CHAPTER IV

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

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ABSTRACT

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

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

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

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

INTRODUCTION

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

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

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

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

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

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

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

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

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

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

METHODS

Study design

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

Subjects and power calculation

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

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

Procedure

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

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

Treatments

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

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

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

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

Outcome measures

PHARMACOKINETIC MEASUREMENTS

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

PHARMACODYNAMIC ASSESSMENTS

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

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

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

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

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

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

Bioanalyses

SURINABANT SAMPLES

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

THC SAMPLES

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

Statistical Analyses

ADVERSE EFFECTS

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

NONCOMPARTMENTAL PHARMACOKINETICS

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

PHARMACODYNAMICS

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

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

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

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

Population PK-PD modelling

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

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

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

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

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

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

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

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

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

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

(4) Pjk = TVP · e (ηj+τk)

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

(5) Cobs = Cpred · (1+ε)

(6) Cobs = Cpred + ε

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

RESULTS

Subject demographics

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

Adverse effects

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

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

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

PK analysis

SURINABANT

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

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

THC

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

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

Pharmacodynamics

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

Population PK-PD

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

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

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

DISCUSSION

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

Pharmacokinetics

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

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

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

Pharmacodynamics

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

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

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

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

PK-PD

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

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

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

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

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

* median (minimum, maximum)

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

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

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

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

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

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

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

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

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

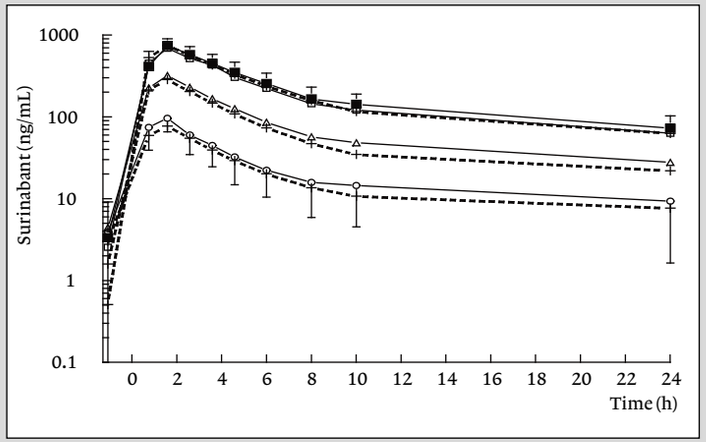
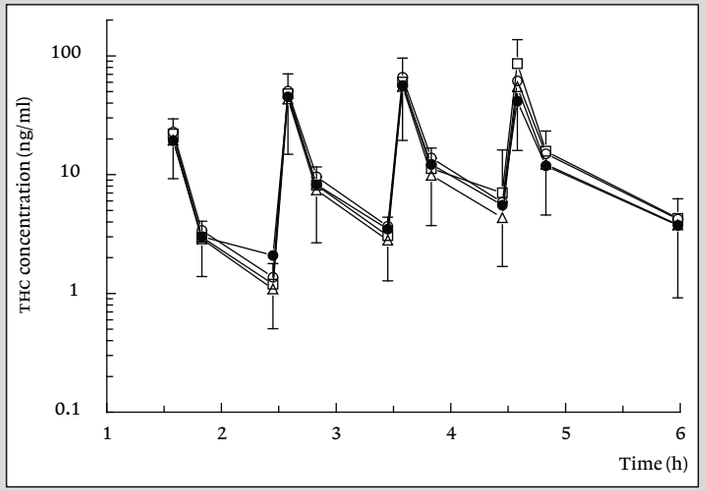


FIGURE 2 Mean plasma concentration-time curve of THC with standard deviations. The arrows indicate the time points of THC administration. The closed circles are the THC concentrations after placebo surinabant + THC treatment, the open circles are surinabant 5 mg + THC, the triangles are surinabant 20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



CHAPTER V

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

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