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Original papers

Effect of intrapulmonary tetrahydrocannabinol administration in humans

L Zuurman *Centre for Human Drug Research, Pharmacology CNS, Leiden, The Netherlands.*
C Roy *Sanofi-aventis, Recherche-Développement, Paris, France.*
RC Schoemaker *Centre for Human Drug Research, Statistics, Leiden, The Netherlands.*
A Hazekamp *Department of Pharmacognosy, Leiden University, Leiden, The Netherlands.*
J den Hartigh *Hospital Pharmacy, Leiden University Medical Center, Leiden, The Netherlands.*
JCME Bender *Farmalyse BV, Zaandam, The Netherlands.*
R Verpoorte *Department of Pharmacognosy, Leiden University, Leiden, The Netherlands.*
JL Pinquier *Sanofi-aventis, Recherche-Développement, Paris, France.*
AF Cohen *Centre for Human Drug Research, Pharmacology CNS, Leiden, The Netherlands.*
JMA van Gerven *Centre for Human Drug Research, Leiden, The Netherlands.*

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-modeling of heart rate displayed a relatively short equilibration half-life of 7.68 min. CNS parameters showed equilibration half-lives ranging between 39.4 - 84.2 min. 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

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 (Ameri, 1999; Ashton, 2001; Baker, *et al.*, 2003), whereas they are expressed at low levels in the brainstem (Baker, *et al.*, 2003). CB2 receptors are predominantly present in the spleen and in haematopoietic cells (Ameri, 1999). 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

Corresponding author: Lineke Zuurman, Centre for Human Drug Research, Zernikedreef 10, 2333 CL Leiden, The Netherlands. Email: linekezuurman@gmail.com

agonists might be of therapeutic use for muscle relaxation, immunosuppression, sedation, improvement of mood, neuroprotection, analgesia and reduction of intra-ocular pressure (Grotenhermen, 2003). Dronabinol (δ-9-tetrahydrocannabinol) and Nabilone®, synthetic THC analogues, 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 (Grotenhermen, 2003).

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-characterized 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 (Ohlsson, *et al.*, 1980; Hollister, *et al.*, 1981; Wall, *et al.*, 1983). However, adequate injection fluids are difficult to manufacture because of the highly lipophilic properties of THC. In man, plasma THC concentration profiles are similar after smoking or intravenous administration with prompt onset and steady decrease (Noyes Jr., *et al.*, 1975; Husain and Khan, 1985; Mathew, *et al.*, 2002).

Although smoking cannabis provides a reliable pharmacokinetic profile (Husain and Khan, 1985; Grotenhermen, 2003), 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 (Hazekamp, *et al.*, 2006). In this study, we investigated the pharmacokinetic and pharmacodynamic effects after inhalation of pure THC using a Volcano® vaporizer.

Although many 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. However, the tests that are particularly sensitive to the acute effects of THC are not clear (Kiplinger and Manno, 1971; Chesher, *et al.*, 1990; Heishman, *et al.*, 1997),

and few studies have investigated the pharmacodynamic time profiles following THC administration. The most conspicuous effects of cannabis are subjective and psychomimetic changes (Zeidenberg, *et al.*, 1973; Vachon, *et al.*, 1974; Grotenhermen, 2003). In some studies, a reduction in smooth pursuit eye movements was observed (Fant, *et al.*, 1998) and changes in pupil size have been reported by several other authors (Brown, *et al.*, 1977). THC increases heart rate by 20–60% (Hall and Solowij, 1998; Sidney, 2002). The effects on blood pressure are complex, with reports of both increases and decreases (Hall and Solowij, 1998; Sidney, 2002; Randall, *et al.*, 2002). 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 (Steveninck, *et al.*, 1999; Visser, *et al.*, 2002). 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 1 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 2 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 the principles of ICH-GCP, the Helsinki declaration and Dutch regulations.

Subjects

Twelve healthy men (21–27 years) with a history of mild cannabis use for at least 1 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 6 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

Table 1 Study design

	8.00–10.00	10.00	11.30	13.00	14.30
Study day 1	Arrival at unit and study preparations	2 mg THC	4 mg THC	6 mg THC	8 mg THC
Study day 2		Placebo	Placebo	Placebo	placebo

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 *C. sativa* grown under Good Agricultural Practice (Bedrocan BV Medicinal Cannabis, Veendam, The Netherlands) (Choi, *et al.*, 2004; Hazekamp, *et al.*, 2004; Hazekamp, *et al.*, 2005). Each dose (2, 4, 6 or 8 mg) of THC (>98% purity by HPLC/GC) was dissolved in 200 µL of 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-min 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 (Hazekamp, *et al.*, 2006). 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 transparent bag was covered with a black plastic bag to prevent unblinding. Subjects were not allowed to speak, and were instructed to inhale deeply and hold their breath for 10 s after each inhalation. Within 2–3 min, 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 approximately 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 (AEs) and physical signs. Because of the long half-life of THC, study days were separated by a washout period of at least 2 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 min after each THC administration. Additional samples were taken at 5, 35 and 55 min after administration of 6 mg THC and at 375, 425, 495, 545 and 1440 min after the first THC administration. After blood collection, the tubes were placed in ice water (0–4 °C) and centrifuged within 1 h for 10 min at $2000 \times g$ 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 standardized 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.

Plasma peak concentration was followed by a short distribution phase (approximately 25 min) and a longer elimination phase (roughly 250 min). Average plasma THC concentrations, 10 min after the fourth dose (50.3 ± 14.4 ng/mL), exceeded 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

Blood pressure and heart rate were measured in supine position after a rest of approximately 5 min, 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, Tokyo, Japan) using a flashlight after at least 5 min 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 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 the registration of the electro-oculographic signals. Skin resistance was reduced to less than 5 kΩ 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

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, RI, 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° to either side. Fifteen saccades were recorded with interstimulus intervals varying randomly between 3 and 6 s. 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 were kept below 5 kΩ. 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) with a time constant of 0.3 s and a low pass filter at 100 Hz. Data collection and analysis were performed using a validated Spike2 script. Per session eight consecutive blocks of 8 s were recorded. The signal was AD-converted using a CED 1401 Power AD-converter 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 δ- (0.5–3.5 Hz), θ- (3.5–7.5 Hz), α- (7.5–11.5 Hz) and β- (11.5–30 Hz) frequency ranges. 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 (Wright, 1971). With a string attached to the waist, all body movements in the anteroposterior direction

over a period of 2 min were integrated and expressed as millimetre 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 were 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 (1984), 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 joystick. 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-min period. Each test was preceded by a run-in period. After four to six practice sessions, learning effects are limited. The adaptive tracking test is more sensitive to the 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 (Steveninck, *et al.*, 1993; Steveninck, *et al.*, 1999).

Visual analogue scales

From the visual analogue scales (VAS), as originally described by Norris (1971) (16 items), three factors can be derived, as described by Bond and Lader (1974), 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 VAS (13 items), originally described by Bowdle *et al.* (1998).

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 before bioanalysis. Analysis was performed using a validated high-performance liquid chromatography with tandem mass spectrometric detection. Calibration range was 1.00–500 ng/mL for all compounds. 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 deviation as error bars. The pharmacodynamic endpoints were analysed separately by mixed model analyses of variance (using SAS PROC MIXED, SAS for Win-

dows, V9.1.2; SAS Institute, Inc., Cary, North Carolina, 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 h was calculated within the statistical model. Additionally, the average response of the values obtained in the 90 min 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 VAS 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 showed 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.

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). Because the $\log + 2$ transformation makes back-transformation problematic and the resulting scales have favourable statistical properties, it was decided not to 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 showing 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, Maryland,

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

	Estimate of difference (U)	95% CI
External perception[a]	0.616	(0.371, 0.860)
VAS 1: my body parts seemed to change their shape or position	0.223	(−0.005, 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[b]	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	(−0.019, 0.274)
VAS 13: I felt anxious	0.144	(−0.062, 0.349)

Data are population average, 95% confidence interval (CI) and *P* value.

THC, tetrahydrocannabinol.

[a]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.

[b]Internal perception = [^{10}log(VAS4 + 2) + ^{10}log(VAS8 + 2) + ^{10}log(VAS9 + 2) + ^{10}log(VAS10 + 2) + ^{10}log(VAS13 + 2)]/5.

USA) was used. The PK/PD modelling is described in detail by Strougo *et al.* (2007).

Results

Subjects

Twelve healthy men 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, six subjects used it two to three times a month, three 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 AEs 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 such as drowsiness, sleepiness, attention deficit and feeling high. In addition, also minor AEs such as headache and eye irritation were reported. During THC inhalation, five subjects had to cough, whereas other subjects were required to hold their breath for 10 s. This was not reported after inhalation of the alcohol-vehicle during placebo occasions. Two of 12 subjects experienced side effects severe enough to decide not to administer the last dose of 8 mg THC. One of these subjects was too sleepy to perform any test, and the other subject vomited just after administration of the third dose.
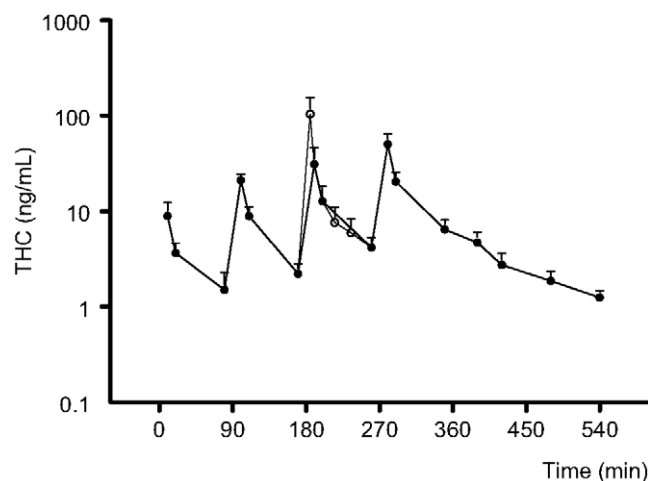

Figure 1   Mean (SD) observed profile of plasma THC: closed circles, common measurement points for all four doses; open circles, extra assessments for third dose. THC administration: 2 mg at *T* = 0; 4 mg at *T* = 90; 6 mg at *T* = 180, 8 mg at *T* = 270.
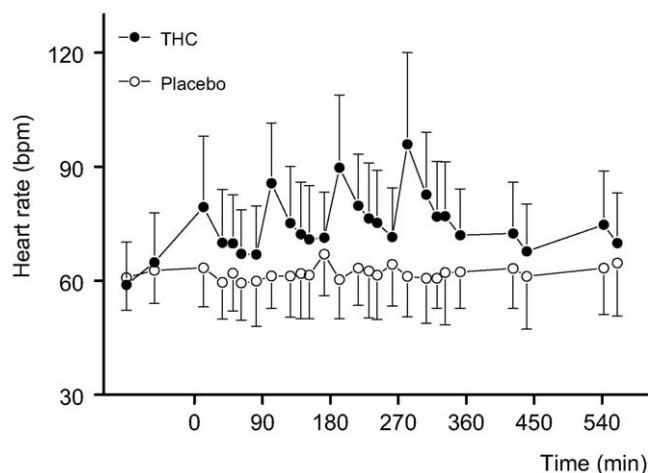

Figure 2   Mean (SD) time profile of heart rate. THC administration: 2 mg at *T* = 0; 4 mg at *T* = 90; 6 mg at *T* = 180, 8 mg at *T* = 270.

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 consequently had deviating concentration and effect–time profiles that would have distorted the average graphs.

Pharmacokinetics

THC plasma peak levels were reached within few minutes (Figure 1).

Heart rate and blood pressure

Heart rate increased in a dose-related manner compared with placebo (Figure 2). The average increase after the fourth dose of 8 mg was 19 bpm (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 with 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 with placebo, sac-
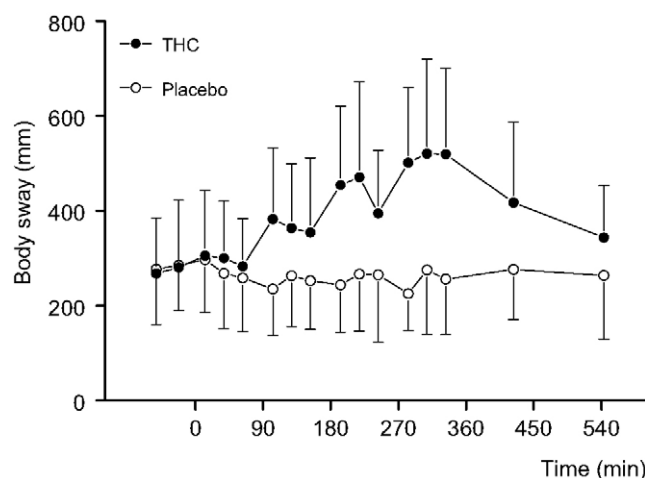
Figure 3 Mean (SD) time profile of body sway. THC administration: 2 mg at *T* = 0; 4 mg at *T* = 90; 6 mg at *T* = 180, 8 mg at *T* = 270.

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.

cadic latency (20 ms: 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/s: 95% CI −4, 32).

Electro-enchephalography (EEG)

After the highest dose of THC, there were decreases in the power of Pz-Oz δ- (−16%: 95% CI −24, −7), Pz-Oz θ- (−15%: 95% CI −24, −5) and Pz-Oz β-activity (−12%: 95% CI −18, −4). No changes were found in α-activity (−6%: 95% CI −17%, 5%). In the Fz-Cz region, changes in β-activity were predominant. No changes in δ- and θ-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 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 with placebo, no changes were observed in adaptive tracking performance (fourth dose effect: −1%: 95% CI −3, 1).

Visual analogue scales

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
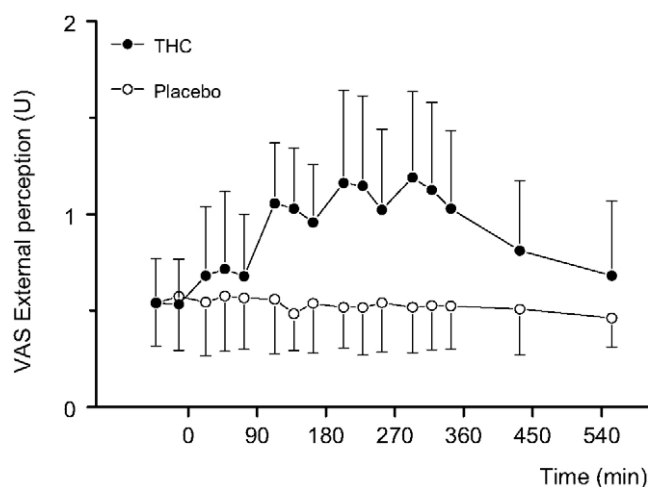
was seen in VAS 'contendness ' after the fourth dose (−7 mm 95% CI −13, −1) but no change was seen in VAS 'calmness' (−3 mm: 95% CI −10, 4).

VAS Bowdle – internal and external perception Many of the individual VAS measuring psychedelic effects showed 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 reported for the 'internal perception' composite scale
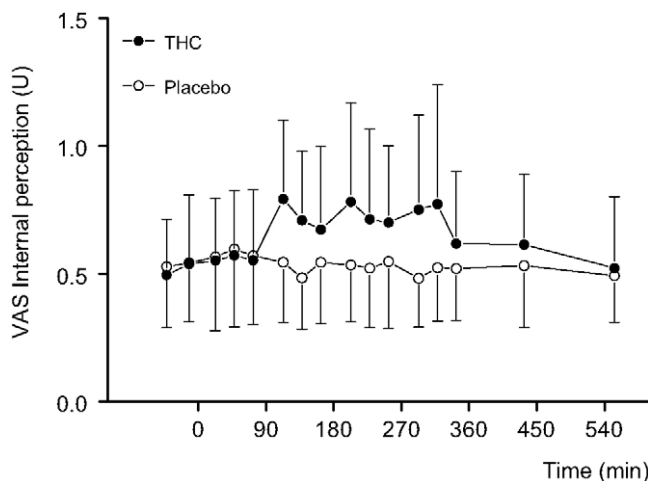

Figure 5 Mean (SD) time profile VAS 'internal perception'. THC administration: 2 mg at *T* = 0; 4 mg at *T* = 90; 6 mg at *T* = 180, 8 mg at *T* = 270.

(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, showing hysteresis. Equilibration half-lives that quantify hysteresis varied from 7.68 min for heart rate and from 39.2 to 84.8 min for the effects on the CNS. The PK/PD modelling is described in detail by Strougo *et al.* (2007).

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 (Hazekamp, *et al.*, 2006). Therefore, in our study an estimated average cumulative dose of 11 mg of THC was inhaled from the balloon. This is comparable with the doses used in the literature because 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, characterizing 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 (Grotenhermen, 2003). Although 11-OH-THC is equipotent or twice as potent as THC (Perez-Reyes, *et al.*, 1972; Howlett, *et al.*, 2004), 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 (Ashton, 2001; Mackie, 2005). 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 marijuana effects (Liguori, *et al.*, 2003).

The sensitive subjective parameters included in particular 'alertness' of the 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 (Steveninck, *et al.*, 1999; Visser, *et al.*, 2002) 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-know effect of THC and has been observed after oral administration of 15 mg THC (Zeidenberg, *et al.*, 1973), 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 naive subjects (Dittrich and Woggon, 1974; D'Souza, *et al.*, 2004). In this study, subjects familiar with the effects of cannabis were included and possibly the doses in our study were not high enough to elicit such effects. Interestingly, all observed CNS effects showed accumulation of the effects because the effect of the previous dose had not faded before the next dose was administered.

In the current study, limited decreases in EEG δ, θ and β-activity were reported. One of the earliest signs of drowsiness is the disappearance of the occipital dominant α-activity (Visser, *et al.*, 2002). Although subjects reported being drowsy, no changes in α-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 (Iversen, 2000).

The literature reports conflicting results on tracking tests, which is probably because of differences in tasks (Roth, *et al.*, 1973). The critical tracker task used 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 (Stoller, *et al.*, 1976). Because the pulmonary administration of THC is on average approximately 2.6–3 times more potent than oral administration (Isbell, *et al.*, 1967), 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 (Straiker, *et al.*, 1999). 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 (Weil, *et al.*, 1968; Zeidenberg, *et al.*, 1973; Brown, *et al.*, 1977).

CB1 receptors are sparsely found in the brainstem (Herkenham, *et al.*, 1990; Mackie, 2005), 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 (Leigh and Zee, 1991). The lower brain stem areas also control cardiovascular function. Orthostatic hypotension has been reported in literature (Hall and Solowij, 1998; Sidney, 2002). 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 bpm after the fourth dose, without any indications for blood pressure reductions. On the contrary, 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 (Zuardi, *et al.*, 1982; Heishman, *et al.*, 1997; Hall and Solowij, 1998; Sidney, 2002). The faster response in heart rate before the onset of subjective effects has also been observed after oral administration of 15 mg THC (Zeidenberg, *et al.*, 1973). Literature also reported that THC plasma concentration already dropped significantly before maximum psychotropic effects were achieved (Ohlsson, *et al.*, 1980; Chiang and Barnett, 1984). 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-life for heart rate of 7.68 min (Strougo, *et al.*, 2007). This is much shorter than the equilibration half-lives found on CNS effects, which varied from 39.2 to 84.8 min. In addition, CB1 receptors are present in human atrial muscle (Bonz, *et al.*, 2003) but they are sparse in the lower brainstem areas controlling cardiovascular function (Herkenham, *et al.*, 1990). In combination, these results suggest that the increase in heart rate seen after THC administration is not mediated by brain stem centres, but is established by a direct effect of THC on the heart.

Lipophilic compounds such as 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 min for the effects on the CNS. 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 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.

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