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CLINICAL PHARMACOLOGY OF CANNABINOIDS IN EARLY PHASE DRUG DEVELOPMENT LINEKE ZUURMAN

# Clinical pharmacology of cannabinoids in early phase drug development

PROEFSCHRIFT

ter verkrijging van de graad van Doctor aan de Universiteit Leiden,
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door **Hillie Henka Zuurman**, geboren te Hoogezand-Sappemeer in 1972

PROMOTIECOMMISSIE

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# 1 Introduction

# Recreational and medical use of cannabis

*Cannabis sativa* L. is one of the oldest plants used for industrial, recreational and medical purposes. The fibers of the cannabis plant have been used for the production of rope, cloths and paper, and its seeds for soap and oil. Even a 'hemp car' was constructed by Henry Ford (Figure 1). Cannabis is especially known for its recreational use as a 'soft drug' for its appreciated psychoactive effects, like relaxation and euphoria. Cannabis is also known as marihuana, hashish, weed, hemp, charas and dagga among others.

Cannabis is the most widely used illicit drug in the western world. At least 45 million people in the European Union have tried cannabis at least once in their lives. Experience with this drug is less common in Europe than in the USA and Australia (www.trimbos.nl). Besides its recreational use, through the ages cannabis has also been used as a medicine for the treatment of nausea, loss of appetite, pain, pre-menstrual symptoms, and insomnia. The flowers of the female plants are used for recreational and medicinal purposes. These flowers contain high quantities of the psychoactive substance delta-9-tetrahydrocannabinol, or simply THC. Two oral formulations, dronabinol (Marinol®) and nabilone (Cesamet®, a synthetic THC analogue) are registered in several countries as anti-emetic and anti-anorexic agents for patients with cancer or HIV. These products are not registered in the Netherlands.

According to the Dutch law on controlled substances (Opium Act, 'Opiumwet') cannabis is considered a controlled substance. There is however a policy of tolerance towards use and possession of small quantities of cannabis. Since September 1st, 2003 patients in the Netherlands can obtain medicinal cannabis on doctor's prescription for the treatment of spasticity with pain (multiple sclerosis and spinal cord injury), nausea and vomiting (induced by chemotherapy/radiotherapy or treatment with HIV medication), chronic neuralgic pain, Gilles de la Tourette syndrome and the palliative treatment of cancer and HIV/AIDS (www.cannabisbureau.nl). The Office of Medicinal Cannabis ('Bureau Medicinale Cannabis') of the Ministry of Health, Welfare and Sport ('Ministerie van Volksgezondheid, Welzijn en Sport') is responsible for the supply of medicinal cannabis to all pharmacies in the Netherlands and monitors the origin and its composition. For research purposes cannabis can be obtained from the 'Bureau Medicinale Cannabis' as well. This is cannabis grown under Good Agricultural Practice conditions in greenhouses, using hydroculture, artificial light, a fixed regime of day and night temperatures, growth period and day length, and without the use of pesticides. Although smoking cannabis provides a reliable pharmacokinetic profile,[1] cannabis smoke has the disadvantage that it contains a mixture of psychoactive and partly noxious compounds, and that the active drug is partly lost by heat. To overcome these issues pure THC instead of cannabis was used in this thesis. THC was purified according

to GMP-compliant procedures (Farmalyse BV, Zaandam, The Netherlands) from the flowers of *Cannabis sativa* grown under Good Agricultural Practice (Bedrocan BV Medicinal Cannabis, Veendam, The Netherlands).

## Cannabis effects

One of the best known effects of cannabis is euphoria, commonly known as 'feeling high' or 'being stoned'. Besides euphoria people feel relaxed, have an impairment of short term memory, an increase in heart rate, may have uncontrollable fits of laughter, and experience changes in the awareness of their surroundings. Colours seem brighter, sounds are enhanced, and even mild visual and auditory hallucinations may occur. In a recreational setting these symptoms are mostly mild and appreciated. For inexperienced users or after the consumption of high doses these symptoms can be more severe and may induce uncontrollable movements, anxiety, derealization and even psychosis. Another well-known effect of cannabis is that it stimulates appetite. Cannabis users often use the term 'having the munchies'. Mostly this is a desire for fast foods and sweets or other high caloric foods. This high caloric intake may contribute to weight gain. Table 1 summarizes the physiological effects of THC which demonstrates that cannabis has an extensive effect on mental and physiological functions.

## Endocannabinoid system

Although cannabis was used and studied throughout the ages its main psychoactive component was not identified until 1964. In 1964 Raphael Mechoulam and his team isolated THC in a pure form from *Cannabis sativa* and described in detail its chemical structure.[2] Mechoulam's discovery led to new research programs all over the world. In the meanwhile several dozens of cannabinoids have been identified in *Cannabis sativa*.[3] The term 'cannabinoid' refers to chemical compounds that are structurally related to THC or bind to cannabinoid receptors.

Cannabinoids induce their pharmacological effects by binding to cannabinoid receptors, which are inhibitory G-protein coupled receptors. Until now two cannabis receptors (CB1 and CB2) have been identified with certainty. The CB1 receptor was cloned in 1990[4] and the CB2 receptor in 1993[5]. The CB1 receptors are predominantly situated in the brain with the highest densities in the hippocampus, cerebellum and striatum, which account for the well-known effects of cannabis on motor coordination and short term memory processing.[6-8] CB1 receptors are expressed at low levels in the brainstem.[8] Lower densities of CB1 receptors have also been found on immune and fat cells, in heart, lung, reproductive and gastrointestinal tissues

and in the urinary bladder.[9,10] CB2 receptors are predominantly present in the spleen and in haematopoietic cells.[6] In 2006 Onaivi *et al.* reported the discovery and functional presence of CB2 receptors in the rodent brain.[11] These CB2 receptors seem to be widely distributed in the brain and their function is still not clear.

CB1 (Figure 2) and CB2 receptors are both negatively coupled to adenylate cyclase and positively to mitogen-activated protein kinase. CB1 receptors are also coupled to ion channel, negatively to N-type and P/Q type calcium channels and positively to A-type and inwardly rectifying potassium channels.[1] They may also mobilize arachidonic acid and close serotonin (5-HT3) receptor ion channels, and some CB1 receptors are negatively coupled to M-type potassium channels.[1]

The discovery of the cannabinoid receptors initiated research to identify its natural ligands. In 1992 the endocannabinoid anandamide (Figure 3) was discovered by Raphael Mechoulam and his team.[12] Anandamide refers to the Sanskrit word 'ananda', meaning bliss. The effects of anandamide parallel those caused by psychotropic cannabinoids like THC.[13] 2-arachyldonyl glycerol (2-AG) (Figure 3), arachyldonyl glycerol ether, virodhamine and N-arachidonyl dopamine were also identified as endocannabinoids.[14] The physiological significance of endocannabinoids is not fully elucidated. However, endocannabinoid receptors form one of the most widely distributed pharmacological systems in the central nervous system, which provides many opportunities for new pathophysiological perceptions and for the development of new medicines.

## Pharmacokinetics of THC

Smoking is the preferred route of cannabis use. THC is a highly lipophilic compound which is rapidly absorbed and distributed to highly vascularized tissues and the brain, causing its pleasurable effects. In humans, plasma THC concentration profiles are similar after smoking or intravenous administration with prompt onset and steady decline.[15-17] Limited and variable bioavailability is observed after oral administration,[18-20] which is probably due to an extensive first pass effect. Metabolism of THC occurs mainly in the liver by microsomal hydroxylation, and oxidation catalyzed by enzymes of the cytochrome P450 complex. Nearly 100 metabolites have been identified for THC.[1] Besides the liver, other tissues like the heart and the lungs are also able to metabolize cannabinoids albeit to a much lesser degree.[1] The two major metabolites of THC are 11-OH-THC and 11-nor-9-carboxy-THC (Figure 4). 11-OH-THC is the most important psychotropic metabolite of THC, which is equipotent[1] or twice as potent as THC [21,22] and has a similar kinetic profile as the parent molecule[1]. 11-nor-9-carboxy-THC is a non-psychotropic metabolite of THC.[1,20] The plasma half-lives of THC and its metabolites are

long, ranging from 12-36 hours for THC and 11-OH-THC and 25-75 hours for 11-nor-9-carboxy-THC.[1,23] The slow elimination of these compounds is due to the slow rediffusion from body fat and other tissues into the blood. The true elimination half-live of THC is difficult to calculate since it rapidly penetrates highly vascularized tissues resulting in a rapid decrease in plasma concentration which are difficult to analyze.[1] In addition, the rediffusion of THC from the body fat and other tissues is a slow process contributing to low plasma concentrations as well.

Metabolism is the major route for the elimination of THC from the body (Figure 4). Only negligible amounts of THC are excreted as unchanged THC.[24] Most of the absorbed THC (65-80%) is excreted as metabolites in the faeces and a lesser amount is secreted in the urine (20-35%).[1] Among the metabolites, 11-nor-9-carboxy-THC is a major metabolite identified in both urine and faeces.[24] Reported urinary excretion half-lives for 11-nor-9-carboxy-THC vary from 18-60 hours.[25] 11-nor-9-carboxy-THC can be detected in urine up to 18 days.[23]

## Early development of cannabinoids as medicine

As described in the paragraph on 'cannabis effects', THC has an extensive effect on mental and physiological functions (Table 1). These observed desired and undesired effects led after the discovery of cannabinoid receptors and endocannabinoids to the development of synthetic cannabinoids. These synthetic drugs have been used extensively in pre-clinical research to further investigate the role of the endocannabinoid system in health and disease. We seem to be at the beginning of a new era of medicine based on the endocannabinoid system. Therapeutic indications are mainly based on the observed effects of cannabis and the distribution of cannabinoid receptors. CB1/CB2 agonist may therefore be of therapeutic use for muscle relaxation, immunosuppression, sedation, improvement of mood, neuroprotection, analgesia, and reduction of intra-ocular pressure.[1] However, the role of endocannabinoids for these indications is largely unknown. The effects of THC, the main psychoactive ingredient of cannabis can be used in several ways to guide the development of novel drugs that act on the endocannabinoid system.

In 2006 the first 'cannabinoid drug' rimonabant, a selective CB1 antagonist, was registered for the treatment of obesity. Feeling hungry is a well-known effect of cannabis and preclinical studies showed that activation of CB1 receptors by endogenous cannabinoids, such as anandamide, stimulates eating behavior.[26] Blockade of CB1 receptors by rimonabant leads to a decrease in appetite and has shown to be effective in the treatment of obesity.[27,28] In addition, CB1 receptor antagonists may also be useful in the treatment of smoking cessation, cognitive impairments in Alzheimer's

disease and schizophrenia and for advanced Parkinson's disease,[29-31] but this still requires clinical confirmation. Finally, high doses of cannabis may induce psychiatric effects like anxiety, hallucinations, derealization, paranoia and psychosis.[1,32] In theory, cannabinoid antagonism may pose a new mechanism of action for antipsychotic drugs.

The selective CB1 antagonists rimonabant[33] and AVE1625 (unpublished data) are devoid of measurable central nervous system effects. Cannabinoid antagonist activity can be demonstrated by showing inhibitory activity on the effects of a CB1/CB2 agonist like THC. Although a large number of studies have been performed with cannabis, it is not clear which biomarkers most accurately reflect the activities of the cannabinoid system. **Chapter 2** describes a systematic review of studies with cannabis and THC in healthy volunteers and reveals tests that show a clear, consistent response to cannabis or THC across studies. This information may be useful to enhance the drug development programme of a new cannabinoid drug at an early stage.

To study the inhibitory activity of CB1 antagonists on THC-induced effects a reproducible and practical mode of THC administration with a reliable pharmacokinetic and pharmacodynamic time profile is required. In **chapters 3, 4 and 5** the dose- and concentration-related effects of a novel mode of THC administration is described after intrapulmonary administration. This information can be used to demonstrate the ability of the selective CB1 antagonist AVE1625 to antagonize THC-induced effects on the central nervous system and heart rate (**chapters 5 and 6**). In addition, effects that show a clear PK/PD relationship to THC are eminently suited as pharmacodynamic parameters for novel CB1 agonists. Cannabis has sedative, amnestic and analgesic effects (Table 1). CB1/CB2 agonists with a combination of those properties may be useful for a range of indications, such as outpatient surgical procedures or adjuvant analgesic therapy. In **chapters 7 and 8** the sedative and amnestic properties of two novel intravenous CB1/CB2 agonists from different chemical classes, Org 28611 and Org 26828, are evaluated. Mutual comparison of the pharmacodynamic effect profiles of THC, Org 28611 and Org 26828 can demonstrate pharmacological differences and similarities between these CB1/CB2 agonists (**chapter 9**). Compounds from a similar drug class are expected to have similar proportional effects on different CNS parameters.

## Conclusions

Although cannabis is especially known for its recreational use as a 'soft drug', its potential therapeutic properties have been recognized for hundreds of years. Since the isolation of THC from *Cannabis sativa*, the discovery of cannabinoid receptors and their natural ligands (endocannabinoids) led to the acceleration of the development of novel cannabinoids as medicine.

This thesis describes useful cannabis biomarkers and the clinical pharmacology of some cannabinoid agonists and antagonists in early drug development. This includes a novel mode of pure intrapulmonary THC administration that can be used as a benchmark for novel CB1/CB2 agonists, or to demonstrate inhibitory activity of CB1 antagonists. In addition, the pharmacodynamics and pharmacokinetics of two novel CB1/CB2 agonists are evaluated and compared with the pharmacodynamic effect profile of THC.

**Figure 1** Henry Ford demonstrates his experimental automobile with a plastic body, better known as Henry Ford's 'hemp car' (1941). *(From: http://memimage.cardomain.net/member_images/8/web/2900000-2900999/2900475_178_full.jpg)*


**Figure 2** Pre- and post-synaptic nerve terminal of a CB1 receptor.

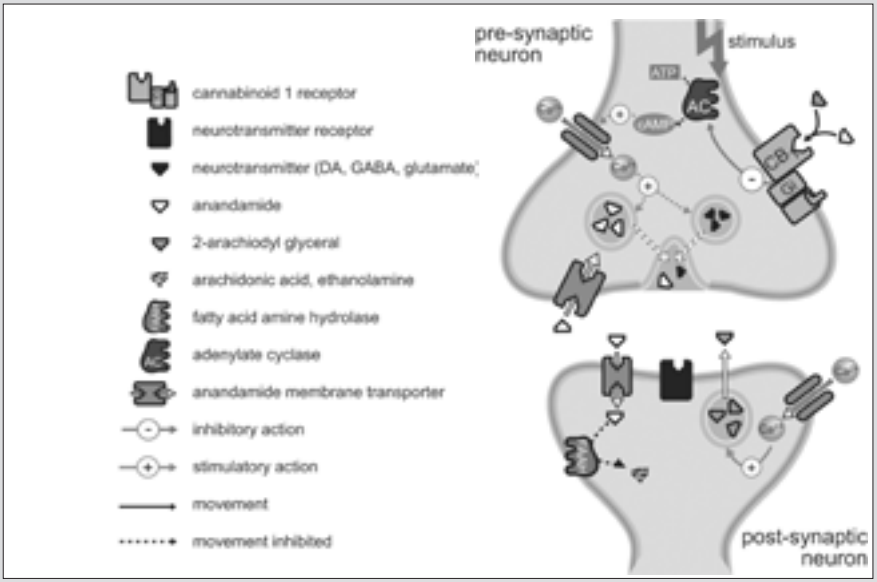


**Figure 3** Chemical structures of the two best characterized endocannabinoids: anandamide (left) and 2-arachidonyl glycerol (2-AG) (right).

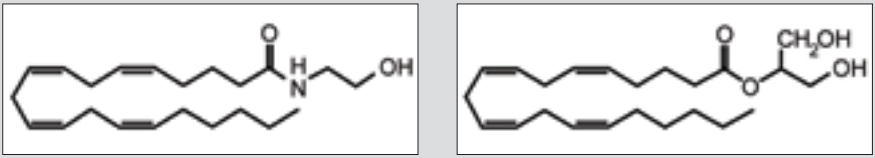
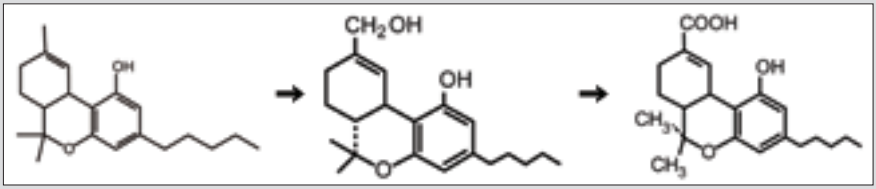



**Figure 4** THC's major metabolic route.




THC 11-OH-THC 11-NOR-9-COOH-THC

**Table 1** Physiological effects of THC. These dose-dependent effects have been observed in clinical studies, in vivo or in vitro (*From: Grotenhermen, Clinical Pharmacokinetics 2003; 42 (4): 327-360*).

| Body system | Effects |
|---|---|
| Psyche and perception | Fatigue, euphoria, enhanced well-being, dysphoria, anxiety, reduction of anxiety, depersonalization, increased sensory perception, heightened sexual experience, hallucinations, alteration of time perception, aggravation of psychotic states, sleep |
| Cognition and psychomotor performance | Fragmented thinking, enhanced creativity, disturbed memory, unsteady gait, ataxia, slurred speech, weakness, deterioration or amelioration of motor coordination |
| Nervous system | Analgesia, muscle relaxation, appetite stimulation, vomiting, antiemetic effects, neuroprotection in ischemia and hypoxia |
| Body temperature | Decrease of body temperature |
| Cardiovascular system | Tachycardia, enhanced heart activity, increased output, increase in oxygen demand, vasodilation, orthostatic hypotension, hypertension (in horizontal position), inhibition of platelet aggregation |
| Eye | Reddened conjunctivae, reduced tear flow, decrease of intraocular pressure |
| Respiratory system | Bronchodilation |
| Gastrointestinal tract | Hyposalivation and dry mouth, reduced bowel movements and delayed gastric emptying |
| Hormonal system | Influence on luteinising hormone, follicle-stimulating hormone, testosterone, prolactin, somatotropin, thyroid-stimulating hormone, glucose metabolism, reduced sperm count and sperm motility, disturbed menstrual cycle and suppressed ovulation |
| Immune system | Impairment of cell-mediated and humoral immunity, immune stimulation, anti-inflammatory and antiallergic effects |
| Fetal development | Malformations, growth retardation, impairment of fetal and postnatal cerebral development, impairment of cognitive functions |
| Genetic material and cancer | Antineoplastic activity, inhibition of synthesis of DNA, RNA and proteins |

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# 2 Biomarkers for the effects of cannabis and THC in healthy volunteers

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## Abstract

**Background**: An increasing number of novel therapeutic agents is targeted at cannabinoid receptors. Drug development programs of new cannabinoid drugs may be facilitated by the identification of useful biomarkers.
**Aim**: This systematic literature review aims to assess the usefulness of direct biomarkers for the effects of cannabis and THC in healthy volunteers.
**Methods**: 165 useful articles were found that investigated the acute effects of cannabis or THC on the central nervous system (CNS) and heart rate in healthy volunteers. 318 tests (or test variants) were grouped in test clusters and functional domains, to allow their evaluation as a useful biomarker and to study their dose response effects.
**Results**: THC/cannabis affected a wide range of CNS domains. In addition to heart rate, subjective effects were the most reliable biomarkers, showing significant responses to cannabis in almost all studies. Some CNS domains showed indications of stimulation at higher doses.
**Summary**: Subjective effects and heart rate are currently the most reliable biomarkers to study the effect of cannabis. Cannabis affects most CNS domains, but too many different CNS tests are used to reliably quantify the drug-response relationships.


## Introduction

The discovery of cannabinoid receptors and endocannabinoids has pointed to the physiological and possibly pathophysiological relevance of cannabinoids in humans. This has stimulated the development of synthetic cannabinoids, which have been used in pre-clinical research to further investigate the role of the endocannabinoid system in health and disease. However, the clinical development of cannabinoids as medicines is only just beginning. Although a large number of studies have been performed with cannabis and THC (a CB1/CB2 agonist) in healthy volunteers, it is not clear which biomarkers are useful in early cannabinoid drug development, and how cannabis affects different central nervous system (CNS) functions. A biomarker is a characteristic that is measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.[1] A validated biomarker in early phase I studies that provides useful information on the potential therapeutic effects of the investigational drug could support the drug development programme of the new compound. In general, a useful biomarker for activity of a drug class should meet the following criteria: 1) a clear, consistent response across studies (from different research groups) and drugs from the same class; 2) a clear response of the biomarker to therapeutic doses; 3) a dose (concentration)-response relationship; 4) a plausible relationship between

the biomarker, the pharmacology of the drug class and / or the pathogenesis of the therapeutic area. Previously, these criteria were used to evaluate the literature for the usefulness of biomarkers for the effects in healthy volunteers of antipsychotic drugs[2], benzodiazepines[3], selective serotonin reuptake inhibitors[4] and 3,4-methylene-dioxy-methamphetamine (MDMA, ecstasy)[5]. In the current review, the effects of cannabis and THC in healthy volunteers were systematically evaluated using the same methodology.

## Methods

### STRUCTURED LITERATURE EVALUATION

A literature search was performed up to 15 November 2007 using MedLine, Web of Science and Embase. The following keywords were used: marijuana, marihuana, cannabis, THC, tetrahydrocannabinol and delta-9-tetrahydrocannabinol. The searches were limited to healthy adults and papers in English. The resulting studies were subject to several selection criteria.

This review aimed to assess the usefulness of direct CNS biomarkers and heart rate for studies of cannabinoids in healthy volunteers. Reviews, studies in experimental animals or patients, and studies of interactions of cannabis use with personality features, behavioural characteristics, metabolic variations, other drugs, pain models or environmental factors (including secondary or subgroup analyses) were excluded from this review.

Studies with fewer than 10 subjects were not included in this review. Study participants were divided into non-users and users. No distinction was made according to the levels of previous or current usage, which ranged from occasional to chronic frequent use. Frequent and infrequent users were grouped as users. The review was restricted to the effects of acute cannabis exposure. Hence, abstinence effects, 'morning after effects' (including sleep effects after dosing on the preceding day), long-term effects in chronic users or effects of repeated dosing were not incorporated in this review.

The study characteristics and each individual test result of all articles that complied with the criteria were put into a database (Microsoft Excel). The following items were recorded: number of subjects, sex (male; female), age, past cannabis use (users; non-users; unknown), abstinence period (yes; no; unknown), blinding (double blind; single blind; open; unknown), design (cross-over; partial cross-over; parallel; unknown), drug name (cannabis, including hashish and marijuana; THC (dronabinol)), dose, route of administration (oral; intrapulmonary; intravenous; unknown), THC equivalence (<7 mg; 7-18 mg; >18 mg), test name, test effect, test cluster and functional domain. Most studies used different tests on different doses of cannabis, which were all regarded as independent measures of the canna-

bis effect. Thus, the total number of evaluated tests (cases) was a product of the numbers of articles, drugs, doses and tests (including secondary outcomes).

### Individual test results

Based on previous reviews, it was anticipated that in most cases no consistent quantitative results could be recorded for individual tests, because of the large diversity of methods, parameters and treatments. Therefore, the ability of a test to show a statistically significant difference from placebo or baseline was scored as + (improvement/increase), = (no significant effect) or - (impairment/decrease). Subjective assessments with a desirable effect (e.g. increase of a high scale) were scored as an improvement/increase, and unwanted effects (e.g. increase of sedation) as an impairment/decrease. Heart rate was expected to be an easily quantifiable exception, but for this parameter a concentration-effect-relationship has recently been described.[6] Since it would be redundant to repeat this effort cross-sectionally based on the literature, heart rate effects were scored quantitatively, similar to other tests in this review. In this way, heart rate served as an internal control of the methodological approach of this systematic review.

Some studies explicitly reported the use of several different tests in the methods section, without presentation of the results for no apparent reason. In these cases, it was assumed that these tests had not shown any significant effects. In some studies with different drug doses, overall significances were reported for drug effects, without (post hoc) quantifications of the statistical significance levels for each individual dose. In these cases, efforts were made to estimate the individual dose effects from graphs or tables provided in the article. If this was impossible, only the effect of the highest dose was assumed to be significant (in case of overall statistical significance) and lower doses were considered non-significant.

### Grouping of individual test results

Because of an apparent lack of standardisation between the studies even for the same tests, a structured procedure described previously[2-5] was adopted in order to obtain an overview. This approach allowed the preservation of individual study data in early stages, followed by a progressive condensation of results into logical test clusters and functional domains. For the subjective assessments, visual analogue scales can for example be grouped under scales of feeling high, craving, alertness, general drug effect etc. A compendium of neuropsychological tests from Strauss *et al*.[7] was primarily consulted to group functional tests into clusters of related tests or test variants. If necessary, the compendium of Lezak was consulted.[8] Sometimes, these compendia did not mention the test. In these cases, the author's clas-

sification was followed or if necessary the test was looked up in other literature and classified by consensus. Tests and clusters were grouped further into domains that represent higher aggregates of integration of subjective, neuropsychological, neuroendocrine, neurophysiological or autonomic functions. For each test (cluster), the compendia and other literature were used to determine which function was principally assessed by the test. Neuropsychological domains consisted of executive functions, memory, attention, motor functions, language and perception. Some tests provided provided different parameters with information on more than one functional domain. The results of the effects of a single test on different domains were scored separately, and the secondary effects were marked.

Results from tests that were used only occasionally or tests used only by a single research group could not be generalised. Therefore, these were not analysed individually, but grouped with other comparable tests. This step started with the grouping of tests that could be regarded as variants of a basic form (e.g. individual scores that are also part of more comprehensive tools like Profiles of Mood States (POMS), Addiction Research Center Inventory (ARCI) or Bond and Lader Visual Analogue Scales (VAS)[9]). Subscales of such inventories were grouped if they fell in the same cluster. Within such clusters, all scales showing a significant effect were grouped, whereas all scales showing no effect were grouped separately. In this way, scales within the same cluster that showed mixed results were scored equivocally. Comprehensive scoring instruments like Waskow's Drug Effect Questionnaire can often be subdivided into different subjective clusters (e.g. drug effect, high effect, etc.), but these subscales were not always reported separately. In these cases, the results were presented as part of the overall Scale Drug Effect cluster. In a few articles, a couple of composite scores of different CNS functions were presented, which could not be grouped according to the clusters or domains used in this review. These tests were not included in the analysis.

All effect scores and subdivisions of the tests were initially performed by two of the authors (EM and AEI), and subsequently checked and discussed by the other authors (LZ, AEI and JVG).

### DOSE-EFFECT RELATIONSHIPS

The chance that a test will detect a difference from placebo is expected to increase with dose. For each test that was used ten times or more and for all clusters, potential dose response relationships were determined. Dose-related increases or decreases of the average percentages of tests or clusters were reported without formal statistical analyses. Since the review yielded no immediately quantitative test effects, dose-relationships were represented by the proportions of statistically significant results for a given test or cluster. Similarly, since THC doses were not reported uniformly, THC/

cannabis dosages were pooled into 'lower', 'medium' and 'higher' dosages. The 'lower' dose was chosen to be a dose lower than 7 mg (roughly corresponding to half a cigarette), the 'medium' dose lay between 7 mg and 18 mg (approximately corresponding to one to one-and-a-half cigarette), and the 'higher' doses were all dosages above 18 mg (comparable with two cigarettes or more).[6,10,11]

Cigarette smoking was the predominant administration form. In many articles the exact THC content of a cigarette was mentioned. However, some articles mentioned the THC contents in percentage without the weight of the cigarette. In these cases a cigarette weight of 700 mg was assumed since most cigarettes weight between 500 and 900 mg. In other articles the number of puffs taken was documented. In these instances the dose was calculated as eight puffs corresponding with one marijuana cigarette.[11] Some studies provided weight-adjusted doses, without specifying the (average) body weight. In these cases, the 70 kg adult general population body weight was used to calculate the average administered dose.

To be able to compare the test results obtained for oral and intravenous administration with the results obtained for smoking, all doses were normalized to smoking. After smoking, roughly 50% of the THC contents of a cigarette is delivered into the smoke[12] and another 50% of the inhaled smoke is exhaled again[13]. In addition, the bioavailability after oral administration was assumed to be around 10%.[14,15] Therefore, all oral doses were divided by 2.5 to calculate the equivalent intrapulmonary THC doses. The THC plasma concentrations after smoking a 19 mg marijuana cigarette are equal to intravenous administration of 5 mg THC.[16] Therefore, all intravenous dosages were multiplied by four for dose normalization. In this way all routes of administration could be compared.

## Results

### STUDY DESIGN

The literature search yielded 165 different studies on cannabis and THC that met all criteria, published between 1966 and November 15th, 2007. The numbers of participants ranged from 10 to 161, where 115 studies (70%) included 10-20 subjects and 6 studies included more than 75 subjects (9%). Ages ranged from 18 to 59, but the vast majority were young adults between 18 and 35 years of age. In 57% of the studies only healthy males were included in the study and 2% of the studies included only females. Thirty-three percent of the studies included males and females while the sex of the subjects was not mentioned in 8%.

Most studies (80%) included subjects that were familiar with the effects of cannabis. In contrast, non-users were included in only 3%. Eleven percent of the studies reported inclusion of both cannabis users and non-

users. Previous cannabis use was not mentioned in 6% of the studies. A small majority of the studies (53%) described an abstinence period or the use of a THC drug screen. Four percent of the studies reported the lack of an abstinence period, while 44% did not mention this topic.

Fifty-seven percent of the reviewed studies had a double-blind design; 26% was single-blinded; 7% had an open design and for 10% the blinding was unknown. In addition, a small majority of the studies had a cross-over design (60%); 3% had a partial cross-over design; 33% had a parallel design and from 4% of the studies the study design was not mentioned in the article.

### STUDY DRUG AND DOSING

Cannabis is also known as marijuana, and dronabinol is an analogue of THC, the predominant psychoactive component of cannabis. Cannabis was used in 63% of the studies and THC in 34% of the studies. Intrapulmonary administration was the preferred route of administration in 71% of the studies. Oral administration of the drug was mentioned in 25% of the studies and intravenous administration was only used in 3%. Three percent of the studies did not describe which form of cannabis was used and 1% did not mention the route of administration. In these cases it could be inferred from the doses and the design that cannabis was smoked.

### TESTS, CLUSTERS AND DOMAINS

In total 318 different tests were used. Table 1 presents the frequency distribution of the different tests, and Table 2 presents the frequency of the test used ten times or more. This distribution shows that only a couple of tests were used frequently enough to allow individual analysis. The majority of the tests (196 tests, 61.6%) were only used once, and only heart rate (0.3%) was used over 50 times (in 92 articles). VAS scale high/stoned was studied in 30 articles, while the subjective effect rating scale high/stoned/euphoria was assessed in 28 articles. Taken together, the subjective high phenomenon was measured in more than 50 (35.2%) articles as well. The Digit Symbol Substitution Test (DSST) or variants like the Symbol Digit Substitution Tests was the most frequently used neuropsychological test (22 times). The Addiction Research Center Inventory (ARCI) was used in 18 articles.

Although many different tests and test variants were used to evaluate the effects of cannabis, most actually measured a limited number of core features. Therefore, tests were grouped further into clusters and subsequently in domains. Table 3a-d is a progressive condensation of all reported tests; from test to cluster to domain. This table includes the overall calculated significant drug effects on each cluster (impairment/decrease, no change or improvement/increase).

Table 3a-d shows that most drug-sensitive clusters cause a consistent functional impairment, and some an enhancement (heart rate, scale high). A few clusters show both impairments and improvements (e.g., time estimation, EEG alpha and evoked potential measurements, and scales for calmness, craving, mood and performance). Only a few frequently (>10 times) used test clusters showed significant responses to THC/cannabis in more than 80% of studies, notably heart rate (n = 85/92), scale high (n = 67/70) and scale psychotomimetic (n = 14/18). Most other clusters only reported significant drug effects in about 30-50 percent of the studies (Table 3a-d). All tests that were used five times or more showed a significant THC effect in at least one case; except EEG delta, which never responded in any study.

### DOSE-RESPONSE RELATIONSHIPS

Tests and clusters that were used in more than 10 articles were inspected for potential dose-response relationships (Table 4). Heart rate showed a statistically significant increase in 78% of measurements in the THC equivalence dose group <7 mg, which increased to 99% and 98% after the use of 7-18 mg and >18 mg THC, respectively. The subjective high feeling included many different scoring methods, varying from observer rating scales to individual VAS scores, either in isolation or as a part of multidimensional inventories (Table 2d). Despite this variability, the cluster scale high showed very consistent effects for all dose groups. The lowest dose group of <7 mg THC already showed a response of 94%, and the middle (7-18 mg) and highest dose group (>18 mg) scored close to 100%. The related subjective cluster scale psychotomimetic also showed a consistent increase with THC/cannabis of 76-83% without a clear dose-response relationship. A small increase with dose (from 56% to 78%) was observed for the cluster scale drug effect.

The relationship between memory and doses of THC/cannabis were more complex. The impairment increased with dose for auditory/verbal delayed recall (from 23% with the lowest doses to 78% with the highest dose range), but the effects were less clear for immediate recall (Table 4). Auditory/verbal delayed recognition also deteriorated with dose (from 17% to 50%), but this was assessed in only 11 studies. Working memory impairment on the other hand seemed to decrease with dose, from 52% impairments in the lowest dose group to 9% in the highest (Table 4). Other clusters that also appeared to show an inverse dose response association were the DSST-like cluster, focused selective attention and tests of motor and visuomotor control (Table 4). The proportion of significant effects of THC/cannabis within the cluster scale aggression increased slightly with dose (from 20 to 40%). No clear dose-response relationships were observed for inhibition, reasoning/association and reaction time, and for most subjective scales (Table 4). For studies with different doses, we scored significance

for the highest dose only, if significance was merely reported for the overall group effect. Although in such cases we could have artificially induced a dose-response relationship, this was only observed in 3% of all test scores.

## Discussion

This review aimed to systematically evaluate the usefulness of tests for the effects of cannabis and THC in healthy volunteers. The results were quite comparable to those of similar reviews of biomarkers of different CNS-active drugs in healthy volunteers.[2-5] A striking number of 318 different tests or test variants were described, and 61.6% of these were used only once. Grouping of tests in clusters and domains was required to evaluate the general usefulness of functional measurements, but this inevitably led to a loss of information. Even clustering tests with the same name and/or description could have bypassed differences among research groups or tests variants. In addition, this review investigated biomarkers for the effects of cannabis and THC in healthy volunteers, i.e. often with relatively small subject numbers; 70% of the studies had no more than 20 participants. It is possible that some tests will be useful biomarkers in patient studies or studies with large numbers of subjects. The observed variability in test results may have been enhanced by differences in prior cannabis use (non-users, occasional and frequent users). In this review these differences where not taken into consideration. A small majority of articles mentioned an abstinence period, but it is likely that this was also included in many other studies, without being mentioned. Chronic and occasional cannabis users show similar drug effects, although chronic users generally require higher doses and thus seem to be less sensitive.[17] The neglect of prior use intensity or abstinence duration may have confounded the detection of dose-response relationships, which was only roughly possible anyhow because of the many different doses and administration forms.

### USEFUL CANNABINOID BIOMARKERS

The effects of cannabis were observed on all clusters and all domains and in almost all individual tests, which might be due to the wide distribution of cannabinoid receptors in the brain.[18] An increase in heart rate was the most consistent result (Table 1, 2, 3a), and almost all studies with heart rate measurements showed statistically significant effects. This was expected, since heart rate shows a sharp increase and rapid decline after intrapulmonary THC administration that is clearly concentration related.[6,19] Feeling high has previously also been shown to be closely related to THC plasma concentrations.[19] The high phenomenon was measured in many different ways, but despite this variability almost all studies showed statistically significant

subjective drug effects. The predicted and highly consistent effects of THC/cannabis on the most clearly concentration-related effects (heart rate and feeling high)[6,19] in this review also support the methodological approach that was adopted, to integrate the widely variable study designs, drug forms and doses, and tests reported in the literature. Feeling high seems to be the most sensitive CNS biomarker for the effects of cannabis, irrespective of how it is measured. The scales psychotomimetic and drug effect are not quite as sensitive, but they address subjective changes that are less specific for THC/cannabis. This is clearly illustrated by the only negative scores on the drug effect cluster, which are all due to the negative scores on the benzedrine scale (BG scale) of the Addiction Research Center Inventory (ARCI). Most other clusters show a low to medium sensitivity for the effects of THC/cannabis, with significant drug effects in roughly 30-60% of cases (Table 2a-d). These findings are comparable for other drug classes, which show very comparable sensitivities of neurophysiological, neuropsychological, and subjective tests of 30-60% with benzodiazepines[3] and neuroleptics[2]. In these reviews, saccadic peak velocity (SPV) was highly sensitive to benzodiazepines in 100%[3], and prolactin release to neuroleptics in 96%[2]. These parameters were not particularly responsive to THC/cannabis in the current review, where heart rate and subjective high feeling scored 92-96%. This illustrates the differential effect profiles of different pharmacological groups, even among drug classes that are generally considered to be 'CNS depressant'. Such variability should be considered when methods are selected to study the CNS effects of neuropsychiatric agents.

### DOSE-RESPONSE RELATIONSHIPS

A useful biomarker should show a dose response relationship starting at a low therapeutic dose. In this review, doses could be grouped only roughly, and effects could only be scored as either statistically significant or not. Moreover, hardly any test was measured frequently and quantified consistently enough for a meaningful analysis of dose response associations. Perhaps due to these limitations, dose response relationships were found for only a few clusters (Table 4). THC doses were categorized in a low (<7 mg, roughly half a cannabis cigarette), medium (7-18 mg, approximately one to one-and-a-half cigarette) and high (>18 mg, two cigarettes or more) dose. This pragmatic division was not based on well-established relations between doses, plasma concentrations and CNS effects. Nonetheless, it led to roughly similar numbers of tests at the three different dose-levels (623-852 in each dose group), and thus reflects the practical dose-selection in the literature. This practice could however be based on the habit of subjects to smoke enough cannabis to elicit a desirable subjective state that does not cause unpleasant effects. It is not illogical to assume that this is reflected in the dose of one cigarette, and that a 'standard dose' is near the maximum-

tolerated dose for most subjects. In this review, lower doses (<7 mg) were only used in about 30% of the cases, and even this dose range caused subjective high feeling in 94% of cases. In a recent pharmacokinetic/pharmacodynamic (PK/PD) study, heart rate, VAS high and alertness, and postural stability were already sensitive to levels as low as 2 mg of intrapulmonary THC, and PK/PD effect relationships showed that near-maximum effects are reached with THC doses corresponding to roughly 10 mg of cannabis.[6,19] It seems that most doses studied in the literature may have been too high to show clear dose-response-relationships.

The memory effects of cannabis showed some dose response relationships but this differed for the various types of memory tests. Impairments increased with dose for auditory/verbal delayed recall and to a lesser extent for immediate recall and auditory/verbal delayed recognition (Table 4). Working memory on the other hand seemed to improve (i.e. normalize) with dose, with 52% impairments in the lowest dose group to 9% in the highest (Table 4). The clusters of focused selective attention and of motor and visuomotor control also appeared to show an inverse dose response association (Table 4). All these functions are highly influenced by attention and concentration.[7] Decreases in subjective alertness were noted in 43% with the lowest doses and 35% with the highest. This may have been accompanied by some agitation. Significant decreases in subjective calmness were found in 10% of cases with <7 mg and 26% with >18 mg (Table 4). At the same time, dose-related increases in (subjective) aggression (which increased with dose from 20% to 40%) and anxiety (from 11% to 33%) were observed. All this suggests that lower doses of THC/cannabis generally cause pleasant effects of relaxation and reduced attention, whereas with high doses CNS depression is partly overcome by more stimulatory effects. A survey of clusters like judgment and driving or subjective performance suggested that executive functions also tend to diminish at high doses, although these tests were not performed frequently enough for a reliable population dose-response relationship.

## SUMMARY

Biomarkers are useful tools to study drug effects since they can provide information on the potential pharmacological effects of the investigational drug in early phase drug development. However, the number of tests and test variants that is used in studies of THC and cannabis seems excessively large. This abundance thwarts a good assessment of the physiological, neuropsychological and subjective effects of this drug class, and there is a dire need for test standardisation in these areas. In general, the doses studied in the literature reflect the patterns of recreational use, and are often too high to accurately determine pharmacological dose-response relationships. THC/cannabis has an effect on a wide range of central nervous

system domains. At lower doses, THC/cannabis seems to be relaxant and to reduce attention, which is accompanied by an impaired performance on other CNS tests that require active participation. At high doses, the drug seems to be more stimulatory. Subjective effects are the most reliable biomarkers to study the effects of cannabis, in addition to heart rate increases that reflect peripheral cannabinoid activation. This review may facilitate a rational selection of CNS tests in future studies of THC/cannabis and other cannabinoid agonists.

**Table 1** Frequency distribution of the different tests used.

| Test frequency | Number of tests | Frequency (%) |
|---|---|---|
| 1 | 196 | 61.6% |
| 2-5 | 87 | 27.4% |
| 6-10 | 14 | 4.4% |
| 11-25 | 18 | 5.7% |
| 26-50 | 2 | 0.6% |
| >50 | 1 | 0.3% |

**Table 2** Frequency of tests used ten times or more.

| Test name | Frequency |
|---|---|
| Heart Rate | 92 |
| Visual Analogue Scale (VAS) (scales high/stoned) | 30 |
| Subjective Effect Rating Scale (scales high/stoned/euphoria) | 28 |
| Digit Symbol Substitution Test (DSST) | 22 |
| Addiction Research Center Inventory (ARCI) (scale drug effect) | 18 |
| Profiles of Mood States (POMS) (scales anger/friendliness/hostility) | 18 |
| POMS (scales confusion/clear headedness/energy/confused-bewildered/vigour/stimulation) | 18 |
| VAS (scales sedation/stimulation-alertness/attentiveness/interest/clear headed/confused/energetic/ sluggish/ sleepiness/drowsy/concentration/forgetfulness) | 18 |
| POMS (scales anxiety-tension/tension/arousal) | 17 |
| Subjective Effect Rating Scale (scales intoxication/drunk/drug effect/placebo-THC/feel marijuana effect) | 16 |
| POMS (scales anxiety-tension/anxiety) | 15 |
| POMS (scales composure/depression/depression-dejection/elation/(positive)mood) | 15 |
| POMS (scale fatigue) | 14 |
| Potency Rating Scale | 14 |
| VAS (scales (good/bad) drug effect/feel drug/intoxication/drunk/comparison to usual smoke) | 14 |
| Time Estimation Task | 13 |
| VAS (scale anxiety/anxious/panic) | 13 |
| Pleasantness Rating Scale | 12 |
| VAS (scales content/down/mood/withdrawn/sociability feelings) | 11 |
| VAS (scales feelings of tranquility/calm/relaxed/mellow/arousal) | 11 |
| VAS (scales hungry/hunger) | 11 |
| Drug Effect Questionnaire (DEQ) (scales good/bad/strong/feel effect) | 10 |
| Pursuit Meter/Motor/Rotor Task | 10 |

**Table 3a** Progressive condensation of all reported tests, into their corresponding clusters and domains. The overall cluster effects are reported together with the articles in which they are reported.

| Domain<br>Cluster | Tests | Effects (%)<br>(-) | <br>(=) | <br>(+) | Article (fequency; n) |
|---|---|---|---|---|---|
| **(Neuro)Endocrine** | | | | | |
| Cortisol | Cortisol | 0 | 0 | 100 | 20 (n=1) |
| Prolactin | Prolactin | 0 | 100 | 0 | 20 (n=1) |
| **Autonomic** | | | | | |
| Heart rate | Heart Rate | 1 | 7 | 92 | 17, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111 (n=92) |
| Pupil size | Pupil Size | 24 | 59 | 18 | 21, 22, 29, 44, 68, 112, 113 (n=7) |
| Temperature | Temperature | 12 | 88 | 0 | 21, 68, 101, 105 (n=4) |
| **Neurophysiologic** | | | | | |
| EEG | EEG | 29 | 43 | 29 | 17, 43, 114 (n=3) |
| EEG alpha | EEG alpha | 17 | 22 | 61 | 17, 22, 84, 85, 88, 93, 115, 116, 117 (n=9) |
| EEG beta | EEG beta | 59 | 35 | 6 | 17, 22, 84, 88, 93, 115, 117 (n=7) |
| EEG delta | EEG delta | 0 | 100 | 0 | 17, 22, 84, 115, 117 (n=5) |
| EEG theta | EEG theta | 6 | 88 | 6 | 17, 22, 84, 93, 115, 117 (n=6) |
| Evoked potential | Auditory Evoked Potentials, Contingent Negative Variation (CNV), Evoked Potentials, Visually Evoked Potentials | 20 | 45 | 35 | 22, 43, 93, 115, 118, 119, 120, 121, 122 (n=9) |
| Eye movements - nystagmus | Electro-nystagmographic Recordings, Electro-oculographic Recordings | 0 | 100 | 0 | 69, 123 (n=2) |
| Eye movements - pursuit | Electro-oculographic Recordings, Eye Performance System (EPS-100), Eye-Point of Regard System, Tracking Task | 38 | 63 | 0 | 21, 69, 123, 124 (n=4) |
| Eye movements - saccadic | Electro-oculographic Recordings, Eye-Point of Regard System, Saccadic Eye Movement | 0 | 80 | 20 | 123, 124, 125, 126 (n=4) |

**Table 3b** Progressive condensation of all reported tests, into their corresponding clusters and domains. The overall cluster effects are reported together with the articles in which they are reported. * Indicates that the test was also used as a secondary parameter.

| Domain<br>Cluster | Tests | Effects (%)<br>(-) | <br>(=) | <br>(+) | Article (fequency; n) |
|---|---|---|---|---|---|
| **Memory** | | | | | |
| Auditory/verbal memory: delayed recall | Babcock Story Recall Test, Buschke Selective Reminding Test, Color-Number Matching Task, Digit Recall Task, Free Recall of Story Test, Hopkins Verbal Learning Test, Memory assessment of POMS scores, Orienting Word Task, Prose Recall Task, Randt Memory Battery, Recognition Task, Semantic Memory Retrieval Task, Text Learning Task, Verbal Recognition & Recall Task, Word List, Word Recall Task | 53 | 47 | 0 | 20, 23, 51, 52, 53, 55, 64, 66, 91, 94, 107, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136 (n=21) |
| Auditory/verbal memory: delayed recognition | Cued Recall of Story Test, Delayed Story Recognition Task, Hopkins Verbal Learning Test, Name and Address Recognition Task, Verbal Recognition & Recall Task, Word List, Word Recognition Task | 27 | 73 | 0 | 20, 23, 52, 53, 55, 56, 94, 107, 131, 135 (n=10) |
| Auditory/verbal memory: immediate recall | Babcock Story Recall Test, Benton Sentence Repetition Task, Buschke Selective Reminding Test, Color-Number Matching Task, Digit Recall Task, Free Recall of Story Test, Free Recall Test, Hopkins Verbal Learning Test, List Learning Task, Orienting Word Task, Prose Recall Task, Randt Memory Battery, Seashore Tonal Memory Task, Syllable List Learning Task, Text Learning Task, Word Anagram Solution Task, Word List, Word Recall Task | 60 | 40 | 0 | 20, 23, 25, 30, 32, 50, 51, 52, 53, 55, 57, 64, 66, 91, 107, 127, 128, 129, 130, 132, 135, 136, 137, 138, 139, 140 (n=26) |
| Implicit memory | Common Facts Recall Task, Detailed Recall Task, Perceptual Priming Task, Remote Memory Task, Word List | 0 | 100 | 0 | 64, 128, 131, 141 (n=4) |
| Learning | Artificial Conditioned Speech Connections, Colour/Word Presentation Task*, Driving Task, Hopkins Verbal Learning Test*, Intelligence Structure Test, Memory for Designs Test*, Method of Artificial Conditioned Speech Connections, Paired Associate Learning Task, Randt Memory Battery, Repeated Acquisition Task, Tactual Performance Test, Word List* | 38 | 62 | 0 | 20, 25, 28, 45, 54, 66, 75, 91, 93, 129, 132, 138, 139, 142, 143, 144 (n=16) |
| Visual/spatial memory: delayed recognition | Benton Visual Retention Test | 0 | 100 | 0 | 28 (n=1) |
| Visual/spatial memory: immediate recall | Memory for Designs Test, Peterson Visual Memory Test, Picture Recall Test | 100 | 0 | 0 | 32, 54, 138 (n=3) |

| | | | | | |
|---|---|---|---|---|---|
| **Executive** | | | | | |
| Driving | Driving Task, Flight Simulator Task | 62 | 38 | 0 | 24, 45, 79, 97, 145, 146, 147, 148 (n=8) |
| Inhibition | Central and Peripheral Light Flashes Task*, Colour/Word Presentation Task*, Decision Making Task, Delay Discounting Task, Digit Span Test with Signal Detection Task*, Divided Attention Task (DAT)*, Go/No-Go Task, Hopkins Verbal Learning Test*, Memory for Designs Test*, Monetary Stimulation Task, Randt Memory Battery*, Ratings of Narrative Quality, Stop Task, Stroop Color and Word Test, Temporally Controlled Operant Task, Thematic Apperception Test (TAT), Verbal Fluency Task, Word List*, Word Recall Task* | 52 | 48 | 0 | 20, 23, 25, 30, 34, 41, 52, 53, 54, 66, 85, 86, 93, 107, 137, 140, 149, 150, 151, 152, 153 (n=21) |
| Judgement | Flexibility and Closure Test, Iowa Gambling Task, Scores of Willingness to Drive | 25 | 75 | 0 | 105, 110, 146 (n=3) |
| Planning | Goal-Directed Serial Alternation Task, Thematic Apperception Test (TAT) | 67 | 33 | 0 | 152, 154 (n=2) |
| Reasoning/association | Alternate Use Task, Analogy Task, Association IV, Associative Processing Test, Baddeley Reasoning Task, Categorization Task, Concept Formation Task, Contingent Categorization Task, Free and Constrained Associations Test, Halstead Category Test, Hidden Word Test, Iowa Test of Educational Development, Letter Series Test, Logical Reasoning Task, Numerical Reasoning Task, Object Description Test, Object-Match Task, Picture Arrangement Test, Production and Recall of Free Associations Test, Ratings of Narrative Quality, Thematic Apperception Test (TAT), Water-Jar Test, Word Grouping Test | 37 | 63 | 0 | 21, 30, 33, 85, 128, 129, 130, 131, 134, 135, 138, 150, 151, 152, 153, 155, 156, 157, 158, 159 (n=20) |
| Set shifting | Delayed Auditory Feedback Device (DAF), Object-Match Task*, Trail Making Test* | 20 | 80 | 0 | 37, 100, 131, 137, 160 (n=5) |
| Time estimation | Time Estimation Task | 18 | 33 | 48 | 23, 29, 30, 42, 50, 64, 82, 83, 88, 102, 151, 154, 161 (n=13) |
| Working memory | Alphabet Task, Boggle Word Construction Test, Colour/Word Presentation Task, Conceptual Clustering Memory Test, Cued Recall of Story Test, Delayed Auditory Feedback Device (DAF), Digit Recall Task, Digit Span (Backward), Goal-Directed Serial Alternation Task, Matching to Sample Task, Mental Calculation Task, Picture Recognition Test, Rapid Information Task, Rapid Visual Information Processing Task, Repeated Acquisition Task, Running Memory Span, Serial Addition/Subtraction Task, Spatial N-Back Task, Sternberg Memory Scanning Task, Story Recognition Task, Word Anagram Solution Task, Word List*, Word Recognition Task | 40 | 60 | 0 | 12, 20, 21, 23, 26, 30, 33, 37, 52, 55, 58, 66, 70, 85, 93, 100, 107, 116, 121, 127, 128, 131, 132, 138, 139, 154, 156, 160, 161, 162 (n=30) |
| **Motor** | | | | | |
| Motor control | Card Sorting Task, Choice Reaction Time Task, Compensation Apparatus, Finger Tapping Test, Finger Tremor Test, Foot Tapping Test, Klove Grooved Steadiness Task, Klove Static Steadiness Task, Manual Dexterity Test, Minnesota Rate of Manipulation - Block Turning, Pegboard Test, Tapping Task, Toe Tapping Test, Vienna Determination Apparatus (VDA) | 47 | 53 | 0 | 26, 50, 97, 103, 130, 138, 157, 158, 160, 163, 164, 165 (n=12) |
| Postural stability | Body Sway, EquiTest, Finger Tapping Test*, Foot Tapping Test*, Klove Grooved Steadiness Task, Klove Static Steadiness Task, Standing Steadiness Task, Wobble Board | 54 | 46 | 0 | 24, 26, 30, 31, 70, 97, 100, 103, 138, 157, 158, 160 (n=12) |
| Visuo-Motor control | Bender-Gestalt Test, Circulair Lights Task, Compensation Apparatus, Efficiency Test System, Gibson Spiral Maze, Groove Pegboard Task, Hand Maze Task, Hand Steadiness Task, Horizontal Groove Task, Klove Maze Coordination Task, One-Hole Test, Pursuit Meter/Motor/Rotor Task, Rod and Frame Deviation Task, Spiral Rotor Task, Star Tracing Task, Tracking Task, Trail Making Test A and B, Trail Making Test A, Vertical Groove Task, Vienna Determination Apparatus (VDA) | 51 | 49 | 0 | 21, 26, 28, 29, 31, 37, 40, 88, 94, 100, 103, 116, 128, 130, 137, 138, 151, 157, 160, 161, 163, 164, 165, 166, 167 (n=25) |

**Table 3c** Progressive condensation of all reported tests, into their corresponding clusters and domains. The overall cluster effects are reported together with the articles in which they are reported. * Indicates that the test was also used as a secondary parameter.

| Domain<br>Cluster | Tests | Effects (%)<br>(-) | <br>(=) | <br>(+) | Article (fequency; n) |
|---|---|---|---|---|---|
| **Attention** | | | | | |
| Divided attention | Dichotic Listening Task, Digit Span Test with Signal Detection Task, Distraction Task, Divided Attention Task (DAT), Landolt C-Rings Test, Matching to Sample Task, Trail Making Test B | 37 | 59 | 4 | 30, 33, 36, 41, 58, 70, 91, 111, 124, 130, 131, 132, 137, 160 (n=14) |
| DSST-like | Barrage de Signe, Digit Symbol Substitution Test (DSST), Digit Symbol Substitution with Memory Test | 42 | 58 | 0 | 12, 23, 29, 30, 31, 38, 46, 50, 58, 70, 71, 72, 73, 83, 88, 91, 109, 126, 131, 132, 139, 151, 161 (n=23) |
| Flicker discrimination | Critical Flicker Fusion Test, Critical Stimulus Duration Task | 33 | 56 | 11 | 89, 97, 129, 130, 168, 169 (n=6) |
| Focused/selective attention | 3x3 Block Matrix Task, Arbeit und Konzentrationstest Geräte, Arithmetic Task, Auditory Reaction Time Task*, Choice Reaction Time Task*, Continuous Performance Task*, D2 Attention Test, Digit Span (Forward), Digit Span Test with Signal Detection Task*, Double Target Digit Cancellation Task, Number Facility Test, Paced Auditory Serial Addition Test, P-Deletion Test, Single Target Digit Cancellation Task, Stroop Color and Word Test | 35 | 65 | 0 | 20, 23, 26, 41, 58, 66, 70, 85, 105, 111, 126, 128, 131, 136, 137, 158, 161 (n=17) |
| Reaction time | Alerting Task, Auditory Reaction Time Task, Central and Peripheral Light Flashes Task, Choice Reaction Time Task, Complex Reaction Time Task, Contingent Categorization Task*, Contingent Negative Variation (CNV)*, Dichotic Listening Task*, Discrimination Reaction Time Task, Driving Task*, Iowa Gambling Task*, Letter Matching Task, Matching to Sample Task*, Perceptual Speed Task, Peripheral Visual Detection Task*, Rapid Information Task, Reaction Time Task, Simple Auditory Reaction Time Task, Simple Reaction Time Task, Simple Visual Reaction Time Task, Spatial N-Back Task*, Stroop Color and Word Test*, Visual Reaction Time Task, Word Recognition Task* | 48 | 51 | 1 | 12, 26, 31, 33, 34, 40, 66, 84, 85, 91, 93, 94, 97, 103, 110, 111, 118, 121, 126, 128, 129, 130, 131, 132, 139, 145, 157, 158, 160, 170, 171, 172 (n=32) |
| Sustained attention (Vigilance) | Continuous Performance Task, Mackworth Clock-Vigilance Task, Pursuit Meter/Motor/Rotor Task*, Visual Search Task | 14 | 86 | 0 | 12, 20, 29, 31, 35, 83, 124, 160 (n=8) |
| **Language** | | | | | |
| Comprehension | Text Learning Task | 100 | 0 | 0 | 129 (n=1) |
| Production | Cloze Method, Controlled Oral Word Association Test (COWAT), Object Description Test, Spontaneous Speech, Thematic Apperception Test (TAT), Verbal Fluency Task, Word Recall Task* | 23 | 69 | 8 | 20, 39, 66, 128, 140, 150, 152, 159 (n=8) |
| Semantics | Iowa Test of Educational Development, Orienting Word Task | 0 | 100 | 0 | 51, 129 (n=2) |
| **Perception** | | | | | |
| Auditory perception | Auditory Rhythm Test, Auditory Threshold Test | 17 | 83 | 0 | 130, 173 (n=2) |
| Tactile perception | Tactual Performance Test, Vibratory Sense Appreciation Test | 33 | 50 | 17 | 116, 130, 138 (n=3) |
| Visual/spatial perception | Archimedian Spiral After Effect, Binocular Depth Inversion Test, Block Design Test, Clock Faces Task, Closure Speed Test, Dot Tests, Driving Task, Glare Recovery Task, Group Embedded Figures Test, Hidden Figures Task, Mannequin Task, Peripheral Visual Detection Task, Size-Weight Illusion Test, Visual Acuity Task, Visual Autokinetic Motion Task, Visual Brightness Test, Visual Information Processing Task, Visual Recognition Task | 27 | 73 | 0 | 21, 27, 40, 54, 97, 113, 130, 131, 135, 151, 156, 165, 172, 173, 174, 175, 176 (n=17) |

**Table 3d** Progressive condensation of all reported tests, into their corresponding clusters and domains. The overall cluster effects are reported together with the articles in which they are reported.

| Domain<br>Cluster | Tests | Effects (%)<br>(-) | <br>(=) | <br>(+) | Article (fequency; n) |
|---|---|---|---|---|---|
| **Subjective Experience** | | | | | |
| Scale aggression | Brief Psychiatric Rating Scale (Scale Hostility), Clyde Mood Scale (Scales Friendly/Aggressive), Gottschalk-Gleser Content Analysis (Scales Social Alienation/Hostility), Jackson Personality Research Form (Scale Autonomy), Jackson Personality Research Form (Scale Dominance), POMS (Scales Anger/Friendliness/Hostility), Primary Affect Scale (PAS) (Scale Anger), Ratings of Narrative Quality, Thematic Apperception Test (TAT), VAS (Scales Friendly/Social) | 27 | 68 | 5 | 23, 30, 38, 49, 50, 60, 61, 62, 63, 67, 71, 91, 96, 97, 98, 105, 108, 133, 139, 150, 152, 153, 157, 165, 175, 177 (n=26) |
| Scale alertness | Addiction Research Center Inventory (ARCI) (Scale Stimulated), Brief Psychiatric Rating Scale (Scale Activation), Clyde Mood Scale (Scales Sleepy/Clear Thinking), Comprehensive Psychiatric Rating Scale (AMDP) (Scale Alertness), Drug Effect Questionnaire (DEQ) (Scales Sluggish/ Stuffy Feeling/Thinking Clearer/Concentration), Feeling Scale of Janke (Composite Scale Vital), Medical Questionnaire (Scale Impaired Concentration), Observer Rated Signs, POMS Scales Confusion/ Clear Headedness/Energy/Confused-Bewildered/Vigour/Stimulation, Scale Stimulated, Subjective Effect Rating Scale (Scales Concentration Impairment/Interest), VAS Scales Sedation/Stimulation Alertness/Attentiveness/Interest/Clear headed/Confused/Energetic/Sluggish/Sleepiness/Drowsy/ Concentration/Forgetfulness | 39 | 50 | 11 | 21, 23, 24, 28, 30, 37, 38, 40, 46, 49, 50, 58, 60, 61, 62, 63, 67, 70, 71, 72, 73, 96, 97, 105, 108, 126, 128, 130, 131, 132, 133, 139, 146, 149, 157, 164, 175, 178 (n=38) |
| Scale anxiety | Ditman's DWM Scale (6-8), Drug Effect Questionnaire (DEQ) (Scale Anxiety), Gottschalk-Gleser Content Analysis Scales (Scale Anxiety), POMS Scales Anxiety-Tension/Anxiety, Primary Affect Scale (PAS) (Scale Fear), State Trait Anxiety Inventory, Taylor Manifest Anxiety Scale (MAS), Thematic Apperception Test (TAT), VAS (Scale Anxiety/Anxious/Panic) | 29 | 67 | 5 | 20, 23, 24, 30, 33, 38, 49, 50, 58, 59, 60, 61, 62, 70, 71, 72, 73, 96, 97, 101, 108, 133, 139, 150, 152, 157, 165, 179, 180 (n=29) |
| Scale calmness | Ditman's DWM Scale (1-20), Drug Effect Questionnaire (DEQ) (Scales Relaxation/Tension/Excited), Feeling Relaxed, Feeling Scale of Janke (Scale Passive), POMS (Scales Anxiety-Tension/Tension/ Arousal), Primary Affect Scale (PAS) (Scale Arousal), VAS (Scales Feelings of Tranquility/Calm/ Relaxed/Mellow/Arousal) | 23 | 53 | 24 | 20, 23, 24, 30, 33, 38, 49, 50, 58, 59, 60, 63, 67, 71, 78, 91, 96, 97, 98, 106, 108, 128, 130, 131, 132, 133, 146, 149, 157, 164, 165, 178, 179 (n=33) |
| Scale craving | Drug Effect Questionnaire (DEQ) (Scales Like Drug/Want More/Take Drug Again), End-of-Session Questionnaire (Scales Dislike/Like a Lot), Pleasantness Rating Scale, Subjective Effect Rating Scale (Scales Feel Like Smoking/Like Drug Effect/Want More/Price Willing To Pay), VAS (Scales Like Drug/ Like Effect/Desire) | 50 | 18 | 32 | 17, 21, 23, 25, 30, 32, 38, 49, 52, 53, 54, 55, 56, 57, 70, 71, 72, 73, 78, 91, 107, 128, 132 (n=23) |
| Scale dizziness | Clyde Mood Scale (Scale Dizzy), Ditman's DWM Scale (1-20), Drug Effect Questionnaire (DEQ) (Scale Dizzy), Medical Questionnaire (Scales Disturbed Equilibrium/Faintness), Subjective Effect Rating Scale (Scale Dizziness) | 58 | 42 | 0 | 105, 128, 130, 164 (n=4) |
| Scale drug effect | Addiction Research Center Inventory (ARCI) (Scale Drug Effect), Ditman's DWM Scale (6-8), Drug Effect Questionnaire (DEQ) (Scales Good/Bad/Strong/Feel Effect), End-of-Session Questionnaire (Scales Like/Feel/Strength), Estimation of Received Drug, Feeling of Intoxication, Numeric Scale Cannabis, Observer Rated Signs, Potency Rating Scale, Psychological Subjective Effect Ratings (Scale Drug Effect), Scale Intoxication, Subjective Effect Rating Scale (Scales Intoxication/Drunk/ Drug Effect/Placebo-THC/Feel Marijuana Effect), Subjective Psychological Effects Ratings, VAS (Scales Drug Effect/Feel Drug/Intoxication/Drunk/Good Drug Effect/Bad Drug Effect/Comparison to Usual Smoke) | 7 | 26 | 67 | 21, 23, 24, 25, 26, 29, 30, 31, 32, 33, 37, 38, 39, 40, 41, 44, 46, 49, 50, 51, 52, 53, 54, 55, 56, 57, 63, 65, 66, 67, 70, 71, 72, 73, 79, 81, 82, 88, 91, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 107, 108, 109, 110, 128, 130, 131, 132, 139, 145, 146, 149, 151, 160, 165, 167, 178, 179 (n=67) |
| Scale fatigue | Drug Effect Questionnaire (DEQ) (Scale Fatigue), Karolonsika Sleepiness Rating (Scale Tiredness), POMS (Scale Fatigue), VAS (Scale Tired) | 33 | 67 | 0 | 20, 23, 30, 38, 49, 50, 62, 63, 67, 71, 93, 96, 97, 106, 130, 133, 139, 157 (n=18) |

| | | | | | |
|---|---|---|---|---|---|
| Scale high | Addiction Research Center Inventory (ARCI) (Scale High), Drug Effect Questionnaire (DEQ) (Scales High/Euphoria), Feeling High, Subjective Effect Rating Scale (Scales High/Stoned/Euphoria), VAS (Scales High/Stoned) | 0 | 4 | 96 | 17, 20, 21, 23, 24, 27, 30, 33, 38, 39, 40, 42, 44, 47, 48, 49, 50, 58, 59, 60, 61, 64, 65, 70, 71, 72, 73, 74, 76, 77, 78, 79, 83, 84, 85, 86, 87, 88, 89, 91, 92, 93, 96, 97, 104, 106, 110, 111, 116, 117, 118, 119, 125, 128, 130, 131, 132, 134, 135, 139, 144, 146, 153, 155, 156, 160, 173, 178, 181, 182 (n=70) |
| Scale mood | Brief Psychiatric Rating Scale (Scales Anergia-Depression/Anergia), Clyde Mood Scale (Scale Unhappy), Comprehensive Psychiatric Rating Scale (AMDP) (Scales Sexual Desire/Euphoria), Ditman's DWM Scale (1-20), Ditman's DWM Scale (6-8), Drug Effect Questionnaire (DEQ) (Scales Well-Being/Dysphoria/Feel Free/Feel Serious), Jackson Personality Research Form (Scale Exhibitionism), Observer Rated Signs, Pleasantness Rating Scale, POMS (Scales Composure/ Depression/Depression-Dejection/ Elation/Mood/Positive Mood), Positive and Negative Symptom Scale (PANSS), Primary Affect Scale (PAS) (Scale Depression), Primary Affect Scale (PAS) (Scale Happiness), Scale Depression, Subjective Effect Rating Scale (Scales Enjoyability/Pleasantness), Thematic Apperception Test (TAT), VAS (Scales Content/Down/Mood/Withdrawn/Sociability Feelings) | 23 | 61 | 17 | 20, 23, 28, 30, 37, 38, 40, 46, 49, 50, 51, 62, 63, 67, 70, 71, 83, 84, 88, 91, 96, 97, 105, 114, 126, 128, 130, 131, 132, 133, 139, 146, 149, 152, 157, 164, 165, 175, 177 (n=39) |
| Scale performance | Drug Effect Questionnaire (DEQ) (Scales Psychomotor Activity/Control/Control/Accelerated-Improved Cognition), Instructor's Performance Rating, Mental Status Examination (Scale Intellectual Efficiency), Subjective Effect Rating Scale (Scales Difficulty/Driving Ability/Impaired/ Motivation/Memory Impairment/Performance), Subjective Effect Rating Scale (Scales Difficulty/ Driving Ability/Impaired/Motivation/Memory Impairment/Performance), Subjective Performance Rating, Subjective Performance Rating, VAS (Scale Impaired) | 65 | 24 | 12 | 21, 24, 40, 58, 70, 79, 93, 97, 116, 128, 130, 145, 146, 160 (n=14) |
| Scale psychotomimetic | Brief Psychiatric Rating Scale (Scale Thought Disorder), Clinician Administered Dissociative Symptoms Scale (CADSS) (Scale Perceptual Alternations), Comprehensive Psychiatric Rating Scale (AMDP) (Scale Thought Disorder), Depersonalization Inventory, Ditman's DWM Scale (1-20), Ditman's DWM Scale (6-8), Drug Effect Questionnaire (DEQ) (Scales Weird/Silly/Increased Sensitivity/Perceptual and SensorySharpness/Timesense/Dreamlike/ Giddy/Floating/Unreal Perception/Detachment/ Enhanced Awareness/Slow Speech/Fast Thoughts), Mental Status Examination (Scales Illusions/Hallucinations/Paranoid/Delusional), Positive and Negative Symptom Scale (PANSS), Ratings of Narrative Quality, Temporal Disintegration Inventory, Vividness of Imageries | 80 | 20 | 0 | 20, 28, 33, 46, 59, 60, 61, 88, 101, 108, 114, 116, 130, 144, 150, 164, 175, 179 (n=18) |
| Scale satiety | Drug Effect Questionnaire (DEQ) (Scale Hunger), Feeling Hungry, Food Intake, VAS (Scales Hungry/ Hunger) | 52 | 48 | 0 | 30, 38, 46, 49, 50, 70, 71, 72, 73, 80, 127, 131, 132, 139 (n=14) |
| Scale sensory | Comprehensive Psychiatric Rating Scale (AMDP) (Scale Disturbance of Sensory Perception), Medical Questionnaire (Scales Heat Ebulations/Cold Sensation), Scale Taste/Harshness/Draw, Subjective Effect Rating Scale (Scale Enhanced Sensations), VAS (Scale Loud Noise) | 9 | 64 | 27 | 24, 28, 93, 97, 131, 164 (n=6) |
| Scale sleep | Sleep Questionnaire | 0 | 100 | 0 | 49 (n=1) |
| Scale symptoms | Comprehensive Psychiatric Rating Scale (AMDP) (Scales Headache/ Nausea), Cornell Medical Index (CMI), Ditman's DWM Scale (1-20), Drug Effect Questionnaire (DEQ) (Scales Sick Feeling/ Symptoms/Heart Pounding/Dry Throat), Medical Questionnaire (Scales Tremor/Headache/ Dysphagia), Observer Rated Signs, Somatic Sensation Scale, Subjective Effect Rating Scale (Scales Heart Pounding/Dry Mouth), VAS (Scale Nauseous/Symptoms) | 52 | 46 | 2 | 28, 33, 37, 46, 59, 60, 70, 71, 80, 85, 87, 91, 100, 108, 128, 130, 131, 132, 160, 164, 179 (n=21) |

**Table 4** Dose-response relationship of clusters studied in more than 20 articles. Results are given in % per THC dose group for each cluster and listed with their functional domain.

| Domain | < 7 mg | | | 7-18 mg | | | >18 mg | | |
|---|---|---|---|---|---|---|---|---|---|
| Cluster | - | = | + | - | = | + | - | = | + |
| **Autonomic** | | | | | | | | | |
| Heart rate | 0 | 22 | 78 | 0 | 1 | 99 | 2 | 0 | 98 |
| **Motor** | | | | | | | | | |
| Motor control | 71 | 29 | 0 | 50 | 50 | 0 | 27 | 73 | 0 |
| Visuo-motor control | 68 | 32 | 0 | 64 | 36 | 0 | 19 | 81 | 0 |
| **Memory** | | | | | | | | | |
| Auditory/verbal memory delayed recall | 23 | 77 | 0 | 63 | 38 | 0 | 78 | 22 | 0 |
| Auditory/verbal immediate recall | 50 | 50 | 0 | 75 | 25 | 0 | 45 | 55 | 0 |
| **Attention** | | | | | | | | | |
| DSST-like | 31 | 69 | 0 | 50 | 50 | 0 | 47 | 53 | 0 |
| Focused selective attention | 57 | 43 | 0 | 33 | 67 | 0 | 14 | 86 | 0 |
| Reaction time | 46 | 54 | 0 | 52 | 45 | 3 | 47 | 53 | 0 |
| **Executive** | | | | | | | | | |
| Inhibition | 50 | 50 | 0 | 52 | 48 | 0 | 57 | 43 | 0 |
| Working memory | 52 | 48 | 0 | 42 | 58 | 0 | 9 | 91 | 0 |
| Reasoning/association | 33 | 67 | 0 | 37 | 63 | 0 | 43 | 57 | 0 |
| **Subjective experiences** | | | | | | | | | |
| Scale aggression | 20 | 80 | 0 | 24 | 71 | 5 | 40 | 50 | 10 |
| Scale alertness | 43 | 50 | 7 | 43 | 50 | 7 | 35 | 51 | 14 |
| Scale anxiety | 11 | 83 | 6 | 35 | 62 | 4 | 33 | 63 | 4 |
| Scale calmness | 10 | 60 | 30 | 31 | 50 | 19 | 26 | 48 | 26 |
| Scale craving | 53 | 22 | 25 | 61 | 11 | 28 | 20 | 20 | 60 |
| Scale drug effect | 12 | 32 | 56 | 4 | 18 | 78 | 3 | 21 | 76 |
| Scale high | 0 | 6 | 94 | 0 | 0 | 100 | 0 | 5 | 95 |
| Scale mood | 29 | 61 | 10 | 17 | 66 | 17 | 19 | 59 | 22 |
| Scale psychotomimetic | 83 | 17 | 0 | 81 | 19 | 0 | 76 | 24 | 0 |
| Scale symptoms | 64 | 36 | 0 | 58 | 37 | 5 | 41 | 59 | 0 |

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# 3 Effect of intrapulmonary THC administration in humans

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## Abstract

This randomised, double-blind, placebo-controlled, cross-over study was designed to identify which pharmacodynamic parameters most accurately quantify the effects of delta-9-tetrahydrocannabinol (THC), the predominantly psychoactive component of cannabis. In addition, we investigated the acceptability and usefulness of a novel mode of intrapulmonary THC administration using a Volcano® vaporizer and pure THC instead of cannabis.

Rising doses of THC (2, 4, 6 and 8 mg) or vehicle were administered with 90 minutes intervals to twelve healthy males using a Volcano® vaporizer. Very low between-subject variability was observed in THC plasma concentrations, characterising the Volcano® vaporizer as a suitable method for the administration of THC.

Heart rate showed a sharp increase and rapid decline after each THC administration (8 mg: 19.4 bpm: 95% CI 13.2, 25.5). By contrast, dose dependent effects of body sway (8 mg: 108.5%: 95% CI 72.2%, 152.4%) and different subjective parameters did not return to baseline between doses (Visual Analogue Scales of alertness (8 mg: -33.6 mm: 95% CI -41.6, -25.7), feeling high (8 mg: 1.09 U: 95% CI 0.85, 1.33), external perception (8 mg: 0.62 U: 95% CI 0.37, 0.86)). PK/PD modelling of heart rate displayed a relatively short equilibration half-life of 7.68 minutes. CNS parameters showed equilibration half-lives ranging between 39.4 - 84.2 minutes. Some EEG-frequency bands, and pupil size showed small changes following the highest dose of THC. No changes were seen in saccadic eye movements, smooth pursuit and adaptive tracking performance.

These results may be applicable in the development of novel cannabinoid agonists and antagonists, and in studies of the pharmacology and physiology of cannabinoid systems in humans.


## Introduction

Delta-9-tetrahydrocannabinol (THC), a partial CB1/CB2 agonist, is the most abundant and major psychoactive cannabinoid identified in the plant *Cannabis sativa*. Cannabinoids cause their pharmacological effects by binding to cannabinoid receptors, which are G-protein coupled receptors. At the moment two cannabis receptors (CB1 and CB2) have been identified. The CB1 receptors are predominantly situated in the brain with the highest densities in the hippocampus, cerebellum and the striatum, which accounts for the well-known effects of cannabis on motor coordination and short-term-memory processing[1-3], whereas they are expressed at low levels in the brainstem[3]. CB2 receptors are predominantly present in the spleen and in haematopoietic cells.[1] CB1 receptors are only present in these tissues in low density.

An increasing number of novel drugs in development are targeted at cannabinoid receptors, although their exact role in health and disease has not been fully elucidated. CB1/CB2 agonists might be of therapeutic use for muscle relaxation, immunosuppression, sedation, improvement of mood, neuroprotection, analgesia, and reduction of intra-ocular pressure.[4] Dronabinol (delta-9-tetrahydrocannabinol) and nabilone, a synthetic THC analogue, are registered in different countries as anti-emetic and anti-anorexic agents for patients with cancer or HIV. Recently rimonabant, a CB1 antagonist, was registered for the treatment of obesity. CB1 antagonists might also be useful for the treatment of smoking cessation, Parkinson's disease, and cognitive impairments in Alzheimer's disease and schizophrenia.[4]

The availability of a CB1 agonist, with well-described pharmacokinetics and pharmacodynamics could be of use as a pharmacological tool in the clinical development of CB1 agonist and antagonists. Such a well-characterised CB1 agonist could serve as a positive control for studies with novel CB1 agonists, provide responsive biomarkers or potency benchmarks for new drugs, or be used to show evidence of CB1 antagonist activity in humans. THC would be the most appropriate candidate, but its use as a model cannabinoid is currently hampered by the lack of a reproducible and practical mode of THC administration with a reliable pharmacokinetic and pharmacodynamic time profile.

Intravenous administration would overcome the unfavourable characteristics of orally administered cannabinoids, such as limited and variable bioavailability.[5-7] However, adequate injection fluids are difficult to manufacture due to the highly lipophilic properties of THC. In man, plasma THC concentration profiles are similar after smoking or intravenous administration with prompt onset and steady decline.[8-10]

Although smoking cannabis provides a reliable pharmacokinetic profile,[8,9] cannabis smoke has the disadvantage that it contains a mixture of psychoactive and partly noxious compounds, and that the active drug is partly lost by heat. The Volcano® vaporizer is a novel mode of intrapulmonary THC administration that overcomes these issues.[11] In this study, we investigated the pharmacokinetic and pharmacodynamic effects after inhalation of pure THC using a Volcano® vaporizer.

Although a huge number of studies have been performed with cannabis, many of these have addressed the consequences of chronic cannabis use and after acute administration a wide variety of tests was used. It is far from clear however, which tests are particularly sensitive to the acute effects of THC[12-14] and few studies have investigated the pharmacodynamic time profiles following THC administration. The most conspicuous effects of cannabis are subjective and psychomimetic changes.[4,15,16] In some studies, a reduction in smooth pursuit eye movements was observed[17] and changes in pupil size have been reported by several other authors.[18] THC increases heart rate by 20-60%.[19,20] The effects on blood pressure are complex, with

reports of both increases and decreases.[19-21] In the current study, the pharmacodynamic effects of pure THC were measured using a battery of central nervous system (CNS) assessments that have been shown to be sensitive to a wide range of CNS-active agents.[22,23] In addition, heart rate and blood pressure were measured frequently.

## Methods

### DESIGN

This was a double-blind, randomized, two-way balanced placebo-controlled, cross-over study of inhaled rising doses of THC (Table 1). Informed consent was obtained in writing before any study-specific procedure was carried out. After a general health screen, eligible subjects were enrolled in the study. Subjects were acquainted with the experimental methods and conditions, and with the inhalation procedure using alcohol-vehicle, in a training session within one week before the first study day. Pharmacodynamic and pharmacokinetic measurements were performed frequently on both study days. A follow-up visit (medical screening) was scheduled within two weeks after the second study day. The study protocol was approved by the Medical Ethics Review Board of Leiden University Medical Center and performed according to principles of ICH-GCP, the Helsinki declaration and Dutch regulations.

### SUBJECTS

Twelve healthy males (21-27 years) with a history of mild cannabis use for at least one year were included in the study. Subjects were not allowed to use cannabis more than once a week (the average was calculated over the last six months), and had to be able to refrain from using cannabinoids during the study. Use of other drugs or any medication was not allowed. Subjects with a positive THC test at screening were tested again, and were required to be negative before the first study day. Subjects with a positive drug test on a study day were excluded. Subjects had to refrain from smoking and use of coffee and tea on study days. The subject had to maintain a normal day-night-rhythm in the week before each study day. Severe physical exercise shortly before the study days had to be avoided. Subjects were financially compensated for their participation.

### TREATMENTS

THC was purified according to GMP-compliant procedures (Farmalyse BV, Zaandam, The Netherlands) from the flowers of *Cannabis sativa* grown under

Good Agricultural Practice (Bedrocan BV Medicinal Cannabis, Veendam, The Netherlands).[24-26] Each dose (2, 4, 6 or 8 mg) of THC (>98% purity by HPLC/GC) was dissolved in 200 µl 100 vol% alcohol. THC was stored in the dark at -20°C in 1 ml amber glass vials containing a teflon screw-cap secured with Para film to minimize evaporation. The solvent was used as placebo.

On each study day, rising doses of THC (2, 4, 6 and 8 mg) or placebo were administered by inhalation at 90 minute intervals using a Volcano® vaporizer (Storz-Bickel GmbH, Tüttlingen, Germany). Before the start of the study the efficiency and reproducibility of THC delivery into the balloon of the Volcano was evaluated.[11] Five to ten minutes before administration THC was vaporized at a temperature of about 225°C and the vapour was stored in a transparent polythene bag equipped with a valved mouthpiece, preventing the loss of THC in between inhalations. The transparant bag was covered with a black plastic bag to prevent unblinding. Subjects were not allowed to speak, were instructed to inhale deeply and hold their breath for 10 seconds after each inhalation. Within 2-3 minutes the bag was to be fully emptied. The inhalation procedure was practiced at screening using the solvent as a placebo.

The inhalation schedule was predicted to cause incremental THC plasma concentrations and effects, with cumulative peak plasma levels corresponding to a single dose of around 11 mg, which roughly corresponds to the THC contents in one or two marijuana cigarettes. The decision to proceed to the next highest THC dose was made by a physician, based on adverse events and physical signs. Because of the long half-live of THC study days were separated by a washout period of at least two weeks.

## PHARMACOKINETIC MEASUREMENTS

### BLOOD SAMPLING AND THC LABORATORY ANALYSES

For determination of the concentration of plasma THC and its two most important metabolites (11-OH-THC and 11-nor-9-carboxy-THC), venous blood was collected in aluminium foiled EDTA tubes of 4.5 ml. Blood samples were taken at baseline and at 10, 20 and 80 minutes after each THC administration. Additional samples were taken at 5, 35 and 55 minutes after administration of 6 mg THC and at 375, 425, 495, 545 and 1440 minutes after the first THC administration. After blood collection the tubes were put in ice water (0-4 °C) and centrifuged within one hour for 10 minutes at 2000G at 4°C. The THC samples were handled sheltered from light. Plasma samples were stored at a temperature of -20°C for less than 3 months before laboratory analysis. Concentrations of THC and the metabolites were shown to be stable over this period.

## PHARMACODYNAMIC MEASUREMENTS

Pharmacodynamic assessment was performed in a quiet and temperature controlled room with standardised illumination with only one subject per session in the same room. All tests were measured twice pre-dose and obtained frequently at fixed timepoints after each consecutive THC dose.

### HEART RATE AND BLOOD PRESSURE

Blood pressure and heart rate were measured in supine position after a rest of approximately 5 minutes, twice pre-dose and repeatedly post-dose on each of the two study days. All measurements were carried out with an automated sphygmomanometer (Nihon Kohden, Life Scope EC, Tokyo, Japan).

### PUPIL SIZE

For pupil size (pupil/iris ratio) measurements, a picture of both eyes was taken using a digital camera (Minolta DiMAGE) using a flashlight after at least five minutes adaptation in subdued lighting. For each eye, the diameters of the pupil and the iris in millimetres were determined. The pupil/iris ratio was subsequently calculated as a measure of pupil size.

### SMOOTH PURSUIT AND SACCADIC EYE MOVEMENT

Recording and analysis of saccadic and smooth pursuit eye movements was conducted with a personal computer using a validated Spike2 script (Cambridge Electronic Design Limited, Cambridge, UK). Disposable silver-silver chloride electrodes (Mediscore, VDP Medical, Nieuwegein, The Netherlands) were applied on the forehead and beside the lateral canthi of both eyes of the subject for registration of the electro-oculographic signals. Skin resistance was reduced to less than 5 kOhm before application of the electrodes. Head movements were restrained using a fixed head support. The equipment used for stimulus display was manufactured by Nihon Kohden (Nihon Kohden Corporation, Tokyo, Japan). For signal collection and amplification, a CED 1401 Power AD-converter (Cambridge Electronics Design, Cambridge, UK), a Grass telefector (F-15EB/B1) and a 15LT series Amplifier Systems (Grass-Telefactor, Braintree, USA) was used.

For recording and analysis of smooth pursuit eye movements the target moved sinusoidally at frequencies ranging from 0.3 to 1.1 Hz, increased by eight steps of 0.1 Hz. The amplitude of target displacement corresponded to 22.5 degrees eyeball rotations to both sides. Four cycles were recorded for each stimulus frequency. The average time during which the eyes were in smooth pursuit of the target, expressed as a percentage of stimulus duration, was used as the measurement parameter.

The target for the saccadic eye movements consisted of an array of light emitting diodes on a bar, fixed at 50 cm in front of the head support. Saccadic eye movements were recorded for stimulus amplitudes of approximately 15 degrees to either side. Fifteen saccades were recorded with interstimulus intervals varying randomly between 3 and 6 seconds. Average values of latency (reaction time), saccadic peak velocity and inaccuracy of all artefact-free saccades were used as parameters. Saccadic inaccuracy was calculated as the absolute value of the difference between the stimulus angle and the corresponding saccade, expressed as a percentage of the stimulus angle.

### PHARMACO-EEG

EEG recordings were made using silver chloride electrodes, fixed with collodion at Fz, Cz, Pz and Oz positions, with the same common ground electrode as for the eye movement registration (international 10/20 system). The electrode resistances was kept below 5 kOhm. EEG signals were obtained from leads Fz-Cz and Pz-Oz and a separate channel to record eye movements (for artefacts). The signals were amplified by use of a Grass telefector (F-15EB/B1) and a 15LT series Amplifier Systems (Grass-Telefactor, Braintree, USA) with a time constant of 0.3 seconds and a low pass filter at 100 Hz. Data collection and analysis were performed using a validated Spike2 script (Cambridge Electronics Design, Cambridge, UK). Per session eight consecutive blocks of eight seconds were recorded. The signal was AD-converted using a CED 1401 Power (Cambridge Electronics Design, Cambridge, UK) and stored on hard disk for subsequent analysis. Data blocks containing artefacts were identified by visual inspection and these were excluded from analysis. For each lead, fast Fourier transform analysis was performed to obtain the sum of amplitudes in the delta (0.5-3.5 Hz), theta (3.5-7.5 Hz), alpha (7.5-11.5 Hz) and beta (11.5-30 Hz) frequency ranges. Outcome parameters were the square root of the total power in each band for each lead.

### BODY SWAY

The body sway meter allows measurement of body movements in a single plane, providing a measure of postural stability. Body sway was measured with an apparatus similar to the Wright ataxia meter.[27] With a string attached to the waist, all body movements in the antero-posterior direction over a period of 2 minutes were integrated and expressed as mm sway on a digital display. The contribution of vision to postural control was eliminated by asking subjects to close their eyes. Subjects were not allowed to talk during the measurement, and asked to wear the same comfortable low-heeled shoes at all measurements.

### ADAPTIVE TRACKING

The adaptive tracking test was performed as originally described by Borland and Nicholson,[28] using customized equipment and software (Hobbs 2000, Hertfordshire, UK). Adaptive tracking is a pursuit tracking task. A circle moved randomly about a screen. The subject had to try to keep a dot inside the moving circle by operating a joy stick. If this effort was successful, the speed of the moving circle increased. Conversely, the velocity was reduced if the test subject could not maintain the dot inside the circle. Average performance was scored after a 3 minute period. Each test was preceded by a run-in period. After 4 to 6 practice sessions, learning effects are limited. The adaptive tracking test is more sensitive to impairment of eye-hand coordination by drugs than compensatory pursuit tasks or other pursuit tracking tasks, such as the pursuit rotor. The adaptive tracking test has proved to be useful for measurement of CNS effects of alcohol, various psychoactive drugs and sleep deprivation.[23,29]

## VISUAL ANALOGUE SCALES (VAS)

From the visual analogue scales as originally described by Norris[30] (16 items), three factors can be derived, as described by Bond and Lader[31], corresponding to alertness, contendness and calmness. Increased scores of these scales indicate enhanced subjective feelings of alertness, contendness (in general) and calmness. Psychedelic effects were monitored by an adapted version of the visual analogue scales (13 items), originally described by Bowdle *et al.*[32]

## ANALYSIS

### PHARMACOKINETIC ASSAY

Plasma samples for determination of THC, 11-OH-THC and 11-nor-9-carboxy-THC were stored at a temperature of -20°C prior to bioanalysis. Analysis was performed using a validated high performance liquid chromatography with tandem mass spectrometric detection. Calibration range was for all compounds 1.00 – 500 ng/ml. Over this range the intra-assay coefficient of variation was between 4.0 and 6.5%. The inter-assay coefficient of variation was between 1.4 and 9.4%.

### STATISTICS

All pharmacodynamic endpoints were summarized by treatment and time, and were presented graphically as mean over time, with standard devia-

tion as error bars. The pharmacodynamic endpoints were analyzed separately by mixed model analyses of variance (using SAS PROC MIXED, SAS for Windows V9.1.2, SAS Institute Inc., Cary, NC, USA) with treatment, period, time and treatment by time as fixed effects, with subject, subject by time and subject by treatment as random effect, and with the (average) baseline value as covariate. Treatment effect was reported as the contrast between the placebo and THC treatment where the average of the measurements up to (and including) 10 hours was calculated within the statistical model. Additionally, the average response of the values obtained in the 90 minutes after the final administration of THC (identified as the 'fourth dose effect') was compared between treatments within the statistical model. Contrasts were reported along with 95% confidence intervals. EEG and body sway data were analysed after log-transformation and all other parameters were analysed without transformation except for THC Bowdle (see below). Log-transformed contrasts were back-transformed resulting in geometric mean ratios with associated confidence intervals. These were re-expressed as percentage change from placebo.

Examination of average graphs (and summary measures over time) indicated that the VAS measuring psychedelic effects demonstrated a very skewed frequency distribution. As zeroes can naturally occur for these data (response from 0 to 100), a $^{10}$log transformation was applied after first adding 2 to all values. The rationale for log(x+2) instead of the more common log(x+1) transformation was that, after examining scatter plots of the psychedelic variables, a clear gap was observed between the log(1) values and the remaining values. After implementing the log(x+2) transformation, the gap decreased and a more homogenous distribution was obtained.

In order to reduce the number of VAS Bowdle scales and facilitate the interpretation of the results, cluster analysis and factor analysis was performed on the transformed psychedelic VAS scales. Two distinct clusters were found. VAS feeling drowsy was removed from the first cluster because this was not really considered a psychedelic effect, and drowsiness is more properly assessed using Bond and Lader VAS alertness. The two resulting clusters can be interpreted as two modalities of psychedelic effects roughly corresponding to 'external perception' and 'internal perception'. Changes in external perception reflect a misperception of an external stimulus or a change in the awareness of the subject's surroundings. Internal perception reflects inner feelings that do not correspond with reality. Table 2 gives an overview of the parameters included in external and internal perception.

A subsequent factor analysis indicated that the factor loadings were more or less the same for factors in the two clusters. This means that the two new composite factors can be derived by simply averaging the (transformed) psychedelic VAS Bowdle scales (Table 2). Since the log+2 transformation makes back-transformation problematic and the resulting scales

have favourable statistical properties, it was decided to not back-transform the results. To avoid confusion, the unit 'U' was used instead of 'average (log+2 mm)' in reporting the results.

### PK/PD MODELLING

PD parameters demonstrating a significant treatment effect and clear concentration-dependency were analysed using pharmacokinetic-pharmacodynamic (PK/PD) modelling. Nonlinear mixed effect modelling as implemented in the NONMEM program (Version 5, Globomax LLC, Ellicot City, MD, USA) was used. The PK/PD modelling is described in full detail by Strougo *et al*.[33]

## Results

### SUBJECTS

Twelve healthy males were included in the study. Their ages were in the range 21-27 years with a mean of 23 ± 2 years. The mean height and weight were respectively 185 ± 6 cm (range 174 – 194 cm) and 83 ± 8 kg (range 73 – 100 kg). All subjects were familiar with the effects of cannabis. Two subjects used cannabis four times a month, 6 subjects used it two to three times a month, 3 subjects used cannabis just once a month and two subjects used cannabis less than once a month. All subjects completed the study.

### CLINICAL EFFECTS

Most adverse events (AE) were mild, transient and did not require medical intervention, except for occasional use of paracetamol. The most frequently observed events were well-known THC effects like drowsiness, sleepiness, attention deficit and feeling high. In addition, also minor adverse events like headache and eye irritation were reported. During THC inhalation five subjects had to cough while subjects were required to hold their breath for 10 seconds. This was not reported after inhalation of the alcohol-vehicle during placebo occasions. Two out of 12 subjects experienced side effects severe enough to decide not to administer the last dose of 8 mg THC. One of these subject was too sleepy to perform any test, and the other subject vomited just after administration of the third dose.

### PHARMACOKINETIC AND PHARMACODYNAMIC DATA ANALYSIS

All data were used for the pharmacodynamic and pharmacokinetic analysis. However, for the average figures shown in this article, two subjects were excluded. These subjects did not receive the highest THC dose and conse-

quently had deviating concentration and effect time-profiles that would have distorted the average graphs.

### PHARMACOKINETICS

THC plasma peak levels were reached within a few minutes (Figure 1). Plasma peak concentration was followed by a short distribution phase (approximately 25 minutes) and a longer elimination phase (roughly 250 minutes). Average plasma THC concentrations 10 minutes after the fourth dose (50.3 ± 14.4 ng/mL) exceded the 11-OH-THC concentrations (6.8 ± 2.8 ng/mL) by 7.4-fold, and the 11-nor-9-carboxy-THC concentrations (21.8 ± 4.8 ng/mL) by 2.3-fold. There was a very small between-subject variability in THC plasma concentrations as illustrated by the low standard deviations.

### HEART RATE AND BLOOD PRESSURE

Heart rate increased in a dose-related manner compared to placebo (Figure 2). The average increase after the fourth dose of 8 mg was 19 beats per minute (95% CI 13.2, 25.5 bpm). After the initial increase, heart rate decreased rapidly after each dose, and hardly any accumulation was seen with repeated dosing (Figure 2). Blood pressure did not change after THC administration (fourth dose effect: systolic blood pressure: -1 mmHg: 95% CI -8, 6; diastolic blood pressure: -0.5 mmHg: 95% CI -8, 7).

### PUPIL SIZE

Compared to placebo, slight increases were seen in pupil/iris ratio that were only significant after the fourth dose of 8 mg THC (0.025: 95% CI 0.003, 0.047).

### SMOOTH PURSUIT AND SACCADIC EYE MOVEMENT

No changes in smooth pursuit eye movements occurred (fourth dose effect: -3%: 95% CI -9, 3). Compared to placebo, saccadic latency (20 msec: 95% CI 10, 30) and saccadic inaccuracy (3.1% : 95% CI 1, 5) increased only after the fourth dose of 8 mg THC. No changes were found in saccadic peak velocity (fourth dose effect: 14 deg/sec: 95% CI -4, 32).

### ELECTRO-ENCEPHALOGRAPHY (EEG)

After the highest dose of THC there were decreases in the power of Pz-Oz delta (-16%: 95% CI -24, -7), Pz-Oz theta (-15%: 95% CI -24, -5) and Pz-Oz beta activity (-12%: 95% CI -18, -4). No changes were found in alpha activity (-6%: 95% CI -17%, 5%). In the Fz-Cz region, changes in beta activity were predominant. No changes in delta and theta activity were seen in Fz-Cz region.

Although EEG was affected significantly by active treatment, the average time profiles did not indicate a clear dose and concentration dependency.

#### BODY SWAY

After THC administration, dose-related increases were seen in body sway, which decreased only slowly after each dose and did not return to baseline between doses (Figure 3). Consequently, the effect accumulated with repeated dosing to a 109% increase over placebo: (95% CI 72, 152) after the highest dose.

#### ADAPTIVE TRACKING

Compared to placebo no changes were observed in adaptive tracking performance (fourth dose effect: -1%: 95% CI -3, 1).

### VISUAL ANALOGUE SCALES (VAS)

#### VAS BOND AND LADER

The VAS alertness was affected by THC in a dose related manner. The decrease accumulated to -34 mm: 95% CI -42, -26 after the fourth dose. A decrease was seen in VAS contendness after the fourth dose (-7 mm 95% CI -13, -1) but no change was seen in VAS calmness (-3 mm: 95% CI -10, 4).

#### VAS BOWDLE – INTERNAL AND EXTERNAL PERCEPTION

Many of the individual visual analogue scales measuring psychedelic effects demonstrated treatment effects (Table 2), with VAS feeling high as one of the most responsive scales (1.1 U: 95% CI 0.9, 1.3). The composite score of 'external perception' showed a dose response effect of THC (Figure 4) and an increase of 0.6 U after the fourth dose (95% CI 0.4, 0.7). Although a significant treatment effect was also demonstrated for the 'internal perception' composite scale (0.2 U: 95% CI 0.1, 0.4 after the fourth dose), concentration- and dose-dependency were much less pronounced than the effect for 'external perception' and seemed to be associated with an on/off effect or at least a very steep dose-response curve (no response after 2 mg, maximum response at doses of 4 mg and higher) (Figure 5).

#### PK/PD MODELLING

The effects of THC lagged behind the THC plasma concentration, revealing hysteresis. Equilibration half-lives that quantify hysteresis varied from 7.68 minutes for heart rate and from 39.2 to 84.8 minutes for the effects on the

central nervous system. The PK/PD modelling is described in full detail by Strougo *et al*.[33]

## Discussion

This study was designed to investigate the acceptability and usefulness of a novel mode of intrapulmonary THC administration using a Volcano® vaporizer and pure THC instead of cannabis. A recent study showed that the vapour contains 98% THC and that about 54% (SD ±8%) of this was delivered to the vapour collection balloon of the administration system by the Volcano® vaporizer.[11] Therefore in our study an estimated average cumulative dose of 11 mg of THC was inhaled from the balloon. This is comparable to the doses used in the literature, since most studies report effects of 1-2 marijuana cigarettes, containing between 2.5-30 mg THC, of which roughly half is lost by heat. In this study the average plasma THC profiles indicate very limited inter-individual variability, characterising the Volcano® vaporizer as a suitable method for the administration of pure THC.

Unlike 11-OH-THC, 11-nor-9-carboxy-THC is a non-psychotropic metabolite of THC.[4] Although 11-OH-THC is equipotent or twice as potent as THC,[34,35] the observed plasma THC concentrations are roughly 25 times higher than the observed plasma concentrations of 11-OH-THC. This indicates that the observed effects are due to THC itself.

The effect of THC on different CNS and non-CNS tests was investigated. Many of the THC effects were dose-dependent after administration of repeated doses of 2, 4, 6 and 8 mg. High densities of CB1 receptors are found in the basal ganglia, cerebellum amygdala and forebrain.[2,36] This may explain why THC had clear dose-dependent effects on postural stability and a number of subjective parameters after administration of rising doses of THC (2, 4, 6 and 8 mg). Body sway clearly increased with dose, which agrees with previous reports of the effects marijuana.[37]

The sensitive subjective parameters included in particular alertness of the Visual Analogue Scales (VAS) of Bond and Lader; the newly derived 'external perception' scale, which is a composite subscale of VAS Bowdle's for psychedelic effects, and the VAS scale for feeling high. Alertness is closely related to the ability to pay attention, to concentrate on a specific issue, and attention deficit is a well-known effect of cannabis. The Bond and Lader VAS scales for contendness and calmness are rarely affected by CNS-active drugs[22,23] and they do not seem to be prominently affected by THC. The changes in the 'external perception' reflect a misperception of an external stimulus or a change in the awareness of the subject's surroundings. This is a well-known effect of THC and has been observed after oral administration of 15 mg THC,[16] making the composite scale of 'external perception' a useful tool for assessing the effects of THC. Limited changes were seen on

'internal perception', which reflects inner feelings not corresponding with reality. Feelings of unreality, hallucinations, paranoia and anxiety have been observed after use of high doses of cannabis and in cannabis naïve subjects.[38,39] In this study subjects familiar with the effects of cannabis were included and possibly the doses in our study were not high enough to ellicit such effects. Interestingly, all observed CNS effects showed accumulation of the effects since the effect of the previous dose had not faded before the next dose was administered.

In the current study, limited decreases in EEG delta, theta and beta activity were demonstrated. One of the earliest signs of drowsiness is the disappearance of the occipital dominant alpha activity.[22] Although subjects reported being drowsy, no changes in alpha rhythm were seen in this study. In the literature, the EEG results obtained after cannabis use are often contradictory. Acute reactions to the drug have sometimes been compatible with a waking type activation of the EEG pattern, but increased slow wave EEG characteristics of a resting or sleep state have also been seen, and there seem to be no obvious localizations of the EEG changes to any particular brain region.[40]

The literature reports conflicting results on tracking tests, which is probably due to differences in tasks.[41] The critical tracker task employed by Stoller *et al.* resembles the tracker test used in this study. They reported a statistically significant effect on the critical tracking test after the oral administration of 22.5 mg THC.[42] Since the pulmonary administration of THC is on average approximately 2.6-3 times more potent than oral administration,[43] this result should resemble the cumulative effect after the fourth dose in this study. However, we did not observe significant changes.

The presence of CB1 receptors in the sphincter pupillae muscle provides a possible site of action by cannabinoids on pupil dilation or contraction.[44] This study showed a slight increase in pupil/iris ratio after the fourth dose of 8 mg THC. Conflicting results have been published after administration of THC, which do not seem to be clearly related to differences in dosing.[16,18,45]

CB1 receptors are sparsely found in the brainstem[36,46] which may explain why few changes in smooth pursuit and saccadic eye movements were seen. Smooth pursuit eye movements are primarily steered by the paramedian pontine reticular formation, and saccadic eye movements by the superior colliculus.[47] The lower brain stem areas also control cardiovascular function. Orthostatic hypotension has been reported in literature.[19,20] In this study no changes in blood pressure have been seen, which may be due to the supine blood pressure measurements. In this respect, the sharp dose dependent increase in heart rate could be considered as a compensatory mechanism for a loss of vascular tone. The increase in heart rate was clearly dose dependent and closely associated with THC plasma concentrations. Tachycardia was significant, with an average increase of 19 beats per minute

after the fourth dose, without any indications for blood pressure reductions. In contrast with different CNS parameters hardly any accumulation was seen in heart rate after rising doses of THC. These results correspond to data found in literature.[13,19,20,48] The faster response in heart rate prior to the onset of subjective effects has also been observed after oral administration of 15 mg THC.[16] Literature also reported that THC plasma concentration already dropped significantly before maximum psychotropic effects were achieved.[6,49] These observations make it likely that a peripheral mechanism is involved in the increase in heart rate. This is supported by PK/PD modelling of the current study, which showed a relatively short equilibration half-live for heart rate of 7.68 minutes.[33] This is much shorter than the equilibration half-lives found on central nervous system effects, which varied from 39.2 to 84.8 minutes. In addition, CB1 receptors are present in human atrial muscle[50] but they are sparse in the lower brainstem areas controlling cardiovascular function.[46] In combination, these results suggest that the increase in heart rate seen after THC administration is not mediated by brain stem centers but is established by a direct effect of THC on the heart.

Lipophilic compounds like THC that cross the blood-brain barrier tend to accumulate in the brain, which explains the prolongation of the CNS effects, in contrast to the much faster response of heart rate. The equilibration half-lives that quantify hysteresis varied from 39.2 to 84.8 minutes for the effects on the central nervous system. This range may reflect various mechanisms of action, in which receptor density and receptor distribution between different brain regions, activation of secondary neurotransmitters systems, or perhaps yet unidentified CB receptors may play a role.

Only limited and transient side effects were seen. We therefore consider administration of rising doses up to 6 or 8 mg pure THC using the Volcano® vaporizer a safe method of THC administration. Two out of 12 subjects experienced side effects severe enough to decide not to administer the last dose of 8 mg THC. Therefore, a study design with rising doses up to 6 mg is preferable as it seems to allow CNS testing on all doses, at least for all subjects with previous experience with THC.

In conclusion, this study showed a range of pharmacodynamic effects of THC, using a novel mode of intrapulmonary THC administration. Some of these effects were clearly dose- and concentration-related, and started with the lowest dose of 2 mg. These dose-related effects include impairments of subjective alertness and postural stability, feeling high and psychedelic effects, and an increase in heart rate. The most sensitive effects seem to correspond to brain regions that have the highest densities of cannabinoid receptor localization. These results can be useful in the development of therapeutically beneficial cannabinoid agonists and antagonists, and in studies of the pharmacology and physiology of cannabinoid systems in humans.

**Figure 1** Mean (SD) observed profile of plasma THC; closed circles: common measurement points for all 4 doses, open circles: extra assessments for third dose. THC administration: 2 mg at T = 0, 4 mg at T = 90, 6 mg at T = 180, 8 mg at T = 270.

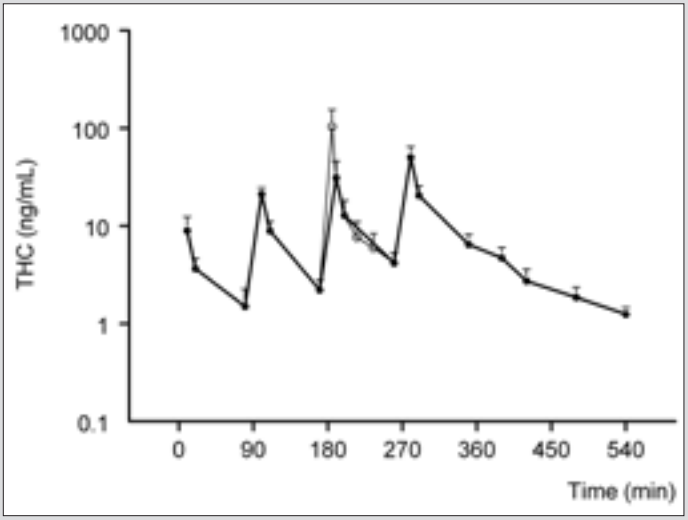


**Figure 2** Mean (SD) time profile of heart rate. THC administration: 2 mg at T = 0,4 mg at T = 90,6 mg at T = 180,8 mg at T = 270.

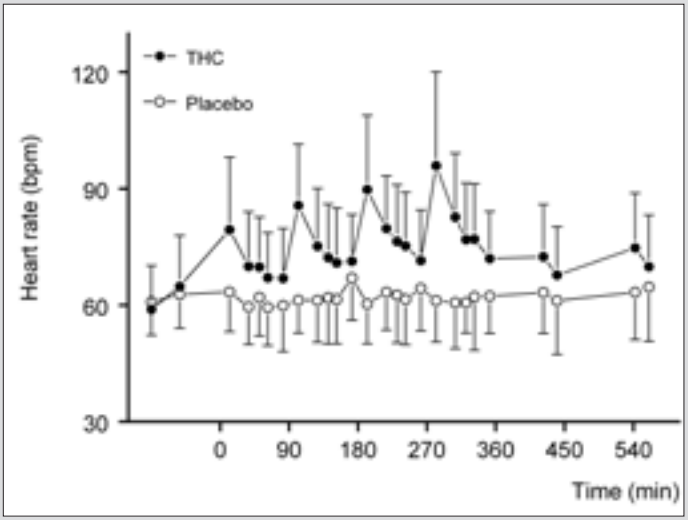

**Figure 3** Mean (SD) time profile of body sway. THC administration: 2 mg at T = 0,4 mg at T = 90,6 mg at T = 180,8 mg at T = 270.

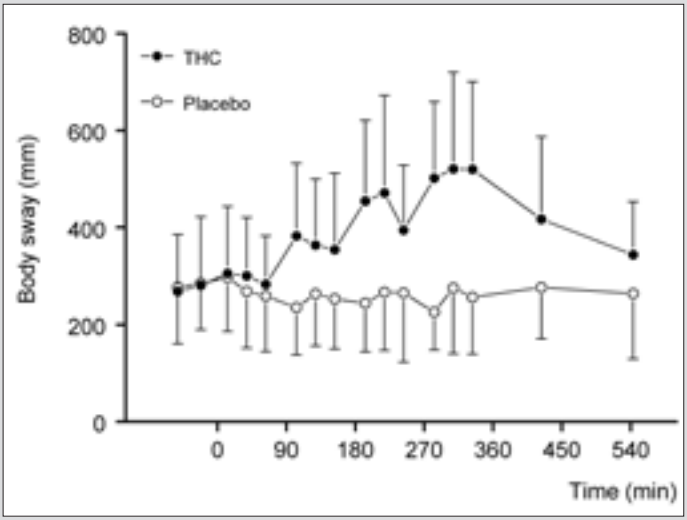


**Figure 4** Mean (SD) time profile VAS 'external perception'. THC administration: 2 mg at T = 0,4 mg at T = 90,6 mg at T = 180,8 mg at T = 270.

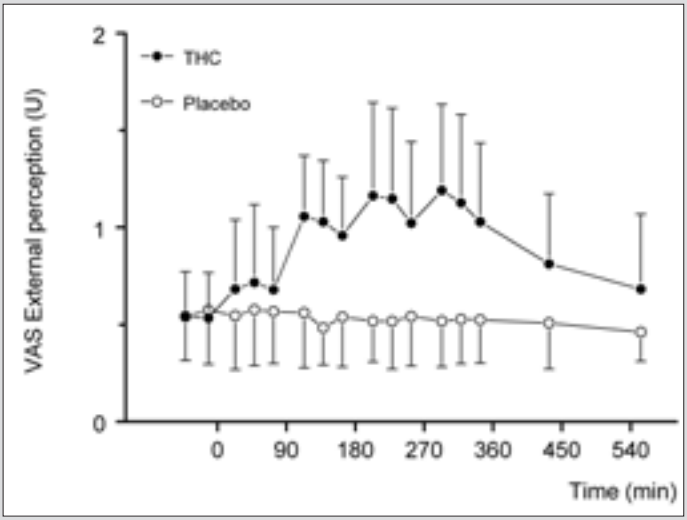

**Figure 5** Mean (SD) time profile VAS 'internal perception'. THC administration: 2 mg at T = 0,4 mg at T = 90,6 mg at T = 180,8 mg at T = 270.

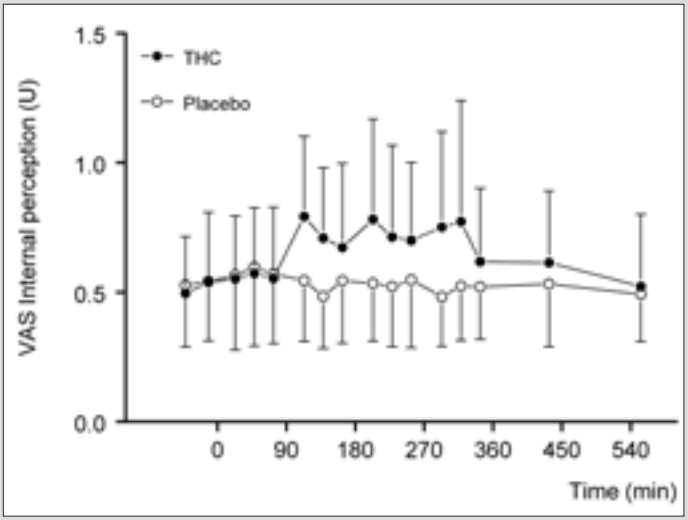


**Table 1** Study design

| | 8:00 – 10:00 | 10:00 | 11:30 | 13:00 | 14:30 |
|---|---|---|---|---|---|
| Study day 1 | Arrival at unit and | 2 mg THC | 4 mg THC | 6 mg THC | 8 mg THC |
| Study day 2 | study preparations | placebo | placebo | placebo | placebo |

**Table 2** Fourth dose treatment effect (8 mg THC) of the different parameters of the Visual Analogue Scales of psychedelic effects, which are also presented as two composite scales: 'external perception' and 'internal perception'.

| | Estimate of difference (U) | 95% CI |
|---|---|---|
| **External perception*** | 0.616 | (0.371, 0.860) |
| VAS 1: my body parts seemed to change their shape or position | 0.223 | (-.005, 0.451) |
| VAS 2: my surroundings seemed to change in size, depth, or shape | 0.408 | (0.144, 0.671) |
| VAS 3: the passing of time was altered | 0.808 | (0.479, 1.137) |
| VAS 5: it was difficult to control my thoughts | 1.047 | (0.705, 1.388) |
| VAS 6: the intensity of colours change | 0.448 | (0.180, 0.716) |
| VAS 7: the intensity of sound changes | 0.761 | (0.487, 1.034) |
| **Internal perception**** | 0.212 | (0.066, 0.357) |
| VAS 4: I had feelings of unreality | 0.502 | (0.249, 0.754) |
| VAS 8: I heard voices and sounds that were not real | 0.144 | (0.021, 0.266) |
| VAS 9: I had the idea that events, objects, or other people had particular meaning that was specific for me | 0.149 | (0.017, 0.281) |
| VAS 10: I had suspicious ideas or the belief that others were against me | 0.127 | (-.019, 0.274) |
| VAS 13: I felt anxious | 0.144 | (-.062, 0.349) |

Data are population average, 95% confidence interval (CI) and p-value.
* external perception = $[^{10}\log(VAS1+2)+^{10}\log(VAS2+2)+^{10}\log(VAS3+2)+^{10}\log(VAS5+2)+^{10}\log(VAS6+2)+^{10}\log(VAS7+2)]/6$
** internal perception = $^{10}\log(VAS4+2)+^{10}\log(VAS8+2)+^{10}\log(VAS9+2)+^{10}\log(VAS10+2)+^{10}\log(VAS13+2)]/5$

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# 4 Modelling of the concentration-effect relationship of THC on central nervous system parameters and heart rate – insight into its mechanisms of action and a tool for clinical research and development of cannabinoids

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# Abstract

Pharmacokinetics after pulmonary administration of delta-9-tetrahydrocannabinol (THC) and its major metabolites 11-OH-THC and 11-nor-9-COOH-THC was quantified. Additionally, the relationship between THC and its effects on heart rate, body sway and several visual analogue scales was investigated using PK/PD modelling. This provided insights useful for the research and development of novel cannabinoids, and the physiology and pharmacology of cannabinoid systems. First, the PK/PD model gave information reflecting various aspects of cannabinoid systems. The delay between THC concentration and effect was quantified in equilibration half-lives of 7.68 minutes for heart rate and from 39.2 to 84.8 minutes for the CNS responses. This suggests that the effect of THC on the different responses could be due to different sites of action or different physiological mechanisms. Differences in the shape of the concentration-effect relationship could indicate various underlying mechanisms. Second, the PK/PD model can be used for prediction of THC concentration and effect profiles. It is illustrated how this can be used to optimise studies with entirely different trial designs. Third, many new cannabinoid agonists and antagonists are in development. PK/PD models for THC can be used as a reference for new agonists, or as tools to quantify the pharmacological properties of cannabinoid antagonists.


# Introduction

## CANNABINOID SYSTEM

The cannabinoid system may be implicated in a range of disease states, and is considered a potential target for a variety of new drugs. An accurate characterisation of the pharmacology and physiology of the cannabinoid system is of use in the development of these compounds. This can be achieved with challenge tests, which have been used to study a wide range of pharmacological systems in health and disease, including serotonergic systems[1,2], dopaminergic systems[3], cholinergic systems[4] and many others. So far, clinical research of cannabinoid systems in humans has been hampered by the lack of a reproducible cannabinoid challenge test. Marijuana cigarettes have been used, but smoking has the disadvantage that the active drug is partly lost by heat and partly escapes from the burning cigarette. Additionally, cannabis contains a wide range of psychoactive compounds, and these potentially noxious compounds are inhaled as well.

The most abundant and major active cannabinoid identified in the plant *Cannabis sativa* L. is delta-9-tetrahydrocannabinol (THC). Various preparations of THC are available for oral administration, but the bioavailability of these compounds is low and variable.[5,6] Intrapulmonary administration

of purified THC led to reproducible plasma concentrations and a range of dose-dependent effects,[7] which allows an accurate analysis of the effects of changing levels of THC on different physiological responses. The aims of the current article are to provide some insights into the physiology and pharmacology of cannabinoid systems, and to demonstrate how such analyses can be used to support the research and development of novel cannabinoids. By modelling the relationships between plasma concentrations and the dose-dependent responses, it is possible to derive quantitative information about different systems that are influenced by cannabinoids. The models also allows the prediction of the concentration and effect profiles of alternative dosing regimens, which can be used to optimise studies by achieving stable central nervous system effects, or reducing inter-subject variability. Another application of concentration-effect-models is to facilitate the design and analysis of studies with other cannabinoid agonists or antagonists.

## PHARMACOKINETIC-PHARMACODYNAMIC MODELLING

Pharmacokinetic/pharmacodynamic (PK/PD) modelling attempts to characterise the concentration time profile and the relationship between concentrations and effects using a mathematical model. The parameters that shape such a structural model can be estimated using non-linear regression techniques. Estimation can be either on an individual basis by estimating the parameters separately for each individual, or on a population basis by simultaneously estimating all subjects. Simultaneous population estimation assures a common structural model and allows the sharing of information across individuals, which is important for estimating parameters that are ill-defined for a single individual. Additionally, competing models can be compared using a single test for the population as a whole, instead of having to test each individual separately with the risk of ending up with a mixture of structural models. The assumption that all individual concentration-effect relationships can be described with the same structural model, is based on the notion that the drug activates the same pharmacological system in all subjects (or systems for different responses). In this respect, the PK/PD parameters are quantitative measures of different aspects of this pharmacological system.

Non-linear mixed effect modelling is one of these population analysis techniques and the most popular implementation is in the NONMEM program.[8] This non-linear modelling approach provides estimates of the population average parameters assuming each individual can be described using the same structural model, and their associated inter-individual variability which allows individuals to differ from each other. Finally, a residual error describing the variability of the difference between predicted values and the observations is estimated. After estimating population parameters, empirical Bayes estimates can be generated, which are individual estimates

conditional on the previously obtained population information. These empirical Bayes estimates allow the sharing of information between subjects, and the generation of individual predicted profiles. The basic principles of the non-linear mixed effects modelling approach are extensively discussed elsewhere.[8-11] The current article describes the development of a PK/PD model for THC, and how this is used to study different cannabinoid systems, and to optimise the research and development of cannabinoid agonists and antagonists.

## Methods

The comprehensive description of the applied methods and clinical results of this clinical trial are described elsewhere.[7] In short, a double blind, randomised, placebo-controlled, two-way cross-over design was used involving twelve healthy male subjects who received rising doses of THC (2, 4, 6 and 8 mg) or a matching placebo administered at 1,5 hour intervals starting around 11:00 in the morning (range 10:30-12:00), by inhalation using a Volcano® vaporizer (Storz-Bickel GmbH, Tüttlingen, Germany). The cumulative dose of administered THC was chosen to correspond to smoking one or two marihuana cigarettes. Blood samples for determination of THC, 11-OH-THC and 11-nor-9-COOH-THC were collected frequently.

Although cannabis displays many CNS effects, relatively little is known about the functional relevance of the endocannabinoid system in the human brain, and about the tests that best reflect the effects of cannabinoid stimulation. The effect parameters in this study were elements of a comprehensive CNS test-battery, that have allowed the establishment of concentration-effect relationships for a wide range of CNS-active drugs. The effects of THC on the complete test battery are described in the companion article.[7] In the current article, PK/PD analyses were only performed on those parameters that showed a statistically significant overall response to THC (see Zuurman *et al*. 2007[7] for a detailed description of these and other parameters). Subjective CNS effects were assessed using visual analogue scales (VAS) on alertness[12], feeling high and VAS external perception[7]. To assess postural stability, body sway was measured using an apparatus similar to the Wright ataxia meter.[13] Additionally, heart rate was determined by using an automated sphygmomanometer (Nihon Kohden, Life Scope EC, Tokyo, Japan) in supine position after a rest of approximately 5 minutes.

### DATA ANALYSIS

The analysis of the concentration-effect relationship of THC followed a two-stage approach. In the first stage, a pharmacokinetic model was developed to describe the disposition of THC and its major metabolites (11-OH-THC

and 11-nor-9-COOH-THC). In the second stage, a concentration-effect model was developed using the individual empirical Bayes estimates generated with the model developed in the first stage.

During model development, models gradually increased in complexity. In order to test if the increase in complexity was sustained by the data, models were compared for the entire population by using the likelihood ratio test. This test examines whether the more complex model results in a better model fit by calculating the difference in the minimum value of the objective function (dMVOF). The minimum value of the objective function is a goodness of fit statistic defined as minus two times the logarithm of the likelihood. This value is provided in the output of each model. Conventional critical values were applied which means for instance that a decrease for a single fixed parameter in MVOF of 10.83 was considered statistically significant at $p = 0.001$.

Throughout model development the first order conditional estimation (FOCE) was used. FOCE is a numerical method for parameter estimation in NONMEM. Within NONMEM, this is the most accurate estimation procedure available that is implemented by generating individual empirical Bayes estimates during each iteration. Inter-individual variability was estimated using an exponential error model that results in a log-normal distribution of the parameters thereby avoiding negative estimates. The variability is described using coefficients of variation (CV), corresponding to the ratio of the standard deviation and the associated average, multiplied by 100.

### MODELLING OF PLASMA CONCENTRATIONS OF THC AND ITS METABOLITES

The disposition of THC and its major metabolites (11-OH-THC and 11-nor-9-COOH-THC) was performed using a compartmental approach. As neither our data nor the literature contained quantitative information regarding the THC fraction metabolised or the THC fraction excreted, the metabolite models assumed that the full fraction of THC was converted into the metabolite. This assumption is supported by the literature that reports the excretion of unchanged THC in faeces and urine to be insignificant.[14]

Saturable elimination of THC into its metabolites was tested by introducing the Michaelis-Menten equation into the model. Population averages and individual empirical Bayes estimates were estimated as initial and terminal half-life, volume of distribution and clearance. By introducing Michaelis-Menten elimination into the model, the clearance is replaced by the maximum rate of THC metabolism ($V_{max}$) and THC plasma concentration at its half-maximum rate of metabolism (Km). For residual variability a proportional error structure was used. This means that the difference between predicted values and observed concentrations is proportional to the predicted concentration, and this proportionality is described by a coefficient of variation (CV).

THC-induced effects were assumed to be exclusively due to THC plasma concentrations. The metabolites were ignored, because their concentrations or intrinsic activity were too low to explain much of the effects. After visual inspection of the concentration-time and the effect-time plots, hysteresis was apparent as a clear time delay between changes in concentrations and associated effect changes, as well as an accumulation of the effects over time despite rapidly declining plasma concentrations. Hysteresis is commonly dealt with by assuming an extra hypothetical effect compartment linked to the observation compartment, where the degree of hysteresis is characterised by the equilibration half life. The equilibration half-life determines the shape of the concentration profile in this effect compartment; the effect compartment concentrations become more flattened out and accumulated with an increase in equilibration half-life. The estimation of this equilibration half-life aims to abolish the hysteresis between effect and concentration. The relationship between the effect and the concentration in the hypothetical effect compartment can then be assessed using for instance a straight line (linear model) where the slope of the line determines the sensitivity of the effect, or a relationship with a maximum effect ($E_{max}$ model) where the sensitivity is determined by the concentration ($EC_{50}$) required to obtain 50% of the maximum change ($E_{max}$). The most appropriate model (linear or $E_{max}$) was determined by comparing the model results using the likelihood ratio test.

Since no circadian rhythm was observed in the average placebo profile of all the modelled effects, the data were not corrected for the placebo treatment (see Figures 2, 3 and 4). For residual variability, an additive error structure was used. This means that the variability of the difference between predicted values and observed responses is independent of the predicted values.

### SOFTWARE

Data management was performed using SAS for Windows V9.1.2 (SAS Institute Inc., Cary, NC, USA) and calculations using nonlinear mixed effect modelling were implemented using NONMEM Version V software (GloboMax, Ellicot City, MD, USA).

## Results

All data were used for the modelling process, but for the reported average graphs, two subjects were excluded. These subjects did not tolerate (and therefore did not receive) the highest THC dose and as a result had deviat-

ing concentration and effect time-profiles that would have distorted the average graphs. When using a modelling approach, such deviating subjects do not pose problems, while a more traditional analysis might be flawed.

### MODELLING OF PLASMA CONCENTRATIONS OF THC AND ITS METABOLITES

THC plasma concentration levels decline bi-exponentially, in accordance with fast distribution followed by a gradual elimination (Figure 1). In order to describe this concentration time profile, a two-compartment model was required, with initial and terminal half-life estimates of 3.81 and 68.4 minutes respectively. Inter-individual variability could not be estimated for these (and other) parameters and was therefore fixed to 0%. This does not necessarily mean that there is no actual variation in half lives between subjects, but rather that the description of the data does not improve when these parameters are varied between subjects. Incorporation of an absorption compartment into the two-compartment model did not improve the description of the THC plasma concentration profiles because drug appeared in the plasma without an observable delay. Therefore, bolus administration in the observation compartment was modelled.

Inspection of individual concentration time profiles of THC revealed that individuals could not be adequately described using a single set of pharmacokinetic parameters for all doses; sometimes higher and sometimes lower peaks were observed for some of the doses. This could be due to differences in bioavailability between doses within subjects (i.e. one dose is absorbed better than another), which could be related to coughing or exhalation during the intrapulmonary administration. This variability was assessed by incorporating an intra-individual variability term, allowing the relative bioavailability to vary within a subject for each of the four different doses administered. The introduction of this term resulted in an improvement of the model fit, expressed by a significant decrease in MVOF ($p < 0.001$). A CV of 24% was estimated for the relative bioavailability using THC data alone (Table 1).

Description of the metabolite concentration as a function of time also required two-compartment models. The observation compartment of the two-compartment model of THC was initially linked to the observation compartment of the two-compartment model of one the metabolites (11-OH-THC or 11-nor-9-COOH-THC) through a first order constant (describing both the elimination of THC and the formation of metabolite). Thereby we assumed that full fraction of THC was converted into the metabolite. This assumption does not affect either the description of the pharmacokinetic profiles or the parameter estimates, except for the volume of distribution of the metabolites. These may be overestimated by the actual model.

These models resulted in a small but systematic under-prediction of the concentration of the metabolites of the initial lower doses and an over-

prediction for the later higher doses. This bias disappeared when saturable elimination of THC was introduced into the model by means of a Michaelis-Menten equation. In addition, a significant improvement in the goodness of fit was obtained ($p < 0.001$ for both metabolites).

In the final model developed for 11-OH-THC, slight underestimation of the observed concentrations in the elimination phase prior to the third and after the fourth dose was observed, but overall the data were well-described (Figure 1). The model descriptions for THC and 11-nor-9-COOH-THC were unbiased (Figure 1). The pharmacokinetic parameters estimates for THC and metabolites are shown in Table 1.

### MODELLING OF CONCENTRATION-EFFECT PROFILES OF THC

In all the responses analysed, a pronounced treatment effect and an apparently THC concentration-dependent time profile with a time delay was observed. Additionally, a persistence of the effect in relation to the THC plasma concentrations was observed for all responses, combined with an accumulation of the effect, which was especially observed with the CNS responses. These differences were effectively described by the estimation of the equilibration half-lives, which define the change in shape of the effect profile. For heart rate, the average population equilibration half-life was estimated at 7.68 minutes, whereas for the CNS responses it varied from 39.2 minutes to 84.8 minutes (Table 2).

The effects produced by THC on heart rate, VAS feeling high, VAS external perception and body sway were best described by an $E_{max}$ model, while VAS alertness was best described by a linear model. The increase in goodness of fit provided by the $E_{max}$ model for heart rate, VAS feeling high and VAS external perception was statistically significant at $p < 0.001$ and for body sway at $p < 0.05$.

The estimates for the $EC_{50}$ parameters themselves are not very meaningful and can only be interpreted in combination with the concentrations achieved in the effect compartment. Table 2 for example shows equal $EC_{50}$ values for heart rate and body sway (30.7 ng/mL) which would suggest the same sensitivity. However, the pronounced difference in equilibration half-lives (7.68 and 84.8 minutes) results in a large difference in predicted maximum concentrations (lower for body sway) and as a consequence, the maximum effect is approached with lower doses for heart rate than for body sway The right panels of Figures 2 (heart rate) and 3 (VAS feeling high) illustrate the concentration effect relationship, where the maximum on the x-axis corresponds to the maximum concentration in the effect compartment that was achieved in this study. The shape of both these curves also illustrates that increasing the dose further will probably not lead to a much higher response. This is in sharp contrast with the effect on VAS alertness (Figure 4) for which no approach of a maximum can be detected at these concentrations.

# Discussion

Although THC is the most extensively studied cannabinoid, this is the first time that several effects evoked after administration of pure THC were quantitatively characterised by means of a non-linear mixed effect modelling approach. By using this analysis approach we aimed to provide some insight into the complexity of the mechanism of action of THC. Further, we will also discuss how this model can be useful to support research and development of novel cannabinoids.

The first stage of the analysis was to develop a model capable of characterising the concentration time profile of THC. The results show that the biphasic concentration-time profile was best described by a two-compartment model with bolus administration, resembling the model developed by Harder and Rietbrock.[15] According to the estimates of the model, the short initial half-life of approximately 4 minutes quantitatively characterised the rapid distribution observed. This rapid distribution is in accordance with the high lipophilicity of THC, resulting in rapid disappearance from the plasma and rapid penetration into highly vascularised tissues, among which are heart and brain.[14,16,17] The rapid distribution phase was followed by an elimination phase with a half-life quantified at approximately 70 minutes. It should be noted that these two compartments only provide an empirical description of the body and are therefore not necessarily related to actual physiological compartments.

The pharmacokinetic model also yields information about the variability attributable to the use of the Volcano® vaporizer to administer THC. The between-dose variability in bioavailability was quantified at 24% (Table 1). This variability is acceptable, and probably mostly related to inadequate inhalation due to coughing, evoked by the slightly irritant vapour. This indicates the need to carefully instruct the volunteer, practice the dosing procedure with placebo (ethanol vapour) before the actual drug administration, and closely monitor the inhalation of THC vapour. The very low inter-individual variability in the other pharmacokinetic parameters (≤6% for all parameters) illustrates the very reproducible THC profiles observed.

THC major metabolites (11-OH-THC and 11-nor-9-COOH-THC) were each described in a two-compartment model with Michaelis-Menten elimination of THC, indicating that THC follows saturable enzymatic metabolism. Although to date no similar report has been made, the clinical significance seems limited; only modest model misspecification was observed when using a first order elimination model.

The slight model misspecification observed for 11-OH-THC prior to the first and after the fourth dose (Figure 1) could be caused by the presence of time varying entero-hepatic circulation of this metabolite as demonstrated in bile cannulated rats[18] and in humans[6,19]. As this deviation did not seem to have a marked influence on the exposure of 11-OH-THC, and since this

metabolite is not relevant for the evoked effects, no efforts were made to further improve the model.

In the second stage of model development, the concentration-effect profile was modelled based only on the predicted concentration of THC. The metabolites were assumed to have an insignificant contribution to the overall effect after THC inhalation. 11-nor-9-COOH-THC has been reported to have no psychotropic or cardiovascular effects.[14,20] The concentrations of 11-OH-THC were negligible, even though it is equipotent[14] or twice as potent as THC [21,22]. The fourth dose showed a peak concentration ratio for 11-OH-THC and THC of roughly 1:7. Ignoring of metabolite effects is in agreement with the models developed by Chiang and Barnett[23] and Harder and Rietbrock[15].

The THC evoked effects lagged behind the THC plasma concentration, revealing hysteresis. Moreover, the effects lasted significant longer than the THC plasma concentrations. This was accurately empirically described with the incorporation of a hypothetical effect compartment. Physiologically, hysteresis can be caused not only by the physicochemical characteristics of actual effect compartments (e.g. accumulation of lipophilic compounds in fatty tissues), but also by processes that delay the development of the effect, like production of proteins or the initiation of a process with its own independent dynamics. The calculated equilibration half-life is composed of the summation of all these processes for a given outcome parameter. Various outcome parameters may have different equilibration half-lives, which is an indication that different physiological systems are involved. The reverse is not true: different physiological systems may have similar equilibration half-lives.

The equilibration half-life estimate was 7.68 minutes for heart rate and varied from 39.2 to 84.8 minutes for the CNS parameters (Table 2). Effects in CNS therefore developed somewhat slower and lasted longer than the effect on the heart rate. This clear difference in equilibration half-lives suggests that THC acts in two different physiological compartments, which is reasonable based on the cannabinoid receptor distribution[16,24] and partition coefficient of THC. As the partition coefficient between brain and plasma may be greater than between heart and plasma (as reported by Fuseau and Sheiner[25], the highly lipophilic THC would tend to accumulate in the brain, causing the prolongation of the duration of the effect observed for the CNS parameters.

For heart rate, limited effect prolongation occurs, compatible with the possibility that the increase in heart rate may be a direct consequence of an agonist action on CB1 receptors present on peripheral sympathetic nerve terminals of the heart[26,27]. However, the differences observed in equilibration half-life estimate could be also partially explained by the existence of different receptor domains[28], receptor (sub)types[26,29] or amplification factors for cannabinoid receptors[30]. Although it is difficult to identify the

causes for differences in equilibration half-lives, these values provide valuable measures of different physiological and/or pharmacological systems, which can be used to characterise the biology or pathology of cannabinoid systems in humans.

Similarly, different structures of the PK/PD models can be a reflection of different underlying physiological responses, and the parameters that describe these models can be considered as quantitative descriptors of these systems. This is apparent for VAS feeling high, which showed a clear approach of maximum effects (Figure 3). This could indicate that THC has a limited inherent potency to elicit psychotomimetic changes in healthy subjects. In contrast, the maximum effect for VAS alertness was not even approached with the maximum cumulative dose. Apparently, THC will continue to reduce alertness without reaching an $E_{max}$ until the subject falls asleep. In this way, characteristics of different aspects of the cannabinoid system can be quantified in terms of average or individual PK/PD parameters.

Modelling the concentration-effect of THC does not only provide insights into its mechanism of action, but it can also be useful in the prediction of THC concentration-effect profiles in subsequent studies with an entirely different trial design and dosing regime. For many studies, it will be useful to maintain stable THC effects throughout the experiment, for instance during studies of memory processing or functional imaging. This cannot be achieved with stabilisation of the plasma concentrations, since this would lead to a rapid accumulation of the central nervous system effects. Stable CNS effects can be achieved using PK/PD modelling. This is illustrated in Figure 5, which gives an example of a predicted concentration and effect profile for a hypothetical study with an entirely different dosing regime, aimed to arrive at a maximum effect quickly that is maintained for 1,5 hours. Based on the model predictions, doses of 6, 1 and 1 mg with an interval of 30 minutes would be a rational choice. These predictions are based on the VAS feeling high response and choice of other endpoints may influence the optimal dosing regime, but simulation of a number of responses may lead to an overall optimal choice of doses.

Availability of a PK/PD model may also allow dose optimisation on an individual level. After administering a test dose, PK/PD parameters may be estimated to allow simulation of an individually optimised dosing regime based on pharmacodynamic response. Knowledge of actual concentrations is not even essential for such a procedure.[31] The developed PK/PD model can be also useful during the development of novel compounds with related mechanisms of action. The developed model for THC allows a comparison of the concentration-effect relationships of agonist leads with that of THC as benchmark. The concentration-effect characteristics can be used to compare the effect profiles of the different compounds, and may elucidate different sensitivities for various effects. This can be helpful for the selection

of compounds with improved efficacy and tolerability, or optimisation of dosing schedules for subsequent studies or treatment regimens.

Finally, PK/PD models may enable prediction of the time course of the analysed effects, after co-administration of THC and antagonist leads in pharmacological proof-of-mechanism counteraction studies. Such studies have been used to confirm the pharmacological activity of rimonabant in humans.[32] The problem with this approach however, is that the antagonistic effect depends on the levels of both the agonist and the antagonist: higher doses of THC require more of the antagonist to achieve the same effect reduction, and vice versa. PK/PD analyses can help to generalize the conclusions concerning receptor affinity and potency.[33] Moreover, the use of this approach might allow more efficient dose range selection for efficacy studies at earlier phases of clinical development.[33,34]

With all of these features, this integrated modelling approach offers insights into the physiology and pharmacology of the human cannabinoid systems, and allows the optimisation of study designs using THC. In this way, PK/PD modelling can contribute to the development of novel cannabinoid agonist and antagonist.

**Figure 1** Average graph of predicted (dark line) and observed (open circles) concentration-time profiles of log THC, 11-OH-THC and 11-nor-9-COOH-THC (ng/mL). SD error bars.

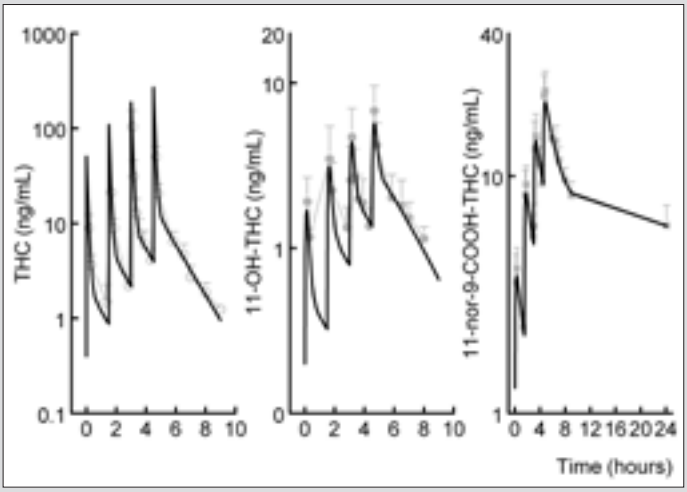


**Figure 2** Left: Average graph of predicted (dark line) and observed (open circles) effect-time curve of hear rate (bpm). Placebo curve is added as reference (open squares). SD error bars. Right: Predicted population average response relationship between the hypothetical effect compartment concentration and heart rate.

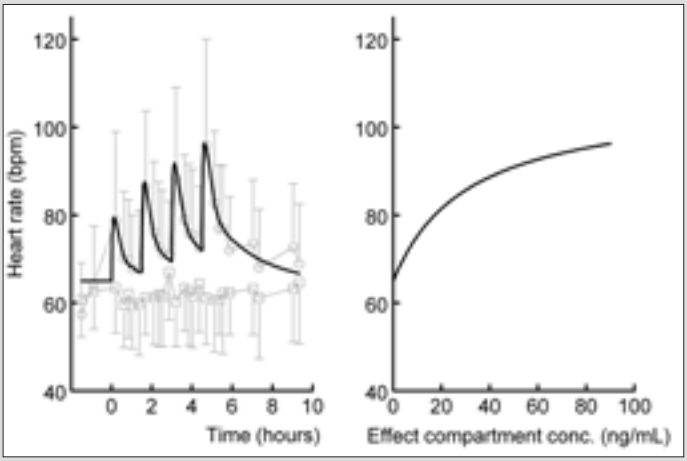

**Figure 3** Left: Average graph of predicted (dark line) and observed (open circles) effect-time curve of VAS feeling high (U). Placebo curve is added as reference (open squares). SD error bars. Right: Predicted population average response relationship between the hypothetical effect compartment concentration and VAS feeling high.

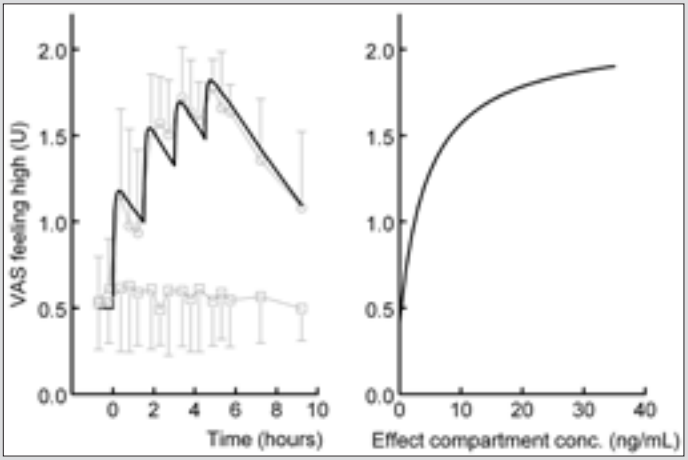


**Figure 4** Left: Average graph of predicted (dark line) and observed (open circles) effect-time curve of VAS alertness (mm). Placebo curve is added as reference (open squares). SD error bars. Right: Predicted population average response relationship between the hypothetical effect compartment concentration and VAS alertness. A decrease corresponds to a decrease in alertness.

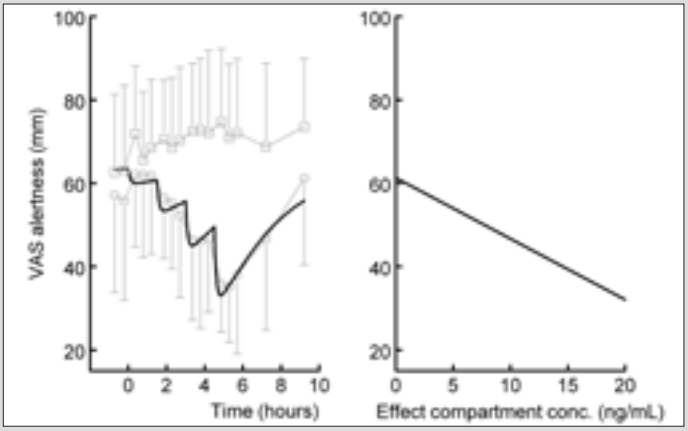

**Figure 5** Mean THC concentration (left) and predicted VAS feeling high (right) for a dosing regime aimed at maintaining a stable effect compartment level for 90 minutes (6, 1 and 1 mg THC with 30 minutes intervals). SD error bars.

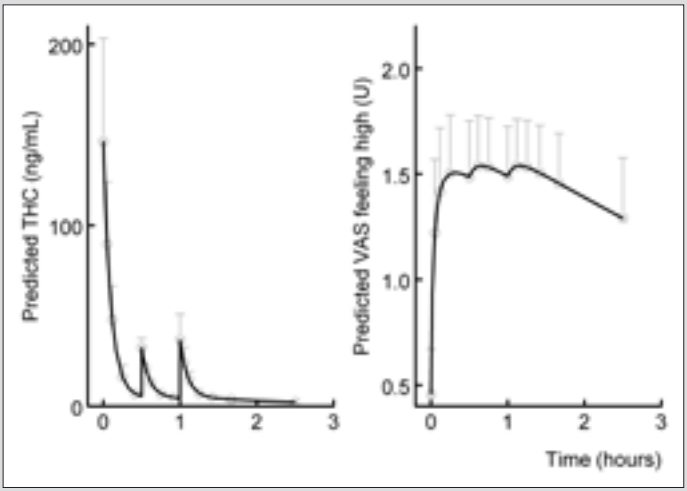


**Table 1** Mean (CV) of NONMEM pharmacokinetic population parameter estimates.

| **THC alone** | | |
|---|---|---|
| Cl/F (L/min) | 3.45 (5%) | |
| Initial half-life (min) | 3.81 (0%*) | |
| Terminal half-life (min) | 68.4 (0%*) | |
| Central volume/F(L) | 32.2 (6%) | |
| Relative bioavailability | (24%) | |
| Residual variability (%) | 20.9% | |
| **THC with MM#** | **with 11-OH-THC** | **with 11-nor-9-COOH-THC** |
| Vmax (µg/min) | 583 (10%) | 828 (9%) |
| Km (µg/L) | 134 (0%*) | 196 (0%*) |
| K12 (/min) | 0.0853 (0%*) | 0.0741 (0%*) |
| K21 (/min) | 0.0184 (3%) | 0.0178 (2%) |
| Central volume/F(L) | 32.9 (17%) | 36.3 (19%) |
| Relative bioavailability | (15%) | (18%) |
| Residual variability (%) | 21.9% | 20.6% |
| **Metabolite PK** | | |
| Initial half-life (min) | 5.22 (0%*) | 32.6 (4%) |
| Terminal half-life (min) | 92.1 (0%*) | 54.8 (32%) |
| Central volume/F (L) | 352 (29%) | 279 (16%) |
| K43 (/min) | 0.0176 (15%) | 0.00252 (6%) |
| Residual variability (%) | 22.6% | 15.1% |

*Inter-individual variability fixed at 0%, #MM: Michaelis-Menten elimination

**Table 2** Mean (CV) of NONMEM PK/PD population parameter estimates

| | Heart rate | Body sway | VAS external perception | VAS feeling high | VAS alertness |
|---|---|---|---|---|---|
| Equilibration half-life (min) | 7.68 (35%) | 84.8 (0%) | 39.2 (44%) | 46.8 (50%) | 84.2 (47%) |
| Intercept | 65.3 (17%) | 2.39 (7%) | 0.472 (41%) | 0.414 (53%) | 61.3 (27%) |
| Emax | 41.5 (32%) | 0.759 (34%) | 1.38 (56%) | 1.68 (34%) | NA |
| EC50 (ng/mL) | 30.7 (79%) | 30.7 (0%) | 23.7 (138%) | 4.54 (126%) | NA |
| Slope | NA | NA | NA | NA | -1.46 (45%) |
| Residual variability (SD) | 6.53 | 0.0803 | 0.158 | 0.206 | 9.42 |

NA not applicable

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# 5 Evaluation of THC-induced tachycardia in humans using heart rate variability

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## Abstract

Cannabis use induces tachycardia, but its mechanism is unexplained. Heart rate variability (HRV) can provide information concerning effects of drugs on parasympathetic and sympathetic tone. HRV data of healthy male volunteers were used from two separate double-blind and placebo-controlled studies. Rising doses of pure THC were administered by inhalation with or without co-administration of the selective CB1 antagonist AVE1625. After THC administration, significant dose-related changes compared to placebo were seen in the 'time domain' on heart rate and SDSD. In the 'frequency domain' dose-related changes were seen on total power, low frequency power and high frequency power. Overall, normalized LF and HF and the LF/HF ratio did not change significantly. However, with the two highest THC doses, average values increased for LF and decreased for HF, leading to an average increase in LF/HF ratio. Co-administration of the selective CB1 antagonist AVE1625 had no effect on HRV under placebo conditions, but completely antagonized THC-induced effects on HRV. This indicates that HRV is mediated by CB1 receptors. These findings confirm the involvement of CB1 receptors in THC-induced tachycardia and suggest that the increase in heart rate caused by acute THC administration may be caused by a peripheral mediated reduction in the vagal tone.


## Introduction

Delta-9-tetrahydrocannabinol (THC), a CB1/CB2 agonist, is the most abundant and major psychoactive cannabinoid identified in the plant *Cannabis sativa* L. Cannabis causes a pronounced increase in heart rate,[1,2] but its mechanism has not been fully elucidated.

An increase in heart rate can be established by direct or indirect effects. In the case of THC-induced tachycardia it may be caused by direct stimulation of CB1 receptors in human atrial muscle.[3] Indirect adaptation of heart rate is mainly mediated by the central nervous system and involves a change in the interaction between sympathetic and parasympathetic stimulation on heart rate. Although CB1 receptors are located in human atrial muscle,[3] CB1 receptors are predominantly situated in the brain with the highest densities in the hippocampus, cerebellum and striatum, which account for the well-known effects of cannabis on motor coordination and short term memory processing.[4-6] However, CB1 receptors are also expressed at low levels in the brainstem[6] where the cardiovascular control centers are located, making it possible that THC exerts its effects on heart rate via this route.

Propranolol, a non-selective beta-adrenergic blocking agent without sympaticomimetic activity, is able to antagonize tachycardia induced by THC.[7,8] This suggests that THC may increase heart rate by activation of the

sympathetic nervous system either centrally or peripherally. Pretreatment with atropine, a parasympatholytic drug, attenuates THC-induced tachycardia as well.[8] Pretreatment with both propranolol and atropine abolishes THC-induced tachycardia completely.[8] These findings suggest that both anticholinergic and beta-adrenergic effects contribute to the increase in heart rate after THC administration.[8] However, these data do not elucidate the balance between the parasympatic and sympatic nervous system involved in the increased heart rate. In addition, it cannot be determined from these experiments if the tachycardia is controlled by centers in the brain and spinal cord or that a direct effect on CB1 receptors in the heart is involved in THC-induced tachycardia.

Heart Rate Variability (HRV) analyses can provide information concerning the effects of drugs on parasympathetic and sympathetic tone.[9] In this study, the sympathovagal balance in THC-induced tachycardia was evaluated. In addition, co-administration of the selective CB1 antagonist AVE1625 will elucidate the role of CB1 and CB2 receptors in THC-induced tachycardia.

## Methods

### DESIGN

The heart rate variability (HRV) data originate from two double-blind and placebo-controlled studies. For both studies pure HRV was purified from *Cannabis sativa* according to GMP-compliant procedures and administered by inhalation using a Volcano® vaporizer (Storz-Bickel GmbH, Tüttlingen, Germany).[10]

**Study 1**[10]: Twelve healthy males (average 23 ± 2 years, range 21-27) with a history of mild cannabis use for at least one year were included in the study. On one study day, rising doses of THC (2, 4, 6 and 8 mg) were administered at 90 minute intervals. On a separate occasion, vehicle was administered in the same way, as double-blinded placebo. This study is described in full detail by Zuurman *et al.*[10] Heart rate was measured at baseline and 10, 35, 45, 55 and 85 minutes after each THC administration. HRV measurement was performed at baseline and 25 minutes and 85 minutes after each THC administration.

**Study 2**[11]: Thirty-six healthy males (average 21 ± 3 years, range 18-31) with a history of mild cannabis use for at least one year were included in the study. During each study period a single oral dose of the CB1 receptor antagonist AVE1625 (20, 60 or 120mg) or matching placebo was administered three hours prior four consecutive rising doses of THC (2, 4, 6 and 6 mg) or placebo were administered by inhalation at 60 minute intervals using a Volcano® vaporizer. Each subject received four out of the six available treatment

combinations. The treatment combination 'placebo AVE1625 + placebo THC' was used as a negative control (24 subjects received this treatment). All three single AVE1625 doses (20, 60 and 120 mg) were administered in combination with the rising doses of THC, but only the highest dose of 120 mg AVE1625 was administered in combination with 'placebo THC' to study the effects of the antagonist itself. This study is described in full detail by Zuurman *et al*.[11] Heart rate was measured at baseline and 11, 23, 32 and 43 minutes after each THC administration. HRV measurement was performed at baseline, after AVE1625 administration and 25 minutes after each THC administration. After the last THC administration 3 additional measurements were performed.

### HEART RATE

Heart rate was measured in sitting position after a rest of approximately 5 minutes, twice pre-dose and repeatedly post-dose at fixed time-points. All measurements were carried out with an automated sphygmomanometer (Nihon Kohden, Life Scope EC, Tokyo, Japan).

### HEART RATE VARIABILITY

Five-minutes ECG recordings using lead II were made using a CardioPerfect ECG machine (Welch Allyn, Delft, The Netherlands). Recordings were made at baseline, 32, 92 and 152 minutes after oral administration of AVE1625 and 37 minutes after each consecutive dose of THC. In addition, after the last THC administration two additional ECG recordings were made to study the decline of the effects. The recordings were analyzed using the software provided with the device which employs the methodology as described by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology.[9]

The parameters in the time-domain were the average RR-interval (corresponding to heart rate) and SDSD (standard deviation of consecutive RR-intervals, a measure of vagal activity).[9] For the frequency-domain we analyzed the total power (TP, total variability), low frequency power (LF, 0.01-0.08 Hz, measure of sympathetic activity), high frequency power (HF, 0.15-0.5 Hz, associated with respiratory sinus arrhythmia, and almost exclusively due to parasympathetic activity), and LF/HF (measure of sympathovagal balance).[9]

### STATISTICS

All parameters were summarized by treatment and time, and were presented graphically as mean over time, with standard deviation as error bars. The parameters were analyzed separately by mixed model analyses of

variance (using SAS PROC MIXED, SAS for Windows V9.1.2, SAS Institute Inc., Cary, NC, USA) with treatment, period, time and treatment by time as fixed effects, with subject, subject by time and subject by treatment as random effect, and with the (average) baseline value as covariate.

Treatment effect was estimated using the average response of the values obtained in the 90 minutes after the final administration of THC. Contrasts were reported along with 95% confidence intervals. All HRV parameters were analyzed after log-transformation. Log-transformed contrasts were back-transformed resulting in geometric mean ratios with associated confidence intervals. These were expressed as percentage change from placebo.

## Results

After THC administration, dose-related changes compared to placebo were seen in heart rate (Figure 1), SDSD (Figure 2), Total Power (TP), Low Frequency power (LF) and High Frequency power (HF) (Table 1). In the first study (n = 12), normalized LF power and normalized HF power and the LF/HF ratio did not change (Table 1). However, with the two highest THC doses, average values increased for LF and decreased for HF, leading to an average increase in LF/HF ratio (Table 1). In the second study normalized LF power increased with THC (Table 1). Normalized HF and the LF/HF ratio (Figure 3) did not change in study two (Table 1). Comparable to study one, with the two highest THC doses in study two, average values increased for LF and decreased for HF, leading to an average increase in LF/HF ratio (Table 1).

Co-administration of the selective CB1 antagonist AVE1625 in study two did completely antagonize THC effects on heart rate variability (Table 2). AVE1625 did not have an effect on heart rate variability itself (Table 2).

## Discussion

This study evaluated the sympathovagal balance in THC-induced tachycardia using heart rate variability (HRV) analysis. THC (a CB1/CB2 agonist) increased heart rate in a dose-related manner in comparison to placebo (Figure 1). After the initial increase, heart rate decreased rapidly after each dose.

Time domain analysis showed that this increase in heart rate was associated with a decreased standard deviation of consecutive RR-intervals. This indicates a reduction in vagal tone.[12] The analyses of the frequency domain were more ambiguous, and both studies only showed average changes with the highest two THC doses. However, both studies demonstrated an increase in LF/HF ratio, a measure of sympathovagal balance. Although these

changes did not reach statistical significance, this finding also suggests that a lowering of vagal tone occurred. This is in agreement with findings from Newlin *et al.*,[13] who used vagal tone index, which is another non-invasive measure of tonic vagal inhibition of the heart. These investigators showed that heart rate was significantly increased and vagal tone significantly decreased at 5 and 30 minutes after smoking a 2.7% marijuana cigarette. This dose is comparable to the cumulative THC dose in our studies. The 5 minutes recording yielded a much greater decrease in vagal tone compared to the 30 minutes recording. The lack of a significant effect in the present studies may be due to the timing of the measurement of the vagal activity. Scheduling the HRV measurement closely after THC administration, e.g. after 5 minutes instead of after 25 minutes, may provide stronger evidence that THC-induced tachycardia is established by a reduction in the vagal tone.

Although our data indicate a reduction of the vagal tone, a direct effect of THC on the heart cannot be excluded. Also other indirect mechanism by THC cannot be excluded although these are unlikely. For instance, THC may induce orthostatic hypotension and accompanying sign of vasodilatation like facial flushing and conjunctival reddening.[1,14,15] In young healthy males a decrease in blood pressure may be directly compensated by an increase in heart rate. In these two studies blood pressure did not change and other observable signs of vasodilatation were not observed.[10,11] Another indirect effect by which THC may induce tachycardia could be via activation of PPARγ receptors for which THC is a ligand. Activation of these receptors leads to vasorelaxation through increased bioavailability of nitric oxide and hydrogen peroxide production.[16,17] This effect is mediated by a nuclear receptor resulting in altered gene expression. However, tachycardia in our study appeared to be a direct receptor-mediated pharmacological effect, because heart rate increased very rapidly in response to changing THC concentration. In the rat isolated aorta, these vasorelaxant effects were not inhibited by the selective CB1 antagonist rimonabant, but were inhibited by the selective CB2 receptor antagonist SR144528.[16] In the present study the selective CB1 antagonist AVE1625 completely antagonized THC-induced effects on HRV parameters. These observations demonstrate the involvement of the CB1 receptor in THC-induced tachycardia. However, it does not elucidate if they are involved in a direct or an indirect regulatory mechanism on cardiac functioning. Together with the observation that CB1 receptors are expressed at only low levels in the brainstem[6] where the cardiovascular control centre is located, the above mentioned observations favors an indirect and peripheral mediated regulatory mechanism involved in THC-induced tachycardia.

In summary, our findings confirm the involvement of CB1 receptors in THC-induced tachycardia and suggest that the increase in heart rate caused by acute THC administration may be caused by a peripheral mediated reduction in the vagal tone.

**Figure 1** Least square means graph (SD) of heart rate time profile. AVE1625 administration at T = 0. THC administration: 2 mg at T = 3 hours; 4 mg at T = 4 hours; 6 mg at T = 5 hours; 6 mg at T = 6 hours. Arrows indicate drug administration.

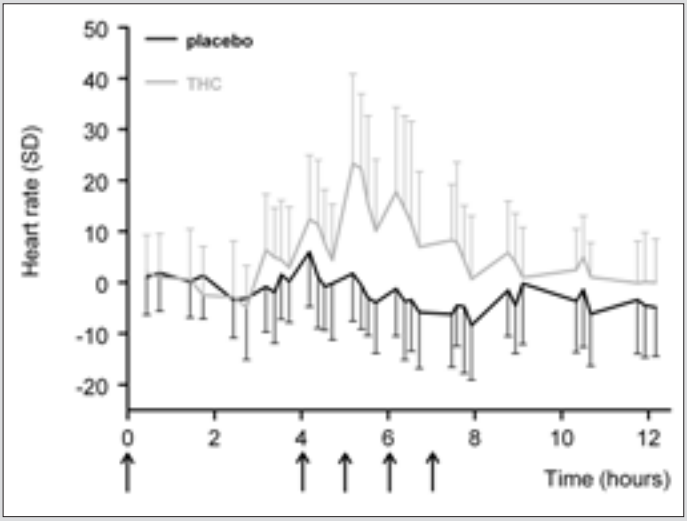


**Figure 2** Least square means graph (SD) of SDSD time profile. AVE1625 administration at T = 0. THC administration: 2 mg at T = 3 hours; 4 mg at T = 4 hours; 6 mg at T = 5 hours; 6 mg at T = 6 hours. Arrows indicate drug administration.

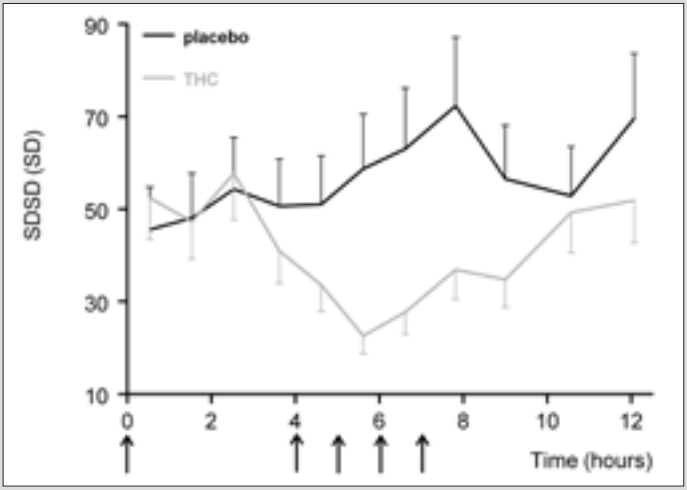

**Figure 3** Least square means graph (SD) of LF/HF time profile. AVE1625 administration at T = 0. THC administration: 2 mg at T = 3 hours; 4 mg at T = 4 hours; 6 mg at T = 5 hours; 6 mg at T = 6 hours. Arrows indicate drug administration.

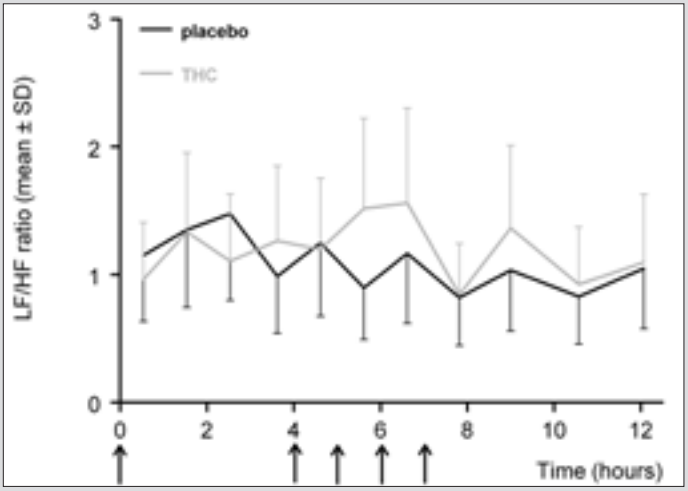


**Table 1** Heart rate variability measurements after THC administration with 95% CI. Bold indicates a significant result.

| Description | Variable | Study 1 (n=12) | Study 2 (n = 36) |
|---|---|---|---|
| | Heart rate (bpm) | **+19 (+13, +26)** | **+15 (+12, +19)** |
| Total variability | Total Power ($ms^2$) | **-71 (-81, -57)** | **-54 (-65, -40)** |
| Vagal activity | SDSD (ms) | **-49 (-58, -38)** | **-33 (-41, -24)** |
| Sympathetic activity | Low Frequency power (LF) ($ms^2$) | **-70 (-81, -52)** | **-55 (-66, -39)** |
| | Normalized Low Frequency power | +7 (-14, +34) | **+16 (+0.2, +35)** |
| Parasympathetic activity | High Frequency power (HF) ($ms^2$) | **-80 (-88, -67)** | **-67 (-77, -53)** |
| | Normalized High Frequency power | -20 (-42, +9) | -10 (-24, +6) |
| Sympathovagal balance | LF/HF ratio | +33 (-20, +121) | +29 (-5, +75) |

**Table 2** Heart rate variability measurements after co-administration of THC and AVE1625 with 95% CI (data from study 2). Bold indicates a significant result.

| Description | Variable | Placebo + 120 mg AVE1625 | THC + 120 mg AVE1625 |
|---|---|---|---|
| | RR-interval (bpm) | +1% (-2, +5) | **+14% (+11, +19)** |
| Total variability | Total Power ($ms^2$) | +10% (-14, +41) | **+138% (+85, +206)** |
| Vagal activity | SDSD (ms) | +1% (-10, +14) | **+51% (+34, +70)** |
| Sympathetic activity | Low Frequency power (LF) ($ms^2$) | +1% (-23, +34) | **+124% (+70, +196)** |
| | Normalized Low Frequency power | -4% (-16, +11) | **-17% (-28, -4)** |
| Parasympathetic activity | High Frequency power (HF) ($ms^2$) | +7% (-25, +52) | **+224% (+128, +359)** |
| | Normalized High Frequency power | +6% (-10, +25) | **+18% (+0, +39)** |
| Sympathovagal balance | LF/HF ratio | -9% (-32, +22) | **-29% (-48, -6)** |

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# 6 Inhibition of THC-induced effects on the central nervous system and heart rate by a novel CB1 receptor antagonist AVE1625

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## Abstract

CB1 antagonists like AVE1625 are potentially useful in the treatment of obesity, smoking cessation and cognitive impairment. Proof of pharmacological action of AVE1625 in the brain can be given by antagonizing the effects of THC, a CB1/CB2 agonist. Inhibition of THC-induced effects by AVE1625 was observed on VAS alertness, feeling high, external perception, body sway and heart rate. The lowest dose of AVE1625 20 mg inhibited already most of THC-induced effects. AVE1625 did not have any effect on psychological and behavioural parameters or heart rate by itself. This study demonstrates a useful method for studying the effects of CB1 antagonists. AVE1625 penetrates the brain and antagonizes THC-induced effects with doses at or above 20 mg.

## Introduction

AVE1625 is a new selective CB1 antagonist with very high affinity for the CB1 receptor (Figure 1). Activation of CB1 receptors by endogenous cannabinoids, such as anandamide, stimulates eating behavior.[1] Rimonabant (SR141716) is the first CB1 antagonist developed for the treatment of obesity.[2,3] In addition, CB1 receptor antagonists may also be useful in the treatment of smoking cessation,[4] cognitive impairments in Alzheimer's disease and schizophrenia;[5-7] and for the treatment of advanced Parkinson's disease.[8-10]

Despite the emergence of potential new indications for CB1 agonists or antagonists, the physiological role of the cannabinoid system is still not clear. No reproducible subjective or objective pharmacodynamic effects on the central nervous system have been reported or could be observed after administration of CB1 antagonists.[11] As a result, no direct pharmacodynamic parameters have yet been identified that show brain penetration or cannabinoid antagonism in the central nervous system (CNS). Without any pharmacodynamic tests that are able to directly demonstrate an effect of AVE1625 in humans, the prediction of therapeutically active and safe doses for early patient studies would completely rely on preclinical pharmacological and disease models, and on maximum tolerability in healthy subjects.

In principle, two research strategies can be used to show whether a CB1 antagonist actually displays its expected pharmacological activity in the brain, and to establish the time-effect-profile of endocannabinoid inhibition at a given dose. Positron Emission Tomography (PET) scan can be a useful method to study receptor occupancy. Recently, a validated PET ligand for the CB1 receptor showed inhibition of CB1 receptors in the human brain.[12] However, the level of receptor occupancy by the CB1 antagonist required to obtain therapeutic efficacy is unknown, and receptor binding does not equal antagonism. Functional CB1 inhibition can be demonstrated by showing antagonism of the effects of an externally administered CB1/CB2 agonist like delta-9-tetrahydrocannabinol (THC), the psychoactive ingredi-

ent of cannabis. Inhibition of THC-induced effects confirms that the drug penetrates the brain, and that it behaves like a CB1 antagonist in humans. This is useful information for the development of a novel compound, but inhibition of THC-induced effects on the central nervous system does not by itself preclude the possibility of excessive inhibition of CB1 systems that are not involved in the disease but may be needed for normal functioning. High doses of an agonist require higher doses of an antagonist; external CB1 stimulation by THC may exceed the activity of the endocannabinoid system even under pathophysiological conditions; and the pharmacodynamic effects induced by THC may not be representative of cannabinoid systems that are related to the disease or to therapeutic efficacy. Not all these considerations can be solved with the present state of knowledge.

Recently we examined the effects of intrapulmonary rising doses of pure THC (2, 4, 6 and 8 mg) using a Volcano® vaporizer.[13,14] Dose- and concentration-related effects could be measured with low intersubject variability on a number of CNS measurements and heart rate. This new method of THC administration was used in the current study, to determine the ability of three doses of AVE1625 to antagonize the effects of THC. The aims were to demonstrate that AVE1625 penetrates the central nervous system and acts as a functional CB1 antagonist in humans, and to identfy the doses at which these effects occur.

## Methods

### DESIGN

This was a single-centre, double-blind, randomized, six-way balanced, placebo-controlled, partial cross-over study in healthy male volunteers. Each subject received four out of the six available treatment combinations, with a washout period of at least two weeks between treatments. The incomplete cross-over study design, rather than a complete cross-over design, was chosen in order to reduce the risk of having subjects drop out due to an inability to refrain from smoking cannabis for the duration of the study (cannabis users are, in contrast to other volunteers, not as reliable). The study protocol was approved by the Medical Ethics Review Board of Leiden University Medical Center and performed according to principles of ICH-GCP and Dutch clinical trial law.

### SUBJECTS

Thirty-six males were included in the study. Their ages ranged from 18-31 years with a mean of 22 ± 3 years. The mean height and weight were respectively 183 ± 6 cm (range 167 – 192 cm) and 76 ± 11 kg (range 60 – 95 kg). Mean BMI was 23 ± 3 (range 19 – 27). All subjects were familiar with the effects of cannabis with an average use of less than once a week. Fif-

teen subjects used cannabis once a week, two subjects used it three times a month, eleven subjects twice a month, five subjects once a month and three subjects used cannabis less than once a month. All urinary drug screens (One Step®, Instruchemie, The Netherlands), including THC, were negative at screening and at baseline on all study days. Subjects refrained from smoking and use of coffee and tea on study days. Subjects who admitted to expecting withdrawal symptoms (e.g. headache) if they did not use these product for a day were excluded from the study. Twenty-five subjects completed the study. Eleven (30.6%) discontinued from the study. Six subjects discontinued because they did not wish to continue, 4 subjects did not show up for the next study period and were lost to follow-up. One subject discontinued because of an adverse event (diarrhoea). The primary reason for discontinuation was that subjects considered the study procedures to be too burdensome.

### TREATMENTS

THC was purified according to GMP-compliant procedures (Farmalyse BV, Zaandam, The Netherlands) from the flowers of *Cannabis sativa* grown under Good Agricultural Practice (Bedrocan BV Medicinal Cannabis, Veendam, The Netherlands).[15-17] Each dose (2, 4, 6 or 6 mg) of THC (>98% purity by HPLC/GC) was dissolved in 200 µL 100 vol% alcohol. THC was stored in the dark at -20°C in 1 ml amber glass vials containing a teflon screw-cap secured with Para film to minimize evaporation. Stability data of the THC solution demonstrate stability of at least 29 months. The solvent was used as placebo.

On each study day a single oral dose of AVE1625 (20, 60 or 120 mg) or matching placebo was administered in fed condition (standardized 530 Kcal breakfast) as 6 soft capsules. The administration of THC was started 3 hours after administration of the CB1 antagonist (Table 1). This delay was based on maximum AVE1625 plasma concentrations in healthy subjects in the window between 3 and 6 hours after AVE1625 administration and on brain pharmacokinetics in animals. In the same time frame repeated THC administration was performed.

Four fixed consecutive rising doses of THC (2, 4, 6 and 6 mg) or placebo (THC vehicle) were inhaled at one-hour intervals, using a Volcano® vaporizer (Storz-Bickel GmbH, Tüttlingen, Germany) (Table 1). Five minutes before administration THC was vaporized at a temperature of about 225°C and the vapor was stored in an opaque polythene bag equipped with a valved mouthpiece, preventing the loss of THC in between inhalations. Subjects were not allowed to speak, and were instructed to inhale deeply and hold their breath for 10 seconds. Within 2-3 minutes the bag was to be fully emptied. The inhalation procedure was practiced at screening using the solvent as a placebo. This method of intrapulmonary THC administration was validated by Zuurman *et al.*,[13] Strougo *et al.*[14] and Hazekamp *et al.*[18]

The study had a partial cross-over design, where each subject received four out of the six available treatment combinations. This partial cross-over design was chosen to avoid a high drop out rate due to a long study duration. The treatment combination 'placebo AVE1625 + THC vehicle' was used as a negative control and 'placebo AVE1625 + THC' as positive control. All three single AVE1625 doses (20, 60 and 120 mg) were administered in combination with the rising doses of THC. Only the highest dose of AVE1625 120 mg was administered in combination with 'THC vehicle' to study the effects of the antagonist itself.

## PHARMACOKINETIC MEASUREMENTS

For determination of the concentration of plasma AVE1625, venous blood was collected in heparinized polypropylene tubes (lithium heparin) of 4 ml. Blood samples were taken at baseline and 1, 2, 3, 4, 5, 6, 12 and 24 hours after oral administration of AVE1625 or matching placebo. After blood collection the tubes were centrifuged within 30 minutes for 15 minutes at 2000G at 4°C. Plasma samples were stored at a temperature of -20°C.

### HAEMATOLOGY, BIOCHEMISTRY, URINALYSIS AND BLOOD PRESSURE

Blood samples for routine haematology and biochemistry were taken at screening, in the morning before each drug administration and at follow up. In addition, routine urinalysis was performed by dipstick (Multistix 10 SG®, Bayer, Mijdrecht, The Netherlands) using 10 ml urine. Blood pressure was measured for safety reasons in sitting position after a rest of approximately 5 minutes with an automated sphygmomanometer (Nihon Kohden, Life Scope EC, Tokyo, Japan).

## PHARMACODYNAMIC MEASUREMENTS

Pharmacodynamic parameters were chosen based on the observed THC effects as reported by Zuurman *et al.*[13] Subjects were acquainted with the experimental methods and conditions in a training session within one week before the first study period. Pharmacodynamic assessment was performed in a quiet and temperature controlled room with standardised illumination with only one subject per session in the same room. All tests were measured twice pre-dose and obtained frequently at fixed time points after AVE1625 administration (T = 0) and after each consecutive THC dose. Relative to AVE1625 administration heart rate was measured at 25 and 40 minutes post time points 0, 1 and 2 hours; 10 and 30 minutes post time points 3, 4, 5, 6 and 7 hours; and 20 and 45 minutes post time points 3, 4, 5, 6, 7 and 10

hours. Relative to AVE1625 administration body sway, VAS Bond and Lader, VAS Bowdle and EEG were measured in succession at the following time points: 20 and 40 minutes post time points 0, 1 and 2 hours; 5, 20 and 30 minutes post time points 3, 4, 5 and 6 hours; 30 and 40 minutes post time point 7 hours; 40 and 50 minutes post time point 8 hours; 15 and 25 minutes post time point 10 hours; and 40 and 50 minutes post time point 11 hours.

### HEART RATE

Heart rate was measured in sitting position after a rest of approximately 5 minutes. The measurement was carried out with an automated sphygmomanometer (Nihon Kohden, Life Scope EC, Tokyo, Japan).

### ELECTRO-ENCEPHALOGRAPHY (EEG)

EEG is often quite sensitive to the effects of a wide range of CNS-active drug classes. Since the direct pharmacodynamic effects of AVE1625 were not been examined exhaustively, pharmaco-EEG was added to the THC responsive tests, to form a broad CNS test battery. EEG recordings were made using silver chloride electrodes, fixed with collodion at Fz (frontal), Cz (central), Pz (parietal) and Oz (occipital) positions, with the same common ground electrode as for the eye movement registration (international 10/20 system). The electrode resistances was kept below 5 kOhm. EEG signals were obtained from leads Fz-Cz and Pz-Oz and a separate channel to record eye movements (for artefacts). The signals were amplified by use of a Grass Telefactor (F-15EB/B1) and a 15LT series Amplifier Systems (Grass-Telefactor, Braintree, USA) with a time constant of 0.3 seconds and a low pass filter at 100 Hz. Data collection and analysis were performed using a validated Spike2 script (Cambridge Electronics Design, Cambridge, UK). Per session eight consecutive blocks of eight seconds were recorded. The signal was AD-converted using a CED 1401 Power (Cambridge Electronics Design, Cambridge, UK). Data blocks containing artefacts were identified by visual inspection and these were excluded from analysis. For each lead, fast Fourier transform analysis was performed to obtain the sum of amplitudes in the delta (0.5-3.5 Hz), theta (3.5-7.5 Hz), alpha (7.5-11.5 Hz) and beta (11.5-30 Hz) frequency ranges. Outcome parameters were the square root of the total power in each band for each lead.

### BODY SWAY

Postural stability was measured with a string attached to the waist connected to a measurement device. All body movements in the antero-posterior direction over a period of 2 minutes were integrated and expressed as mm sway on a digital display. Subjects were required to keep their eyes

closed and not allowed to talk during the measurement, and were asked to wear the same comfortable low-heeled shoes during all measurements.

## VISUAL ANALOGUE SCALES (VAS)

From the visual analogue scales as originally described by Norris[19] (16 items), three factors can be derived, as described by Bond and Lader[20], corresponding to alertness, contentedness and calmness. Increased scores of these scales indicate enhanced subjective feelings of alertness, contentedness (in general) and calmness. Psychedelic effects were monitored by an adapted version of the visual analogue scales (13 items), originally described by Bowdle *et al*.[21] From the Bowdle scale two composite scales could be identified, corresponding to 'internal' and 'external perception', two separate modalities of psychedelic effects.[13] 'External perception' reflects a misperception of an external stimulus or a change in the awareness of the subject's surroundings. It is calculated as the average (after log-transformation +2) of the following VAS scores: changing of body parts, changes of surroundings, altered passing of time, difficulty controlling thoughts, changes in colour intensity and changes in sound intensity. The 'internal perception' reflects inner feelings that do not correspond with reality, and is composed of feelings of unreality, hearing voices/sounds, things have a specific particular meaning, paranoia and feeling anxious. In addition to these scales VAS high was assessed.

## ANALYSIS

### PHARMACOKINETICS

The Tubulent Flow Chromatography - Mass Spectrometry/Mass Spectrometry (TFC-MS/MS) was a validated method to analyse plasma AVE1625 concentrations. The validation of this method included evaluation of selectivity for AVE1625. In each run standards (known amount of AVE1625) were included after every ten samples. The limit of quantification was 0.2 ng/mL. The intra-assay coefficient of variation was between 1.0 and 5.4%. The inter-assay coefficient of variation was between 2.0 and 6.5%. AVE1625 plasma pharmacokinetic parameters ($t_{max}$, $C_{max}$, $AUC_{0-24}$) were determined using non-compartmental analysis from the individual plasma concentration-time profiles.

### PHARMACODYNAMICS

All pharmacodynamic endpoints were summarized by treatment and time, and were presented graphically as mean over time, with standard deviation as error bars. All pharmacodynamic endpoints were analyzed by mixed

model repeated measurement analyses of variance (using SAS PROC MIXED, SAS for Windows V8.2, SAS Institute Inc., Cary, NC, USA) with treatment, period, time and treatment by time as fixed effects, with subject and subject by treatment as random effect, and with the average baseline value as covariate. EEG were analyzed as the natural logarithm of the square root of each frequency range, body sway as natural logarithm of body sway and VAS Bowdle as natural logarithm of (score +2) for each individual item.

The effect of THC alone was estimated by comparing the effect of 'placebo AVE1625 + THC vehicle' with the effect of 'placebo AVE1625 + THC'. The peak THC effect was defined as the effect after the third inhalation (THC 6 mg) until one hour after the fourth inhalation (THC 6 mg). The inhibitory effect of AVE1625 on THC-induced effects on the central nervous system parameters and heart rate were estimated at THC peak effect by [(AVE1625 + THC) - (placebo AVE1625 + THC)] / [(placebo AVE1625 + THC vehicle) - (placebo AVE1625 + THC)]. The effect of AVE1625 120 mg alone was estimated by comparing the effect of 'placebo AVE1625 + THC vehicle' with the effect of 'AVE1625 120 mg + THC vehicle' from AVE1625 administration until 12 hours post dose. All contrasts were reported along with 95% confidence intervals.

# Results

### CLINICAL EFFECTS

After administration of AVE1625 alone, the reported adverse events (AE) were similar to placebo, namely fatigue, headache and somnolence. Consistently with the pharmacodynamic results (VAS Bowdle feeling high), AVE1625 decreased THC-induced effects (euphoric mood and dizziness). No changes in clinical chemistry, haematology or urinanalysis were observed. Blood pressure was not affected by THC or AVE1625.

One subject experienced THC effects that were strong enough to decide not to administer the fourth dose of THC 6 mg. For this treatment period, this subject was co-administered with AVE1625 placebo. The subject was feeling high, dizzy and sleepy and had complaints of mild paresthaesia. He recovered without sequelae soon after the third THC dose.

### PHARMACOKINETIC AND PHARMACODYNAMIC RESULTS

Twenty-five of the 36 enrolled subjects in the study completed the study. All subjects were evaluable for safety analysis and 34 were included in PK and PD analysis (i.e. completing at least one treatment period). The subject who did not receive the fourth THC dose of 6 mg had deviating concentration and effect time-profiles that would have distorted the analyses of the overall results. For this subject, only the data up to one hour after the third dose were included.

### PHARMACOKINETICS

A non-linear dose dependent increase in plasma AVE1625 concentrations was observed with moderate to high intersubject variability, with higher variability at the 120 mg dose of AVE1625 (Figure 2). No significant differences were observed between treatment periods and therefore pharmacokinetic parameters were analyzed per treatment. The $t_{max}$ was around four hours for all doses of AVE1625. Exposure to AVE1625 increased proportionally between 20 mg and 60 mg. However, a 3-fold increase in $C_{max}$ and a 2.5-fold increase in $AUC_{0-24}$ were observed between AVE1625 60 and 120 mg. There was no evidence that the pharmacokinetics of AVE1625 was influenced by the presence of THC (Figure 2).

## PHARMACODYNAMIC MEASUREMENTS

### HEART RATE

Heart rate increased significantly in a dose-dependant manner after THC administration (Figure 3). The average THC peak effect consisted of an increase of 15 beats per minute (95% CI +12, +19 bpm). Co-administration of AVE1625 20, 60 and 120 mg significantly inhibited THC-induced effects (Figure 3). The inhibition ratios are presented in Table 2. No changes in heart rate were observed after administration of AVE1625 120 mg + THC vehicle compared to placebo + THC vehicle.

### ELECTRO-ENCEPHALOGRAPHY (EEG)

At the peak THC effect, the power of delta (-15%: 95% CI -6, -21), theta (-16%: 95% CI -10, -23) and beta activity (-16%: 95% CI -10, -23) decreased in the Pz-Oz region in comparison with THC vehicle (Table 2). No changes were found in alpha activity. In the Fz-Cz region, only a decrease in beta activity (-10%: 95% CI -2, -15) was seen (Table 2). Inhibition of THC-induced effects by AVE1625 was observed on different EEG parameters (Table 2). After administration of AVE1625 120 mg + THC vehicle, an increase in the power of Fz-Cz beta activity (+13%: 95% CI +6, +20) and Fz-Cz delta activity (+6%: 95% CI +2, +13) was observed in comparison to placebo AVE1625 + THC vehicle.

### BODY SWAY

THC alone increased body sway in a dose-related manner in comparison with THC vehicle. At the peak THC effect the increase accumulated to +43% (95% CI +27, +62). Statistically significant inhibition of THC-induced effects on body sway was observed after co-administration of AVE1625 20, 60 and

120 mg (Table 2). Administration of AVE1625 120 mg + THC vehicle did not change body sway in comparison to placebo AVE1625 + THC vehicle.

### SUBJECTIVE EFFECTS BY VISUAL ANALOGUE SCALES

THC alone decreased VAS 'alertness' in a dose-related manner in comparison with THC vehicle. The decrease accumulated to -17 mm (95% CI -11, -22) at the peak THC effect. Statistically significant inhibition of THC-induced changes on VAS alertness were seen after co-administration of all doses of AVE1625 (Table 2). For this parameter a statistically significant dose dependent effect of AVE1625 was observed. Administration of AVE1625 120 mg + THC vehicle did not change VAS alertness in comparison to placebo AVE1625 + THC vehicle.

No significant changes in VAS calmness were observed after administration of AVE1625 or THC, neither alone nor in combination. A slight decrease was seen in VAS contentedness after administration of THC alone (-6 mm: 95% CI -0.2, -12) compared to in comparison to THC vehicle. No changes in VAS contentedness were observed after administration of AVE1625 120 mg alone or in combination with THC.

VAS 'feeling high' (Figure 4) is one of the most responsive scales to the effects of THC. THC alone increased VAS 'feeling high' (+2.0 U: 95% CI +1.8, +2.3) at the peak THC effect in comparison to placebo AVE1625 + THC vehicle. Co-administration of AVE1625 20, 60 and 120 mg inhibited THC-induced effects (Table 2 and Figure 4)). Administration of AVE1625 alone did not change VAS feeling high in comparison to placebo AVE1625 + THC vehicle.

A dose-response effect on the composite score of 'external perception' (Figure 5) was observed after administration of THC with an increase of +1.1 U at the peak THC effect (95% CI +0.9, +1.3) in comparison to placebo AVE1625 + THC vehicle. Although a significant THC effect was also observed on the composite scale 'internal perception' at THC peak effect (+0.6 U: 95% CI +0.4, +0.8), concentration- and dose-dependency were much less pronounced than the increase in 'external perception'. 'Internal perception' seemed to be associated with an on/off effect or at least a very steep dose-response curve (no response at 2 mg, maximum response at doses of 4 mg and higher).

Statistically significant inhibition of THC-induced effects on VAS 'internal perception' and 'external perception' was observed after co-administration of all doses of AVE1625 (Table 2). Administration of AVE1625 + THC vehicle did not change 'internal' and 'external perception' in comparison to placebo AVE1625 + THC vehicle.

## Discussion

Although CB1 antagonists have been shown to have beneficial effects on body weight in obese patients and possibly on lipid profile,[3] so far no acutely measurable objective pharmacodynamic effects on the central nervous system have been reported. This study revealed that CB1 antagonist activity of AVE1625 could be demonstrated by inhibition of THC-induced effects on the central nervous system and on heart rate. The effects of THC alone were typical cannabis-like effects as reported in a previous study.[13] Statistically significant inhibition of THC-induced effects was observed after co-administration of the selective CB1 antagonist AVE1625 on almost all chosen subjective and objective measures (except EEG Pz-Oz delta activity at AVE1625 20 mg). High inhibition ratios were found on most parameters (Table 2). These findings provide useful information on the mechanisms of central and cardiac activity of THC and about the pharmacologically active dose of AVE1625.

THC is an agonist at both CB1 and CB2 receptors. In animals it has been firmly established which of the wide range of THC effects are due to activation of the CB1 or the CB2 receptors. However, this is only partially corroborated in humans. AVE1625 is a selective CB1 antagonist with very high affinity for the CB1 receptor that antagonized almost all THC-induced effects. This indicates that both the central nervous system effects (subjective changes and impaired postural stability) and the probably peripheral effects (heart rate acceleration) are mostly if not completely mediated by CB1 activation.

AVE1625 dose selection for this study was based on tolerability in humans and pre-clinical data. The 20 mg dose was in the lower range of an equivalent active dose in animals, and 120 mg was a safe dose in humans. High inhibition ratios were observed after the administration of THC in combination with AVE1625. Endocannabinoids have a much lower affinity for the CB1 receptor than THC,[22] and even the lowest dose of THC that was used in this study has probably exceeded the effects of a physiologically stimulated endocannabinoid system. This suggests that an active single dose of AVE1625 may be less than the lowest dose of 20 mg that was used in this study. However, this has to be confirmed in clinical trials. Unfortunately the inhibition ratios were too high to be able to perform PK/PD modelling to quantitatively estimate a minimally inhibitory dose. This was due to the lack of sufficient data that showed partial inhibition with AVE1625 20 and 60 mg, instead of almost complete inhibition of THC-induced effects with these doses. Although CB1 antagonists can be demonstrated to inhibit the effects of external THC administration, it should be realized that it is unclear whether endocannabinoid CB1 receptors have similar characteristics in patient as in healthy controls.

A recent study with rimonabant suggested that a CB1 antagonist may even be therapeutically active at a dose that causes an incomplete suppres-

sion of THC-induced effects. Huestis *et al*. studied the effects of the CB1 antagonist rimonabant on marijuana-induced effects in 63 healthy male volunteers with a history of smoking marijuana.[11] Volunteers were given either a single oral dose of rimonabant (1 - 90 mg) or placebo. Two hours later subjects smoked one marijuana (2.64% THC ≈20 mg THC) or placebo cigarette. Rimonabant reduced the effects of marijuana smoking on psychological effects and on heart rate in a dose-related manner. Contrary to the much higher inhibition ratios observed in our study (Table 2), the highest dose of rimonabant 90 mg demonstrated only 38% to 43% reductions in subjective effects and of 59% in heart rate. Similar results were found in an other interaction study of Huestis *et al*. after single or repeated doses of rimonabant in combination with marijuana cigarettes.[23] The concentrations attained with a single dose of rimonabant 90 mg seem clinically relevant, considering that the recommended dose of rimonabant for the treatment of obesity of 20 mg daily accumulates up to 3.3-fold during prolonged treatment.[24] The findings of Huestis *et al*. would suggest that perhaps 50% inhibition of THC-induced effects would be sufficient for a therapeutic (anti-obesity) effect. However, there are some differences between the study of Huestis *et al*. and the results presented here which make the comparison difficult. The total administered THC dose is roughly comparable between our study and the study of Huestis *et al*. In our study 18 mg THC was administered using a Volcano® vaporizer. Since the recovery of the vaporizer is slightly more than 50%,[18] in total a dose of almost 10 mg THC was inhaled from the plastic bag equipped with a valved mouthpiece, preventing the loss of THC in between inhalations. In the study of Huestis *et al*. the marijuana cigarette contained 20 mg THC from which about half is lost in the side stream smoke.[25] Considering the difference in time-profiles of drug administation the THC doses of both studies were roughly comparable. In addition, the THC plasma concentrations are considered comparable. In the study of Huestis *et al*. maximum THC plasma concentrations of 139 ng/ml were found two minutes after starting smoking. These finding are roughly comparable with the plasma THC concentrations using the same methodology as this study.[13,14] Pharmacokinetic modelling data of our previous study[14] suggest a THC plasma concentration of ≈180 ng/ml (two minutes after the start of the inhalation) if a single dose of 10 mg THC would have been administered. This plasma concentration is higher than in the study of Huestis *et al*.[11] The puffing procedures may explain some of the differences. In our study THC was inhaled within 2-3 minutes while in the study of Huestis *et al*. 8 puffs were taken with 60 seconds intervals, while distribution processes where already going on. In addition, smoking marijuana enables experienced subjects to influence their total administered THC dose, with the aim of achieving the expected and desired subjective effects. Subjects who were treated with the CB1 antagonist may have tried to achieve the expected 'high feeling', by inhaling deeper or longer – despite efforts to maintain a standardized inhalation

method. In the present study a validated and much more precise mode of pure THC administration was used[13] with a fixed dose of THC, and all subjects were instructed to fully inhale the contents of the balloon through a valve that avoided drug leakage. Moreover, marijuana cigarettes contain a mixture of psychoactive compounds, which in combination may contribute differently to the psychological and physical effects of marijuana.[26] If not all effects of marijuana are established by activation of CB1 receptors, this could explain why not all effects were inhibited in the study of Huestis *et al*. Also, rimonabant and AVE1625 belong to different chemical series, and their receptor efficacy cannot be directly compared due to lack of published pre-clinical data. In the future, a prediction of therapeutically active doses may be possible, when the inhibitory effects on THC have been established for different CB1 antagonists with established therapeutic doses. In addition, THC plasma concentrations and PK/PD modelling are needed to fully understand the relationship between levels of CB1 antagonists and shifts in the concentration-effect curves for THC.

The therapeutic indications for CB1 antagonists like anxiety and schizophrenia are partly based on the well-known effects of cannabis. In contrast to the pleasant effects of relaxation and mild euphoria seen after recreational cannabis use, high doses of THC may induce untoward psychiatric (anxiety, derealisation, hallucinations, altered body perception) and psychotropic (feeling high) effects.[27-29] In this study we demonstrated that CB1 antagonists are able to antagonize THC-induced effects on the composite VAS Bowdle scales 'internal' and 'external perception', which may represent aspects of CB1 mediated psychosis. Changes in the 'external perception' reflect a misperception of an external stimulus or a change in the awareness of the subject's surroundings. The 'internal perception' reflects inner feelings that do not correspond with reality. This may indicate that CB1 antagonist may be helpful in the treatment of psychosis. Since cannabis effects are used to inspire potential indications for CB1 antagonists, reversal of THC-induced effects on subjective and psychotropic measurements could provide useful information concerning these indications and the therapeutic doses. This could also be the case for other THC-induced effects, for example on memory[30] or feeding behaviour.[1,3] However, one clinical study was performed in patients with schizophrenia or schizoaffective disorder in which rimonabant showed no difference from placebo on any of the psychiatric outcomes measures reflecting antipsychotic effects.[5]

In summary, this study demonstrates a useful method for studying the central nervous system effects of CB1 antagonists, which by themselves are devoid of measurable acute pharmacodynamic effects. AVE1625 penetrates the brain and antagonizes THC-induced effects with doses at or above 20 mg with high ratios of inhibition. Lower doses of AVE1625 may suffice to suppress endocannabinoid activity and may also be sufficient for therapeutic activity, but this requires confirmation from clinical trials.

**Figure 1** Chemical structure of the CB1 antagonist AVE1625

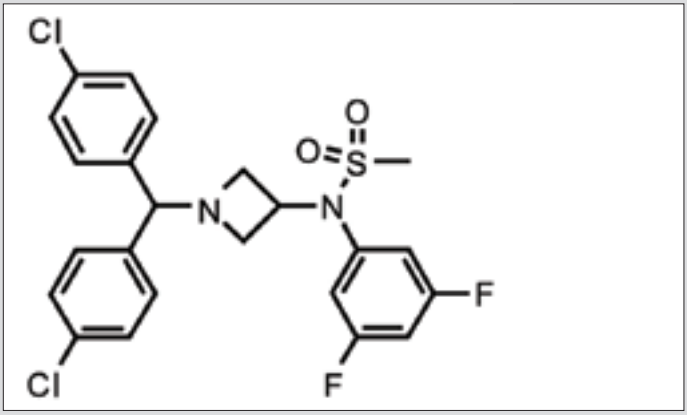


**Figure 2** Mean (SD) time profile of plasma AVE1625 concentration-time profiles. AVE1625 administration at T = 0. THC vehicle administration T = 3, 4, 5 and 6 hours.

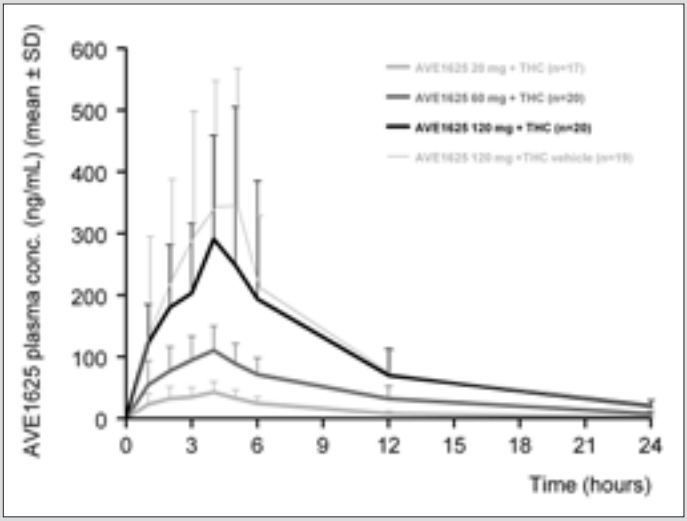

**Figure 3** Mean (SD) time profile of heart rate. (Placebo) AVE1625 administration at T = 0. THC (vehicle) administration: 2 mg at T = 3 hours; 4 mg at T = 4 hours; 6 mg at T = 5 hours; 6 mg at T = 6 hours. Arrows indicate drug administration.

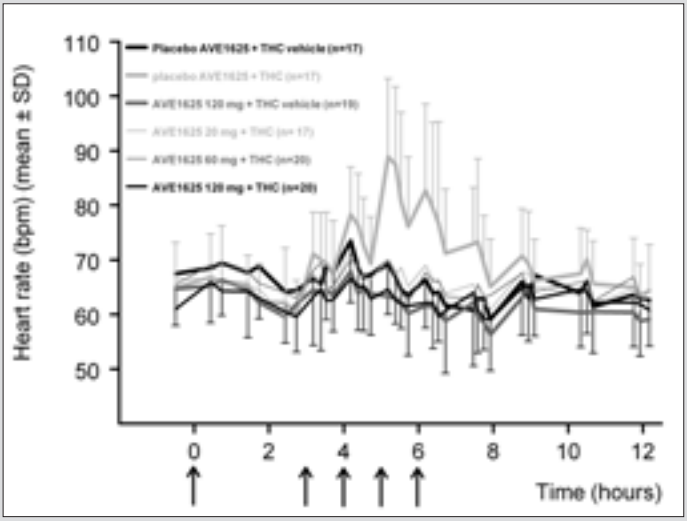


**Figure 4** Mean (SD) time profile of feeling high-time profiles with 95% CI as error bars. (Placebo) AVE1625 administration at T = 0. THC (vehicle) administration: 2 mg at T = 3 hours; 4 mg at T = 4 hours; 6 mg at T = 5 hours; 6 mg at T = 6 hours. Arrows indicate drug administration.

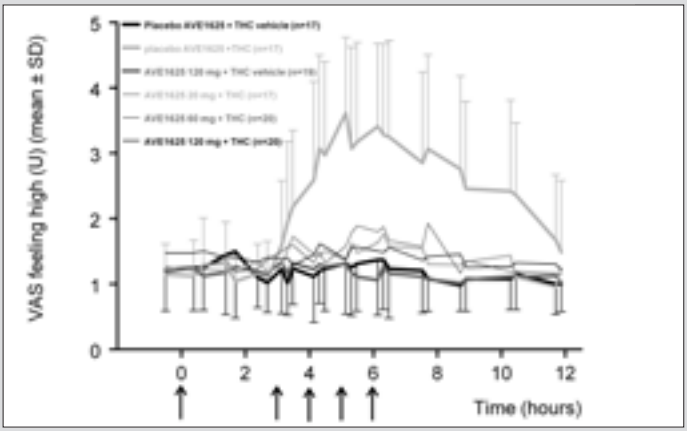

**Figure 5** Mean (SD) time profile of 'external perception'-time profiles with 95% CI as error bars. (Placebo) AVE1625 administration at T = 0. THC (vehicle) administration: 2 mg at T = 3 hours; 4 mg at T = 4 hours; 6 mg at T = 5 hours; 6 mg at T = 6 hours. Arrows indicate drug administration.

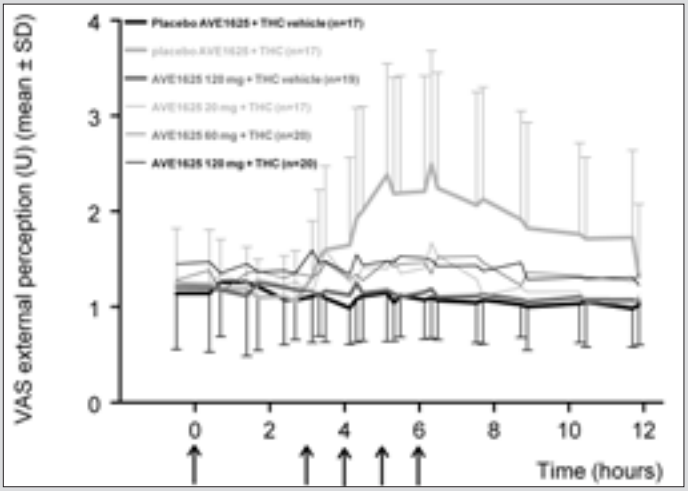


**Table 1** Study design. The order of AVE1625 was randomized across study days. If a subject was randomized to receive rising doses of THC (instead of placebo) the order of the THC doses was fixed on each study day (2 mg, 4mg, 6 mg and 6 mg).

| Oral | Intrapulmonary | | | |
|---|---|---|---|---|
| 9:00 | 12:00 | 13:00 | 14:00 | 15:00 |
| AVE1625 (20 or 60 or 120 mg) or placebo | 2 mg THC or placebo | 4 mg THC or placebo | 6 mg THC or placebo | 6 mg THC or placebo |

**Table 2** Effects of AVE1625 on THC-induced effects: estimates of the inhibition ratios with 95% CI at THC peak effect. The inhibition ratios are calculated with the following formula: [(AVE1625 + THC) - (placebo AVE1625 + THC)] / [(placebo AVE1625 + THC vehicle) - (placebo AVE1625 + THC)]. For further details see analyses section of the methods.

| | Inhibition ratios | | |
|---|---|---|---|
| | **20 mg AVE1625** | **60 mg AVE1625** | **120 mg AVE1625** |
| Heart rate | 89% (61, 118) | 96% (66, 126) | 109% ( 78, 140) |
| EEG Pz-Oz delta | 44% (-16, 104) | 88% (16, 160) | 85% (14, 156) |
| EEG Pz-Oz theta | 98% (32, 164) | 69% (12, 125) | 91% (28, 154) |
| EEG Pz-Oz beta | 75% (15, 135) | 74% (15, 133) | 129% (51, 206) |
| EEG Fz-Cz beta | 90% (-1, 181) | 117% (14, 221) | 218% (56, 381) |
| Body sway | 61% (22, 100) | 73% (32, 113) | 74% (33, 114) |
| VAS alertness | 61% (25, 97) | 76% (37, 114) | 94% (52, 136) |
| VAS feeling high | 90% (72, 107) | 83% (66, 99) | 101% (83, 120) |
| Internal perception | 103% (62, 144) | 86% (49, 122) | 71% (37, 105) |
| External perception | 83% (60, 105) | 90% (67, 113) | 88% (65,111) |

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# 7 Pharmacodynamic and pharmacokinetic effects of the intravenously administered CB1 receptor agonist Org 28611 in healthy male volunteers

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## Abstract

CB1/CB2 agonists are reported to have sedative, amnestic, analgesic and anti-emetic properties, which would make them ideal drugs for outpatient treatments under conscious sedation. The main objective of this first in human study was to assess the sedative properties of Org 28611, a potent water-soluble CB1 agonist. Single ascending doses were administered during a slow 25 minute infusion and after a 1 minute bolus administration to healthy male volunteers. In addition, the pharmacokinetics, amnestic properties, postural stability, EEG, behavioural and cardiovascular effects were studied. Midazolam 0.1 mg/kg was used as a positive control. The pharmacokinetic parameters were proportional to dose. No effects were observed after intravenous administration of doses up to Org 28611 1 µg/kg. Dose related effects were observed at higher doses. Although subjects reported subjective sedation after administration of Org 28611 3-10 µg/kg, the observed sedation was considerably less than after midazolam. Org 28611 is therefore not suitable for providing sedation for outpatient surgical procedures and doses above the maximum-tolerated dose of 3 µg/kg (either administered as a slow infusion or a bolus dose) can cause untoward psychotropic effects.


## Introduction

Many surgical procedures are carried out under conscious sedation in an outpatient environment. Conscious sedation can be defined as a minimally depressed level of consciousness that retains the patient's ability to independently and continuously maintain an airway and respond appropriately to physical stimulation or verbal commands.[1] Under conscious sedation the patient does not receive general anaesthesia but is treated with drug combinations such as midazolam and an opioid, or with low sedative doses of propofol and an opioid to produce sedation and analgesia, respectively. Although these combinations are usually quite safe, they have a small chance of causing respiratory or cardiac depression, nausea and vomiting or psychomotor effects. The identification of a drug that could be used for conscious sedation with both sedative and analgesic properties but without these potential detrimental effects, would be valuable. The cannabinoid type 1 (CB1) agonist Org 28611 might have such properties.

Delta-9-tetrahydrocannabinol (THC), the main active ingredient of cannabis is a CB1 (cannabinoid receptor type 1) and CB2 agonist and known for its sedative, amnestic, analgesic and anti-emetic properties.[2-4] In addition, THC induces tachycardia.[5] However, THC produces unwanted psychiatric side effects at high doses[6-9] and therefore is not ideal for inducing conscious sedation for outpatient procedures. The water-soluble CB1 receptor

agonist Org 28611, which is structurally unrelated to THC, may have a pharmacological profile suitable for outpatient surgical procedures. Although the affinity of Org 28611 is similar for the CB1 and CB2 receptor, the efficacy of Org 28611 for the CB2 receptor has not been clarified in detail (Organon, data on file).

Cannabinoid activation in rodents produces a consistent decrease in spontaneous locomotor activity (indication for sedation) as well as hypothermia, analgesia and catalepsy.[3] Rodent models showed that Org 28611 has sedative properties in addition to analgesic effects. Org 28611 causes dose dependent bradycardia and hypotension but does not induce respiratory depression (Organon, data on file). These data suggest that Org 28611 might have both sedative and analgesic properties in humans as well.

Important aims of the current first in human study were to asses the pharmacokinetic (PK) and pharmacodynamic (PD) properties of intravenously administered Org 28611. The primary objective of the study was to assess the sedative potential of Org 28611. Other useful properties of a sedative compound for outpatient treatments would be amnesia (the patient does not remember the procedure), lack of postural instability (patient can go home soon after treatment), a short time to maximum effect ($t_{max}$) and a short half-life (related to fast onset and disappearance of the effects), positive subjective effects, and a lack of undesirable systemic effects (hypotension, respiratory depression, nausea/vomiting). These properties of Org 28611 were studied as well. Midazolam (a benzodiazepine) has sedative and amnestic properties and is frequently used in outpatient surgical procedures. It has some desirable properties for outpatient procedures, such as anxiolysis, amnesia, sedation, and a fast onset and short duration of action. However, midazolam's $t_{max}$ may vary and it can cause balance impairment and respiratory depression at higher doses or in combination with other central nervous system (CNS) depressants. Midazolam was used as a positive control, as a benchmark for the effect profile of Org 28611.

## Methods

### DESIGN

This was a single-centre, double-blind, partially randomized, placebo- and active-controlled, 5-way cross-over intravenous single ascending dose study, where Org 28611 was administered for the first time to healthy male volunteers. Subjects were dosed on five consecutive days and stayed at the clinic until the morning after the last dosing. The study protocol was approved by the Medical Ethics Review Board of Leiden University Medical Center and performed according to principles of ICH-GCP, and Dutch clinical trial law.

### SUBJECTS

Twenty-one male healthy volunteers were screened and found to be healthy determined from medical history, physical examination, laboratory and urine screens and electrocardiography. All subjects had negative urinary drug screens at screening and prior to each study occasion, except for one subject who was excluded on his first study day before medication was administered, because of a positive cocaine screen. The study was restricted to males since the reproductive toxicology data were not yet available. The ages of the twenty enrolled subjects were in the range 18-40 years with a mean of 23 ± 6 years. Their mean height and weight were 182 ± 6 cm (range 174 – 199 cm) and 77 ± 9 kg (range 62 – 99 kg), with a mean body-mass index of 23 ± 3 kg/m$^2$ (range 18 – 28 kg/m$^2$). Cannabis was not used within one month before screening and did not exceed a life-time use of five times. None of the subjects had a prior negative experience with cannabis use, a history of psychosis or had a first degree relative with psychosis. Subjects were excluded if they smoked more than five cigarettes a day and they had to refrain from smoking on study days. In addition, they had to be able to refrain from use of (methyl)xanthines (e.g. coffee, tea, coke, chocolate) from 48 hours prior to the first dose until the last pharmacokinetic blood sample was taken. Subjects were not allowed to use any medication. A follow-up visit was scheduled within 2-7 days after the last blood sample was taken.

### TREATMENTS

The study consisted of two phases in which on five consecutive days medication was administered intravenously. During the first phase Org 28611 was administered as a slow infusion and during a subsequent second phase as an intravenous bolus. Fifteen subjects were included in Phase I (group I-III) and five in Phase II (group IV). Between each consecutive group was one week for interim analysis (Table 1). During the first phase each subject received three ascending doses of Org 28611 with a constant infusion pump rate for up to 25 minutes. Placebo (vehicle of Org 28611; Mannitol 5% was used as a solvent) and midazolam (0.1 mg/kg) were randomly interspersed between the three single ascending doses of Org 28611 (Table 1). The infusion was stopped after 25 minutes (full dose administered) or when a subject was asleep as determined by the modified Observer's Assessment of Alertness/Sedation (OAA/S) scale (score > 6) (Table 3). In this way, doses of Org 28611 were increased from 0.3 to 10.0 µg/kg in three consecutive groups of five subjects each. The actual doses (which partly overlapped among groups) are presented in Table 2. After each group (I, II and III) an interim analysis was performed to select the dose range for the next group.

After the slow 25 minute infusion phase, PK/PD analysis was performed to predict the optimal bolus dose of Org 28611. In this subsequent phase (Phase II), five subjects received three ascending bolus doses of Org 28611 (0.3, 1 and 3 µg/kg) intravenously in one minute. Placebo was randomly interspersed (Table 1). Midazolam was omitted from phase II because Org 28611 had shown an almost complete lack of objective sedation during slow 25 minute infusion, refuting the use of a sedative agent as a positive control.

### BLOOD SAMPLING

Blood samples were taken once at baseline; during the 25 minute infusion at 1, 6, 9, 14, 17 and 22 minutes after the start of the infusion. In addition, samples were obtained 1, 2, 5, 10, 15, 40, 75, 125, 265 and 365 minutes after the 25 minute infusion was stopped or after the bolus dose was administered. For determination of the concentration of plasma Org 28611, venous blood was collected in EDTA tubes of 4 ml. After blood collection the tubes were put in ice water (0-4 degrees Celsius) and were centrifuged at 2000G at 4 degrees Celsius for 15 minutes. Plasma samples were stored at a temperature of -20 degrees Celsius.

### HAEMATOLOGY, BIOCHEMISTRY AND URINANALYSIS

Blood samples for routine haematology and biochemistry were taken at screening, in the morning before each drug administration and at follow up. In addition, routine urinanalysis was performed by dipstick (Multistix 10 SG®, Bayer, Mijdrecht, The Netherlands) using 10 ml urine.

### ECG AND $SPO_2$ MONITORING

ECG monitoring was conducted for 70 minutes after the start of the infusion using a 12-lead continuous registration (Cardioperfect ECG recorder, Welch Allyn, Delft, The Netherlands). In addition, 10 seconds 12-lead ECGs were recorded using Nihon Kohden Cardiofax with ECaps 12 software devices (Nihon Kohden, Tokyo, Japan). Measurements were taken twice at baseline and 20, 48, 50, 80, 130, 270 and 370 minutes after the end of the slow infusion or after the bolus dose was administered. An adult TL-101T (Nellcor) probe (Nihon Kohden, Life Scope EC, Tokyo, Japan) was attached to a finger to measure $SpO_2$ continuously for 70 minutes after the start of the infusion.

### HEART RATE AND BLOOD PRESSURE

Blood pressure and heart rate were measured in supine position after a rest of approximately 5 minutes. All measurements were carried out with an

automated sphygmomanometer (Nihon Kohden, Life Scope EC, Tokyo, Japan). Measurements were taken twice at baseline; during the 25 minute infusion at 0, 8, 16 and 25 minutes after the start of the infusion. In addition, measurements were taken 9, 19, 39, 49, 79, 129, 269 and 369 minutes after the slow infusion was stopped or after the bolus dose was administered.

## PHARMACODYNAMIC MEASUREMENTS

Subjects were acquainted with the experimental methods and conditions in a training session within one week before the first study day. Pharmacodynamic assessment was performed in a quiet and temperature controlled room with standardised illumination with only one subject per session in the same room. All tests were measured twice pre-dose and obtained frequently at fixed time points after the start of the infusion. Table 4 describes the set of assessments conducted at baseline and directly following the start of the infusion. Tests were always measured in the same order.

### SACCADIC EYE MOVEMENT

Saccadic peak velocity is an objective measure of sedation. Recording and analysis of saccadic eye movements was conducted with a personal computer using a validated Spike2 script (Cambridge Electronic Design Limited, Cambridge, UK). Disposable silver-silver chloride electrodes (Mediscore, VDP Medical, Nieuwegein, The Netherlands) were applied on the forehead and beside the lateral canthi of both eyes of the subject for registration of the electro-oculographic signals. Skin resistance was reduced to less than 5 kOhm before application of the electrodes. Head movements were restrained using a fixed head support. The equipment used for stimulus display was manufactured by Nihon Kohden (Nihon Kohden, Life Scope EC, Tokyo, Japan). For signal collection and amplification a Grass Telefactor (F-15EB/B1) and a 15LT series Amplifier Systems (Grass-Telefactor, Braintree, USA) was used.

The target consisted of an array of light emitting diodes on a bar, fixed at 50 cm in front of the head support. Saccadic eye movements were recorded for stimulus amplitudes of approximately 15 degrees to either side. Fifteen saccades were recorded with interstimulus intervals varying randomly between 3 and 6 seconds. Average values of saccadic peak velocity of all artefact-free saccades were used as parameters. Measurements were taken twice at baseline; during the 25 minute infusion at 2, 10 and 18 minutes after the start of the infusion. In addition, measurements were taken 3, 13, 23, 33, 43, 73, 133, 263 and 363 minutes after the slow infusion was stopped or after the bolus dose was administered.

### ELECTRO-ENCEPHALOGRAPHY (EEG)

EEG recordings were made using silver chloride electrodes, fixed with collodion at Fz, Cz, Pz and Oz positions, with the same common ground electrode as for the eye movement registration (international 10/20 system). The electrode resistances was kept below 5 kOhm. EEG signals were obtained from leads Fz-Cz and Pz-Oz and a separate channel to record eye movements (for artefacts). The signals were amplified by use of a Grass Telefactor (F-15EB/B1) and a 15LT series Amplifier Systems (Grass-Telefactor, Braintree, USA) with a time constant of 0.3 seconds and a low pass filter at 100 Hz. Data collection and analysis were performed using a validated Spike2 script (Cambridge Electronics Design, Cambridge, UK). Per session eight consecutive blocks of eight seconds were recorded. The signal was AD-converted using a CED 1401 Power (Cambridge Electronics Design, Cambridge, UK). Data blocks containing artefacts were identified by visual inspection and these were excluded from analysis. For each lead, fast Fourier transform analysis was performed to obtain the sum of amplitudes in the delta (0.5-3.5 Hz), theta (3.5-7.5 Hz), alpha (7.5-11.5 Hz) and beta (11.5-30 Hz) frequency ranges. Outcome parameters were the square root of the total power in each band for each lead. Measurements were taken twice at baseline; during the 25 minute infusion at 5, 13 and 21 minutes after the start of the infusion. In addition, measurements were taken 4, 14, 24, 34, 44, 74, 124, 264 and 364 minutes after the slow infusion was stopped or after the bolus dose was administered.

### BODY SWAY

Postural stability was measured with a string attached to the waist with an apparatus similar to the Wright ataxia meter.[10] All body movements in the antero-posterior direction over a period of 2 minutes were integrated and expressed as mm sway on a digital display. The contribution of vision to postural control was eliminated by asking subjects to close their eyes. Subjects were not allowed to talk during the measurement, and asked to wear the same comfortable low-heeled shoes at all measurements. Measurements were taken twice at baseline. Body sway was not measured during drug infusion. Measurements were taken 11, 21, 31, 51, 81, 131, 271 and 371 minutes after the slow infusion was stopped or after the bolus dose was administered. Body sway was not measured during drug administration.

### VISUAL ANALOGUE SCALES (VAS) AND OAA/S

To asses objective sedation, a modified Observer's Assessment of Alertness/Sedation (OAA/S) scale was scored on paper by trained attending physicians

(Table 3).[11] Measurements were taken twice at baseline and at 0, 8, 16 and 24 minutes after start of the 25 minute infusion. In addition, measurements were taken 9, 19, 29, 39, 49, 79, 129, 269 and 369 minutes after the slow infusion was stopped or after the bolus dose was administered. Subjective effects were indicated on sixteen Visual Analogue Scales according to Bond and Lader (100 mm scale, electronic version). From these measurements, three factors were derived: alertness (from nine scores), contentedness (often called mood, from five scores) and calmness (from two scores).[12] Psychotropic effects were monitored by an adapted version of the VAS described by Bowdle (100 mm scale, electronic version).[13] Previous studies with THC showed that two separate modalities of psychedelic effects can be derived from 11 Bowdle VAS scores,[5] namely external and internal perception. External perception reflects a misperception of an external stimulus or a change in the awareness of the subject's surroundings. It is calculated as the average (after log-transformation +2) of the following VAS scores: changing of body parts, changes of surroundings, altered passing of time, difficulty controlling thoughts, changes in colour intensity and changes in sound intensity. The internal perception reflects inner feelings that do not correspond with reality, and is composed of feelings of unreality, hearing voices/sounds, things have a specific particular meaning, paranoia and feeling anxious.

VAS Bond and Lader and VAS Bowdle were measured twice at baseline and at 7, 15 and 23 minutes after the start of the 25 minute infusion. In addition, measurements were taken 7, 17, 27, 37, 47, 77, 127, 267 and 367 minutes after the end of drug administraion. In addition, the subjects indicated their subjective qualification of the drug's effects on five different VAS (100 mm scale, paper version) at 1 hour and 51 minutes after termination of the infusion. Subjects were asked the following questions: 'have you felt any good drug effects?', 'have you felt any bad drug effects?', 'have you felt any hangover effects?', 'have you recovered from the effects of the study drug?' and 'how much do you like the drug effects?'.

### VISUAL VERBAL LEARNING TEST

The Visual Verbal Learning Test (VVLT) contains three different subtests of learning behaviour, namely immediate recall, delayed recall and delayed recognition.[14] In this study six different versions of the VVLT were used to ensure that each subject received a new test version on each of the five study days, each different from the training version. Thus, learned effects were prevented. On each study day the test was performed once.

55 minutes after the drug infusion stopped subjects were presented 30 words in three consecutive word learning trials, i.e. word learning test. Each trial ended with an immediate free recall test to determine acquisition and short-term retention of information. The instructions were to mention

each word they recalled only once. From each trial the following scores were counted: number of correct words, number of incorrect words as well as words that were mentioned more than once and words that were not presented. If a (in)correct word was mentioned twice, the overall score included 1 (in)correct response and 1 double response. Forty minutes after start of the first trial, the volunteers were asked to recall as many words as possible. This is a delayed free recall test which measures active retrieval from long term memory. Immediately thereafter, the volunteers underwent a delayed memory recognition test, which consisted of 15 previous presented words and 15 distracters to test memory storage.

## ANALYSIS

### PHARMACOKINETICS (PK)

Plasma samples for the determination of Org 28611 concentrations were analysed at Organon NV, Oss, The Netherlands. Org 28611 plasma levels were measured using a validated LC-MS-MS assay in full compliance with GLP regulations. Lower Limit of quantification was 5.0 pg/ml and upper limit of quantification was 5000 pg/mL. The inter-assay coefficient of variation was between 2.2 and 6.8%. Non-compartmental pharmacokinetic analyses were performed using SAS version 8.2 on a PC running under Windows XP v5.1.

### PHARMACODYNAMICS (PD)

Data from the slow infusions (groups I-III) were analysed separately from data from from the bolus infusions (group IV). Some doses were administered in more than one group (groups I-III). The data from a particular dose, administered in different groups, were grouped and analysed together. Analysis of the pharmacodynamic data was performed using mixed model analysis of variance (using SAS PROC MIXED) with treatment, study day, time and treatment by time as fixed effects and subject, subject by time and subject by treatment as random effect, and with the average baseline value as covariate. If necessary to meet requirements of the ANCOVA, the data were log-transformed. In case of a significant treatment effect, contrasts between the treatments and placebo were calculated. Log treatment estimates were back-transformed resulting in geometric mean treatment estimates corrected for potential differences in baseline values. Contrasts and 95% confidence intervals between treatments were back-transformed resulting in geometric mean ratios which were subsequently translated into percentage increase of the treatment relative to the placebo. All reported significant effects are mean treatment effects or significant contrasts between a certain dose and placebo.

### PHARMACOKINETIC/PHARMACODYNAMIC ANALYSES (PK/PD)

The purpose of the PK/PD analysis was to support the selection of doses for each new dosing group, and after phase I (slow 25 minute infusions) to predict the effect (PK and PD) of a bolus administration. The effect of Org 28611 plasma concentrations on internal and external perception and heart rate was investigated since these parameters demonstrated a clear response to Org 28611, and they provided information on potentially undesirable and clinically relevant effects. Individual empirical Bayes estimates for elimination half-life and clearance were determined for all occasions separately, and predicted individual Org 28611 concentration profiles were obtained using these estimates. Model choice was based on the goodness of fit test results.

Pharmacokinetic modelling was performed using SAS® Version 8.02, S-PLUS version 6.2 and NONMEM version V, level 1.1 software (NONMEM Project Group, UCSF, San Francisco, CA, USA) under Windows XP v5.1.

## Results

The available data of all withdrawn subjects were included in the analyses if they completed at least one study day.

### CLINICAL EFFECTS

After administration of Org 28611 (either administered as a slow 25 minute infusion or a bolus dose) the most frequently reported adverse events were somnolence, dizziness, disturbance in attention, depressed level of consciousness, thirst, palpitations, headache and nausea. These adverse events were of mild or moderate severity and disappeared rapidly. After slow infusion, the highest doses of 6 and 10 µg/kg Org 28611 caused psychiatric and undesirable central nervous system effects. Org 28611 6 µg/kg (n = 5) caused the following symptoms in different subjects: paraesthesia (3/5), delusional perception (1/5), euphoric mood (1/5), sensory disturbance (1/5), derealisation (1/5), confusional state (1/5), hallucinations (1/5) and motor dysfunction (1/5). Org 28611 10 µg/kg (n = 5) caused: paraesthesia, sensory disturbance, derealisation, confusional state, hallucinations, inappropriate affect and disturbance in attention (all in one subject). In addition, in group II subject 6 was withdrawn due to a serious adverse event during infusion of 10 µg/kg on his second study day. The infusion was stopped after 19 minutes. He suffered from tachycardia (up to about 110 beats per minute), akathisia, lancinating pains and a panic attack. He recovered without sequelae within 5.5 hours from all symptoms. For safety reasons, all subjects treated in the same week as subject 6 were withdrawn after completion of the second study day. Since it was not clear if the serious adverse event of subject 6

was a rare event or dose-related to the drug, it was approved by the ethical committee to repeat the slow 25 minute infusion of Org 28611 10 µg/kg with 6 µg/kg as an intermediate dose.

In the last slow infusion group (group III), subject 11 was withdrawn from the study due to adverse effects including tachycardia (up to 160 beats per minute) and psychiatric symptoms after infusion of Org 28611 6 µg/kg. Subject 13 of the same group discontinued the study due to a mild central nervous system adverse events after infusion of Org 28611 10 µg/kg. All subjects recovered without sequelae. The maximum-tolerated dose was 3 µg/kg Org 28611.

None of the subjects felt asleep as determined by the modified Observer's Assessment of Alertness/Sedation (OAA/S) scale (score > 6) (Table 3) and therefore all infusions (except for subject 6) were stopped after 25 minutes when the full dose of Org 28611, midazolam or placebo was administered. The lack of clearly detectable sedative effects after the highest infused dose of Org 28611 also refuted the use of midazolam (which was highly sedative as expected) as a positive control. Therefore, bolus medication in the subsequent phase of the study (group IV) was restricted to Org 28611 or its placebo.

The most frequently reported adverse events for midazolam were mild in intensity and included somnolence, fatigue, disturbance in attention, dizziness and blurred vision. The adverse events under placebo conditions were mild in intensity and all occurred only once: dizziness, infusion site reaction, headache, hunger, depressed level of consiousness and bradyphrenia.

### PHARMACOKINETICS

The pharmacokinetic parameters seemed proportional to dose although mean parameters tended to decrease with dose. Maximum Org 28611 concentrations were reached at the end of the infusion indicating that steady state was not reached. This is confirmed by the mean concentration versus time profiles during and after infusion (Figure 1). The maximum concentration of Org 28611 that was found in a 24 hour blood sample was 0.4 ng/ml in a single subject.

For the 25 minute infusion, the following population mean (approximate SE of population mean ± SEM) pharmacokinetic parameters were estimated: elimination half-life 3.5-5.5 hours (inter-individual coefficient of variance (IICV) 10-64%), clearance 13.7 L/h (SE 0.422, IICV 35.1%). For the bolus dose administration the pharmacokinetic parameters were: elimination half-life 6-10 hours (inter-individual coefficient of variance (IICV) 31-44%), clearance 11.4 L/h (SE 0.220, IICV 24.6%).

During the serious symptoms of subject 6, his maximum plasma concentration of Org 28611 was 6.5 ng/mL, which was at the lower end of the expected concentration range. The maximum-tolerated dose of 3 µG/kg corresponds to a plasma concentration of 4.0 ± 1.7 ng/mL (mean ± SD) for

the 25 minute infusion. Administered as a 1 minute bolus dose the maximum plasma concentration was 24 ± 16.8 ng/mL (mean ± SD).

### HAEMATOLOGY, BIOCHEMISTRY AND URINANALYSIS

There were no clinically significant abnormal values in this trial for haematology, biochemistry and urinalysis parameters.

### ECG, $SPO_2$ MONITORING, HEART RATE AND BLOOD PRESSURE

No clinically significant changes were observed in ECG recordings and $SpO_2$ monitoring. Blood pressure was not changed by Org 28611 or midazolam administration. Heart rate increased significantly in comparison with placebo after slow 25 minute infusion of Org 28611 6 µg/kg (+16 bpm: 95% CI +8, +24) and 10 µg/kg (+17 bpm: 95% CI +8, +25) (Figure 2). Heart rate did not change after infusion of doses Org 28611 ≤ 3 µg/kg (25 minute infusion or 1 minute bolus) or after midazolam administration.

### SACCADIC EYE MOVEMENT

Saccadic peak velocity did not change after administration of Org 28611 (Figure 3). By contrast, midazolam decreased this parameter significantly (-62 deg/sec: 95% CI -84, -41) (Figure 3).

### ELECTRO-ENCEPHALOGRAPHY (EEG)

After Org 28611 administration no changes were observed in any of the EEG parameters. Midazolam decreased the power of Pz-Oz alpha (-34.6%: 95% CI -41.5, -26.8%) and in Fz-Cz theta (-14.0%: 95% CI -21.4, -5.9%), and increased beta activity in the Fz-Cz region (+72.9%: 95% CI +57.3, +90.0%) and in the Pz-Oz region (+18.7%: 95% CI +6.4, +32.3%). No changes were observed in delta activity.

### BODY SWAY

Postural stability was not assessed during the infusions. After the slow 25 minute infusion of Org 28611 was stopped a dose-related average increase in body sway compared to placebo was observed: Org 28611 3 µg/kg (+31.9%: 95% CI +6.6, +63.2%), 6 µg/kg (+91.7%: 95% CI +41.7, +159.4%) and 10 µg/kg (+59.0%: 95% CI +12.2, +125.3%) (Figure 4). Body sway did not change after administration of doses Org 28611 ≤1 µg/kg (25 minute infusion) or after infusion of the three bolus doses of Org 28611. Compared to placebo, infusion of midazolam showed a significant increase in body sway (+83.1%: 95% CI +46.8, +128.2%) (Figure 4).

## VISUAL ANALOGUE SCALES (VAS)

### OBSERVER'S ASSESSMENT OF ALERTNESS/SEDATION (OAA/S)

No significant changes have been observed on OAA/S after infusion of any of the doses of Org 28611 (Figure 5). However, a higher score on OAA/S was observed after infusion of midazolam (+0.91: 95% CI +0.67, +1.15) (Figure 5).

### VAS ALERTNESS

Compared to placebo, the slow 25 minute infusion of Org 28611 caused a dose-related decrease in VAS alertness. Significantly lower scores were seen after slow 25 minute infusion of Org 28611 1 µg/kg (-14 mm: 95% CI -24, -4), 3 µg/kg (-14 mm: 95% CI -21, -7), 6 µg/kg (-39 mm: 95% CI -49, -29) and 10 µg/kg (-32 mm: 95% CI -43, -22) (Figure 6). No significant changes were observed after administration of the three bolus doses. Midazolam caused a decrease in VAS alertness (-20 mm: 95% CI -27, -12) (Figure 6). These data indicate subjective sedation after slow infusion of Org 28611 1, 3, 6 and 10 µg/kg, but not after bolus administration of 0.3, 1 or 3 µg/kg.

### VAS CALMNESS

Lower scores on VAS calmness (signifying reduced calmness) were observed after slow 25 minute infusion of Org 28611 6 µg/kg (-16 mm: 95% CI -26, -5) and 10 µg/kg (-32 mm: 95% CI -44, -21). VAS calmness was not changed after infusion of doses of Org 28611 3 µg/kg or less (either slow infusion or 1 minute bolus) or with midazolam.

### VAS MOOD

Org 28611 decreased VAS mood scores after slow 25 minute infusion of 6 µg/kg (-26 mm: 95% CI -36, -17) and 10 µg/kg (-32 mm: 95% CI -42, -22), indicating that contentedness was decreased. No changes were observed after infusion of doses of Org 28611 3 µg/kg or less (slow infusion or 1 minute bolus). Lower scores on VAS mood were also found after infusion of midazolam (-9 mm: 95% CI -16, -2).

### VAS INTERNAL PERCEPTION

The composite score of internal perception were higher after slow 25 minute infusion of Org 28611 6 µg/kg (+88%: 95% CI +29, +172%) and 10 µg/kg (+112%: 95% CI +41, +219%) (Figure 7). No significant changes were observed after infusion of Org 28611 3 µg/kg or less (slow infusion or 1 minute bolus). Higher scores were also observed after infusion of midazolam (+37%: 95% CI +4, +80%) (Figure 7).

### VAS external perception

The composite score of external perception had a similar time profile as the internal perception. External perception showed higher scores after slow 25 minute infusion of Org 28611 6 µg/kg (+186%: 95% CI +99, +311%) and 10 µg/kg (+118%: 95% CI +47, 223%). No significant changes were observed after slow or bolus infusion of doses lower than Org 28611 3 µg/kg. Midazolam also produced higher scores (+51%: 95% CI +16, +97%).

### VAS for assessment of subjects' qualification of drug effect

Table 5 provides a summary of the subjective qualifications of Org 28611 and midazolam. No effect was seen after slow 25 minute infusion of Org 28611 0.3 µg/kg or placebo and after the bolus doses of Org 28611 0.3 and 1 µg/kg. These data are therefore not presented in the table.

### Visual verbal learning test

On average, fewer words were recalled after the midazolam infusion (average 9 words) than after placebo (average 18 words) (Table 6). During active delayed recall, on average 4 words could be retrieved during the midazolam occasion, compared to 14 words with placebo. The difference was smaller during delayed recognition, when 22 words were correctly identified during placebo and 17 with midazolam.

The memory effects of Org 28611 differed from midazolam. Immediate word recall was somewhat reduced by the highest two doses of Org 28611, albeit slightly less than with midazolam (Table 6). This was also observed during delayed recall, which showed that the highest doses of Org 28611 caused a significant reduction of actively retrieved words, compared to placebo. Contrary to midazolam, just as many words were recognized during delayed recognition (22-24 words on average), as with placebo (22 words).

### Pharmacokinetic/pharmacodynamic (PK/PD) relationships

The concentration-effect-relationships for the slow 25 minute infusion and bolus data could not be captured in a single PK/PD model. The effect of Org 28611 plasma concentrations on internal and external perception was investigated using a linear concentration-effect model with additive residual error. For heart rate a direct model was used. The Org 28611 concentrations during and after 25 minute infusion were best fitted by a two-compartmental model with a random effect structure for all pharmacokinetic parameters and a combined proportional and additive residual error model. The bolus dose data were best fitted by a three compartmental model.

Exposure ($AUC_{0\text{-}inf}$) was comparable for the 25 minute infusion and the 1 minute bolus dose regimen.

The effect of slow 25 minute intravenous infusion of Org 28611 on internal and external perception showed hysteresis signifying that the effects were delayed relative to the plasma concentrations of Org 28611 with an equilibrium half-life of approximately ten minutes. A clear linear concentration effect relationship on heart rate was observed during and after the 25 minute infusion. An increase in plasma concentration of 1 ng/mL increased heart rate by approximately 3 beats per minute. Upon a 1 minute bolus dose the effect on heart rate is less pronounced. No hysteresis was seen for the effects on heart rate.

## Discussion

An ideal sedative compound for outpatient treatments should have at least a rapid onset of action, a short duration without accumulation and no or only mild side effects. Midazolam (0.1 mg/kg), which was used as a positive control in this study, has many of these properties.[15,16] Other useful properties for short treatments under conscious sedation are amnesia, postural stability, positive subjective effects and no inadvertent respiratory or cardiovascular effects. Midazolam is not analgesic, and for surgical procedures it is often combined with anaesthetics like opiods. Midazolam is used to produce sedation and opiods to produce analgesia during out-patient procedures. Such drug combinations have limitations such as respiratory or cardiac depression, nausea and vomiting and psychomotor effects. A drug with both sedative and analgesic properties would avoid these drug interactions. The CB1 agonist Org 28611 was expected to have these properties. The current study did not assess the analgesic properties of Org 28611, but it was shown that the pharmacokinetic and pharmacodynamic profile of Org 28611, a CB1 receptor agonist, differs considerably from midazolam.

### PHARMACOKINETIC AND PHARMACOKINETIC/PHARMACODYNAMIC ANALYSES

Org 28611 did not have a rapid onset of action like midazolam.[16,17] The effect of slow 25 minute intravenous infusion of Org 28611 on internal and external perception showed hysteresis signifying that the effects were delayed relative to the plasma concentrations of Org 28611 with an equilibrium half-life of approximately ten minutes. In addition, Org 28611 had a much longer half life than midazolam.[16,17] These characteristics make Org 28611 less suitable for short out patient treatments.

Although the AUCs of the 3 µg/kg are comparable for the slow 25 minute infusion and the bolus dose administration, the pharmacodynamic effects

differed. The maximum-tolerated dose of 3 µg/kg induced some effects on body sway and VAS alertness after slow infusion. In contrast, the 1 minute bolus only caused some changes in the VAS of the subjects' qualification of drug effect. Higher doses (6 and 10 µg/kg) had a much more pronounced effect on the pharmacodynamic measurements and caused untoward psychotropic effects, but these doses were only given as a slow infusion. The reasons for the apparent difference between the effects of the slow infusion and the bolus administration are not immediately apparent. The CNS effects do not seem to be directly related to plasma concentrations. The bolus had more limited effects than the infusion, despite much higher peak plasma concentrations (Figure 1). There is also no immediate relationship between AUCs (which were similar among the two administration modes) and CNS effects (which differed). The rate of infusion was much higher with the bolus, but fast rates would be expected to cause more CNS effects rather than less. Consequently, the concentration-effect-relationships for the slow 25 minute infusion and bolus data could not be captured in a single PK/PD model. The pharmacokinetic differences between the slow infusion and bolus dose administration are most likely caused by the higher variability observed in the 25 minute infusion concentration versus time profiles, and by the fact that the fast distribution processes are hard to identify when the compound is administered as a slow infusion.

### SEDATION

Org 28611 did not cause the same type of conscious sedation as midazolam. Midazolam induced conscious sedation within ten minutes, which lasted for about 30-45 minutes. In contrast to midazolam, Org 28611 showed a discrepancy between subjective and objective sedation. The scores for VAS alertness, an indication for subjective sedation, were significantly lower scores after infusion of Org 28611 (at doses above 1 µg/kg) compared to placebo. However, subjects were awake and reacted quickly to verbal stimuli as observed on the OAA/S. Subjects also clearly reported a difference in the character of the sensation: midazolam caused drowsiness, whereas Org 28611 induced feelings of tiredness. In addition, Org 28611 did not change saccadic peak velocity movements, an objective measure of sedation for benzodiazepines.[18] There is a possibility that higher doses of Org 28611 would have induced conscious sedation, but this was precluded by pronounced subjective effects, including reduced calmness and psychomimetic effects.

### ANTEROGRADE AMNESIA

Another well-known effect of midazolam is anterograde amnesia, which is revealed by the disruption of the storage of information (evidenced by

significantly reduced immediate word learning) and retrieval difficulties of newly stored information (shown by significant reductions of delayed word recall and delayed recognition). Consequently, the patient does not remember much of the procedure. Although the highest two doses of Org 28611 also caused an impairment of active immediate and delayed recall, there was a remarkable preservation of the number of recognized (and hence stored) words during the delayed recognition phase of the memory test. Although impaired retrieval may reduce the capacity of the patient to recall the details of a surgical or diagnostic procedure, it is unclear how retained storage with diminished retrieval would affect the subconscious impact of a traumatic experience. However, this only seems to be relevant for higher doses of Org 28611, since no memory effects were observed with doses up to 3 µg/kg.

### POSTURAL STABILITY

The advantage of treatments under conscious sedation is discharge of patients on the same day. This implies that patients should be able to go home independently, without increased risks of falls or accidents. Midazolam causes postural instability as measured with the body sway, which returns to baseline after 100 minutes. This indicates that patients can walk stably fairly soon after treatment. Org 28611 also induces postural instability, although not as much as midazolam (+59% and +83% respectively). Compared to midazolam, the effects on the body sway lasted one hour longer after infusion of Org 28611 (>6 µg/kg). However, three hours is still short enough to be useful in outpatient treatments and bolus doses up to 3 µg/kg caused no changes in body sway.

### SUBJECTIVE EFFECTS

Org 28611 caused pronounced subjective and psychomimetic effects that did not seem to agree with the usual pleasant effects of relaxation and mild euphoria seen after recreational cannabis use. In this study Org 28611 was administered to subjects whose life-time use did not exceed five times, which may explain the observed adverse effects after Org 28611 administration compared to the effects usually seen after recreational cannabis use. However, literature is not consistent in reporting kinetic and subjective differences between users and non or infrequent-users.[7,19-22] Doses higher than the maximum-tolerated dose of Org 28611 3 µg/kg caused untoward psychiatric (anxiety, derealisation, hallucinations, altered body perception) and psychotropic (feeling high) effects and diminished mood and calmness. These effects were seen in various subjects, most severely in subject 6, who suffered from severe mental, motor and sensory symptoms that could be largely attributed to a panic attack. There were no clear signs of psychotic

restlessness. Similar effects were observed after intravenous administration of THC, the main active ingredient of cannabis.[9,23]

Org 28611 caused a range of subjective effects characterised by changes in internal and external perception on the Bowdle VAS, and reductions of calmness and mood (or contentedness) on the Bond and Lader VAS, and by an unfavourable assessment on the subjects' own qualification of Org 28611. However, these effects were mainly noted after infusion of doses of 6 µg/kg or higher, and no significant adverse subjective, pharmacodynamic or clinical effects were observed after any bolus dose of Org 28611 up to 3 µg/kg. In comparison, midazolam induced only small subjective changes, with the exception of considerably reduced alertness.

### CARDIOVASCULAR EFFECTS

No cardiovascular effects of midazolam were observed in this study, although it has been described that higher doses of midazolam decrease blood pressure and increase heart rate.[24] In general the cardiovascular effects of Org 28611 in rodents were comparable to the effects of THC,[25] the reference compound in the pre-clinical studies of Org 28611. In humans, the cardiovascular effects of THC are well-known and include an increase in heart rate by 20-60% (smoking, oral or intravenously administered). Maximal heart rate increase occurs 10 to 15 minutes after peak plasma THC concentration.[26] Similar changes in heart rate (16-17%) were observed after infusion of Org 28611 (>6 µg/kg). However, no changes in heart rate were observed after bolus administration of Org 28611 (≤3 µg/kg). The effects of THC on blood pressure are complex, both increases and decreases in blood pressure have been reported.[26-28] In this study blood pressure was not changed by Org 28611.

### SUMMARY

This study shows that a first in human study can provide a range of valuable data for the further development of a drug. Org 28611 is does not provide enough sedation for outpatient surgical procedures, does not induce anterograde amnesia and causes undesirable subjective effects at higher doses. However, bolus doses up to 3 µ/kg (with maximum initial plasma concentrations of 24 ng/mL) or mean plasma levels up to 4 ng/mL are well-tolerated and make it worthwhile to further explore the analgesic or anti-emetic properties of this CB1 agonist.

**Figure 1** Mean (SD) observed time profile of plasma Org 28611 (ng/mL). Left: during and after 25 minute infusion of Org 28611 (group I–III). Right: 1 minute bolus administration. Please note the different concentration-scale for the intravenous and bolus administrations. T = 0 corresponds to the start of the infusion. The arrow indicates the stop of the infusion at T = 25 minutes. Since hardly any effect was observed after bolus administration, for clarity purposes no bolus data are presented in the other graphs.

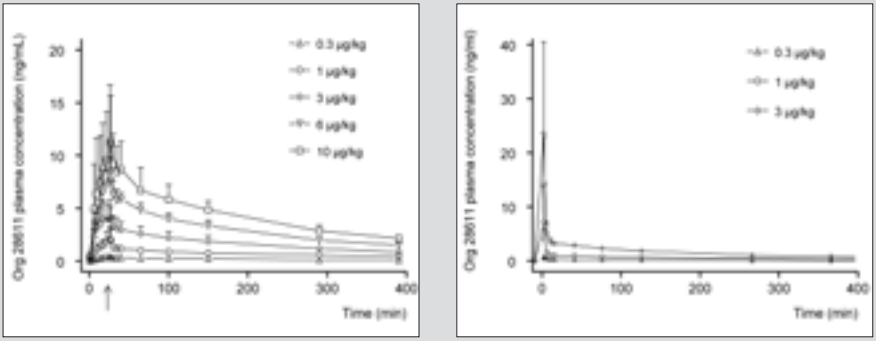


**Figure 2** Mean (SD) time profile of heart rate during and after 25 minute infusion of Org 28611 (group I–III). T = 0 corresponds to the start of the infusion. The arrow indicates the stop of the infusion at T = 25 minutes.

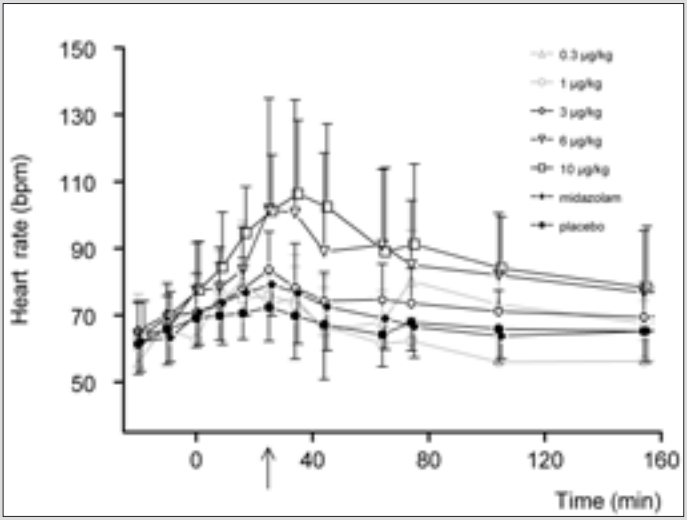

**Figure 3** Mean (SD) time profile of saccadic peak velocity during and after 25 minute infusion of Org 28611 (group I–III). T = 0 corresponds to the start of the infusion. The arrow indicates the stop of the infusion at T = 25 minutes.

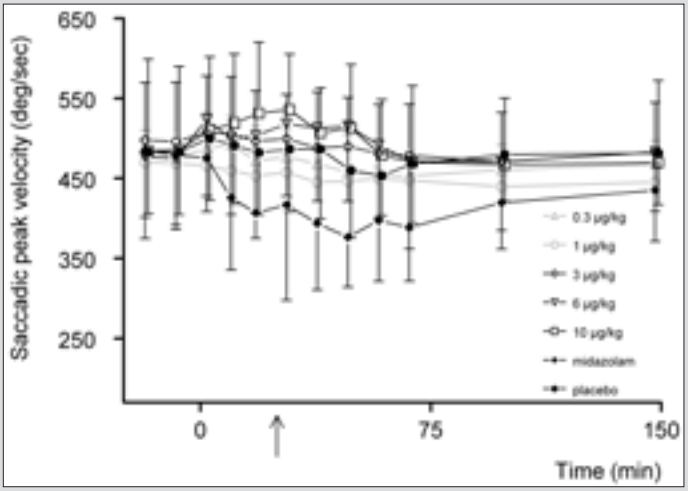


**Figure 4** Mean (SD) time profile of body sway during and after 25 minute infusion of Org 28611 (group I–III). T = 0 corresponds to the start of the infusion. The arrow indicates the stop of the infusion at T = 25 minutes. During the infusion no body sway measurements were performed.

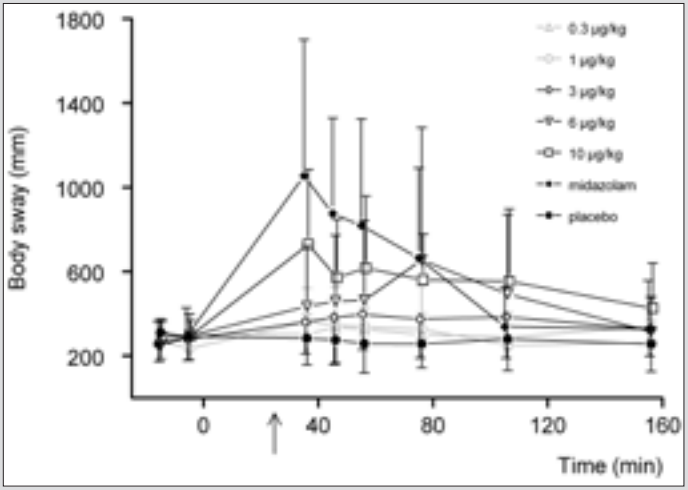

**Figure 5** Mean (SD) time profile of Observers Assessment of Alertness/ Sedation during and after 25 minute infusion of Org 28611 (group I–III). T = 0 corresponds to the start of the infusion. The arrow indicates the stop of the infusion at T = 25 minutes.

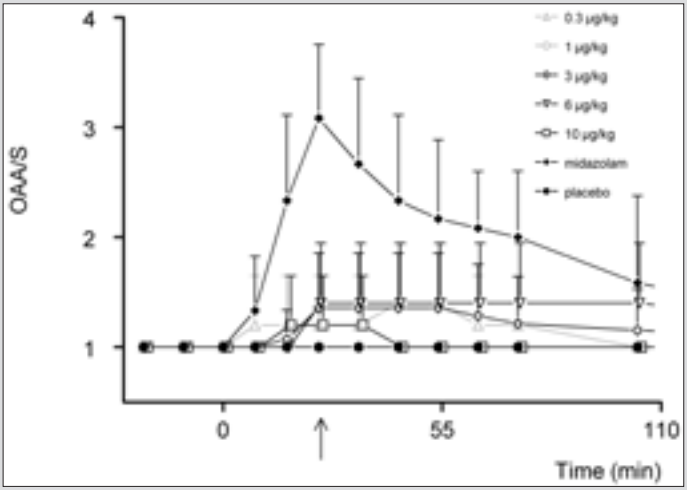


**Figure 6** Mean (SD) time profile of VAS alertness during and after 25 minute infusion of Org 28611 (group I–III). T = 0 corresponds to the start of the infusion. The arrow indicates the stop of the infusion at T = 25 minutes.

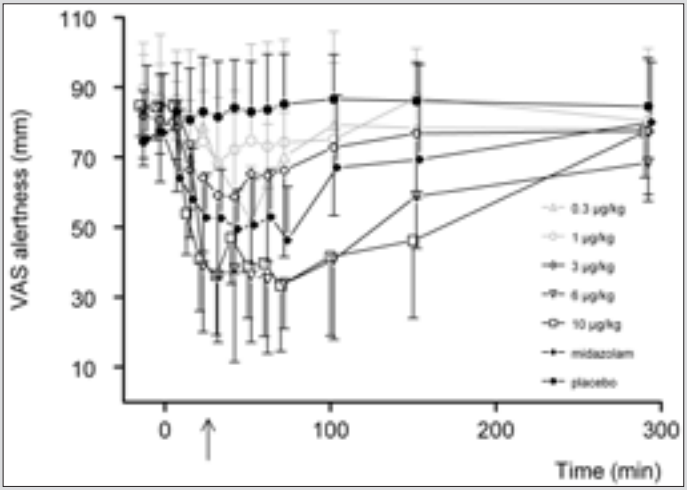

**Figure 7** Mean (SD) time profile of internal perception during and after 25 minute infusion of Org 28611 (group I–III). T = 0 corresponds to the start of the infusion. The arrow indicates the stop of the infusion at T = 25 minutes.

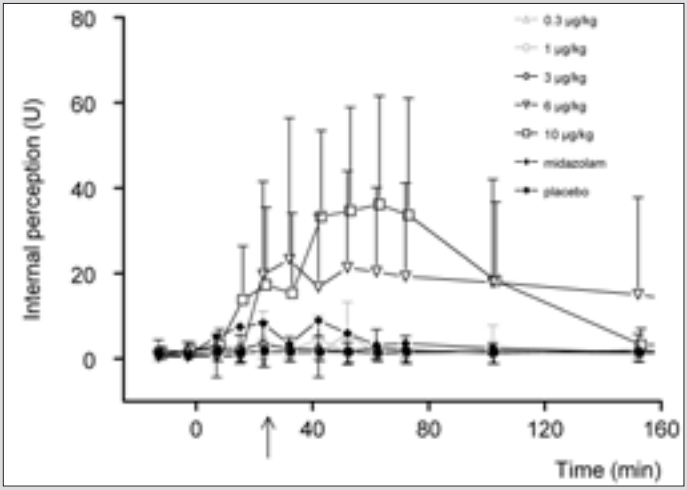


**Table 1** Study design of part I (25 minute infusion) and part II (1 minute bolus). D1-3 and Da-c represent Org 28611, P placebo and M midazolam administation. One subject participated per sequence.

| **Part I:** Group I, II, III (25 minute infusion) | | | | | **Part II:** Group IV (1 minute bolus) | | | |
|---|---|---|---|---|---|---|---|---|
| Mo | Tue | Wed | Thur | Fri | Mo | Tue | Wed | Thur |
| M | $D_1$ | $D_2$ | $D_3$ | P | Da | Db | Dc | P |
| $D_1$ | M | $D_2$ | P | $D_3$ | Da | Db | P | Dc |
| $D_1$ | $D_2$ | P | M | $D_3$ | Da | Db | P | Dc |
| $D_1$ | P | M | $D_2$ | $D_3$ | Da | P | Db | Dc |
| P | $D_1$ | $D_2$ | $D_3$ | M | P | Da | Db | Dc |

**Table 2** Doses of Org 28611 and midazolam during slow 25 minute infusion (part I) and 1 minute bolus (part II). Number between the brackets is the actual number of subjects dosed.

| | Part I | | | Part II |
|---|---|---|---|---|
| | Group I | Group II | Group III | Group IV |
| **Org 28611 (µg/kg)** | 0.3 (5)<br>1.0 (4)<br>3.0 (4) | 3.0 (5)<br>10.0 (1)<br>-* | 3.0 (5)<br>6.0 (5)<br>10.0 (4) | 0.3 (5)<br>1.0 (5)<br>3.0 (5) |
| **Midazolam (mg/kg)** | 0.1 (5) | 0.1 (2) | 0.1 (5) | - |

* The subjects in this group were withdrawn and the subsequent dosing regimen was revised because of a serious adverse event on this group's second study occasion.

**Table 3** Modified Observer's Assessment of Alertness/Sedation Score

| | |
|---|---|
| 1 | awake, orientated |
| 2 | reports feeling drowsy, quick reaction verbal stimulus, clear look, normal speech |
| 3 | slow reaction to verbal stimulus |
| 4 | inability to perform 2 saccades correctly, lethargic response to verbal stimulus, glazed look, slowing of speech |
| 5 | reacts to soft touch and repeated verbal stimulus |
| 6 | reacts to repeated loud verbal stimulus, glazed look and ptosis, muscle relaxation, slurred speech |
| 7 | reacts to non-painful stimulus |
| 8 | no reaction to non-painful stimulus, eyes closed, few recognizable words |
| 9 | reacts to painful stimulus |
| 10 | no reaction to painful stimulus |

**Table 4** Set of assessments conducted at baseline and directly following the start of the infusion. During the slow 25 minute infusion this section was repeated 3 times. An empty cell means no measurement.

| Time (minute) | |
|---|---|
| 1 | OAA/S, heart rate, blood pressure |
| 2 | Blood sample |
| 3 | Saccadic eye movement |
| 4 | |
| 5 | EEG |
| 6 | Blood sample |
| 7 | VAS Bond and Lader |
| 8 | VAS Bowdle |
| 9 | |
| 10 | |
| 11 | |
| 12 | |

**Table 5** VAS for assessment of subjects' qualification of Org 28611. Results are presented as the average effect (mm) compared to placebo with 95% CI. Bold numbers indicate a significant result.

| Measurement | infusion (25 minutes) | | | | bolus (1 minute) | midazolam |
|---|---|---|---|---|---|---|
| | 1 µg/kg (n=4) | 3 µg/kg (n=14) | 6 µg/kg (n=5) | 10 µg/kg (n=4) | 3 µg/kg (n=5) | 0.1 mg/kg (n=12) |
| Have you felt any bad drug effect? | +24 (-3, +51) | **+22 (+3, +40)** | **+81 (+55, +107)** | **+84 (+53, +115)** | **+48 (+15, +81)** | **+24 (+5, +44)** |
| Have you felt any hangover effect | +14 (-10, +38) | **+18 (+2, +34)** | **+50 (+27, +73)** | **+76 (+49, +102)** | **+25 (+12, +38)** | **+21 (+4, +38)** |
| Have you felt any good drug effect? | +12 (-15, +40) | +9 (-10, +28) | **-37 (-63, -10)** | **-39 (-70, -9)** | **-18 (-28, -9)** | +13 (-7, +32) |
| Have you recovered from the effects of the study drug? | **-39 (-72, -7)** | -12 (-34, +11) | **-53 (-85, -22)** | **-38 (-74, -2)** | +11 (-1, +23) | -19 (-42, +4) |
| How much do you like the drug effect? | +6 (-26, +23) | +7 (-11, +24) | **-32 (-57, -8)** | **-39 (-67, -10)** | -11 (-26, +4) | +3 (-15, +21) |

**Table 6** Average number of correct words with 95% CI after different treatments on the Visual Verbal Learning Test (30 words). Bold numbers indicate a significant result compared to placebo.

| Treatment | Storage (working memory and learning) Immediate recall | | | Retrieval Delayed recall | Consolidation Delayed recognition |
|---|---|---|---|---|---|
| | Recall 1 | Recall 2 | Recall 3 | | |
| placebo | +10 | +14 | +18 | +14 | +22 |
| midazolam | **+4 (-6: -8, -4)** | **+7 (-7: -9, -4)** | **+9 (-9: -12, -7)** | **+4 (-10: -13, -6)** | **+16 (-6: -9, -3)** |
| Org 28 611 0.3 µg/kg (n=5) | +8 (-1: -4, +2) | +14 (+0: -3,+3) | **+14 (-4: -8, -1)** | +11 (-3: -8, +3) | +23 (+1: -4, +5) |
| Org 28 611 1.0 µg/kg (n=4) | +8 (-2: -5, +1) | +12 (-1: -4, +2) | +16 (-2: -6, +2) | +9 (-5: -10, +0) | +23 (+1: -4, +5) |
| Org 28 611 3.0 µg/kg (n=14) | +10 (+1: -1, +3) | +15 (+1: -1, +4) | +18 (-1: -3, +2) | +13 (+0: -4, +3) | +23 (+0: -3, +4) |
| Org 28 611 6.0 µg/kg (n=5) | **+6 (-4: -7, -1)** | **+10 (-4: -7, -1)** | **+11 (-7: -10, -3)** | **+8 (-6: -11, -1)** | +22 (+0, -5, +5) |
| Org 28 611 10 µg/kg (n=4) | +6 (-3: -7, +1) | **+8 (-6: -10, -1)** | **+10 (-8: -13, -3)** | **+6 (-8: -15, -1)** | +24 (+1: -5, +8) |

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# 8 Pharmacodynamic and pharmacokinetic effects of the intravenous CB1 receptor agonist Org 26828 in healthy male volunteers

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## Abstract

**Background**: An ideal drug for outpatient treatments under conscious sedation would have both sedative and analgesic properties. CB1/CB2 agonists are expected to have sedative, amnestic, analgesic and anti-emetic properties similar to THC, the main active ingredient of cannabis. Intravenous CB1 agonists are not yet available in humans, and an infusible agent could offer new therapeutic possibilities for this drug class.
**Aim**: In this first in human study the sedative properties of intravenously administered Org 26828 were assessed. In addition, pharmacokinetics, amnestic properties, postural stability, behavioural and cardiovascular effects were studied. Midazolam 0.1 mg/kg and placebo were used as controls.
**Results**: The pharmacokinetic parameters were proportional to dose. No effects were observed after doses up to 0.3 µg/kg Org 26828. Dose-related effects were observed at higher doses. Although subjects reported subjective sedation after administration of 3 and 6 µg/kg Org 26828, the observed sedation was considerably less than after midazolam. In addition, unlike midazolam (-52 deg/sec: 95% CI -69, -35) a dose of 1 µg/kg Org 26828 (-10 degrees/second: 95% CI -27, 7) did not affect saccadic peak velocity. Doses higher than the maximum-tolerated dose of 1 µg/kg Org 26828 caused unpleasant central nervous system effects (anxiety, paranoia, hallucinations).
**Summary**: The maximum-tolerated dose of 1 µg/kg Org 26828 is not suitable for providing sedation for outpatient surgical procedures and can cause untoward psychotropic effects at high doses. However, Org 26828 is suitable for intravenous administration and well-tolerated up to 1 µg/kg, which may warrant development for other indications.


## Introduction

Delta-9-tetrahydrocannabinol (THC), the main psychoactive ingredient of cannabis, is a CB1 (cannabinoid receptor type 1) and CB2 agonist and known for its sedative, amnestic, analgesic and anti-emetic properties.[1-3] These properties by themselves would be suitable for outpatient surgical procedures. However, THC produces psychological side effects at high doses[4] and although this compound is available for oral dosing (as dronabinol), there are no registered formulations for intravenous administration. The CB1 receptor agonist Org 26828 is infusible and may have a pharmacological profile suitable for outpatient surgical procedures. Org 26828 and its major metabolite Org 26761 are structurally related to THC. Both demonstrate high nanomolar affinity for human CB1 and CB2 receptors (pKi range 8.6-9.8). Although the affinity of Org 26828 is similar for the CB1 and CB2 receptor, the efficacy of Org 26828 for the CB2 receptor has not been clarified in detail. Its efficacy for the human CB1 receptor assessed in a cell-based assay system is 66%,

which is higher than that observed for THC in the same experiments. The potency and efficacy of Org 26761 for CB1 receptors is equivalent to that of Org 26828. Org 26888 (a second metabolite of Org 26828) has no affinity for the CB1 and CB2 receptors (Organon, data on file).

Cannabinoid activation in rodents produces a consistent decrease in spontaneous locomotor activity (indicating sedation) as well as hypothermia, analgesia and catalepsy.[2] Rodent models showed that Org 26828 has sedative properties in addition to analgesic effects. Org 26828 causes dose-dependent bradycardia and hypotension but does not induce respiratory depression (Organon, data on file). THC has a similar effect profile in animals, but does not cause cardiovascular depression in humans. These data suggest that Org 26828 might have both sedative and analgesic properties in humans as well, without the ventilatory or cardiovascular depressive effects of benzodiazepine or opiods.

Important aims of this first in human study were to asses the pharmacokinetic and pharmacodynamic properties of intravenously administered Org 26828. The primary aim of the study was to assess the sedative potential of Org 26828, because of its primarily intended clinical use. Other useful properties of a sedative compound for outpatient treatments are amnesia (the patient does not remember the procedure), lack of postural instability (the patient can go home soon after treatment), a rapid $t_{max}$ and a short half-life (related to fast onset and rapid disappearance of activity), positive subjective effects and a lack of undesirable systemic effects (hypotension, respiratory depression, nausea/vomiting). These properties of Org 26828 were studied as well. Midazolam has sedative and amnestic properties and is frequently used in outpatient surgical procedures. This benzodiazepine was used as a positive control for its sedative and amnestic properties.

## Methods

### DESIGN

This was a first in human, single-centre, double-blind, partially randomized, placebo- and active-controlled, five-way cross-over intravenous single rising dose study with Org 26828 in healthy male volunteers. Subjects were dosed on five consecutive days and stayed at the clinic until the morning after the last dosing. The study protocol was approved by the Medical Ethics Review Board of Leiden University Medical Center and performed according to principles of ICH-GCP, and Dutch clinical trial law.

### SUBJECTS

Fifteen healthy males were dosed in the study, since the reproductive toxicology data were not yet available. Their ages were in the range 19-31 years

with a mean of 21 ± 3 years. The mean height and weight were 187 ± 7 cm (range 171 – 197 cm) and 79 ± 8 kg (range 60 – 91 kg), respectively. Mean BMI was 23 ± 2 (range 18 – 26). Cannabis was not used within one month before screening and did not exceed a life-time use of five times. All urinary drug screens, including THC, were negative. None of the subjects had a history of psychosis or had a first degree relative with psychosis. Subjects were excluded when they smoked more than five cigarettes a day and they had to refrain from smoking on study days. In addition, they had to be able to refrain from use of (methyl)xanthines (e.g. coffee, tea, cola, chocolate) from 48 hours prior to the first dose until the last pharmacokinetic blood sample was taken. Subjects were not allowed to use any medication. A follow-up visit was scheduled within 2-7 days after the last blood sample was taken.

### TREATMENTS

The study consisted of two parts in which on five consecutive days medication was administered intravenously. Ten subjects were included in part I (5 in group I and 5 in group II) and five in part II (group III). Each subject could only participate in one group. Between each consecutive group there was one week for interim analysis (Table 1). During part I rising doses of Org 26828 0.1-6.0 µg/kg (Table 2) were administered intravenously with a constant infusion pump rate for up to 25 minutes, which if necessary allowed the interruption of dosing in case of an adverse event. Placebo (vehicle of Org 26828) and the positive control midazolam (0.1 mg/kg) were randomly interspersed between the three single rising doses of Org 26828 (Table 1). Mannitol 5% and solutol were used as solvents. The infusion was stopped after 25 minutes (full dose administered) or when a subject was asleep as determined by the modified Observers Assessment of Alertness/Sedation (OAA/S) scale (score > 6) (Table 3).

After group I and II an interim analysis was performed. The results were used to support the selection of doses for the next group. Pharmacokinetic/pharmacodynamic (PK/PD) analysis was performed using the most clearly drug-related effect obtained from part I (which proved to be heart rate) to predict the plasma concentrations and effects when Org 26828 was administered as a 1 minute bolus dose instead of a 25 minute infusion. In part II four rising bolus doses of Org 26828 (0.1, 0.3, 0.6 and 1 µg/kg) were intravenously administered in 1 minute, according to the intended clinical administration mode. Placebo was randomly interspersed (Table 1). The interim analysis of part I showed an almost complete lack of objective sedation after Org 26828. Considering the primary aims of the study, this indicated that midazolam would not be an informative positive control, when administered along with the 1 minute bolus doses of Org 26828 in part II.

Immediately after intravenous administration Org 26828 breaks down to Org 26761 and Org 26888 due to enzymatic hydrolysis. Org 26761 is the

active metabolite. As Org 26761 is not water soluble by itself, a solvent was added to the infusible formulation to prevent precipitation of Org 26761.

### BLOOD SAMPLING

For determination of the concentration of plasma Org 26761, venous blood was collected in EDTA tubes of 4 ml. After blood collection the tubes were put in ice water (0-4 degrees Celsius) and were centrifuged at 2000G at 4 degrees Celsius for 15 minutes. Plasma samples were stored at a temperature of -20 degrees Celsius.

### HAEMATOLOGY, BIOCHEMISTRY AND URINANALYSIS

Blood samples for routine haematology and biochemistry were taken at screening, in the morning before each drug administration and at follow-up. In addition, routine urinalysis was performed by dipstick (Multistix 10 SG®, Bayer, Mijdrecht, The Netherlands) using 10 ml urine.

### ECG AND $SPO_2$ MONITORING

ECG monitoring was conducted for 70 minutes after the start of the infusion using a 12-lead continuous registration (Cardioperfect ECG recorder, Welch Allyn, Delft, The Netherlands). In addition 10 seconds 12-lead ECGs were recorded using Nihon Kohden Cardiofax with ECaps 12 software devices (Nihon Kohden, Tokyo, Japan). An adult TL-101T (Nellcor) probe (Nihon Kohden, Life Scope EC, Tokyo, Japan) was attached to a finger to measure $SpO_2$ continuously for 70 minutes after the start of the infusion.

### HEART RATE AND BLOOD PRESSURE

Blood pressure and heart rate were measured in supine position after a rest of approximately 5 minutes. All measurements were carried out with an automated sphygmomanometer (Nihon Kohden, Life Scope EC, Tokyo, Japan).

## PHARMACODYNAMIC MEASUREMENTS

Subjects were acquainted with the experimental methods and conditions in a training session within one week before the first study day. Pharmacodynamic assessment was performed in a quiet and temperature-controlled room with standardised illumination with only one subject per session in the same room. All tests were measured twice pre-dose and obtained frequently at fixed time points after the start of the infusion. The measurement times can be derived from the effect time-profiles presented in the results.

### SACCADIC EYE MOVEMENT

Saccadic peak velocity is an objective measure of sedation following administration of benzodiazepines.[5] Recording and analysis of saccadic eye movements were conducted with a personal computer using a validated Spike2 script (Cambridge Electronic Design Limited, Cambridge, UK). Disposable silver-silver chloride electrodes (Mediscore, VDP Medical, Nieuwegein, The Netherlands) were applied on the forehead and beside the lateral canthi of both eyes of the subject for registration of the electro-oculographic signals. Skin resistance was reduced to less than 5 kOhm before application of the electrodes. Head movements were restrained using a fixed head support. The equipment used for stimulus display was manufactured by Nihon Kohden (Nihon Kohden, Life Scope EC, Tokyo, Japan). For signal collection and amplification a Grass Telefactor (F-15EB/B1) and a 15LT series Amplifier Systems (Grass-Telefactor, Braintree, USA) was used.

The target consisted of an array of light emitting diodes on a bar, fixed at 50 cm in front of the head support. Saccadic eye movements were recorded for stimulus amplitudes of approximately 15 degrees to either side. Fifteen saccades were recorded with interstimulus intervals varying randomly between 3 and 6 seconds. Average values of saccadic peak velocity of all artefact-free saccades were used as parameters.

### BODY SWAY

Postural stability was measured with a string attached to the waist with an apparatus similar to the Wright ataxia-meter.[6] All body movements in the antero-posterior direction over a period of 2 minutes were integrated and expressed as mm sway on a digital display. The contribution of vision to postural control was eliminated by asking subjects to close their eyes. Subjects were not allowed to talk during the measurement, and asked to wear the same comfortable low-heeled shoes at all measurements. Body sway was only measured after the intravenous administration of the compounds was completed.

## VISUAL ANALOGUE SCALES (VAS)

To asses objective sedation, a modified Observer's Assessment of Alertness/Sedation (OAA/S) scale was used (Table 3).[7] Alertness was assessed using the Visual Analogue Scales according to Bond and Lader (100 mm scale).[8] Psychotropic effects were monitored by an adapted version of the VAS described by Bowdle (100 mm scale).[9] Previous studies with THC showed that two separate modalities of psychedelic effects can be derived from the VAS Bowdle scores, namely 'internal' and 'external perception'.[10] Changes in

'external perception' reflect a misperception of an external stimulus or a change in the awareness of the subject's surroundings. The 'internal perception' scores reflect inner feelings that do not correspond with reality.

#### VISUAL VERBAL LEARNING TEST

The Visual Verbal Learning Test (VVLT) contains three different subtests, namely immediate recall, delayed recall and delayed recognition and was performed as described by Zuurman *et al.*[11,12] On each study day the test was performed once and started 55 min. after the drug infusion was stopped.

#### ADDITIONAL METHODS

EEG measurements were performed as described by Zuurman *et al.*[11] Mood and calmness were assessed using the Visual Analogue Scales according to Bond and Lader (100 mm scale).[8]

## ANALYSIS

#### PHARMACOKINETICS (PK)

Immediately after intravenous administration Org 26828 is metabolized to Org 26761 and Org 26888. Org 26828 is not measurable in plasma and therefore its active metabolite Org 26761 is presented in the results. Plasma samples for the determination of Org 26761 plasma levels were sent to Organon NV, Oss, The Netherlands. Org 26761 plasma levels were measured by using a validated LC-MS-MS assay in full compliance with GLP regulations. Limit of quantification was 0.4 ng/mL. The inter-assay precision of QC samples was between 2.6 and 5.4%. The assay accuracy of QC samples expressed as percentage deviation of the nominal concentration was between -2.6 and 0.8%. Non-compartmental pharmacokinetic analyses were performed using SAS version 8.2 on a PC running under Windows XP v5.1.

#### PHARMACODYNAMICS (PD)

Data from the slow infusions (groups I-II) were analysed separately from data from from the 1 minute bolus infusions (group III). Analysis of the pharmacodynamic data was performed using mixed model analysis of variance (using SAS PROC MIXED) with treatment, study day, time and treatment by time as fixed effects, with subject, subject by time and subject by treatment as random effect, and with the average baseline value as covariate. If necessary to meet requirements of the ANCOVA, the data were log transformed. In case of a significant treatment effect, contrasts between the treatments and placebo were calculated. Log treatment estimates

were back-transformed resulting in geometric mean treatment estimates corrected for potential differences in baseline values. Contrasts and 95% confidence intervals between treatments were back-transformed resulting in geometric mean ratios which were subsequently translated into percentage increase of the treatment relative to the placebo. All reported significant effects are mean treatment effects or significant contrasts between a certain dose and placebo.

### PHARMACOKINETIC/PHARMACODYNAMIC (PK/PD) ANALYSIS

The purpose of the PK/PD analysis was to support the selection of doses for the next group, and after part I to predict the plasma concentrations and effects when Org 26828 was administered as a 1 minute bolus instead of a 25 minute infusion. The effect of Org 26761 (active metabolite of Org 26828) plasma concentrations on heart rate was investigated since this parameter demonstrated a clear response to Org 26828, and provided information on potentially undesirable and clinically relevant effects. Individual empirical Bayes estimates for elimination half-life were determined for all occasions separately, and predicted individual Org 26761 concentration profiles were obtained using these estimates. Model choice was based on the goodness of fit test results.

Pharmacokinetic modelling was performed using SAS® Version 8.02, S-PLUS version 6.2 and NONMEM version V, level 1.1 software (NONMEM Project Group, UCSF, San Francisco, CA, USA) under Windows XP v5.1.

## Results

The available data of all withdrawn subjects were included in the analyses if they completed at least one study day.

### CLINICAL EFFECTS

After administration of Org 26828 (either as a slow 25 minute intravenous infusion or a 1 minute bolus dose) the most frequently reported adverse events were somnolence, dry mouth, dizziness, headache and nausea. These adverse events were of mild or moderate severity and disappeared rapidly. After slow infusion, the highest doses of Org 26828 3 and 6 μg/kg caused unpleasant central nervous system effects. These symptoms included for 3 μg/kg Org 26828 (n = 4): somnolence (4/4), dry mouth (4/4), paraesthesia (3/4), hyperacusis (2/4), visual hallucinations (1/4), inappropriate affect (1/4), hypaesthesia (1/4), involuntary muscle contractions (1/4), blurred vision (1/4), tremor (1/4) and feeling abnormal (1/4). These effects were seen in different subjects. For 6 μg/kg Org 26828 (n = 2) these symp-

toms were (all 1/2): anxiety, auditory hallucinations, inappropriate affect, feeling nervous, paranoia, disturbance in attention, involuntary muscle contractions, scotoma, visual disturbance and hyperacusis.

In group II three subjects discontinued the study after administration of Org 26828 3 µg/kg. All subjects completed their study day. Subject 7 was withdrawn from the study due to tachycardia (up to 151 beats per minute) and related symptoms of shortness of breath and angina pectoris. Subject 9 was also withdrawn because of tachycardia (up to 144 beats per minute). Subject 8 discontinued the study due to severe nausea and vomiting. Subject 10 suffered from a serious adverse event after infusion of Org 26828 6 µg/kg. The infusion was stopped after 25 minutes. His neuropsychiatric condition consisted of signs of anxiety, derealization and paranoia, bordering on psychosis. Subject 10 recovered fully within 3 days. All other subjects also recovered without sequelae within one day.

The most frequently reported adverse events for midazolam were mild in intensity and included somnolence (10/10) and dizziness (3/10). There were no clinically significant abnormal values in this trial for haematology, biochemistry and urinanalysis parameters.

None of the subjects fell asleep as determined by the modified Observer's Assessment of Alertness/Sedation (OAA/S) scale (score > 6) (Table 3) and therefore all infusions were stopped after 25 minutes when the full dose was administered. The lack of clearly detectable sedative effects after the highest infused dose of Org 26828 also refuted the use of midazolam (which was highly sedative as expected) as a positive control. Therefore, 1 minute bolus medication in part II (group IV) was restricted to Org 26828 or its placebo.

### PHARMACOKINETICS

Plasma Org 26761 concentrations were not detectable, or only slightly above the lower limit of quantification up to Org 26828 0.3 µg/kg. Therefore, these data were not included in the analysis. The pharmacokinetic parameters seemed proportional to dose. Maximum Org 26761 concentrations were reached at the end of the infusion indicating that steady state was not reached which is confirmed by the mean concentration versus time profiles during and after infusion (Figure 1). For the 25 minute infusion, the population mean (approximate SE of population mean ± SEM) of the elimination half-life was 1 hour (inter-individual coefficient of variance: 18-30%). The 1 minute bolus infusion of 1 µg/kg produced short-lasting detectable plasma concentrations, with an average maximum level of 1.8 ± 1.0 ng/mL (mean ± SD) at T = 2 minutes. Plasma concentrations rapidly became undetectable after T = 5 minutes, and the calculated exposure ($AUC_{0\text{-}inf}$) was more than 50% lower for the 1 minute bolus dose than for to the 25 minute infusion. No elimination half-life could be calculated for the bolus infusion.

During the serious symptoms of subject 10 after slow infusion of Org 26828 6 µg/kg, his maximum plasma concentration of Org 26761 was 7.9 ng/mL. The maximum-tolerated dose of 1 µg/kg after slow intravenous administration corresponded to a maximum plasma concentration of 1.4 ± 0.4 ng/mL (mean ± SD) at the end of the infusion.

### ECG, $SpO_2$ MONITORING, HEART RATE AND BLOOD PRESSURE

No clinically significant changes were observed in ECG recordings and $SpO_2$ monitoring. Compared to placebo, heart rate increased after slow 25 minute infusion of Org 26828 1 µg/kg (mean increase 12 bpm: 95% CI 6, 18), 3 µg/kg Org 26828 (+29 bpm: 95% CI 21, 37) and 6 µg/kg Org 26828 (+17 bpm: 95% CI 6, 28) (Figure 2). Figure 2 shows that peak increases reached values of +60 beats per minute or higher. Note that the average increase in heart rate is higher after Org 26828 3 µg/kg (n = 4) compared to 6 µg/kg (n = 2). In one of the subjects heart rate did not increase after infusion of 6 µg/kg (subject 10). Heart rate did not change after any 1 minute bolus dose of Org 26828 or after midazolam administration.

Systolic blood pressure decreased after slow 25 minute infusion of 1 µg/kg Org 26828 (-5 mm Hg: 95% CI -9, -1), 3 µg/kg Org 26828 (-10 mm Hg: 95% CI -16, -3) and 6 µg/kg Org 26828 (-12 mm Hg: 95% CI -19, -4). Diastolic blood pressure decreased after slow 25 minute infusion of 1 µg/kg Org 26828 (-6 mm Hg: 95% CI -10, -1) and 6 µg/kg Org 26828 (-11 mm Hg: 95% CI -19, -3). A slight decrease in systolic blood pressure was observed after midazolam administration (-4 mm Hg: 95% CI -8, -0). Diastolic blood pressure did not change. One minute bolus dose administration of Org 26828 did not change blood pressure in comparison with placebo.

### SACCADIC EYE MOVEMENT

Saccadic peak velocity did not change after administration of Org 26828. By contrast, midazolam decreased this parameter significantly (-52 deg/sec: 95% CI -69, -35).

### BODY SWAY

Postural stability was not assessed during the infusions. Compared to placebo, slow 25 minute infusion of Org 26828 induced a dose-related increase in body sway: Org 26828 3 µg/kg (+56%: 95% CI +19, +105). No results are available for Org 26828 6 µg/kg since there are only body sway data from one subject. Body sway did not change after administration of the four bolus doses of Org 26828. Body sway increased significantly after infusion of midazolam in comparison to placebo (+100%: 95% CI +68, +137).

## VISUAL ANALOGUE SCALES (VAS)

### OBSERVER'S ASSESSMENT OF ALERTNESS/SEDATION (OAA/S)

No significant changes have been observed on OAA/S after administration of any of the doses of Org 26828 (Figure 3). However, a higher score on OAA/S was observed after infusion of midazolam (+1.0: 95% CI +0.8, +1.2) (Figure 3). These values are mean treatment effects over the observation period, which explains the higher peak effect (+3.7) presented in Figure 3.

### VAS ALERTNESS

Slow 25 minute infusion of Org 26828 caused a dose-related decrease in VAS alertness in comparison to placebo. Significant lower scores were seen after slow 25 minute infusion of Org 26828 3 µg/kg (-16 mm: 95% CI -26, -5) and 6 µg/kg (-29 mm: 95% CI -43, -16) (Figure 4). No significant changes have been observed after administration of the four bolus doses. Compared to placebo a lower score was observed on VAS alertness after infusion of midazolam (-15 mm: 95% CI -22, -8) (Figure 4). These data indicate subjective sedation after slow infusion Org 26828 3 and 6 µg/kg, but not after 1 minute bolus administration of 0.1, 0.3, 0.6 or 1 µg/kg.

### VAS INTERNAL AND EXTERNAL PERCEPTION

The composite scores of 'internal perception' and 'external perception' were not changed by Org 26828 or midazolam. Nonetheless, Org 26828-induced changes were observed in several individual VAS Bowdle scales: 'feelings of unreality' (3 µg/kg +94%: 95% CI -4, +292; 6 µg/kg +260%: 95% CI +43, +808), 'difficulty in controlling thoughts' (3 µg/kg +176%: 95% CI +33, +470; 6 µg/kg +552%: 95% CI +144, +1646), 'changing colour intensity' (6 µg/kg +355%: 95% CI +79, +950), 'feeling high' (3 µg/kg +186%: 95% CI +37, +497; 6 µg/kg +369%: 95% CI +73, +1172) and 'feeling drowsy' (3 µg/kg +168%: 95% CI +11, +549; 6 µg/kg +368%: 95% CI +44, +1419).

### VISUAL VERBAL LEARNING TEST

Table 4 is a summary of the average scores on the Visual Verbal Learning Test. Compared to placebo, fewer words were learned during the midazolam occasion (average 7 words) than during placebo (average 18 words). This difference was roughly maintained during the consolidation phase of the memory test. During delayed recall, on average 3 words could be actively recalled after midazolam, compared to 15 words with placebo. The difference between placebo and midazolam during delayed recognition was 27 correctly recognized words compared to 19, respectively.

The memory effects of Org 26828 differed from midazolam. Retrieval of learned words was somewhat reduced by the highest two doses of Org 26828, albeit slightly less than with midazolam. This was also observed during delayed recall, which showed that Org 26828 1, 3 and 6 µg/kg caused a significant reduction of retrieved words in comparison with placebo.

During delayed recognition, fewer words were recognized after administration of Org 26828 3.0 and 6.0 µg/kg (14 and 16 words on average) and midazolam (average 19 words) than after placebo (27 words), which indicates that storage was slightly worse after Org 26828 compared to midazolam. No memory impairment was observed with doses up to Org 26828 0.3 µg/kg or after any bolus dose Org 26828.

### PHARMACOKINETIC/PHARMACODYNAMIC ANALYSIS

Analysis of concentration-effect relationships was limited to consistent dose-related responses, which turned out to be confined to heart rate increases during the occasions with slow infusion of Org 26828. Changes in heart rate were not directly related to plasma concentrations of Org 26761 and showed hysteresis. Heart rate increases were delayed relative to the plasma concentrations of Org 26761 by approximately ten minutes. Exposure ($AUC_{0\text{-}inf}$) was more than 50% lower for the 1 minute bolus dose regimen compared to the 25 minute infusion.

## Discussion

This study was performed to examine the effects of Org 26828 as a sedative for out-patient procedures, in comparison with midazolam, a benzodiazepine, that is often used in such situations. The properties of Org 26828 are similar to those of the CB1 agonist Org 28611 that were evaluated in a previous study with the same design.[11] In summary, at the maximum-tolerated dose Org 26828 did not induce observable sedation as observed on the OAA/S or anterograde amnesia. These effects differed conspicuously from the results after midazolam, which caused both objective and subjective sedation, clear impairment of memory and postural instability. The apparent differences between Org 26828 and midazolam after the slow infusion phase of the study (part I) made us decide to omit the benzodiazepine as a positive control from the 1 minute bolus infusion phase (part II).

Above the maximum-tolerated dose of 1 µg/kg Org 26828 caused anxiety, hallucinations, feeling abnormal, paraesthesia and involuntary muscle contractions. These effects were seen in various subjects, most severely in one subject, who suffered from signs of anxiety, derealization and paranoia, bordering on psychosis. The dose this subject received was 6 times higher than the maximum-tolerated dose of 1 µg/kg that was tolerated well by all

subjects. Only the slow infusion of Org 26828 produced psychological adverse effects, so this was unrelated to a rapid increase in plasma (or brain) concentrations. The dose is clearly relevant for the occurrence of psychological reactions. But the risk of these side effects may also be increased by the lack of control with the intravenous route, which contrary to inhalation, cannot be actively titrated by the subject. These observed symptoms are in contrast with the pleasant effects of relaxation and mild euphoria seen after recreational cannabis use, which in general will be titrated to avoid unpleasant side effects.

Although Org 26828 is not suitable as an intravenous sedative, it has favourable pharmacokinetic characteristics after intravenous administration that may support its use for other indications. Plasma concentrations increase linearly with dose, and its half-life of one hour is short enough for day-time procedures. The pharmacokinetics after 1 minute bolus administration of the maximum-tolerated dose of 1 µg/kg could not be determined accurately, because plasma levels rapidly decreased below the limits of quantitation. The 1 µg/kg bolus and slow infusions had equal doses, but the plasma concentration profiles differed. The maximum plasma concentrations did not differ much (bolus: 1.8 ng/mL and slow infusion: 1.4 ng/mL on average), but plasma concentrations became undetectable a few minutes after the 1 minute bolus dose. In contrast, plasma levels fluctuated around 1 ng/mL throughout the 25 minute period of the slow infusion (Figure 1). These differences are most likely caused by the fact that the fast distribution processes after the 1 minute bolus are difficult to identify, and the first blood sample at T = 2 minutes may actually have missed a much higher peak plasma concentration. As a result, the area under the plasma concentration curve (AUC) was roughly twice as large with the slow infusion than with the bolus despite equal doses. This larger AUC may explain why a slow infusion of 1 µg/kg caused some memory impairments and heart rate increases, which did not reach statistical significance with the bolus infusion of the same dose. The therapeutic consequences of this difference (if any) remain to be established, but it would suggest that a dose will be better tolerated with a bolus administration than after a slow infusion.

An increase in heart rate was the most clearly dose- and concentration-related effect of Org 26828 in this study. The cardiovascular mechanisms of cannabinoids are complex and cannot be explained by a single mechanism. An additional difficulty is that no good animal model is available to study their cardiovascular effects. In contrast to humans, bradycardia is seen in animals after THC[1] or Org 26828 administration (unpublished data). The cardiovascular effects of Org 26828, Org 28611 and THC do not differ from each other.[10,11,13,14] In humans, an increase in heart rate of 20-60% within 15 minutes after administration is one of the most reliable effects of THC and this effect lasts for up to 3 hours.[10,13,14] Similar dose-related changes in heart rate were observed after infusion of Org 26828 (≥1 µg/kg). This was

accompanied by a slight decrease in blood pressure. This suggests that at least a part of the increase in heart rate is caused by vasodilatation with reflex tachycardia in young healthy males. A direct activation of heart rate cannot be excluded however, either directly on the heart or autonomically by vagal inhibition and/or sympathetic stimulation.[15] The increase in heart rate seems to be related to CB1 receptor stimulation since AVE1625, a selective CB1 antagonist, completely antagonizes THC-induced tachycardia in humans,[16] although CB2 receptors may play a modulating or permissive role.[17] For the development of cannabinoids in general it is worthwhile to unravel the complex cardiovascular mechanisms in humans since cardiovascular side effects (tachycardia, hypotension) are a limitation for their therapeutic use. Although the cardiovascular effects at the maximum-tolerated dose of Org 26828 can be considered minor in young healthy males, these might adversely affect elderly patients with cardiovascular comorbidity.

## SUMMARY

This first in human study revealed that at the maximum-tolerated dose (1 µg/kg) Org 26828 is not suitable for providing sedation for outpatient treatments. Org 26828 does not induce anterograde amnesia and at higher doses it can cause untoward psychological effects. However, the compound is an infusible CB1 agonist with favourable pharmacokinetic properties. One minute bolus doses up to 1 µg/kg or mean plasma concentrations up to 1.4 ng/mL lack most of these adverse effects. This makes it worthwhile to further explore the therapeutic (e.g. analgesic or anti-emetic) potential of Org 26828.

**Figure 1** Mean (SD) observed time profile of plasma Org 26761 (ng/mL) during and after 25 minute infusion of Org 26828 (group I and II). For unknown reasons there is a drop in plasma concentration 3 minutes before (3 µg/kg, n = 4) and 1 minute after (6 µg/kg, n = 2) the stop of the infusion. T = 0 corresponds to the start of the infusion. The arrow indicates the stop of the infusion at T = 25 minutes.

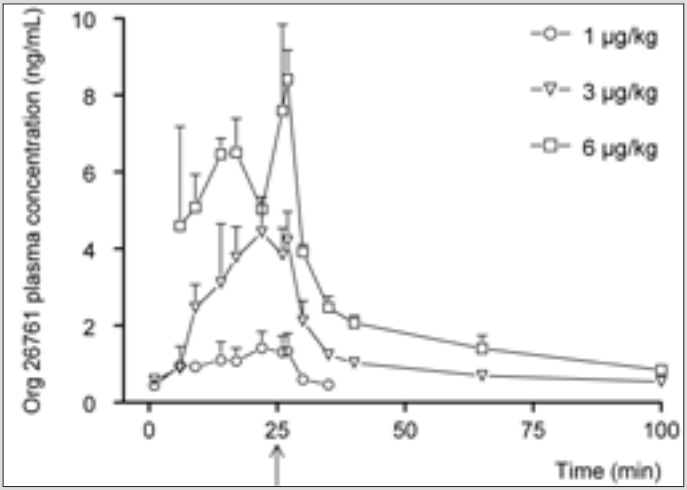


**Figure 2** Mean (SD) time profile of heart rate during and after 25 minute infusion of Org 26828 (group I and II). T = 0 corresponds to the start of the infusion. The arrow indicates the stop of the infusion at T = 25 minutes.

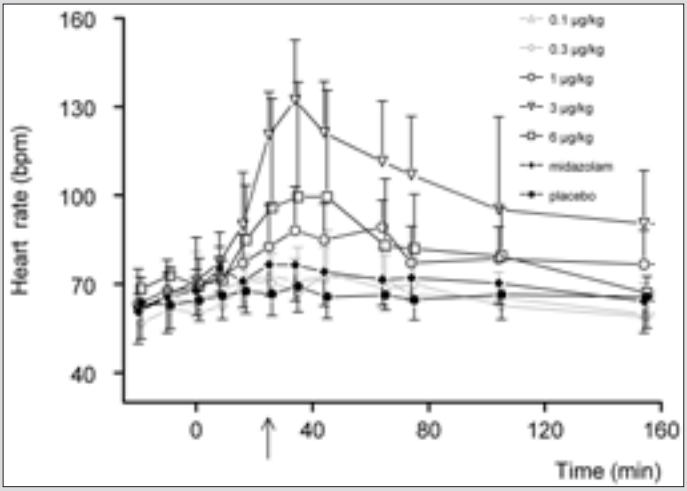

**Figure 3** Mean (SD) time profile of Observers Assessment of Alertness/ Sedation during and after 25 minute infusion of Org 26828 (group I and II). T = 0 corresponds to the start of the infusion. The arrow indicates the stop of the infusion at T = 25 minutes.

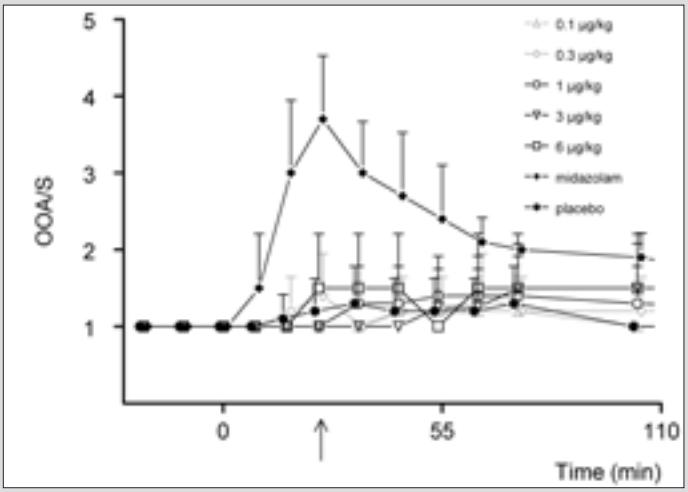


**Figure 4** Mean (SD) time profile of VAS alertness during and after 25 minute infusion of Org 26828 (group I and II). T = 0 corresponds to the start of the infusion. The arrow indicates the stop of the infusion at T = 25 minutes.

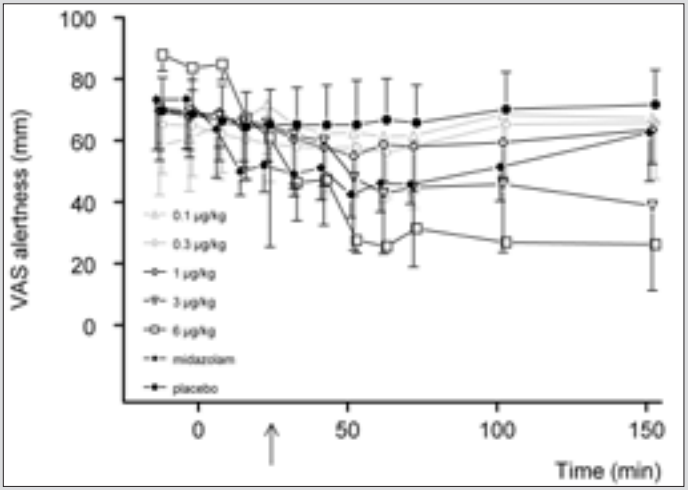

**Table 1** Study design of part I (25 minute infusion) and part II (1 minute bolus). D1-3 and Da-d represent Org 26828, P placebo and M midazolam administration.

| **Part I:** Group I and II (25 minute infusion) | | | | | **Part II:** Group III (1 minute bolus) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Mo | Tue | Wed | Thur | Fri | Mo | Tue | Wed | Thur | Fri |
| M | D1 | D2 | D3 | P | Da | Db | Dc | Dd | P |
| D1 | M | D2 | P | D3 | Da | Db | Dc | P | Dd |
| D1 | D2 | P | M | D3 | Da | Db | P | Dc | Dd |
| D1 | P | M | D2 | D3 | Da | P | Db | Dc | Dd |
| P | D1 | D2 | D3 | M | P | Da | Db | Dc | Dd |

**Table 2** Doses Org 26828 administered during part I (maximally 25 minute infusion) and part II (1 minute bolus). Number between the brackets is the number of subjects dosed.

| | **Part I** | | **Part II** |
|---|---|---|---|
| | Group I | Group II | Group III |
| **Org 26828 (µg/kg)** | 0.1 (5) | 1.0 (5) | 0.1 (5) |
| | 0.3 (5) | 3.0 (4) | 0.3 (5) |
| | 1.0 (5) | 6.0 (2) | 0.6 (5) |
| | | | 1.0 (5) |

**Table 3** Modified Observer's Assessment of Alertness/Sedation Score

| | |
|---|---|
| 1 | awake, orientated |
| 2 | reports feeling drowsy, quick reaction verbal stimulus, clear look, normal speech |
| 3 | slow reaction to verbal stimulus |
| 4 | inability to perform 2 saccades correctly, lethargic response to verbal stimulus, glazed look, slowing of speech |
| 5 | reacts to soft touch and repeated verbal stimulus |
| 6 | reacts to repeated loud verbal stimulus, glazed look and ptosis, muscle relaxation, slurred speech |
| 7 | reacts to non-painful stimulus |
| 8 | no reaction to non-painful stimulus, eyes closed, few recognizable words |
| 9 | reacts to painful stimulus |
| 10 | no reaction to painful stimulus |

**Table 4** Average number of correct words after different treatments on the Visual Verbal Learning Test. Bold numbers, along with 95% CI, indicate a significant result compared to placebo.

| Treatment | Storage (working memory and learning) Immediate recall | | | Retrieval Delayed recall | Consolidation Delayed recognition |
|---|---|---|---|---|---|
| | Recall 1 | Recall 2 | Recall 3 | | |
| placebo | 9 | 14 | 18 | 15 | 27 |
| midazolam | **5 (-4: -6,-2)** | **6 (-8: -10,-5)** | **7 (-11: -14, -8)** | **3 (-12: -15, -10)** | **19 (-7: -10, -4)** |
| Org 26828 0.1 µg/kg | 7 (-2: -5, +1) | 16 (+2: -2, +5) | 18 (+0: -4, +4) | 14 (-1: -5, +3) | 28 (+2: -3, +6) |
| Org 26828 0.3 µg/kg | 10 (+2: -1, +4) | 14 (-0: -4, +3) | 18 (+1: -3, +4) | 15 (-0: -4, +4) | 24 (-3: -7, +2) |
| Org 26828 1.0 µg/kg | 7 (-2: -4, +1) | **11 (-3: -6, -0.4)** | 16 (-2: -5, +1) | **12 (-3: -6, -0.3)** | 26 (-1: -4, +3) |
| Org 26828 3.0 µg/kg | 7 (-2: -5, +1) | 11 (-3: -7, +0) | **12 (-6: -10, -1)** | **10 (-5: -9, -0.5)** | **16 (-11: -15, -6)** |
| Org 26828 6.0 µg/kg | **5 (-4: -8, -0.4)** | **7 (-7: -12, -2)** | **10 (-8: -13, -2)** | **4 (-11: -17, -6)** | **14 (-13, -10, -4)** |

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# 9 Summary and conclusions

## Effects of cannabinoids on the central nervous system

The development of novel cannabinoids as medicine is gaining more and more attention (**chapter 1**). Nevertheless we are just at the beginning of their clinical development. In this thesis the clinical pharmacology of some cannabinoids in early phase drug development is described.

Biomarkers are useful tools to study drug effects since they can provide information on the potential therapeutic effects of the investigational drug in early phase drug development. **Chapter 2** describes a systematic literature review that assesses the usefulness of direct biomarkers for the effects of cannabis and delta-9-tetrahydrocannabinol (THC) (a CB1/CB2 agonist and the main psychoactive ingredient of cannabis) in healthy volunteers. Hundred and sixty-five useful articles were found that investigated the acute effects of cannabis or THC on the central nervous system (CNS) and heart rate in healthy volunteers. Three hundred and eighteen test and test variants were grouped in test clusters and functional domains, to allow their evaluation as a useful biomarker and to study their dose response effects. The number of tests and test variants that was used seems excessively large. This abundance thwarts a good assessment of the physiological, neuropsychological and subjective effects of this drug class, and there is a dire need for test standardisation in these areas. In general, the doses studied in the literature reflect the patterns of recreational use, and are often too high to accurately determine dose-response relationships. THC/cannabis has an effect on a wide range of central nervous system domains. Dose response relationships were found for only a few clusters (e.g. auditory/verbal delayed recall). For some CNS functions inverse dose-response relationships were found, particularly those that are susceptible to concentration and attention (e.g. working memory and motor control). Compared to lower doses, higher doses were also associated with lower scores of subjective calmness and somewhat higher scores for anxiety and aggression. At lower doses THC/cannabis seems to be relaxant, but at high doses the drug seems to be more stimulatory. Subjective effects and heart rate are currently the most reliable biomarkers to study the effect of cannabis, showing significant responses to cannabis in almost all studies. Hopefully, this review will facilitate a rational selection of CNS tests in future studies of THC/cannabis and other cannabinoid agonists.

## Development of a novel THC administration mode

An increasing number of novel drugs in development are targeted at cannabinoid receptors, although their exact role in health and disease has not been fully elucidated. CB1/CB2 agonists might be of therapeutic use for muscle relaxation, immunosuppression, sedation, improvement of mood, neu-

roprotection, analgesia, and reduction of intra-ocular pressure.[1] Recently rimonabant, a CB1 antagonist, was registered for the treatment of obesity. CB1 antagonists might also be useful for the treatment of smoking cessation, Parkinson's disease, and cognitive impairments in Alzheimer's disease and schizophrenia.[1] The CB1 antagonist rimonabant seems to be devoid of acute measurable central nervous system effects.[2] Proof of pharmacological action of CB1 antagonists in the brain like AVE1625 can be given by antagonizing the effects of THC, a CB1/CB2 agonist. This approach needs a reproducible and practical mode of THC administration with a reliable pharmacokinetic and pharmacodynamic time profile. **Chapter 3 and 4** describe the development of a new and useful method for studying the central nervous system effects of CB1 antagonists. Usually, cannabis or THC is orally administered or inhaled by smoking a cigarette. However, oral administration has unfavorable characteristics, such as limited and variable bioavailability[3-5] and smoking has the disadvantage that it contains a mixture of psychoactive and partly noxious compounds, and that the active drug is partly lost by heat. Intravenous administration would overcome the disadvantages of oral administration or smoking a cigarette. However, adequate injection fluids are difficult to manufacture due to the highly lipophilic properties of THC. In this thesis pure THC was administered by inhalation using a Volcano® vaporizer (**chapter 3-6**).

The effect of THC on different CNS and non-CNS tests was investigated (**chapter 3**). Like cannabis, pure intrapulmonary THC administration affects the same neurophysiological domains in healthy volunteers as revealed in the systematic review of cannabis studies described in **chapter 2**. Postural stability, a number of subjective parameters and heart rate showed dose-dependent effects after administration of repeated doses of 2, 4, 6 and 8 mg THC. The sensitive subjective parameters included in particular 'alertness' of the Visual Analogue Scales (VAS) of Bond and Lader; the newly derived 'external perception' scale, which is a composite subscale of Bowdle's VAS for psychedelic effects, and the VAS scale for 'feeling high'. Alertness is closely related to the ability to pay attention, to concentrate on a specific issue, and attention deficit is a well-known acute effect of cannabis.[1] The changes in the 'external perception' reflect a misperception of an external stimulus or a change in the awareness of the subject's surroundings. This is also a well-known effect of THC,[6] making the composite scale of 'external perception' a useful tool for assessing the effects of THC. Limited changes were seen on 'internal perception', which reflects inner feelings not corresponding with reality. In this study no dose-dependent changes were seen in saccadic eye movements, smooth pursuit and adaptive tracking performance. This correponds to the findings on these parameters in the systematic literature review (**chapter 2**).

Pharmacokinetic characterization of the plasma concentration profile of THC and its major metabolites 11-OH-THC and 11-nor-9-COOH-THC are

described in **chapter 4**. Additionally, the concentration-effect relationship of THC and its effects on heart rate, body sway and Visual Analogue Scales (VAS) for alertness, 'feeling high' and 'external perception' were investigated using an integrated modelling approach: pharmacokinetic-pharmacodynamic (PK/PD) modelling. The PK/PD models can be used for the prediction of THC concentration and effect profiles, to optimize entirely different trial designs and dosing regimes. The models also provided information about different peripheral and central cannabinoid systems, which suggests different sites of action and/or different physiological mechanisms, which can be described quantitatively with different PK/PD modelling parameters. The development of such pharmacokinetic and pharmacokinetic-pharmacodynamic models and their applications are described in **chapters 3-6**.

In **chapter 1**, tachycardia was found to be one of the most reliable pharmacodynamic effects of cannabis, but its mechanism is unexplained. Heart rate variability (HRV) analyses can provide information concerning effects of drugs on parasympathetic and sympathetic tone. **Chapter 5** evaluates the sympathovagal balance in THC-induced tachycardia using HRV analysis. An indirect and peripheral mediated regulatory mechanism is probably involved in THC-induced tachycardia. In addition, co-administration of the selective CB1 antagonist AVE1625 confirms the involvement of CB1 receptors in THC-induced tachycardia and suggest that the increase in heart rate caused by acute THC administration may be caused by a peripheral mediated reduction in the vagal tone. These studies were not conclusive in the mechanism by which THC induces tachycardia.

## Inhibition of THC-induced effects with a selective CB1 antagonist

The results of the studies performed in **chapters 3-5** were used to study the effects of the selective CB1 antagonist AVE1625 (**chapter 6**). Evidence of pharmacological action of the selective CB1 antagonist AVE1625 in the brain can be obtained by antagonizing the effects of THC. Inhibition of THC-induced effects on different central nervous system parameters and heart rate was observed following AVE1625 administration with doses at or above 20 mg. Evidence of pharmacological action of AVE1625 in the brain was given by its capability to antagonize THC-induced effects, while AVE1625 did not have any objective measurable central nervous system effects by itself. PK/PD modelling was not possible since the lowest dose of AVE1625 20 mg almost completely antagonized the effects induced by THC. Even a lower dose would likely have caused significant if not complete inhibition as well. It is not very likely that the cannabinoid system in any disease state is stimulated as much as by THC. These findings therefore suggest that an efficacious dose of AVE1625 may be less than the lowest dose of AVE1625

20 mg used in this study. This approach assisted in the determination of a therapeutic dose of AVE1625 for subsequent phase II studies.

## Development of novel intravenous cannabinoid agonists

Cannabis has sedative, amnestic and analgesic effects.[1,7,8] CB1/CB2 agonists with a combination of those properties may be useful in outpatient surgical procedures. Other useful properties for short treatments under conscious sedation are postural stability, appreciated subjective effects and no respiratory or cardiovascular effects. In **chapter 7 and 8** the pharmacodynamic properties of two similar, but not identical novel CB1 agonists, Org 28611 and Org 26828, were evaluated during a first in human administration. Org 26828 and its active metabolite Org 26761 are structurally unrelated to Org 28611 but are related to THC. Midazolam (a benzodiazepine) has sedative and amnestic properties and is frequently used in outpatient surgical procedures and was therefore used as a positive control in these two studies.

Preclinical data suggest that Org 26828 is three times as potent as Org 28611. This is accurately reflected in the maximum-tolerated dose of 1 µg/kg and 3 µg/kg respectively, the accompanying plasma concentrations and the pharmacodynamic effects. The average plasma concentrations of the maximum-tolerated dose of Org 28611 (4 ng/mL) exceeded the levels of Org 26828 (1.4 ng/mL) by a factor of three. Although the half-life of Org 26828 was much shorter (one hour) in comparison to Org 28611 (3.5-5.5 hours), the pharmacodynamic effects were similar and lasted equally long.

Although the pharmacodynamic properties of Org 26828 are similar to the properties of Org 28611 after intravenous infusion, their pharmacodynamic and pharmacokinetic profiles differ from midazolam. The bolus dose was not administered above the maximum-tolerated dose of both compounds, and no effects were observed after bolus administration. Therefore, the comparison below refers to the slow intravenous infusion. At the maximum-tolerated dose both compounds did not cause the same type of 'conscious sedation' as midazolam. Midazolam induced conscious sedation within 10 minutes, which lasted for about 30-45 minutes. In contrast to midazolam, Org 28611 and Org 26828 showed a discrepancy between subjective and objective sedation. The VAS alertness, an indication for subjective sedation, showed significantly lower scores after infusion of Org 28611 (>1 µg/kg) and Org 26828 (>3 µg/kg). However, subjects were awake and reacted quickly to verbal stimuli as observed on the Observers Assessment of Alertness/Sedation (OAA/S) scale. Subjects also reported a clear difference in the character of the sensation: midazolam caused drowsiness, whereas Org 28611 and Org 26828 induced feelings of tiredness. In addition, both cannabinoids did not change saccadic peak velocity movements, an objec-

tive measure of sedation for benzodiazepines.[9,10] These two compounds are therefore not suitable for providing sedation for day-care surgical procedures.

A well-known characteristic of midazolam during outpatient surgical procedures is anterograde amnesia. This is useful, because the patient does not remember much of the procedure. The memory effects of Org 28611 and Org 26828 differed from midazolam. Retrieval of learned words was somewhat reduced at the highest doses, albeit slightly less than with midazolam. This was also observed during delayed recall, which showed that Org 28611 (6 and 10 µg/kg) and Org 26828 (1, 3 and 6 µg/kg) caused a significant reduction of retrieved words in comparison with placebo. However, in contrast to midazolam, delayed recognition was not changed at the maximum-tolerated dose Org 28611 (3 µg/kg) and Org 26828 (1 µg/kg). This indicates that information is still stored in the presence of CB1 agonists, but that it cannot be adequately recuperated. This seems a less desirable effect in case of traumatic events like surgery. Midazolam interfered with both storage (delayed recognition) and retrieval (immediate and delayed recall).

No cardiovascular effects of midazolam 0.1 mg/kg were observed in this thesis (**chapter 7 and 8**), although it has been described that higher doses of midazolam decrease blood pressure and increase heart rate.[11] In general the cardiovascular effects of Org 28611 and Org 26828 in rodents were comparable to the effects of THC,[12] the reference compound in the pre-clinical studies of Org 28611 and Org 26828. In humans, an increase in heart rate is one of the most reliable effects of THC (**chapter 2**). Similar changes in heart rate (16-17%) were observed after infusion of Org 28611 (>6 µg/kg) and Org 26828 (>1 µg/kg). However, no changes in heart rate were observed after bolus administration of Org 28611 (≤3 µg/kg) and Org 26828 (≤1 µg/kg). The effects of THC on blood pressure are complex, both increases and decreases in blood pressure have been reported[13-15] through unknown mechanisms. After administration of Org 28611 and Org 26828 no changes in blood pressure were observed.

Although Org 28611 and Org 26828 do not induce conscious sedation, there is a possibility that this will occur at even higher doses than administered in these studies (**chapter 7 and 8**). However, higher doses were precluded by unpleasant psychiatric and central nervous system effects that were observed above the maximum-tolerated dose administered by intravenous infusion of Org 28611 and Org 26828. They may cause anxiety, paranoia, hallucinations, derealization, feeling abnormal, altered body perception, paraesthesia and involuntary muscle contractions. These effects were seen to a different extent in all subjects, most severely in one subject in each study who suffered from a serious adverse psychiatric event. These observed symptoms are in contrast to the pleasant effects of relaxation and mild euphoria seen after recreational cannabis use or intrapulmonary

THC administration described in this thesis. This raises the possibility that the compounds were pharmacologically dissimilar.

## Comparison of the CB1/CB2 agonists THC, Org 28611 and Org 26828

Preclinically, THC, Org 28611 and Org 26828 are all CB1/CB2 agonists. This would suggest that, depending on the dose, they also show similar pharmacodynamic effects. Org 28611 and Org 26828 were studied using the same design (**chapter 7 and 8**), but the study in which pure THC was administered by inhalation had a completely different design (**chapter 3**) than the intravenous CB1/CB2 studies. A statistical comparison was therefore not possible. Nonetheless, an indication of the relationships in pharmacodynamic effects can be obtained by mutual comparisons of the pharmacodynamic effects. Compounds from a similar drug class are expected to have similar proportional effects on different CNS parameters. At first sight, there were differences between the compounds. As shown in Table 1, high doses of Org 28611 and Org 26828 caused negative effects on VAS mood and calmness and limited blood pressure reductions, which were not found with THC. At least for the central effects, these differences seem to reflect the different side effect profiles. The other CNS pharmacodynamic effects were compared using graphs as presented in Figure 1. In these graphs one average pharmacodynamic effect is plotted against another pharmacodynamic effect. Compounds from the same pharmacological class are expected to produce similar effect relationships. Heart rate, body sway and VAS alertness were determined in each study, and changed significantly after administration of THC, Org 28611 and Org 26828 (Table 1). Although the size of the effect differ considerably among THC, Org 28611 and Org 26828; their effect relationships essentially run parallel to eachother. This indicates that these compounds share similar pharmacological properties and probably belong to the same cannabinoid class. This supports the view that the conspicuous differences in subjective effects were not related to pharmacological differences. Other potential causes lie in differences in dose, subject selection and route of administration.

The doses of the three cannabinoids cannot be directly compared, but in each case they seemed to have been (close to) maximum. The effects of Org 28611 and Org 26828 were both examined during the first in human administration (**chapter 7 and 8**). For both compounds, the maximum-tolerated dose was encountered during these studies. Above the maximum-tolerated dose of Org 28611 and Org 26828, the previously mentioned unpleasant effects were observed. Although THC was also administered in an escalating dose design, the doses were expected to cause pronounced but well-known and well-tolerated effects. The administered THC doses were close to the

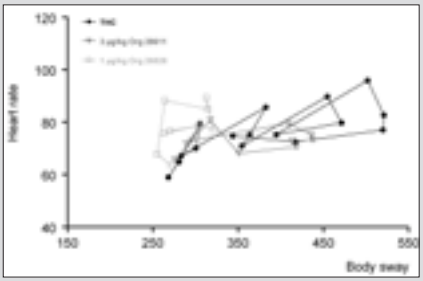
maximum-tolerated dose, since two out of twelve subjects experienced side effects severe enough to decide not to administer the last dose of 8 mg THC (**chapter 3**). One of these subject was too sleepy to perform any test, and the other subject vomited just after administration of the third dose, but no psychiatric events occurred. In general, pure intrapulmonary THC administration induced similar pleasant effects of relaxation and mild euphoria seen after recreational cannabis use. The side effect pattern of THC differed considerably from the two novel CB1/CB2 agonists Org 28611 and Org 26828.

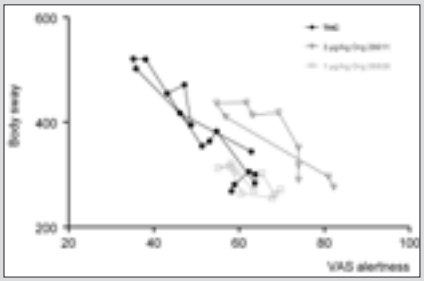
As mentioned above, a second reason for the observed differences in side effects may lie in differences in the subject population. Pure THC was intrapulmonary administered to mild cannabis users with an average use of twice a month (**chapter 3**), while Org 26828 and Org 26828 were administered to subjects whose life-time use did not exceed five times (**chapter 7 and 8**). Literature is not consistent in reporting kinetic differences between users and non or infrequent users.[16-19] This leaves pharmacodynamic sensitivity as a potential explanation, but a general increase in drug responsiveness it not very likely. The pharmacodynamic effect relationships of the three compounds shown in Figure 1 actually indicate that the effects of THC were larger than for the synthetic cannabinoids. This would indicate that THC users were actually more sensitive, which does not agree with their lack of psychiatric side effects. It cannot be excluded that mild or limited users differed in some unknown sensitivity to the psychiatric effects of cannabinoids, which was not measured in these studies. However, the most conspicuous difference between the studies was the route of administration.

The route of administration might be a good explanation for the observed unpleasant effects after intravenous administration of Org 28611 and Org 26828. Similar undesirable effects have been observed after intravenous administration of THC.[9,10] Both intravenous and intrapulmonary administration cause a rapid onset of cannabinnoid effects. In this thesis pure THC administration was performed using a strict inhalation procedure, meaning that the volume of the balloon had to be inhaled in three to four subsequent breaths. Intrapulmonary administration allows for an effect titration and consequently an avoidance of unpleasant exposure levels (and maintenance of pleasant 'high' effects). In contrast, subjects lose the ability to control the dose with intravenous administration. However, it is debatable if inhalation differences are fully responsible for the striking contrast between the observed effects. An unexplained mechanism might be involved in the observed differences between intrapulmonary and intravenous administration. Nevertheless, our findings suggest that the observed unpleasant effects of Org 28611 and Org 26828 are related to the intravenous route of administration of these cannabinoids. Development of patient friendly (partly self-titrated) formulations are worth investigating, if cannabinoids hold their promise as medicine for various indications.

Summarizing, this thesis describes useful cannabinoid biomarkers, which can be of value in early drug development. A reproducible, practical and well-tolerated mode of intrapulmonary THC administration with reliable pharmacokinetic and pharmacodynamic time profiles was described. Accurate pharmacokinetic/pharmacodynamic models were composed, which allow quantitative assessments of endogenous CB1/CB2 systems, and optimization of THC-based study designs. These results were used to confirm the pharmacological effects of a selective CB1 antagonist AVE1625, which led to a reduction of the anticipated therapeutically active dose. In addition, sedative and amnestic properties of two similar, but not identical novel intravenous CB1 agonists, Org 28611 and Org 26828, were evaluated. These compounds did not produce the expected sedation and relaxation that would make them suitable for development in anaesthesia. The compounds are now in development for other indications. Comparisons with THC suggested that the route of administration is a decisive factor in causing unpleasant central nervous system effects. This could have an impact on the desirable galenic and pharmacokinetic properties of new cannabinoid agonists. The studies in healthy volunteers and the models presented in this thesis have been very useful for the early development of different cannabinoids as medicines.

**Figure 1** The different relationships of heart rate versus body sway, heart rate versus VAS alertness and body sway versus VAS alertness for the different compounds (THC, Org 28611 and Org 26828).



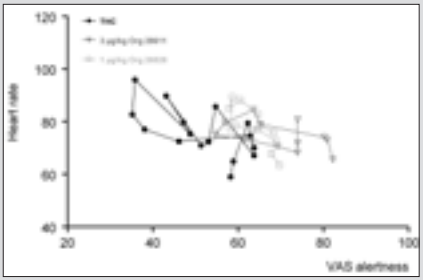




**Table 1** Pharmacodynamic measurements performed after THC, Org 28611 and Org 26828 administration. Blue cells indicate statistically significant changes. Dark cells indicate parameter not measured.

| TEST | THC | Org 28611 (µg/kg) | | | | Org 26828(µg/kg) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 3 | 6 | 10 | 0.3 | 1 | 3 | 6 |
| Heart rate | | | | | | | | | |
| Systolic Blood pressure | | | | | | | | | |
| Diastolic Blood pressure | | | | | | | | | |
| EEG | | | | | | | | | |
| Saccadic peak velocity | | | | | | | | | |
| Body sway | | | | | | | | | |
| VAS alertness | | | | | | | | | |
| VAS calmness | | | | | | | | | |
| VAS mood | | | | | | | | | |
| VAS external perception | | | | | | | | | |
| VAS internal perception | | | | | | | | | |
| VAS feeling high | | | | | | | | | |

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# 10 Nederlandse inleiding, samenvatting en conclusies

## Van cannabis plant tot medicijn

Dit proefschrift beschrijft de klinische farmacologie (werking van geneesmiddelen bij de mens) van een aantal cannabinoïden in de vroege fase van de geneesmiddelenontwikkeling. De term cannabinoïd refereert naar chemische stoffen die qua structuur lijken op TetraHydroCannabinol (THC) of die binden aan cannabinoïd receptoren waardoor de effecten in het menselijke lichaam tot stand komen. THC is de belangrijkste psychoactieve stof in cannabis en zorgt voor het gevoel van 'high' zijn. Cannabis is vooral bekend vanwege het recreatieve gebruik als soft drug waarbij met name de ontspannende en euforische eigenschappen op prijs worden gesteld. Door de eeuwen heen is cannabis ook veel gebruikt als medicijn voor diverse aandoeningen. Sinds 1 september 2003 is het mogelijk om medicinale cannabis op doktersrecept in de apotheek te verkrijgen. Dokters kunnen cannabis voorschrijven voor de behandeling van spasticiteit met pijn (bv. bij multiple sclerosis en ruggemergletsel), misselijkheid en braken (veroorzaakt door chemo- of radiotherapie of door HIV medicijnen), chronische neuropathische pijn, de ziekte van Gilles de la Tourette en voor de palliatieve behandeling van kanker en HIV/AIDS. Niet alleen in de geneeskunde, maar ook in de industrie wordt de cannabis plant gebruikt: de vezels voor het maken van touw, kleding en papier en de zaden voor het maken van zeep en olie. Henry Ford heeft zelfs een auto gemaakt met cannabis als grondstof (zie figuur 1 in hoofdstuk 1). Een algemene introductie over cannabis, de ontdekking van het endocannabinoïd systeem (het systeem van cannabisachtige stoffen in het menselijke lichaam) en de ontwikkeling van cannabisachtige stoffen als medicijn kunt u lezen in hoofdstuk 1.

## Welke testen zijn geschikt voor cannabis en THC studies? *Een literatuurstudie*

Bij het geneesmiddelenonderzoek is de keuze van de testen die tijdens het onderzoek worden afgenomen erg belangrijk. Deze testen worden biomarkers genoemd en kunnen belangrijke informatie geven over de werking van het potentiële geneesmiddel. Al jaren wordt er veel onderzoek gedaan naar de effecten van cannabis en THC, maar welke testen het meest bruikbaar zijn om de effecten van cannabisachtige stoffen te bestuderen is niet duidelijk. Hoofdstuk 2 beschrijft een groot literatuuronderzoek naar de acute effecten van cannabis en THC op het centrale zenuwstelsel in gezonde mensen. Een dergelijk literatuuronderzoek kan duidelijk maken welke testen een duidelijke en consistente respons laten zien na toediening van cannabis of THC en welke niet. Een toename van de hartslag is een van de bekendste effecten van cannabis en is daarom ook meegenomen in het literatuuronderzoek. Er werden 165 artikelen gevonden die de acute effecten van can-

nabis en THC bestudeerden en die voldeden aan de selectiecriteria. In deze artikelen werden 318 verschillende testen en testvarianten gebruikt. Om een uitspraak te kunnen doen over welke test gekozen zou moeten worden om het effect van een cannabisachtige stof te bestuderen, werden vergelijkbare testen gegroepeerd in groepen (clusters) en hun bijbehorende functionele domeinen. Het functionele domein 'geheugen' bestaat bijvoorbeeld uit de clusters korte termijn geheugen, werkgeheugen, leren, enzovoorts. Uit deze literatuurstudie blijkt dat cannabis en THC overal in de hersenen uiteenlopende effecten hebben, hetgeen overeenkomt met het feit dat cannabinoïd receptoren wijdverspreid zijn in de hersenen. Een toename van de hartslag was het meest consistente effect na toediening van cannabis of THC. In bijna alle studies waarin de hartslag is gemeten werd een statistisch significante toename gevonden. Daarnaast blijken subjectieve parameters (bijvoorbeeld zich 'high' voelen, agressief of ontspannen zijn of de mate van verlangen naar cannabis) goed bruikbaar voor het meten van de effecten van cannabis en THC. Er werden teveel verschillende testen gebruikt om een duidelijke uitspraak te kunnen doen over de vraag of de effecten toenamen met hogere doseringen.

## Het endocannabinoïd systeem in het menselijke lichaam

Het endocannabinoïd systeem omvat cannabisachtige stoffen en cannabisreceptoren in het menselijke lichaam. Tot op heden zijn er twee cannabinoïd receptoren bekend, namelijk de cannabinoïd type 1 receptor (CB1) en cannabinoïd type 2 receptor (CB2). Het effect van THC, een CB1/CB2 agonist, treedt onder andere op door binding aan deze receptoren. Momenteel ontwikkelen farmaceutische bedrijven stoffen die cannabis receptoren stimuleren (CB1/CB2 agonisten) of juist blokkeren (CB1/CB2 antagonisten). CB1 antagonisten zijn mogelijk bruikbaar voor de behandeling van vergevorderde ziekte van Parkinson, schizofrenie of geheugenstoornissen die voorkomen bij de ziekte van Alzheimer. CB1 antagonisten kunnen mogelijk ook van nut zijn bij de behandeling van overgewicht door middel van het remmen van de eetlust of ter ondersteuning bij het stoppen met roken. CB1 agonisten kunnen ingezet worden voor de behandeling van neuropatische pijn of glaucoom (hoge oogboldruk).

## Onderzoek van een CB1 antagonist

Rimonabant is een CB1 antagonist en wordt voorgeschreven bij de behandeling van zwaarlijvigheid. AVE1625 is een CB1 antagonist die selectief bindt aan de CB1 receptor. Eerdere studies bij mensen lieten zien dat deze stof geen duidelijk meetbare effecten had op het centrale zenuwstelsel. De ef-

fecten van AVE1625 op het centrale zenuwstelsel kunnen zichtbaar gemaakt worden door gezamenlijke toediening met THC. AVE1625 blokkeert de CB1 receptor waardoor THC (een stimulerende stof) geen effect heeft op de CB1 receptor.

## De effecten van THC en een nieuwe manier van toedienen

Om de interactie tussen THC en AVE1625 te kunnen bestuderen is eerst een studie uitgevoerd waarin de effecten van THC op het centrale zenuwstelsel en het hart bestudeerd zijn (hoofdstuk 3-5). In deze studie is een nieuwe inhalatie methode gebruikt en gevalideerd. Er is gebruik gemaakt van een apparaat waarmee THC verdampt kan worden, de Volcano® vaporizer. Met een interval van 90 minuten werden op de ene studiedag oplopende doseringen van THC (2, 4, 6 en 8 mg) toegediend. Op de andere studiedag werd steeds een placebo toegediend. De toegediende hoeveelheid THC komt ongeveer overeen met het roken van 1-2 cannabis sigaretten. In totaal hebben 12 gezonde, jonge, mannelijke vrijwilligers meegedaan aan de studie.

Toediening van THC veroorzaakt de bekende effecten van cannabis. De meest gevoelige parameters voor THC waren hartslag en subjectieve effecten. Deze subjectieve effecten werden gemeten met vragenlijsten zoals je 'high' voelen, mate van alertheid, interne en externe perceptie. Deze laatste twee zijn nieuwe schalen die van de Visual Analogue Scale van Bowdle zijn afgeleid. De interne perceptie geeft innerlijke gevoelens weer die niet met de werkelijkheid overeenkomen, terwijl de externe perceptie correspondeert met een verkeerde interpretatie van de omgeving. De hartslag nam toe, de vrijwilligers voelden zich high, werden minder alert en scoorden hoger op de externe perceptie schaal. De toename op de interne perceptie schaal was veel kleiner. Gevoelens van onwerkelijkheid, hallucinaties, paranoïde gedachten en angst worden wel eens gezien na het gebruik van hoge doseringen cannabis of bij personen die niet gewend zijn om cannabis te roken. In deze studie (hoofdstuk 3-5) werden alleen personen geïncludeerd die bekend waren met de effecten van cannabis en mogelijk waren de doseringen in onze studie niet hoog genoeg om deze effecten te veroorzaken. Wat wel duidelijk naar voren kwam is dat de effecten toenamen met het toenemen van de dosering. Na elke toediening nam de hartslag snel toe (maximum na 5-10 minuten), nam vervolgens vrij snel weer af en was vrijwel genormaliseerd voor de volgende toediening. De effecten op het centrale zenuwstelsel waren nog niet verdwenen op het moment dat de volgende dosering THC werd toegediend: er was sprake van accumulatie. Dit werd bijvoorbeeld gezien bij diverse vragenlijsten en bij een lichamelijke stabiliteitstest (body sway). Bij deze testen duurde het iets langer voordat het maximale effect bereikt werd (na ca. 20 minuten) en het effect hield ook langer aan. De pupilgrootte en het hersenfilmpje veranderden alleen na

toediening van de laatste en hoogste dosering THC. THC had geen effect op oogbewegingtesten en op een oog-hand-coördinatie test.

## Farmacokinetische / farmacodynamische modellering

Farmacokinetische / farmacodynamische (PK/PD) modellering heeft duidelijk gemaakt dat de effecten van THC op het hart en op het centrale zenuwstelsel waarschijnlijk door verschillende mechanismen veroorzaakt worden (hoofdstuk 4). PK/PD modellering is een wiskundige beschrijving van de verandering van de concentratie van een toegediend geneesmiddel in de tijd, gekoppeld aan de verandering van het farmacologische effect in de tijd. Het PK/PD model laat zien dat er in een persoon weinig variatie is tussen de THC concentraties in het bloed bij diverse doseringen (variatie kleiner dan 6%). Het verschil in THC concentraties tussen verschillende personen is groter (variatie van 24%) en is waarschijnlijk het gevolg van verschillende manieren van inhaleren. Dit onderstreept het belang van het oefenen met de inhalatiemethode en het onder toezicht toedienen van THC.

Het PK/PD model liet ook zien dat bij de effecten op het centrale zenuwstelsel er sprake is van hysterese. Hysterese betekent dat de effecten van THC achterblijven bij de THC concentraties in het bloed. THC wordt in de longen vrijwel direct opgenomen in het bloed en vanuit het bloed komt THC in de hersenen. Hiervoor is tijd nodig en terwijl de concentratie in het bloed afneemt, neemt de concentratie in de hersenen toe. De invloed van THC op testen die effecten meten op het centrale zenuwstelsel wordt groter met het toenemen van de concentratie in de hersenen. Het effect op de hartslag kwam snel tot stand en nam ook weer vrij snel af, hetgeen er op wijst dat dit effect door een ander, veel directer mechanisme veroorzaakt wordt.

## Toename van de hartslag door THC en andere CB1 agonisten

Het is nog onduidelijk hoe de toename van de hartslag na het gebruik van cannabis of THC precies tot stand komt (hoofdstuk 5). De tijd tussen opeenvolgende hartslagen varieert (heart rate variability) en kan onder invloed van medicijnen veranderen. Heart rate variability geeft informatie over de activiteit van het sympathische en parasympathische zenuwstelsel. Beide zijn nauw met elkaar verbonden en onderdeel van het onwillekeurige zenuwstelsel (niet direct te beïnvloeden met je wil). In het algemeen geldt dat het sympathische systeem actief is bij inspanning en als je weer tot rust komt is met name de parasympaticus actief. Een toename in de hartslag kan veroorzaakt worden door stimulatie van de sympaticus, maar kan ook tot stand komen door het onderdrukken van de activiteit van de

parasympaticus. In hoofdstuk 5 staat beschreven dat de activiteit van de nervus vagus (de zwervende zenuw) waarschijnlijk onderdrukt wordt door THC waardoor de hartslag toeneemt. Tevens is duidelijk geworden dat de CB1 receptor betrokken is bij het toenemen van de hartslag na gebruik van THC (zie ook hoofdstuk 6). Ook Org 28611 en Org 26828, twee CB1 agonisten (hoofdstuk 7 en 8), veroorzaken een toename van de hartslag. Voor de verdere ontwikkeling van deze en andere cannabinoïden als geneesmiddel is het van belang dit mechanisme op te helderen aangezien een toename van de hartslag nadelig kan zijn voor mensen met hart- en vaatzieken.

Samenvattend laten hoofdstuk 3-5 zien dat de Volcano® vaporizer een bruikbare methode is om THC toe te dienen en de effecten van THC te bestuderen. Daarnaast kunnen met de PK/PD modellen die beschreven staan in hoofdstuk 4 de opzet van vervolgstudies geoptimaliseerd worden.

## De inhibitie van THC effecten door AVE1625

Door THC (een stimulerende stof) in combinatie met AVE1625 (een blokkerende stof) toe te dienen is de verwachting dat de effecten van THC deels of geheel geblokkeerd worden. Op deze manier zijn de effecten van AVE1625 op het centrale zenuwstelsel en op het hart bestudeerd (hoofdstuk 5 en 6). Zesendertig gezonde, mannelijke vrijwilligers hebben mee gedaan aan deze studie. Er waren vier studiedagen en elke vrijwilliger kreeg vier van de zes beschikbare behandelingen. Op elke studiedag werd eerst AVE1625 (20 of 60 of 120 mg) of een placebo toegediend. Drie uur later werd gestart met de vier THC doseringen (2, 4, 6 en 6 mg) of er werd telkens een placebo toegediend. Het interval tussen de THC of placebo doseringen was één uur. Testen die in de voorgaande studie (hoofdstuk 5) gevoelig bleken te zijn voor de effecten van THC werden gebruikt in deze studie, zoals hartslag, lichamelijke stabiliteit (body sway) en diverse vragenlijsten. THC veroorzaakte dezelfde effecten als in de voorgaande studie, terwijl AVE1625 zelf geen meetbaar had effect op de afgenomen testen. Zoals verwacht bleek AVE1625 de effecten van THC te blokkeren. Zelfs de laagste dosering van 20 mg blokkeerde bijna alle effecten van THC. De concentratie cannabisachtige stoffen die in het lichaam voorkomen en hun affiniteit voor de cannabinoïd receptor is veel lager dan die van de toegediende THC. Dit zou kunnen betekenen dat voor therapeutische doeleinden lagere doseringen voldoende zijn voor het blokkeren van endocannabinoïden. Klinische studies moeten dit echter nog wel bevestigen. Samenvattend laat deze studie zien dat het tegelijk toedienen van de CB1 antagonist AVE1625 met THC een bruikbare methode is om aan te tonen dat AVE1625 inderdaad doordringt in het centrale zenuwstelsel. Dit is een belangrijk gegeven aangezien AVE1625 verder ontwikkeld zal worden voor de behandeling van ziekten van het centrale zenuwstelsel.

## Onderzoek van twee CB1 agonisten

Niet alleen stoffen die de CB1 receptor blokkeren (antagonisten), maar ook stoffen die de CB1 receptor stimuleren (agonisten) worden ontwikkeld voor therapeutische doeleinden. Midazolam, een benzodiazepine, heeft sederende eigenschappen en zorgt ervoor dat je tijdens een korte chirurgische ingreep of een darmonderzoek kortdurend suf bent en na het onderzoek vergeten bent dat het onderzoek onplezierig was. Bij dit soort ingrepen wordt voor de pijnstilling vaak een opiaat zoals morfine toegediend. Diermodellen tonen aan dat THC sederende èn pijnstillende eigenschappen heeft. Daarnaast zorgt THC er ook voor dat het geheugen minder goed werkt. De verwachting is dat CB1/CB2 agonisten deze eigenschappen ook hebben en gebruikt kunnen worden bij korte chirurgische ingrepen of een darmonderzoek. In hoofdstuk 7 en 8 worden twee nieuwe CB1 agonisten (Org 28611 en Org 26828) vergeleken met midazolam.

Org 26828 en zijn actieve metaboliet (afbraakproduct) Org 26761 lijken qua chemische structuur op THC, maar niet op Org 28611. Het voornaamste doel was te onderzoeken of deze stoffen sederend zijn en of ze het geheugen beïnvloeden. Beide stoffen zijn in twee soortgelijke studies (met dezelfde opzet) onderzocht. Eerst zijn Org 28611 en Org 26828 toegediend door middel van een langzaam infuus (25 minuten) en vervolgens door middel van een snel infuus (1 minuut). Dit laatste infuus wordt een bolus toediening genoemd. De bolus dosering werd berekend met behulp van de testresultaten van het langzame infuus. De effecten van Org 28611 en Org 26828 lijken op elkaar, maar verschillen van midazolam. Niet alleen door observatie, maar ook uit een oogbewegingtest en uit vragenlijsten bleek dat de vrijwilligers suf werden van midazolam. De sufheid duurde ongeveer 30-45 minuten. Na toediening van Org 28611 en Org 26828 werd op een vragenlijst wel aangegeven dat men suf was, maar objectief gezien was er geen sprake van sufheid. Na toediening van midazolam moesten vrijwilligers aangespoord worden om de testen te doen, maar na toediening van Org 28611 en Org 26828 was dat niet nodig. Ook werden de gunstige effecten van midazolam op het geheugen niet waargenomen. Bij de maximaal getolereerde dosering (dat is de hoogste dosering die goed verdragen wordt), was er, na toediening van Org 28611 en Org 26828, geen effect op de lichamelijke stabiliteit (body sway). Dit in tegenstelling tot midazolam. Dat betekent dat patiënten zelfstandig kunnen rondlopen en weer snel naar huis kunnen na toediening van Org 28611 en Org 26828. De bolus doseringen van beide stoffen werden goed verdragen en er werden nauwelijks effecten waargenomen. Dit kwam doordat alleen de lagere doseringen door middel van een bolus zijn toegediend.

De effecten van midazolam werden door de meeste vrijwilligers als erg plezierig ervaren. Hoge doseringen van Org 28611 en org 26828, die alleen door middel van een langzaam infuus werden toegediend, bleken onple-

zierige effecten te hebben. Diverse vrijwilligers hadden last van angst of paniek, hallucinaties, zich vreemd voelen, tintelingen of onwillekeurige bewegingen in armen of benen. Op vragenlijsten werd ook aangeven dat de effecten als onplezierig ervaren werden. Deze effecten contrasteren met de veelal plezierige effecten die optreden na het roken van een cannabis sigaret.

## Samenvatting en conclusie

Hoofdstuk 9 is een algemene samenvatting en conclusie van het proefschrift. De effecten van THC, Org 28611 en Org 26828 laten zien dat ze waarschijnlijk tot dezelfde klasse cannabisachtige stoffen behoren. Er wordt tevens een antwoord gezocht op de vraag waarom de bijwerkingen van THC (hoofdstuk 3) enerzijds en Org 28611 en Org 26828 (hoofdstuk 7 en 8) anderzijds behoorlijk van elkaar verschillen. Het vergelijken van de drie stoffen werd bemoeilijkt doordat de studieopzet waarin THC is toegediend anders was dan die voor Org 28611 en Org 26828. De verschillende effecten zouden veroorzaakt kunnen worden door een verschil in dosering, de selectiecriteria waaraan de vrijwilligers die mee deden moesten voldoen en de manier van toedienen.

Ten eerste zou het verschil in bijwerkingenprofiel verklaard kunnen worden door de hoogte van de dosering. Boven de maximaal getolereerde dosering van Org 28611 en Org 26828 werden ongewenste psychische effecten waargenomen. De THC doseringen waren dicht bij de maximaal getolereerde dosering aangezien twee van de twaalf vrijwilligers de hoogste dosering (8 mg) niet kregen toegediend vanwege bijwerkingen (slaperigheid, overgeven, maar geen psychische bijwerkingen). Desondanks werden de effecten van THC, met name het 'high' gevoel, door de meeste vrijwilligers als plezierig ervaren. Dit betekent dat het verschil in bijwerkingenprofiel waarschijnlijk niet toe te schrijven is aan een verschil in dosering. De tweede optie zijn de selectiecriteria waaraan de vrijwilligers moesten voldoen. In de THC studie (hoofdstuk 3) werden matige cannabis gebruikers geïncludeerd (gemiddeld twee keer per maand cannabis gebruik), terwijl in de studies met Org 28611 en Org 26828 vrijwilligers werden geïncludeerd die in totaal niet meer dan 5 keer cannabis hadden gebruikt. Op basis van de literatuur en de gemeten effecten is ook het verschil in gebruikers waarschijnlijk niet de oorzaak van het verschil in bijwerkingen. De manier van toedienen zou echter wel een verklaring kunnen zijn voor de onplezierige psychische bijwerkingen na toediening van Org 28611 en Org 26828. In de literatuur wordt na intraveneuze toediening van THC ook melding gemaakt van angst of paniek, hallucinaties, zich vreemd voelen of tintelingen. Zowel na inhalatie als na toediening via een infuus worden de effecten snel waargenomen. In de THC studie (hoofdstuk 3) werd THC onder toezicht en

volgens een vast inhalatieschema toegediend. De effecten van THC werden overwegend als plezierig ervaren. Bij een inhalatiemethode kan een proefpersoon enige invloed uitoefenen op de toegediende dosering. Bijvoorbeeld door minder diep in te ademen of door de THC via de neus uit te ademen op het moment dat hij de adem moet inhouden. Op deze manier zou een vrijwilliger ongewenste effecten enigszins kunnen voorkomen. Bij intraveneuze toediening kunnen vrijwilligers geen invloed uitoefenen op de toegediende dosering. Het is echter maar de vraag of het enigszins kunnen beïnvloeden van de toegediende dosering het opvallende verschil in bijwerkingen geheel kan verklaren. Een nog onbekend mechanisme dat bij de inhalatie betrokken is, kan misschien de verschillen verklaren. Hoe het ook zij, de toedieningsroute is waarschijnlijk verantwoordelijk voor het optreden van de ongewenste psychische bijwerkingen na intraveneuze toediening van Org 28611 en Org 26828. Bij de verdere ontwikkeling van CB1/CB2 agonisten als medicijn is daarom raadzaam rekening te houden met de manier van toedienen.

Samengevat beschrijft dit proefschrift dat er bruikbare biomarkers zijn om de effecten van cannabisachtige stoffen te bestuderen. De nieuwe inhalatiemethode bleek een bruikbare manier om de effecten van THC te bestuderen. Met behulp van de THC concentraties in het bloed en de gemeten effecten zijn modellen gemaakt waaruit blijkt dat de effecten op het centrale zenuwstelsel en het hart op verschillende manieren tot stand komen. De resultaten van deze studie werden gebruikt om te laten zien dat de CB1 antagonist AVE1625 het centrale zenuwstelsel beïnvloedt. Tevens werden de sederende eigenschappen en het effect op het geheugen van twee CB1 agonisten (Org 28611 en Org 26828) onderzocht. Beide stoffen bleken niet sederend te zijn waardoor ze niet bruikbaar zijn voor kortdurende ingrepen waarbij het gewenst is dat de patiënt suf is. Tevens bleken ze niet de gunstige effecten op het geheugen te hebben zoals die gezien zijn na toediening van midazolam. Org 28611 en Org 26828 worden nu verder ontwikkeld voor andere indicaties. Vergelijking van Org 28611 en Org 26828 met THC suggereert dat de manier van toedienen belangrijk is voor het al dan niet optreden van ongewenste psychische bijwerkingen. De in dit proefschrift beschreven studies in gezonde vrijwilligers en de ontwikkelde modellen zijn niet alleen belangrijk voor de verdere ontwikkeling van de onderzochte cannabonoïden, maar ook voor andere cannabinoïden die in ontwikkeling zijn als medicijn.

# CURRICULUM VITAE

Lineke Zuurman was born in Hoogezand-Sappemeer, The Netherlands, on January 5, 1972. She did not obtain her Atheneum diploma (VWO) at the Wessel Gansfort College in Groningen. Before going to university she worked a couple of years for a temporary employment agency and as a volunteer in a drug rehabilitation centre. In 1994 she passed a Colloquium Doctum Physiotherapy and in 1995 the foundation course Physiotherapy and the Colloquium Doctum Medical Science. In 1999 she completed the doctoral phase of Medical Biology at the Free University of Amsterdam. Her major course was Neuropharmacology. Subsequently she studied Medicine and got her Medical degree in 2003. In October 2003 she started working at the Centre for Human Drug Research (CHDR) in Leiden as Clinical Scientist (CEO: Prof. Dr. A.F. Cohen). The research described in this thesis was performed at CHDR. In June 2007 she was registered as a Clinical Pharmacologist. Currently she works as Clinical Assessor at the Dutch Medicines Evaluation Board. From July 2008 onwards she will work for Hoffmann-La Roche, Basel, Switzerland.