

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 such as 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 antagonising the effects of delta-9-tetrahydrocannabinol (THC), a CB₁/CB₂ agonist. Inhibition of THC-induced effects by AVE1625 was observed on Visual Analogue Scales 'alertness', 'feeling high', 'external perception', 'body sway' and 'heart rate'. Even the lowest dose of AVE1625 20 mg inhibited most of THC-induced effects. AVE1625 did not have any effect on psychological and behavioural parameters or heart rate by itself.

After THC and AVE1625 administration, changes on electroencephalography were observed. This study shows a useful method for studying the effects of CB₁ antagonists. AVE1625 penetrates the brain and antagonises THC-induced effects with doses at or above 20 mg.

Key words

AVE1625; CB₁ antagonist; cannabis; cannabinoid; healthy volunteers; human; pharmacodynamics; volcano[®] vapouriser; THC

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 behaviour (Cooper, 2004). Rimonabant (SR141716) is the first CB₁ antagonist developed for the treatment of obesity (Van Gaal, *et al.*, 2005a,b). In addition, CB₁ receptor antagonists may also be useful in the treatment of smoking cessation (Cahill and Ussher, 2007), cognitive impairments in Alzheimer's disease and schizophrenia (Meltzer, *et al.*, 2004; Di Forti, *et al.*, 2007; Zavitsanou, *et al.*, 2004) and for the treatment of advanced Parkinson's disease (Mesnage, *et al.*, 2004; Fernandez-Espejo, *et al.*, 2005; Benarroch, 2007).

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 (Burns, *et al.*, 2007). 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 such as delta-9-tetrahydrocannabinol (THC), the psychoactive ingredient of

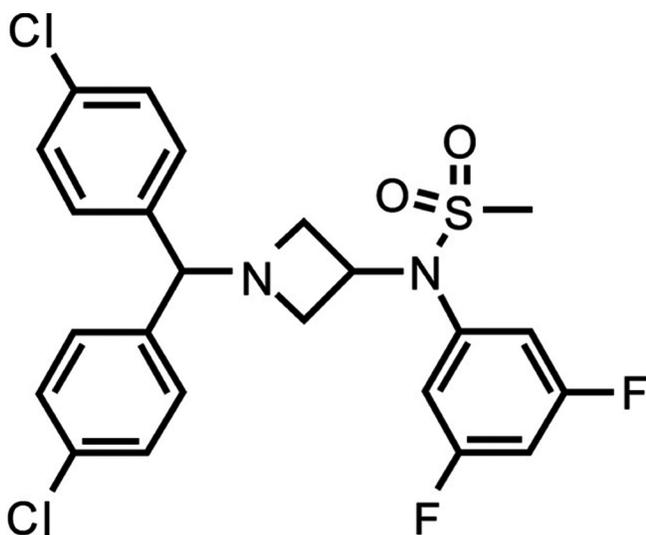


Figure 1 Chemical structure of the CB1 antagonist AVE1625.

cannabis. Inhibition of THC-induced effects confirms that AVE1625 penetrates the brain and that it behaves like a CB1-antagonist in humans.

Recently, we examined the effects of intrapulmonary rising doses of pure THC (2, 4, 6 and 8 mg) using a Volcano® vaporiser (Storz-Bickel GmbH, Tuttlingen, Germany) (Zuurman, *et al.*, 2008; Strougo, *et al.*, 2008). Dose- and concentration-related effects could be measured with low inter-subject variability on a number of central nervous system (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 antagonise the effects of THC. The aims were to show that AVE1625 penetrates the CNS and acts as a functional CB1-antagonist in humans and to identify the doses at which these effects occur.

Methods

Design

This was a single-centre, double-blind, randomised, six-way balanced, placebo-controlled, partial cross-over study in

Table 1 Study design

Oral	Intrapulmonary			
	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

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

healthy male volunteers. Each subject received four of the six available treatment combinations, with a washout period of at least 2 weeks between treatments. The incomplete cross-over study design, rather than a complete crossover design, was chosen to reduce the risk of having subjects drop out due to an inability to refrain from smoking cannabis for the duration of the study. The study protocol was approved by the Medical Ethics Review Board of Leiden University Medical Center and performed according to principles of Conference on Harmonisation -Good Clinical Practice (ICH-GCP) and Dutch clinical trial law.

Subjects

Thirty-six males were included in the study. Their ages ranged from 18 to 31 years with a mean of 22 ± 3 years. The mean height and weight were 183 ± 6 cm (range 167–192 cm) and 76 ± 11 kg (range 60–95 kg), respectively. Mean body mass index 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. Fifteen 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; cut-off concentration of 50 ng/mL), including cannabinoids, were negative at screening and at baseline on all study days. Subjects were excluded if they were unable to refrain from cigarette smoking and use of coffee and tea on study days. In addition, subjects were excluded from the study if they had withdrawal symptoms (e.g., headache) due to not using these products. Twenty-five subjects completed the study. Eleven (30.6%) subjects discontinued from the study. Six subjects discontinued because they did not wish to continue, four 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)

(Choi, *et al.*, 2004; Hazekamp, *et al.*, 2004; Hazekamp, *et al.*, 2005). Each dose (2, 4, 6 or 6 mg) of THC (>98% purity by High-performance liquid chromatography/Gas Chromatography (HPLC/GC)) was dissolved in 200 μ L 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 parafilm to minimise evaporation. Stability data of the THC solution show stability of at least 29 months. No degradation products of THC were found using HPLC, and THC concentration in the vials changed less than 2%. In the present study, THC was used within 5 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 (standardised 530 Kcal breakfast) as six soft capsules. The administration of THC was started 3 h 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 h 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 1-h intervals, using a Volcano[®] vaporiser (Table 1). In all, 5 min before administration THC was vaporised at a temperature of about 225°C , and the vapour 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 s. Within 2–3 min, 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.* (2007), Strougo, *et al.* (2008) and Hazekamp, *et al.* (2006).

The study had a partial cross-over design, where each subject received four 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 heparinised polypropylene tubes (lithium heparin) of 4 mL. Blood samples were taken at baseline and 1, 2, 3, 4, 5, 6, 12 and 24 h after oral administration of AVE1625 or matching placebo. After blood collection, the tubes were centrifuged within 30 min for 15 min at 2000 g at 4°C . Plasma samples were stored at a temperature of -20°C . THC samples were also obtained, but plasma THC

concentrations could not be determined due to instability of THC.

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 min 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.* (2008). Subjects were acquainted with the experimental methods and conditions in a training session within 1 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. Measurements were always executed in the following strict order: body sway, VAS Bond and Lader, VAS Bowdle, electroencephalography (EEG), heart rate, blood pressure and saccadic eye movements. Each block of measurements lasted 11 min. Measurements were repeated twice at baseline and twice each hour (from $T = 0$ to 8 h and after $T = 10$ and 11 h) after administration of AVE1625. To capture the peak effect of THC, one additional measurement block was performed shortly after each THC administration (three measurement blocks in total). In addition, an extra heart rate measurement was obtained after each THC dose (adding up to four measurements after each THC dose) and after $T = 8$ h.

Heart rate

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

EEG

EEG is often quite sensitive to the effects of a wide range of CNS-active drug classes. Because the direct pharmacodynamic effects of AVE1625 have 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 were 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, Massachusetts, USA) 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 (Cambridge Electronics Design, Cambridge, UK). Per session eight consecutive blocks of 8 s 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 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

Postural stability was measured with a string attached to the waist connected to a measurement device similar to the Wright ataxia meter (Wright, 1971). All body movements in the antero-posterior direction over a period of 2 min 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 VAS as originally described by Norris (1971) (16 items), three factors can be derived, as described by Bond and Lader (1974), 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 VAS (13 items), originally described by Bowdle, *et al.* (1998). From the Bowdle scale, two composite scales could be identified, corresponding to 'internal' and 'external perception', two separate modalities of psychedelic effects (Zuurman, *et al.*, 2008). '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 10 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%. All blood samples were handled and analysed according to GCP/GLP. 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 summarised by treatment and time and were presented graphically as mean over time, with standard deviation as error bars. All pharmacodynamic endpoints were analysed by mixed model repeated measurement analyses of variance (using SAS PROC MIXED, SAS for Windows, V8.2, SAS Institute Inc., Cary North Carolina, 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 was analysed 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 1 h after the fourth inhalation (THC 6 mg). The inhibitory effect of AVE1625 on THC-induced effects on the CNS 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 h 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. Consistent with the pharmacodynamic results (VAS Bowdle 'feeling high'), AVE1625 decreased THC-induced effects (euphoric mood and dizziness). No changes in clinical chemistry, haematology or urinalysis 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 paresthesia. He recovered without sequelae soon after the third THC dose.

Pharmacokinetic and pharmacodynamic results

Twenty-four of the 36 enrolled subjects in the study completed the study (for details see method section). All subjects were evaluable for safety analysis and 34 were included in PK and PD analysis (i.e., completing at least one treatment period). One non-completer did not receive the fourth THC dose of 6 mg during one of his four treatment periods. Consequently that period showed deviating concentration and effect time profiles that would have distorted the analyses of the overall results; and therefore, only the data up to 1 h after the third dose were included. Taking cannabis use into consideration, no differences in response (e.g., heart rate or feeling high) were found between weekly users and the less frequent users.

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). The t_{max} was around 4 h for all doses of AVE1625. Exposure to AVE1625 increased proportionally

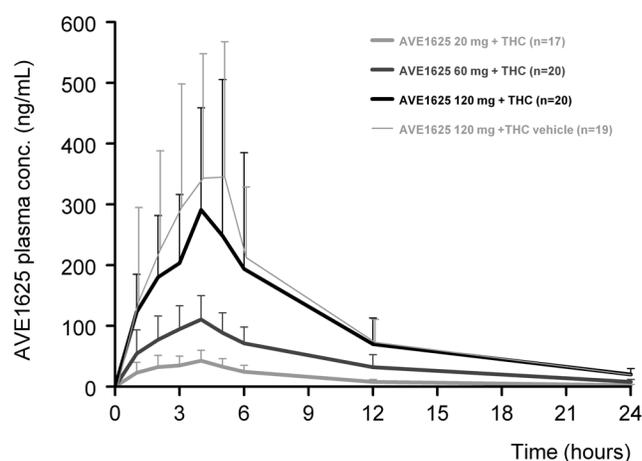


Figure 2 Mean (SD) plasma AVE1625 concentration-time profiles. AVE1625 administration at T = 0. THC vehicle administration T = 3, 4, 5 and 6 hours.

between 20 mg and 60 mg. However, a three-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. The C_{max} was 1.1-fold, and the AUC_{0-24} was 1.2-fold higher with THC vehicle administration compared to the combination with the THC administration.

Pharmacodynamic measurements

Heart rate Heart rate increased significantly in a dose-dependant manner after THC administration (Figure 3). The

Table 2 Effects of AVE1625 on THC-induced effects: estimates of the inhibition ratios with 95% CI at THC peak effect

	Inhibition ratios		
	20 mg AVE1625	60 mg AVE1625	120 mg AVE1625
Heart rate	89% (61, 118)	96% (66, 126)	109% (78, 140)
EEG Pz-0z δ	44% (-16, 104)	88% (16, 160)	85% (14, 156)
EEG Pz-0z θ	98% (32, 164)	69% (12, 125)	91% (28, 154)
EEG Pz-0z β	75% (15, 135)	74% (15, 133)	129% (51, 206)
EEG Fz-Cz β	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)

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.

average THC peak effect consisted of an increase of 15 bpm (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. The inhibition ratio after co-administration of 120 mg was 109%. Effects are subject to measurement variability. If suppression of THC effects is virtually complete, calculation of these ratios can result in values that exceed 100% due to random differences within subjects. No changes in heart rate were observed after administration of AVE1625 120 mg + THC vehicle compared to placebo + THC vehicle.

EEG At the peak THC effect, the power of δ (-15%: 95%CI -6, -21), θ (-16%: 95%CI -10, -23) and β activity (-16%: 95%CI -10, -23) decreased in the Pz-Oz region in comparison with THC vehicle (Table 2). No changes were found in α activity. In the Fz-Cz region, only a decrease in β 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 β activity (+13%: 95%CI +6, +20) and Fz-Cz δ 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

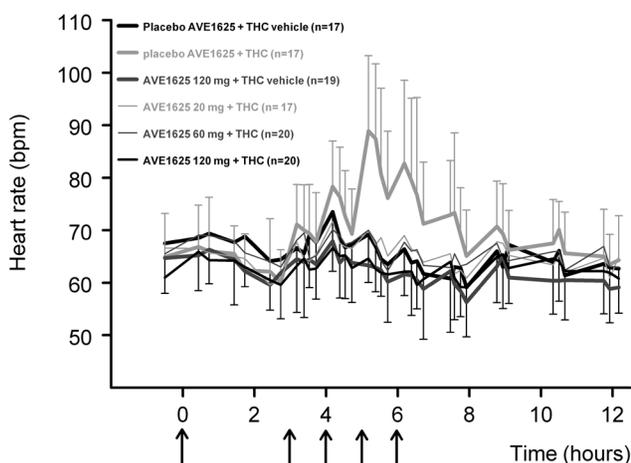


Figure 3 Mean time profile of heart rate with SD as error bars. AVE1625 (or placebo) administration at T = 0. THC (or vehicle) administration: 2 mg at T = 3 hours; 4 mg at T = 4 hours; 6 mg at T = 5 hours; 6 mg at T = 6 hours. Arrows indicate drug administration.

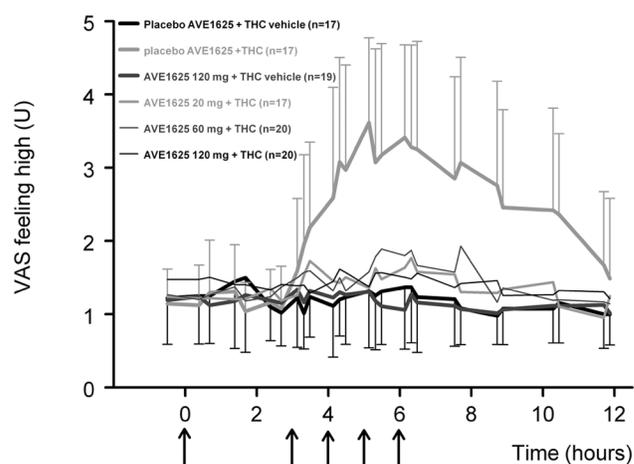


Figure 4 Mean time profile of 'feeling high'-time profiles with SD as error bars. AVE1625 (or placebo) administration at T = 0. THC (or vehicle) administration: 2 mg at T = 3 hours; 4 mg at T = 4 hours; 6 mg at T = 5 hours; 6 mg at T = 6 hours. Arrows indicate drug administration.

120 mg + THC vehicle did not change body sway in comparison to placebo AVE1625 + THC vehicle.

Subjective effects by VAS 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' was seen after co-administration of all doses of AVE1625 (Table 2). For this parameter, a statistically significant dose-dependent effect of

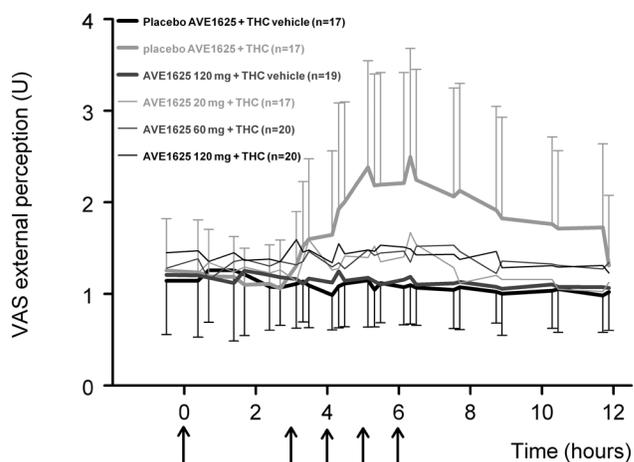


Figure 5 Mean time profile of 'external perception'-time profiles with SD as error bars. AVE1625 (or placebo) administration at T = 0. THC (or vehicle) administration: 2 mg at T = 3 hours; 4 mg at T = 4 hours; 6 mg at T = 5 hours; 6 mg at T = 6 hours. Arrows indicate drug administration.

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 with 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

Thus far, no direct acutely measurable objective pharmacodynamic effects on the CNS have been reported. In the current study, AVE1625 by itself caused only some small EEG changes, which without further indications cannot be taken as direct evidence for CB1-antagonism in the CNS. However, clear proof of CB1 antagonist activity of AVE1625 could be obtained by inhibition of THC-induced effects on the CNS and on heart rate. The effects of THC alone were typical cannabis-like effects as reported in a previous study (Zuurman, *et al.*, 2008). 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 δ 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, the physiology of CB1- and CB2-related systems in humans has only partially been clarified. AVE1625 is a selective CB1 antagonist with very high affinity for the CB1 receptor that antagonised almost all evaluated THC-induced effects. This indicates that both the CNS effects (subjective changes and impaired postural stability) and the probable 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 (Pertwee, 1999), 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. We did not measure THC concentrations in this study, and we cannot exclude a pharmacokinetic interaction with AVE1625 that caused a reduction of THC levels in brain or plasma. It is more likely, however, that the inhibition of the effects of external THC administration by AVE1625 was due to its CB1 antagonist activity in the heart and the CNS. In the present study, healthy mild to moderate male cannabis users were included, and our results may not be generalisable to females, patients or elderly subjects.

Huestis, *et al.* (2001) studied the effects of the CB1 antagonist rimonabant on marijuana-induced effects in 63 healthy male volunteers with a history of smoking marijuana. 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 \approx 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 showed only 38%–43% reductions in subjective effects and of 59% in heart rate. Similar results were found in another interaction study of Huestis, *et al.* (2007) after single or repeated doses of rimonabant in combination with marijuana cigarettes. 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® vaporiser. Because the recovery of the vaporiser is slightly more than 50% (Hazekamp, *et al.*, 2006), 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 approximately 20 mg THC from which about half is lost in the side stream smoke (Casswell, 1975). Considering the difference in time profiles of drug administration, the THC doses of both studies were roughly comparable. However, marijuana cigarettes contain a mixture of psychoactive compounds, which in combination may contribute differently to the psychological and physical effects of marijuana (Elsohly and Slade, 2005). 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.

The therapeutic indications for CB1 antagonists such as 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 (D'Souza, *et al.*, 2004; Naef, *et al.*, 2004; D'Souza, *et al.*, 2005). In this study, we showed that CB1 antagonists are able to antagonise 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. 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 (Meltzer, *et al.*, 2004).

In summary, this study shows a useful method for studying the CNS effects of CB1 antagonists. From the evaluated pharmacodynamic effects, only changes on EEG were observed after AVE1625 administration. AVE1625 penetrates the brain and antagonises 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 evaluation in clinical trials.

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