42)</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (min)</td>
<td>279.0 (51)</td>
<td>196.0 (33)</td>
<td>318.7 (54)</td>
</tr>
</tbody>
</table>

* C<sub>max</sub> and AUC<sub>0-</sub>t<sub>∞</sub> were dose-corrected for treatment p-value calculation.

C<sub>max</sub> = peak plasma concentration; Tmax = time to peak plasma concentration; AUC<sub>0-</sub>t<sub>∞</sub> = area under the curve from t=0 to infinity; t<sub>1/2</sub> = apparent terminal half-life.
### Table 3: THC population pharmacokinetic parameters after oral Namisol®.

<table>
<thead>
<tr>
<th>Parameter (L min⁻¹)*</th>
<th>THC Estimate (rse)</th>
<th>THC iiv Estimate (rse)</th>
<th>11-OH-THC Estimate (rse)</th>
<th>11-OH-THC iiv Estimate (rse)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearance/F</td>
<td>26.5 (10.6)</td>
<td>28.4</td>
<td>9.53 (25)</td>
<td>70.4</td>
</tr>
<tr>
<td>Central volume of distribution/F</td>
<td>889 (22.5)</td>
<td>56.3</td>
<td>52.6 (47.9)</td>
<td>413</td>
</tr>
<tr>
<td>Peripheral volume of distribution/F</td>
<td>1790 (21.9)</td>
<td>-</td>
<td>1010 (15.3)</td>
<td>21.1</td>
</tr>
<tr>
<td>Intercompartmental clearance/F (L min⁻¹)*</td>
<td>13.1 (17)</td>
<td>-</td>
<td>4.46 (34.5)</td>
<td>50.7</td>
</tr>
<tr>
<td>Absorption rate constant (min⁻¹)</td>
<td>0.0401 (22)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Proportional residual error (sv = mean⁻¹)</td>
<td>0.509 (8)</td>
<td>-</td>
<td>0.461 (6.2)</td>
<td>-</td>
</tr>
<tr>
<td>Absorption lag time (min)</td>
<td>11.5 (0.9)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* 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).

<table>
<thead>
<tr>
<th>Panel</th>
<th>I (n=13)</th>
<th>II (n=9)</th>
<th>II (n=9)</th>
<th>II (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>5.0 mg oral vs 5.0 mg sublingual</td>
<td>6.5 mg oral vs placebo</td>
<td>8.0 mg oral vs placebo</td>
<td>8.0 mg vs 6.5 mg oral</td>
</tr>
<tr>
<td>Body sway (%)</td>
<td>7.66 (-4.62, 21.53) p=0.2037</td>
<td>22.06 (-1.05, 50.57) p=0.0610</td>
<td>31.76 (6.53, 62.96) p=0.0145*</td>
<td></td>
</tr>
<tr>
<td>Vas (mm)</td>
<td>-0.3 (-2.0, 1.5) p=0.7124</td>
<td>-1.4 (-3.2, 0.4) p=0.1161</td>
<td>-2.7 (-4.5, -0.9) p=0.0057*</td>
<td>-1.3 (-3.1, 0.5) p=0.1190</td>
</tr>
<tr>
<td>Mood (mm)</td>
<td>0.8 (-0.1, 1.6) p=0.0653</td>
<td>0.1 (-0.3, 0.5) p=0.0686</td>
<td>0.2 (-0.2, 0.6) p=0.0686</td>
<td>0.1 (-0.4, 0.5) p=0.7815</td>
</tr>
<tr>
<td>Calmness (mm)</td>
<td>1.8 (0.1, 3.5) p=0.0443*</td>
<td>0.7 (-0.1, 1.4) p=0.1246</td>
<td>0.3 (-0.2, 1.2) p=0.1246</td>
<td>-0.1 (-0.9, 0.6) p=0.7080</td>
</tr>
<tr>
<td>Feeling high (U)</td>
<td>0.111 (-0.42, 0.265) p=0.1347</td>
<td>0.229 (0.073, 0.384) p=0.0071*</td>
<td>0.256 (0.093, 0.418) p=0.0044*</td>
<td>0.027 (-0.129, 0.183) p=0.7145</td>
</tr>
<tr>
<td>External perception (U)</td>
<td>0.037 (-0.017, 0.090) p=0.1482</td>
<td>0.061 (0.002, 0.121) p=0.0446*</td>
<td>0.078 (0.019, 0.137) p=0.0141*</td>
<td>0.017 (-0.042, 0.076) p=0.5507</td>
</tr>
<tr>
<td>Internal perception (U)</td>
<td>0.006 (-0.014, 0.026) p=0.5247</td>
<td>0.013 (-0.003, 0.029) p=0.1057</td>
<td>0.002 (-0.015, 0.019) p=0.8312</td>
<td>-0.011 (-0.028, 0.005) p=0.1632</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>0.2 (-3.6, 4.0) p=0.9261</td>
<td>5.3 (2.4, 8.2) p=0.0019*</td>
<td>5.6 (2.7, 8.5) p=0.0014*</td>
<td>0.3 (-2.7, 3.2) p=0.8524</td>
</tr>
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</table>

* Statistically significant values

**Figure 1**: THC (A) and 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.

- **A. 5 mg THC sd**
- **B. 6.5 mg THC sd**
- **C. 8 mg THC sd**
- **D. 5 mg THC sd**
- **E. 6.5 mg THC sd**
- **F. 8 mg THC sd**

**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.
Manipulating brain connectivity with ∆9-tetrahydrocannabinol: a pharmacological resting state fMRI study

Neuroimage (2012) volume 63, issue 3, pages 1701-1711

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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 (CB₁)-receptor agonist ∆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 CB₁-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; 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 ∆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...
bonds to two cannabinoid receptors (CB₁ and CB₂) of which CB₁ receptors are predominantly present in various brain areas (Herkenham, 1992). The action of THC on the CB₁ 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/m² 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 1.5h 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°C. THC samples were handled sheltered from light. Plasma samples were stored at –20°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°; 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 (µg/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°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 electrochemoluminescence-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}) 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 frontoparietal 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 FSL 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 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 (6 mg 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 NOI 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 visual external perception (0.225 (U); 95% CI 0.054 - 0.396; p = 0.0149), feeling high (0.768 (U); 95% CI 0.578 - 0.957; p < .0001), and heart rate (10.3 bpm; 95% CI 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% CI 11.9 - 56.3; p = 0.0051) after THC, whereas prolactin decreased with 21.0% (95% CI -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% CI 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 11h28m 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).

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 Boisguilhenec et al., 2006; Rose et al., 2011). In human brain tissue, CB1 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 CB1 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 (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.
REFERENCES


### Table 1: Demographics of the subjects that were included for pharmacodynamic and pharmacokinetic analyses.

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

SD = standard deviation

### Table 2: Pharmacokinetic parameters of THC as assessed by non-compartmental pharmacokinetic analysis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Median</th>
<th>Uncertainty (%Scv)</th>
<th>IIV (%Scv)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL/F (L/hr)</td>
<td></td>
<td>145</td>
<td>11.2</td>
<td>37.4</td>
</tr>
<tr>
<td>V/F (L)</td>
<td></td>
<td>20.1</td>
<td>12.5</td>
<td>37.4</td>
</tr>
<tr>
<td>V peripheral (L)</td>
<td></td>
<td>78.6</td>
<td>15.8</td>
<td>37.4</td>
</tr>
<tr>
<td>Q peripheral (L)</td>
<td></td>
<td>95.6</td>
<td>15.1</td>
<td>37.4</td>
</tr>
<tr>
<td>Q1/2 (hr)</td>
<td></td>
<td>0.986</td>
<td>4.91</td>
<td>NA</td>
</tr>
<tr>
<td>t1/2 (hr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**cv = coefficient of variation, iiv = interindividual variability, Cl = clearance, F = bioavailability, V = distribution volume, Q = intercompartmental clearance, t1/2 = initial half-life, NA = not applicable.**

### 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.

<table>
<thead>
<tr>
<th>Networks</th>
<th>Region (Harvard-Oxford)</th>
<th>t-value</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>Voxel number</th>
<th>THC effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO11: Medial visual</td>
<td>Superior and medial frontal gyri (premotor cortex)</td>
<td>5.32</td>
<td>-24</td>
<td>-2</td>
<td>46</td>
<td>26</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Dorsal acc</td>
<td>5.02</td>
<td>2</td>
<td>12</td>
<td>34</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Temporal occipital fusiform cortex</td>
<td>4.9</td>
<td>-30</td>
<td>-48</td>
<td>-18</td>
<td>45</td>
<td>-</td>
</tr>
</tbody>
</table>

**NO12: Lateral visual**

|          | Temporal occipital fusiform cortex (extending into parahippocampal gyrus) | 4.91 | -30 | -48 | -12 | 163 | + |
|          | Ventromedial cerebellum | 5.03 | -22 | -60 | -44 | 36 | + |
|          | Temporal occipital fusiform cortex (extending into parahippocampal gyrus) | 4.16 | 26 | -38 | -18 | 22 | + |
|          | Middle frontal gyrus, dl pfc | 5.34 | 28 | 20 | 44 | 14 | + |
|          | Posterior precuneous cortex | 4.8 | 2 | -74 | 42 | 203 | - |
|          | Occipital pole, lateral occipital cortex | 4.3 | 34 | -92 | -10 | 27 | - |
|          | Precentral gyrus | 5.54 | 46 | -10 | 58 | 12 | - |

**NO13: Auditory**

|          | Parahippocampal gyrus (extending into hippocampus) | 4.66 | 40 | -34 | -10 | 132 | + |
|          | Pcc, retrosplenial cortex | 5.04 | 0 | -46 | 2 | 20 | + |
|          | Caudate | 5.86 | 20 | 12 | 2 | 16 | + |
|          | Supramarginal gyrus, superior/medial/inferior temporal gyri, temporal pole, parahippocampal gyrus, lateral ofc | 6.07 | 62 | -30 | 28 | 25075 | - |
|          | Superior frontal gyrus, dm pfc | 4.83 | 0 | 50 | 28 | 282 | - |
|          | Frontal pole, dm pfc | 4.13 | 10 | 60 | 22 | 104 | - |
|          | Precentral gyrus, superior parietal cortex | 3.85 | -10 | -16 | 64 | 52 | - |
|          | Mid-cingulate cortex | 4.28 | -4 | -8 | 34 | 38 | - |
|          | Precentral gyrus, superior mid-cingulate cortex | 4.11 | -4 | -20 | 50 | 30 | - |
|          | vm pfc | 4.12 | 16 | 54 | 6 | 28 | - |
|          | Middle frontal gyrus | 3.36 | 46 | 10 | 40 | 19 | - |
|          | Precentral gyrus, superior mid-cingulate | 4.12 | 6 | -26 | 54 | 17 | - |

(Table continues on next page)
### Networks and Region (Harvard-Oxford)

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

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, dmPFC - dorso-lateral PFC, dmPFC - dorso-medial PFC, vmPFC - ventro-medial PFC, OFC - orbito-frontal cortex

### Table 4: Overview of the non-fMRI pharmacodynamic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LSM Treatment</th>
<th>Contrasts</th>
<th>LSM change from baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>THC</td>
<td>P-value</td>
<td>THC vs Placebo</td>
</tr>
<tr>
<td>Placebo</td>
<td>THC</td>
<td></td>
<td>Placebo</td>
</tr>
<tr>
<td>VAS Alertness (mm)</td>
<td>52.5</td>
<td>45.9</td>
<td>0.0646</td>
</tr>
<tr>
<td>VAS Calmness (mm)</td>
<td>53.9</td>
<td>55</td>
<td>0.2248</td>
</tr>
<tr>
<td>VAS Mood (mm)</td>
<td>55</td>
<td>55</td>
<td>0.9787</td>
</tr>
<tr>
<td>VAS External log (mm)</td>
<td>0.32</td>
<td>0.545</td>
<td>0.0149*</td>
</tr>
<tr>
<td>VAS Internal log (mm)</td>
<td>0.308</td>
<td>0.346</td>
<td>0.0718</td>
</tr>
<tr>
<td>VAS feeling high log (mm)</td>
<td>0.285</td>
<td>1.053</td>
<td>-0.001*</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>66.7</td>
<td>77</td>
<td>0.0026*</td>
</tr>
<tr>
<td>fSH (U/L)</td>
<td>2.327</td>
<td>2.291</td>
<td>0.5601</td>
</tr>
<tr>
<td>LH (ng/ml)</td>
<td>4.16</td>
<td>3.15</td>
<td>0.0935</td>
</tr>
<tr>
<td>Cortisol (µmol/ml)</td>
<td>0.36</td>
<td>0.47</td>
<td>0.0051*</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>8.41</td>
<td>6.64</td>
<td>0.0100*</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>4.7</td>
<td>5.1</td>
<td>0.0468*</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.12</td>
<td>1.12</td>
<td>0.8933</td>
</tr>
<tr>
<td>Leptin (µg/l)</td>
<td>3.6</td>
<td>3.7</td>
<td>0.8328</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.98</td>
<td>0.97</td>
<td>0.0806</td>
</tr>
</tbody>
</table>
* 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.
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 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 phylogenetically 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 CB₁ 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 CB₁ 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 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 $t_{\text{MAX}}$) 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 relatively 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 CB1 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 CB1 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 CB1 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 CB1 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 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-
Peripheral 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 CB1 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 neurophysiological 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 CB1 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 (IC50) were similar for TM38837 and rimonabant, whereas for body sway and feeling high the IC50 of rimonabant was 4 times and 36 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 CB1 antagonists: drinabant (AV1625), 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 CB1 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 CB1 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.
AMACouncil (2013) Obesity should be considered a Chronic Medical Disease State.
Al duizenden jaren is cannabis wereldwijd een van de meest populaire drugs. Cannabis wordt vooral gebruikt voor recreatieve en medische doeleinden. De CB₁- en CB₂-receptoren waaraan cannabinoïden (cannabinoiden) zich binden, zijn onderdeel van het endocannabinoïdesysteem, 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 CB₁-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 metabool syndroom, 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 CB₁-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 cannabismeisbruik. Bovendien zijn veel van de potentiële indicaties voor cannabinoïde middelen in discussie; vetzucht 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 receptorsubtypenpecificiteit, locale productie van zeer lipoïele 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 recreatieve cannabismeisbruik. Alhoewel een prettig en ‘high’ gevoel, de ‘munchies’ (hongeraanvallen) en de paniekaanvallen na cannabismeisbruik 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 als of 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 (tmax) en een geringere variabiliteit heeft, wat waarschijnlijk bijdraagt aan snelere 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 CB1-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 CB1-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 kunnen doen met enerzijds optimaal effecten en anderzijds een minimaal risico op ongewenste (centrale) bijwerkingen tijdens patiëntenstudies. Alhoewel surinabant zelf geen effecten liet zien in de verschillende centraal zenuwstelsel (CNS)-tests, concludeerden we dat de dosisgerelateerde remming van THC-effecten wijst op CB1-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 \textit{cb}1-antagonist \textit{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 \textit{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 \textit{pk}- en \textit{pd}-modellering. Vergeleken met rimonabant veroorzaakt \textit{tm38837} een relatief grotere perifere effecten dan centrale effecten vergeleken met rimonabant, surinabant en drinabant. Hieruit concludeerden we dat \textit{tm38837} potentieel heeft om doorontwikkeld te worden voor perifere indicaties, zoals het metabool syndroom, met beperkte risico’s op de centrale bijwerkingen die tot de terugtrekking van rimonabant hadden geleid.

\textbf{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 \textit{czs} manifesteren zich als activiteit van neurale netwerken. Deze activiteiten kunnen direct worden gemeten, door middel van meting van connectiviteitsanalyse. Daarbij worden 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 onderzoek naar de effecten van stoffen in het \textit{czs} worden uitgevoerd.

In hoofdstuk 6 worden de \textit{pk-pd}-analyses in een groter perspectief geplaatst. In dit hoofdstuk werden de resultaten van hoofdstukken 4 en 5 en de resultaten van een eerdere studie met drinabant (\textit{ave1625}) (Zuurman et al., 2010) gebruikt voor het bouwen van een algemeen antagonistenmodel. Deze analyses laten zien dat \textit{tm38837} relatief grotere perifere effecten dan centrale effecten veroorzaakt vergeleken met rimonabant, surinabant en drinabant. Hieruit concludeerden we dat \textit{tm38837} potentieel heeft om doorontwikkeld te worden voor perifere indicaties, zoals het metabool syndroom, met beperkte risico’s op de centrale bijwerkingen die tot de terugtrekking van rimonabant hadden geleid.
In hoofdstuk 3 hebben we veranderingen in hersenconnectiviteit geme-
ten na THC-toediening, met behulp van de zogenoemde 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 ef-
fecten, welke ook werden gevonden in studies van onze groep bij andere 
middelen, welk de suggestie dat RS-FMRI een geschikte methode is voor vroegere fase klinisch geneesmiddelenonderzoek. De hersengebie-
den waarin de connectiviteitsveranderingen werden gevonden waren vergelijkbaar met de functionele hersengebieden die worden geassocie-
eerd met de bekende gedragseffecten na THC- of cannabisgebruik, zo-
as 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 proef-
schrift hebben toegepast. In contrast met de algemeen toegepaste neuronfysiologische, 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 
deuvoering van testen kunnen beïnvloeden. Bovendien biedt deze me-
thodologie de ruimte om in vroege fase klinisch onderzoek met zeer lage 
geneesmiddelenconcentraties te werken, waarvan de effecten eenvoudig 
gecompenseerd worden door compensatoire mechanisen, of in ander-
soortige testen gewoonweg niet kunnen worden waargenomen. Ook kan 
RS-FMRI geschikt zijn om functioneel ‘stille’ geneesmiddel effecten waar 
tene, zoals van CB1-antagonisten die bij gezonde vrijwilligers geen 
aoctuut meetbare veranderingen laten zien van de gangbare neuronfysiolo-
gische testen. Dit moet echter nog wel worden onderzocht. 
Samengevat concluderen we dat THC connectiviteitsveranderingen te-
weeg brengt in hersengebieden die geassocieerd zijn met gedragseffecten 
a THC- of cannabisgebruik. RS-FMRI lijkt een geschikte techniek
voor klinisch geneesmiddelenonderzoek, waaronder de ontwikkeling 
van cannabinöide farmacotherapiën. 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 methodolo-
gie in een breder perspectief te begrijpen, bijvoorbeeld door de relatie 
tussen connectiviteitsveranderingen en hersenfuncties verder te onder-
zoeken. Op deze manier kunnen we de toepassing van de techniek opti-
maal benutten en tegelijkertijd onze nog beperkte kennis over endocan-
babinöide systemen in de hersenen vergroten.

ANALYSES VERBETEREN

In hoofdstuk 5, waarin een studie wordt beschreven met de perifeer se-
lectieve CB₁-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 plasmaconcen-
tratie en een modell 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 hoog gevoel werd een waarschijnlijkheidsmodel (‘probability model’) 
gebouwd, 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 wer-
konding van rimonabant en TM38837. De equilibratie-halfwaardetijd van 
TM38837 was lang vergeleken met rimonabant. Dit veroorzaakte een 
grote verslaving in de aanvang van de effecten van TM38837. Hart-
slag liet half-maximale inhibitoire concentraties (IC₅₀) zien die voor 
TM38837 en rimonabant overeenkomstig waren, terwijl rimonabant bij 
body sway en high gevoel zelfs een IC₅₀ had van respectievelijk 4 en 56 
keer groter dan voor TM38837. Dit doet vermoeden dat TM38837 relatief

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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 CB1-antagonisten, namelijk drinabant (AVE1625), surinabant (SR147778), rimonabant (SR141716) en TM38837, hebben we in hoofdstuk 6PK-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 CB1-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 CB1-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-verschillen tussen de vier antagonisten te genereren. Onderzoek op het gebied van ‘mechanism-based’ PK-PD-modelleren blijft zich ontwikkelen met bijvoorbeeld meer nadruk op systeemanalyse van ziekten. Het is een onderzoeksgebied van grote betekenis en verdere ontwikkeling in de toekomst is belangrijk; niet alleen voor de ontwikkeling van geneesmiddelen, 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 cannabinoiden in gezonde personen en om cannabinoiden effectief te kunnen gebruiken voor de behandeling van ziekten.

In dit proefschrift hebben we een nieuwe orale THC-formulering en een nieuwe CB1-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 CB1-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 neerschuddende klachten. 

**ALGEMENE CONCLUSIES**

Het doel van dit proefschrift was om verschillende manieren te onderzoeken die kunnen bijdragen aan de verbetering van cannabinoidge-
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 CB1-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 CB1-antagonisten. Verder onderzoek is dan ook nodig naar de vraag in welke mate functionele chalengestudies en PK-PD-analyses in staat zijn om een therapeutisch venster te bepalen dat groot genoeg is voor een veilig en effectief gebruik van CB1-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 recreatieve 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 algemene maatschappelijke acceptatie van cannabis, wat een stimulans kan bieden aan verder onderzoek naar cannabisgerelateerde middelen.

**ALGEMENE CONCLUSIE**

Onze resultaten leidden tot de conclusie dat er veel ruimte is voor verbeeteringen 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 endocannabinoïdesysteem. 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.
REFERENCES


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 Pre-doctoral 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.
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ACKNOWLEDGEMENTS

Thank you, (former) colleagues, clients and partners of the CDR for your cooperation, support and the opportunities that you gave to me for research performance, the making of this thesis and for professional and personal development.

Bedankt!

Linda Klumpers
Stellingen behorende bij het proefschrift:

**NOVEL APPROACHES IN CLINICAL DEVELOPMENT OF CANNABINOID DRUGS**

1. Early drug studies that are essentially limited to assessment of tolerability and safety provide insufficient information for go/no-go decisions (*Adam Cohen*)

2. The development of drugs that act on complex systems (such as cannabinoids) requires a pharmacological approach, rather than a focus on safety and tolerability (*adapted from this thesis, chapter 7*)

3. Although the exact mechanism of cannabinoid induced changes in heart rate is unknown, this parameter is a suitable measure in the assessment of effect profiles of cannabinoid agonists and antagonists in addition to CNS parameters

4. The psychiatric adverse effects that led to the desertion of CB1-antagonists can provide important clues to the pathogenesis of psychiatric disorders, which so far have been largely neglected

5. The abandonment of cannabinoid research by many pharmaceutical industries deprived this research field from an important driving force, which is essential to unfold the full therapeutic potential of this important pharmacological system

6. Renaming the cannabinoid system would benefit research and drug development in this field
7 RS-FMRI can possibly be a useful technique for prediction of drug effects (*this thesis, chapter 3*) despite the fact that this technique is (still) unable to link physiological or functional processes.

8 One of the characteristics of successful drug development is the high termination rate in preclinical/phase I stages, which indicates that pharma companies have an early idea of which assets are likely to succeed (*adapted from M. Ringel, P. Tollman, G. Hersch, Boston Consulting Group*).

9 Researchers that can build bridges between business and science can improve the quality and efficiency of scientific research.

10 The progressive and innovative nature of science contrasts with the conservative organization of academic institutions.

11 The dismissal of a leading academic from a governmental drug advisory council*, because he expressed scientific arguments, highlights the danger of political involvement in scientific issues.

12 “[...] music biologically and culturally co-evolved with dance to serve as a technology of social bonding” (*Walter Freeman, in The Origins of Music*); therefore, impoverishment of music education in the Netherlands will negatively influence the social fabric of Dutch society.

*Prof. Dr. David Nutt, former chairman of the Advisory Council on the Misuse of Drugs in the UK*