CHAPTER 2

Does olanzapine inhibit the psychomimetic effects of Δ^9-tetrahydrocannabinol?

J Psychopharmacol 26(10): 1307-1316

Daniël Kleinloog[1,2], Marieke Liem-Moolenaar[1], Gabriël Jacobs[1,2],
Erica Klaassen[1], Marieke de Kam[1], Ron Hijman[3], Joop van Gerven[1,2]

1. Centre for Human Drug Research, The Netherlands
2. Department of Psychiatry, Leiden University Medical Centre, The Netherlands
3. Department of Psychiatry, University Medical Centre, The Netherlands

ABSTRACT

Δ^9-Tetrahydrocannabinol (THC) produces transient psychomimetic effects in healthy volunteers, constituting a pharmacologic model for psychosis. The dopaminergic antagonist haloperidol has previously been shown to reduce these effects. This placebo-controlled, cross-over study in 49 healthy, male, mild cannabis users aimed to further explore this model by examining the effect of a single oral dose of olanzapine (with dopaminergic, serotonergic, adrenergic, muscarinergic and histaminergic properties) or two oral doses of diphenhydramine (histamine antagonist) on the effects of intrapulmonarily administered THC. Transient psychomimetic symptoms were seen after THC administration, as measured on the positive and negative syndrome scale (20.6% increase on positive subscale, $p < 0.001$) and the visual analogue scale for psychedelic effects (increase of 10.7 mm on feeling high). Following the combination of THC and olanzapine, the positive subscale increased by only 13.7% and feeling high by only 8.7 mm. This reduction of THC effects on the positive subscale failed to reach statistical significance ($p = 0.066$). However, one third of the subjects did not show an increase in psychomimetic symptoms after THC alone. Within responders, olanzapine reduced the effects of THC on the positive subscale ($p = 0.005$). Other outcome measures included pharmacokinetics, eye movements, postural stability, pupil/iris ratio, and serum concentrations of cortisol and prolactin.

Introduction

Schizophrenia and other forms of psychosis result from a complex and extensive disruption of the central nervous system (CNS). The pathophysiologic mechanism is not entirely understood and the symptoms of schizophrenia are very heterogenous. Currently available animal models for psychosis and antipsychotic action are not able to adequately model this complex phenomenon (Nestler and Hyman, 2010; Jones et al., 2011).

As an alternative to animal models, several in-human models for psychosis and antipsychotic action have been developed, using psychotropic agents to induce psychomimetic symptoms. Dopaminergic (e.g. amphetamine, see Strakowski et al., 1996), serotonergic (e.g. psilocybin, see Vollenweider et al., 1998) and glutamatergic (e.g. ketamine, see Krystal et al., 1994), as well as cannabinoid compounds (reviewed by Sewell et al., 2010) have been used for this purpose. This idea itself is not new: in the late 1950s one of the registered indications of the partial 5-HT_{2A} agonist lysergic acid (LSD, Delysid®) was to 'experience the nature of psychosis' in normal subjects, patients and psychiatrists (as described by Blewett and Chwelos, 1959).

D'Souza et al. (2004) were the first to show that intravenous administration of Δ^9-tetrahydrocannabinol (THC) induces a transient increase in psychomimetic symptoms in healthy volunteers. These psychomimetic symptoms were measured using the positive subscale of the Positive and Negative Syndrome Scale (PANSS). The results were replicated in healthy volunteers (D'Souza et al., 2008a; Morrison et al., 2009) and patients with schizophrenia (D'Souza et al., 2005).

Subsequently, the potential of the THC-model to measure antipsychotic activity of pharmacologic agents was investigated for the dopamine antagonist haloperidol. In a study by D'Souza et al. (2008a), co-administration of a single dose of haloperidol seemed to show a reduction in psychomimetic symptoms of THC as measured on the PANSS, although this result was not statistically significant. In a study by Liem-Moolenaar et al. (2010b) a significant reduction of THC-induced psychomimetic symptoms as measured on

the PANSS was shown following co-administration of a single dose of haloperidol. The effect of THC on the visual analogue scale (VAS) feeling high was not altered significantly by haloperidol in either study.

The use of THC as a model for psychosis can be supported by the relation between the endocannabinoid system and psychosis. In addition to frequently published links between cannabis use and psychosis (reviewed by D'Souza et al., 2009 and McLaren et al., 2010), several observations connect the endocannabinoid system to the pathophysiology of schizophrenia. Leweke et al. (1999) found increased concentrations of the endocannabinoid anandamide in cerebrospinal fluid of antipsychotic-naïve patients with schizophrenia, which was later replicated by the same group (Giuffrida et al., 2004). It was also found that cerebrospinal fluid concentrations of anandamide were not increased in patients with affective disorders or dementia. Levels of anandamide in cerebrospinal fluid in patients with schizophrenia correlated inversely with the severity of psychotic symptoms as assessed on the PANSS (Giuffrida et al., 2004), suggesting that the endocannabinoid system might be upregulated as a protective mechanism in patients with schizophrenia.

Cerebrospinal fluid concentrations of anandamide were normalized in patients with schizophrenia who were treated with 'typical' (dopaminergic) antipsychotics, but not in patients on 'atypical' (both dopaminergic and serotonergic) antipsychotics (Giuffrida et al., 2004). The authors suggested that 'typical' and 'atypical' antipsychotics may have different effects on the activity of the endocannabinoid system in schizophrenia. The notion that the endocannabinoid system is involved in the pathophysiology of schizophrenia is further supported by neuroimaging studies that found higher expression of cannabinoid type 1 receptors in patients with schizophrenia (Wong et al., 2010; Dalton et al. 2011). In the study by Wong et al. (2010), cerebral expression of CB_1 cannabinoid receptors was correlated with positive symptoms. Additionally, the cannabinoid antagonist rimonabant may reduce positive symptoms in patients with schizophrenia (Kelly et al., 2011).

The pharmacological effect of haloperidol, like most typical antipsychotics, is largely attributed to dopamine receptor antagonism (Agid et al., 2007). Although dopamine antagonism is also important for the activity of atypical antipsychotics, these drugs have a broader pharmacological profile, which includes modulation of serotonergic, glutamatergic, muscarinergic, histaminergic and adrenergic receptors. Among the atypical antipsychotics, olanzapine has relatively low affinity for dopaminergic receptors. However, the antipsychotic efficacy of olanzapine is comparable to that of haloperidol and its effect is assumed to originate from the combination of dopaminergic and serotonergic action. It was hypothesized that the model would be predictive of antipsychotic activity (regardless of the mechanism of action of the antipsychotic drug). The pharmacological differences between olanzapine and haloperidol provide an opportunity to further explore the pharmacologic basis of the PANSS effects of THC as a human psychosis model.

The main objective of the study was to investigate whether olanzapine modulates the (psychomimetic) effects of THC. This could, in addition to the other studies described earlier, further validate the THC model of psychosis. The study also aimed to investigate the influence of drug-induced sedation on psychomimetic symptoms, which is a well known side-effect of olanzapine that is attributed to histamine H_1 receptor antagonism. To this end, the antihistaminergic drug diphenhydramine was incorporated in the design. Also, the effects of THC and olanzapine on the CNS were examined individually.

Methods

Participants

Healthy male subjects aged between 18 and 45 years (inclusive) and with a body mass index between 18 and 30 kg/m^2 (inclusive) were recruited by the Centre for Human Drug Research. Subjects had to be mild cannabis users,

defined as self-reported use of cannabis no more than once a week on average in the last year. After providing written informed consent, subjects received a medical screening within 3 weeks prior to study participation. Clinically relevant abnormalities (in particular a personal or family history of clinically relevant psychiatric illness and/or abnormalities on psychiatric examination) were considered reason for exclusion. More specific: a personal history of attention deficit disorder without the use of medication was allowed; a personal history of depression or psychotic symptoms was not allowed; a family history of psychosis in first or second degree relatives and bipolar disorder in first degree relatives was not allowed. The use of medication and agents (including recreationally used drugs such as cannabis) that were expected to affect central nervous system performance or the pharmacokinetics of the study medication was not allowed during the study period. Subjects were tested for the use of recreational drugs (in urine) and alcohol (in breath) before each study day. Following the medical screening, subjects were trained for the study procedures.

Study design

This was a randomized, double-blind, placebo-controlled, five-way crossover interaction trial with a washout period of minimally two weeks. The study was performed in accordance with Good Clinical Practice and the Dutch Medical Research Involving Human Subjects Act and was approved by the Independent Ethics Committee of the Leiden University Medical Centre.

Interventions

This study investigated the effect of a single oral dose of olanzapine (10 mg tablet, Eli Lilly™) on the psychomimetic effects of THC. A dose of 10 mg olanzapine was expected to show an occupancy rate of dopamine D_2 receptors of 60 to 70% (Kapur et al., 1998). Purified THC was administered intrapulmonary using the Volcano™ vaporizer (Storz-Bickel, Tuttlingen, Germany)

as described in more detail by Zuurman et al. (2008) in three consecutive dosages of 2, 4 and 6 mg with 90 minute intervals. The first dose of THC or placebo was administered 4 hours after olanzapine was given, when the plasma concentration of olanzapine was expected to reach its maximum (Kassahun et al., 1997). Diphenhydramine was used as a positive control for the sedative effects of H_1 antagonism, given as two separate oral doses of 15 mg at 1 and 3 hours after olanzapine administration, to mimic the average expected time-concentration profile of olanzapine.

All treatments and doses were placebo and double-dummy controlled. All subjects were randomized to receive all of the following five treatment arms in a random order: THC + olanzapine (olanzapine 10 mg + placebo diphenhydramine + THC 2, 4 and 6 mg); olanzapine alone (olanzapine 10 mg + placebo diphenhydramine + placebo THC); THC alone (placebo olanzapine + placebo diphenhydramine + THC 2, 4 and 6 mg); placebo (placebo olanzapine + placebo diphenhydramine + placebo THC); and THC + diphenhydramine (placebo olanzapine + diphenhydramine 2 x 15 mg + THC 2, 4 and 6 mg). A treatment arm with diphenhydramine alone was not added as this would increase the burden of the study and diphenhydramine was only added as a positive control.

Outcome measures

Psychomimetic symptoms were measured using the PANSS, as described by Kay et al. (1987). This clinically validated rating scale is based on a structured clinical interview. The interviews were performed four times during a study day: once before the administration of olanzapine (or placebo) and again after each THC (or placebo) administration. To adjust the interview for the repetition of interviews, time frames for symptoms evaluation were limited to 'since this morning' or 'since the last interview'. All interviews were recorded on video and rated by a second blinded person. The PANSS consists of 30 items that are scored on a seven-point scale. The PANSS is subdivided into three subscales: positive, negative and general. The positive subscale,

which consists of 7 items resulting in a total score ranging from 7 to 49, was predefined as the main evaluation endpoint.

To determine other potentially confounding CNS effects, an extensive test battery (NeuroCart) was used, which included VAS, eye movements, postural stability, pupil / iris ratio, Stroop colour word test and the visual verbal learning test (VVLT), extended with measurements of serum cortisol and prolactin. All these measurements were performed repeatedly throughout the study day, including two baseline measurements. The timing of measurements was very similar to the scheme used by Liem-Moolenaar et al. (2010b).

VAS are widely used to quantify subjective effects. In this study, the composite scales described by Bond and Lader (1974) were used to measure alertness, mood and calmness and those described by Bowdle et al. (1998) for psychedelic effects. The VAS for psychedelic effects (VAS Bowdle) is subdivided into the clusters 'internal perception' (5 items), 'external perception' (6 items) and 'feeling high' (1 item). The scores on each individual VAS item can range from 0 to 100 mm.

Both saccadic and smooth pursuit eye movements were recorded through three electrodes placed on the forehead and next to both lateral canthi. The stimulus for saccadic eye movements had amplitude of approximately 15 degrees to either side, with interstimulus intervals varying randomly between 3 and 6 seconds. Smooth pursuit eye movements were stimulated in a sinusoidal manner at frequencies ranging from 0.3 to 1.1 Hz with amplitude of 22.5 degrees to either side. Eye movements are described in greater detail by Zuurman et al. (2008). Saccadic peak velocity is one of the most sensitive parameters for sedation (van Steveninck et al., 1991). The percentage time in which the eye movements are in smooth pursuit of the target is a parameter for motor coordination.

The body sway meter records body movements in a single (sagittal) plane during two minutes while the subjects close their eyes, providing a measure of postural stability, which can be used as a biomarker for drug effect (Liem-Moolenaar et al., 2010a).

The ratio between the diameter of the pupil and the iris forms a measure of the activity of the autonomous nervous system. Diameters were determined using digital photography with flash after adaptation in ambient lighting (Twa et al., 2004).

In the Stroop colour word test, names of colours are formatted in a congruent or incongruent colour. Subjects have to provide the formatted colour of the presented word. Stroop interference effects are helpful in understanding attention, perception and reading (Laeng et al., 2005).

The VVLT is a memory test that uses 30 words in three consecutive trials: immediate recall, delayed recall and delayed recognition. During each study day and at the training, different parallel versions of the test were used to prevent learning effects (Schmitt et al., 2000).

Serum prolactin and cortisol concentrations were measured using electrochemiluminescence immunoassay (ECLIA) as a biomarker for dopaminergic activity.

Repeated blood samples were drawn to determine pharmacokinetic profiles of THC and its main metabolites, olanzapine and diphenhydramine. Samples were analyzed using high performance liquid chromatography with tandem mass spectrometric detection (HPLC-MS).

Sample size

The sample size of the study was based on a power calculation, using the results of a previous study with a comparable design (Liem-Moolenaar et al., 2010b). This study examined the effect of a single oral dose of haloperidol 3 mg on psychomimetic effects of THC. Co-administration of haloperidol was found to reduce the effects of THC as measured on the positive subscale of the PANSS by 1.11 points with an estimated standard deviation of 2.374 points. Using nQuery Advisor v5.0 (Statistical Solutions Ltd, Cork, Ireland) a sample size of 38 was calculated to have 80% power to detect a difference in means of -1.11, assuming a standard deviation of 2.374, using a paired t-test with a 0.05 two-sided significance level. Randomization using Williams

squares for five treatments requires multiples of ten subjects for each group, which required the total number of subjects to be increased to 40. Subjects who did not complete at least three out of five study days were replaced.

Statistical analyses

All pharmacodynamic endpoints were analyzed using a mixed-model analysis of variance (using SAS PROC MIXED). Subject, subject by treatment and subject by time were used as random effects; treatment, study day, time and treatment by time as fixed effects; and the average baseline value as covariate. Parameters of the PANSS, body sway, and neuroendocrine parameters did not have a normal distribution and were analyzed after log-transformation. After analysis these parameters were back-transformed, where the results can be interpreted as percentage change. All outcome measures are presented as estimated means using the least squares method. Parameters of the VAS Bowdle showed a non-normal distribution that could not be corrected by log-transformation. This was largely due to the fact that psychedelic effects are not present under placebo and that a major proportion of the subjects did not show any effect on this outcome measure after THC administration. Results for VAS Bowdle are presented over time as mean ± standard deviation and in a bar graph as mean ± standard deviation for both the average effect and the maximum effect. To further explore the effect of co-administration of olanzapine and diphenhydramine, an exploratory analysis for VAS feeling high was performed using only the treatment arms that included THC administration and only the subjects that showed any response on the VAS feeling high. Within the subpopulation of responders, effects on VAS feeling high showed a normal distribution after log-transformations.

To describe the pharmacokinetic characteristics of THC, olanzapine and diphenhydramine, plasma concentrations were evaluated by data driven compartmental analysis and simulation using NONMEM software (version 7.2.0, Globomax LLC, Ellicot City, MD, USA).

Results

Subjects

A total of 49 subjects were included, 33 (67%) of whom completed all five study days. One subject stopped during the first study day, 5 (10%) stopped after the first study day, 3 (6%) after the second, 5 (10%) after the third and 2 (4%) subjects after the fourth study day. Subjects who did not complete at least three study days were replaced. Premature withdrawal was due to non-compliance in 13 (27%) subjects (two had used cannabis, one did not understand study procedures, seven could no longer attend the planned study days and three did not show up without a reason) and due to adverse events in 3 (6%) subjects (one had paranoid thoughts after THC administration and was discontinued, one had an anxiety attack after THC administration and was discontinued, and one felt too sedated after olanzapine and THC administration and decided to discontinue). All subjects who received at least one administration of THC or its placebo were included in the analysis.

Positive and negative syndrome scale

The least square means (LSM) of the scores on the different subscales of the PANSS are presented in Table 1 and the differences for the main contrasts are presented in Table 2. Compared to placebo, administration of THC induced an average increase on the positive subscale of the PANSS of 20.6% (95%CI 13.1-28.6%; $p < 0.001$). Co-administration of THC and olanzapine caused an average increase on the positive subscale of 13.7% (95%CI 6.5-21.3%; $p < 0.001$). This apparent average reduction of psychomimetic symptoms as measured on the positive subscale of the PANSS by olanzapine did not reach statistical significance in the whole group ($p = 0.066$). However, it appeared that a considerable number of subjects did not show any increase of positive PANSS scores after THC. When no increase was seen, an effect floor occurred

that obviously could not be reduced by any intervention. Therefore, a secondary analysis was performed on the PANSS scores of responders only. For this purpose, responders were conservatively defined as subjects who showed at least one point increase on the positive subscale compared to baseline in any of the measurements following THC administration. In these 33 (67%) responders, THC induced an average increase on the positive subscale of 25.1% (95%CI 16.6-34.1%; $p < 0.001$). Co-administration of olanzapine reduced this average increase to 13.2% (95%CI 5.4-21.5%; $p = 0.001$). This reduction of positive PANSS-increases was highly significant ($p = 0.005$, THC + olanzapine compared to THC alone). The psychomimetic effects as measured on the positive subscale of the PANSS for responders are presented in Figure 1. Co-administration of olanzapine did not significantly alter the effects induced by THC on the general and negative subscale. Diphenhydramine did not affect the effects of THC on any of the subscales of the PANSS.

Visual analogue scales

Table 1 provides an overview of the LSM and differences between contrasts for the different clusters on the VAS. The average score on VAS feeling high increased from 0.0 mm (SD 0.2; range 0.0-1.0) under placebo condition to 10.7 mm (SD 11.9; range 0.0-38.3) following THC administration. Co-administration of olanzapine led to an average score on VAS feeling high of 8.7 mm (SD 14.0; range 0.0-67.4). Olanzapine seemed to mildly inhibit the effects of THC, but this could not be formally tested due to data skewness (because many subjects did not report a high-effect). In an exploratory analysis, using only treatment arms that contained THC and subjects who showed any response on the VAS feeling high, the reduction of VAS feeling high caused by olanzapine was statistically significant ($p = 0.020$). Co-administration of diphenhydramine did not influence the effects of THC on VAS feeling high. The effects are presented in Figure 2. The Bowdle

cluster 'internal perception' increased mildly from an average composite score of 0.0 mm (SD 0.0; range 0.0-0.2) under placebo conditions to 0.4 mm (SD 0.9; range 0.0-4.2) following THC administration. The average score on the cluster 'external perception' increased mildly from 0.0 mm (SD 0.0; range 0.0-0.2) under placebo condition to 1.7 mm (SD 2.5; range 0.0-9.5) following THC administration. The effect of THC on 'internal perception' and 'external perception' was not affected by co-administration of either olanzapine or diphenhydramine.

VAS alertness decreased following administration of THC alone (-2.2 mm; 95%CI -3.8--0.6; $p = 0.006$) and olanzapine alone (-5.4 mm; 95%CI -7.0--3.9; $p < 0.001$). The additional effect of olanzapine to THC administration (-6.2 mm; 95%CI -7.8--4.7; $p < 0.001$) was larger than that of diphenhydramine (-1.4 mm; 95%CI -2.9-0.2; $p = 0.078$). VAS calmness increased mildly after THC alone (+1.7 mm; 95%CI 0.6-2.7; $p = 0.003$) and olanzapine alone (+1.2 mm; 95%CI 0.2-2.3; $p = 0.024$) administration. No significant additional effect of co-administration of either olanzapine or diphenhydramine was found. VAS mood increased non-significantly by 1.1 mm (95%CI -0.0-2.2; $p = 0.054$) following THC alone administration, and decreased by 0.7 mm (95%CI -1.8-0.4; $p = 0.217$) with olanzapine alone. Co-administration of olanzapine and THC decreased VAS mood by 1.1 mm (95%CI -2.2-0.1; $p = 0.068$) compared to placebo and 2.2 mm (95%CI 1.1-3.3; $p < 0.001$) compared to THC alone.

Eye movements

Olanzapine alone caused a decrease in saccadic peak velocity of 98 deg/s (95%CI 85-110; $p < 0.001$), an increase in saccadic reaction time of 28 msec (95%CI 19-36; $p < 0.001$), an increase in saccadic inaccuracy of 2.3% (95%CI 1.6-3.0%; $p < 0.001$) and a decrease in smooth pursuit of 9.4% (95%CI 5.7-13.1%; $p < 0.001$) compared to placebo. THC alone increased saccadic inaccuracy by 0.7% (95%CI 0.1-1.3%; $p = 0.033$) compared to placebo. Other eye movement parameters were not affected by the other treatments.

Body sway

Administration of THC alone increased the body sway by 56% (95%CI 39-76%; $p < 0.001$) compared to placebo. Olanzapine alone increased body sway by 121% (95%CI 95-150%; $p = 0.001$). Co-administration of THC and olanzapine increased body sway by 177% (95%CI 139-221%; $p < 0.001$) compared to placebo and by 77% (95%CI 54-104%; $p < 0.001$) compared to THC alone. Diphenhydramine did not significantly affect the effects of THC on body sway.

Pupil size

Olanzapine alone caused a decrease in pupil / iris ratio of 0.27 (95%CI 0.25-0.29; $p < 0.001$) compared to placebo. The other treatments did not significantly affect pupil size.

Stroop colour word test

THC and olanzapine did not affect the Stroop test by themselves. The number of incorrect answers increased following co-administration of THC and olanzapine (+0.5; 95%CI 0.1-0.8; $p = 0.010$) compared to administration of THC alone.

Visual verbal learning test

Olanzapine caused a strong reduction on the outcome on the VVLT, for the immediate recall, the delayed recall and the recognition trials, both alone when compared to placebo and when co-administration with THC was compared to THC alone. THC alone reduced delayed recall by 2.0 (95%CI 0.4-3.6; p 0.016) compared to placebo. There were no significant differences for other contrasts.

Prolactin and cortisol

THC alone induced a decrease in serum prolactin concentrations by 17% (95%CI 9-24; $p < 0.001$) compared to placebo. Olanzapine alone caused an increase in serum prolactin concentrations by 255% (95%CI 224-289; $p < 0.001$) compared to placebo. Co-administration of THC and olanzapine decreased prolactin concentrations by 10% (95%CI 1-17; $p = 0.026$) compared to olanzapine alone. Compared to placebo, serum cortisol concentrations increased by 21% (95%CI 3-44; $p = 0.024$) following THC alone, and decreased by 45% (95%CI 35-54; $p < 0.001$) after olanzapine alone, and by 25% (95%CI 11-36; $p = 0.001$) when olanzapine was co-administered with THC compared to THC alone. The serum prolactin concentrations are presented in Figure 3.

Pharmacokinetics

The pharmacokinetics of THC were best described using a two compartment linear model with zero-order absorption. The apparent volume of distribution was 10.3 L and the apparent clearance 149 L/hr. The pharmacokinetics of olanzapine could be described with a one compartment model with buffered absorption and an apparent volume of distribution of 7 L and apparent clearance of 0.3 L/hr. The pharmacokinetics of diphenhydramine required a one compartment model with linear absorption and an apparent volume of distribution of 805 L and apparent clearance of 106 L/hr. An overview of the pharmacokinetic parameters is provided in Table 3 and the pharmacokinetic profiles are presented in Figure 4.

Discussion

THC induced a transient psychomimetic effect in healthy volunteers, as measured on the positive subscale of the PANSS and VAS feeling high. This

effect was reduced by olanzapine, but not diphenhydramine. This induction of psychotic-like symptoms and their suppression by anti-psychotic treatments suggests that the model bears resemblance with clinical psychotic symptoms.

The level of induction of the effects on the positive subscale of the PANSS of THC is comparable to previous studies (D'Souza et al., 2004, 2008a, 2008b; Morrison et al., 2009; Bhattacharyya et al., 2010; Liem-Moolenaar et al., 2010b; Barkus et al., 2011). Co-administration of olanzapine leads to a 33.5% reduction (47.4% in responders) of psychomimetic symptoms. This is a smaller reduction than found with haloperidol by Liem-Moolenaar et al. (2010b), but larger than D'Souza et al. (2008a) reported for haloperidol.

THC did not only produce increases in positive PANSS scores, but also well-known euphoric ('high') feelings and other subjective and objective CNS effects. Some of these were also affected by olanzapine. In particular, VAS feeling high effects of THC seemed to be diminished by olanzapine, in apparent contrast to what has previously been reported for haloperidol (D'Souza et al., 2008a; Liem-Moolenaar et al., 2010b). It is difficult to compare the absolute increase on VAS feeling high with previous results. The proportion of non-responders caused considerable skewness of scores on VAS feeling high (leading to many scores of 0, both in the placebo and THC conditions), making even non-parametrical analyses difficult. It is unclear if other studies experienced similar statistical incongruities, but these may have contributed to the differences in analytical approaches that are encountered in the literature. D'Souza et al. (2008a) used a non-parametric approach to describe the effects on VAS feeling high. They found a somewhat stronger effect of THC on VAS feeling high, which can possibly be explained by differences in dose or administration route. Liem-Moolenaar et al. (2010b) used log-transformation to normalize the distribution of the VAS feeling high outcomes and subsequently used a mixed-model analysis of variance. When the same method was applied to data of the current study, a similar increase was found following THC administration. In previous studies, haloperidol did not have a statistically significant influence on

the effects of THC on VAS feeling high (D'Souza et al., 2008a; Liem-Moolenaar et al., 2010b). However, the graph in the publication by Liem-Moolenaar et al. suggests some blunting of the effect. In the results of the current study, olanzapine seems to reduce the effects of THC on VAS feeling high, although this was only formally tested in a post hoc exploratory analysis. The use of different statistical approaches interferes with a quantitative comparison between studies, but it cannot be excluded that antipsychotic drugs can diminish high feelings to some extent.

The VAS calmness was mildly increased following administration of THC. This is the opposite effect as described by Crippa et al. (2009) in a review on the relation between cannabis / THC use and anxiety. During the current study, one subject experienced anxiety and was discontinued for this reason. No other subjects reported anxiety. The absence of THC-induced anxiety could be explained by the selection criteria for this study. Symptoms of anxiety are most common in cannabis-naive individuals (in particular in people who are vulnerable for psychiatric symptoms), whereas people with cannabis dependence typically have higher state anxiety levels and a reduction in anxiety with cannabis use (Crippa et al., 2009). For this study, mild cannabis users (with previous exposure to cannabis and no cannabis dependence) were selected and subjects with a vulnerability for psychiatric symptoms (psychosis, anxiety or other) were excluded during the medical screening.

As is the case with every model, this model also has its limitations. The THC model only represents a small part of the clinical spectrum of schizophrenia, which is a complex chronic syndrome with a range of negative, positive, cognitive, behavioural and emotional symptoms. The absolute increase in psychomimetic symptoms is transient and small, but statistically highly significant. This can be an ethical advantage in studies with healthy subjects, where the induction of psychomimetic symptoms should be reproducible, but mild, safe and rapidly reversible.

The variability and distribution of the outcome measures of the THC model posed some statistical problems with the analysis and interpretation

of the effects. One third of the participants did not show an increase on the PANSS or on the VAS Bowdle and the distribution of the VAS Bowdle among responders is not normal. This may in part be due to the subjective nature of effects like 'feeling high', which can be interpreted differently by different subjects, but it may also reflect individual differences in sensitivity to psychotic decompensation following cannabis (over)use. Differences in proportions of responders and non-responders complicate the design and interpretation of studies using THC or cannabis to induce psychotic-like symptoms or euphoric ('high') feelings, even if regular (albeit non-frequent) users are recruited. In practice, this can be overcome by selecting subjects based on their response to a test-dose of THC, prior to their inclusion into an actual drug-interaction study.

THC induced mild sedation, as measured on the VAS Bond and Lader. There was also a mild decrease in attention as measured by saccadic inaccuracy and the VVLT and in postural stability as measured by the body sway. These effects are comparable with those previously found with THC (Zuurman et al., 2008; Liem-Moolenaar et al., 2010b). Olanzapine showed clear sedative effects, as measured subjectively on the VAS Bond and Lader and objectively through saccadic and smooth pursuit eye movements, comparable with those previously described (Morrens et al., 2007). Furthermore, strong effects were seen on the pupil / iris ratio, body sway and VVLT, suggesting effects on the autonomic nervous system, decreased postural stability and impaired memory or attention. The sedative effects were expected because olanzapine is a potent histamine H_1 receptor antagonist. The sedative and other non-psychomimetic effects of THC and olanzapine were cumulative when both drugs were administered together.

The possibility was considered that the sedative effects of olanzapine 10 mg could have a non-specific impact on its potential antipsychotic-like effects. For this reason, diphenhydramine was added to the study as a positive control. Unfortunately, the dose that we selected for this histamine H_1 antagonist caused less sedation than olanzapine, although the time profile of exposure to both drugs was similar and constant during the

pharmacologic tests. This apparent lack of sedative equipotency was unintended, but the consequence is that we are unable to determine how much of olanzapine's positive PANSS reduction was caused by sedation, and how much by a true antipsychotic effect. However, in the study by Liem-Moolenaar et al. (2010b), haloperidol seemed to cause less sedation and somewhat more PANSS reduction than olanzapine did in the current experiment. This suggests that the contribution of sedation to anti-psychotic-like effects of olanzapine in this model is limited, although an additional contribution of non-specific CNS effects cannot be fully excluded.

The clear and reproducible psychomimetic effects of THC in various studies support the involvement of the endocannabinoid system in psychosis. It should however be acknowledged that the exogenous administration of THC does not necessarily translate to the effect of endocannabinoid system. Most findings of THC challenge studies and many other reports in the literature suggest that dopamine activation by THC plays an essential role in its psychosis-like effects. THC has been shown to increase the release of dopamine in the human striatum, through activation of cannabinoid type 1 (CB_1) receptors, although not all imaging studies have been able to confirm this. Bossong et al. (2009) found a moderate decrease in striatal [^{11}C]-raclopride binding following intrapulmonary THC administration, which suggests an increased dopamine release that would constitute a logical mechanism of action for antidopaminergic antipsychotics. Stokes et al. (2009) however did not find a change in [^{11}C]-raclopride binding after oral administration of THC and Barkus et al. (2011) did not find a change in [^{123}I]-IBZM binding after intravenous administration of THC. This difference might in part be explained by differences in study designs (route of administration, dose, timing of scans) and/or non-response of participants. Interestingly, Bhattacharyya et al. (2010) showed decreased striatal activation on FMRI during a verbal memory task, which was correlated with the severity of psychomimetic symptoms as measured on the PANSS. THC has also been shown to decrease [^{11}C]-raclopride binding in extrastriatal regions, although it can be debated if this represents indirect dopamine release (Stokes et al., 2010).

The degree of dopamine release in the study of Bossong et al. (2009) was only moderate when compared to other drugs (amphetamine, cocaine, alcohol and nicotine), even at a relatively high dose of THC. This might also explain the absence of dopamine release in other THC studies and could represent a mild and indirect effect of CB_1 activation on dopamine release. The moderate degree of dopamine release is consistent with the moderate absolute increase on the positive subscale of the PANSS, which is much less than observed during clinical psychotic episodes.

The neuroendocrine effects that were found in this study provide further support for the involvement of dopamine systems in THC-induced psychomimetic effects. Dopamine has long been known as a 'prolactin inhibiting factor', and an increase in serum prolactin concentrations constitutes an established biomarker for dopamine antagonism (de Visser et al., 2001). Consequently, olanzapine induces a large increase in serum prolactin concentrations, both when administered alone and with THC. THC, however, causes a mild decrease in serum prolactin concentrations compared to placebo, and it reduces olanzapine-induced prolactin release after co-administration. It should be noted that serum prolactin concentrations can more easily be increased than decreased (a so-called 'floor-effect'). These effects on serum prolactin demonstrate that dopamine release in tuberopituitary pathways is increased by THC and support the notion that other dopaminergic systems in the brain are involved in the psychomimetic effects of cannabinoids.

In previous studies, the dopamine antagonist haloperidol reduced the THC-induced increase on the positive subscale of the PANSS (D'Souza et al., 2008a; Liem-Moolenaar et al., 2010b). We used olanzapine to examine the effect of an atypical antipsychotic with a broader relevant pharmacological spectrum than primarily on dopamine alone. Olanzapine has a relatively low affinity for dopamine compared to other neurotransmitter systems, although the antipsychotic efficacy of all currently available antipsychotics, including olanzapine, can be related to their dopaminergic activity (de Visser et al., 2001; Agid et al., 2007). Dopaminergic activation also seems to

be essential in the THC model described in this study. However, olanzapine has several other pharmacological (histaminergic, serotonergic) effects, which may have blunted the psychomimetic effects of THC. Investigations using other antipsychotic drugs, like quetiapine (which also has a relatively low affinity for dopamine receptors, but less affinity for histamine and serotonin receptors than olanzapine), may improve the understanding of the role of different pharmacological systems in the THC psychosis model and the influence of non-specific pharmacological and functional effects.

In conclusion, the current study provides further support for the use of THC in healthy volunteers as a model for psychosis and anti-psychotic drug action in both pathophysiological and pharmacologic research. This model could be used in early phase clinical trials to predict the clinical efficacy of novel antipsychotic drugs.

REFERENCES

Agid O, Mamo D, Ginovart N, Vitcu I, Wilson AA, Zipursky RB and Kapur S (2007) Striatal vs extrastriatal dopamine D_2 receptors in antipsychotic response-a double-blind PET study in schizophrenia. *Neuropsychopharmacology* 32(6): 1209-1215.

Barkus E, Morrison PD, Vuletic D, Dickson JC, Ell PJ, Pilowsky LS, et al. (2011) Does intravenous Δ^9-tetrahydrocannabinol increase dopamine release? A SPET study. *J Psychopharmacol* 25(11): 1462-1468.

Bhattacharyya S, Morrison PD, Fusar-Poli P, Martin-Santos R, Borgwardt S, Winton-Brown T, et al. (2010) Opposite effects of Δ^9-tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. *Neuropsychopharmacology* 35(3): 764-774.

Blewett DB and Chwelos N (1959) Handbook for the therapeutic use of lysergic acid diethylamide-25. Individual and group procedures. Available at: www.maps.org/ritesofpassage/lsdhandbook.html.

Bond A and Lader M (1974) The use of analogue scales in rating subjective feelings. *Br J Med Psychol* 47(3): 211-218.

Bossong MG, van Berckel BNM, Boellaard R, Zuurman L, Schuit RC, Windhorst AD, et al. (2009) Δ^9-tetrahydrocannabinol induces dopamine release in the human striatum. *Neuropsychopharmacology* 34(3): 759-766.

Bowdle TA, Radant AD, Cowly DS, Kharasch ED, Strassman RJ and Roy-Byrne PP (1998) Psychedelic effects of ketamine in healthy volunteers: relationship to steady-state plasma concentrations. *Anesthesiology* 88(1): 82-88.

Crippa JA, Zuardi AW, Martín-Santos R, Bhattacharyya S, Atakan Z, McGuire P and Fusar-Poli P (2009) Cannabis and anxiety: a critical review of the evidence. *Hum Psychopharmacol* 24(7): 515-523.

D'Souza DC, Perry E, MacDougall L, Ammerman Y, Cooper T, Wu YT, et al. (2004) The psychotomimetic effects of intravenous Δ^9-tetrahydrocannabinol in healthy individuals: implications for psychosis. *Neuropsychopharmacology* 29(8): 1558-1572.

D'Souza DC, Abi-Saab WM, Madonick S, Forselius-Bielen K, Doersch A, Braley G, et al. (2005) Δ^9-tetrahydrocannabinol effects in schizophrenia: implications for cognition, psychosis, and addiction. *Biol Psychiatry* 57(6): 594-608.

D'Souza DC, Braley G, Blaise R, Vendetti M, Oliver S, Pittman B, et al. (2008a) Effects of haloperidol on the behavioral, subjective, cognitive, motor, and neuroendocrine effects of Δ^9-tetrahydrocannabinol in humans. *Psychopharmacology (Berl)* 198(4): 587-603.

D'Souza DC, Ranganathan M, Braley G, Gueorguieva R, Zimolo Z, Cooper T, et al. (2008b) Blunted psychotomimetic and amnestic effects of Δ^9-tetrahydrocannabinol in frequent users of cannabis. *Neuropsychopharmacology* 33(10): 2505-2516.

D'Souza DC, Sewell RA and Ranganathan M (2009) Cannabis and psychosis/schizophrenia: human studies. *Eur Arch Psychiatry Clin Neurosci* 259(7): 413-431.

Dalton VS, Long LE, Weickert CS and Zavitsanou K (2011) Paranoid schizophrenia is characterized by increased CB_1 receptor binding in the dorsolateral prefrontal cortex. *Neuropsychopharmacology* 36(8): 1620-1630.

Giuffrida A, Leweke FM, Gerth CW, Schreiber D, Koethe D, Faulhaber J, et al. (2004) Cerebrospinal anandamide levels are elevated in acute schizophrenia and are inversely correlated with psychotic symptoms. *Neuropsychopharmacology* 29(11): 2108-2114.

Jones CA, Watson DJ and Fone KC (2011) Animal models of schizophrenia. *Br J Pharmacol* 164(4): 1162-1194.

Kapur S, Zipursky RB, Remington G, Jones C, DaSilva J, Wilson AA and Houle S (1998) 5-HT_2 and D_2 receptor occupancy of olanzapine in schizophrenia: a PET investigation. *Am J Psychiatry* 155(7): 921-928.

Kassahun K, Mattiuz E, Nyhart E Jr, Obermeyer B, Gillespie T, Murphy A, et al. (1997) Disposition and biotransformation of the antipsychotic agent olanzapine in humans. *Drug Metab Dispos* 25(1): 81:93.

Kay SR, Fiszbein A and Opler LA (1987) The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophr Bull* 13(2): 261-276.

Kelly DL, Gorelick DA, Conley RR, Boggs DL, Linthicum J, Liu F, et al. (2011) Effects of the cannabinoid-1 receptor antagonist rimonabant on psychiatric symptoms in overweight people with schizophrenia: a randomized, double-blind, pilot study. *J Clin Psychopharmacol* 31(1): 86-91.

Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, et al. (1994) Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Arch Gen Psychiatry* 51(3): 199-214.

Laeng B, Låg T and Brennen T (2005) Reduced Stroop interference for opponent colors may be due to input factors: evidence from individual differences and a neural network simulation. *J Exp Psychol Hum Percept Perform* 31(3): 438-452.

Leweke FM, Giuffrida A, Wurster U, Emrich HM and Piomelli D (1999) Elevated endogenous cannabinoids in schizophrenia. *Neuroreport* 10(8): 1665-1669.

Liem-Moolenaar M, Gray FA, de Visser SJ, Franson KL, Schoemaker RC, Schmitt JA, et al. (2010a) Psychomotor and cognitive effects of a single oral dose of talnetant (SB223412) in healthy volunteers compared with placebo or haloperidol. *J Psychopharmacol* 24(1): 73-82.

Liem-Moolenaar M, te Beek ET, de Kam ML, Franson KL, Kahn RS, Hijman R, et al. (2010b) Central nervous system effects of haloperidol on THC in healthy male volunteers. *J Psychopharmacol* 24(11): 1697-1708.

McLaren JA, Silins E, Hutchinson D, Mattick RP and Hall W (2010) Assessing evidence for a causal link between cannabis and psychosis: a review of cohort studies. *Int J Drug Policy* 21(1): 10-19.

Morrens M, Wezenberg E, Verkes RJ, Hulstijn W, Ruigt GS and Sabbe BG (2007) Psychomotor and memory effects of haloperidol, olanzapine and paroxetine in healthy subjects after short-term administration. *J Clin Psychopharmacol* 27(1): 15-21.

Morrison PD, Zois V, McKeown DA, Lee TD, Holt DW, Powell JF, et al. (2009) The acute effects of synthetic intravenous Δ^9-tetrahydrocannabinol on psychosis, mood and cognitive functioning. *Psychol Med* 39(10): 1607-1616.

Nestler EJ and Hyman SE (2010) Animal models of neuropsychiatric disorders. *Nat Neurosci* 13(10): 1161-1169.

Schmitt JA, Jorissen BL, Sobczak S, Van Boxtel MP, Hogervorst E, Deutz NE and Riedel WJ (2000) Tryptophan depletion impairs memory consolidation but improves focussed attention in healthy young volunteers. *J Psychopharmacol* 14(1): 21-29.

Sewell RA, Skosnik PD, Garcia-Sosa I, Ranganathan M and D'Souza DC (2010) Behavioral, cognitive and pathophysiological effects of cannabinoids: relevance to psychosis and schizophrenia. *Rev Bras Psiquiatr* 32(Suppl 1): S15-S30.

van Steveninck AL, Schoemaker HC, Pieters MS, Kroon R, Breimer DD and Cohen AF (1991) A comparison of the sensitivities of adaptive tracking, eye movement analysis and visual analog lines to the effect of incremental doses of temazepam in healthy volunteers. *Clin Pharmacol Ther* 50(2): 172-180.

Stokes PR, Mehta MA, Curran HV, Breen G and Grasby PM (2009) Can recreational doses of THC produce significant dopamine release in the human striatum? *Neuroimage* 48(1): 186-190.
Stokes PR, Egerton A, Watson B, Reid A, Breen G, Lingford-Hughes A, et al. (2010) Significant decreases in frontal and temporal [^{11}C]-raclopride binding after THC challenge. *Neuroimage* 52(4): 1521-1527.
Strakowski SM, Sax KW, Setters MJ and Keck PE Jr (1996) Enhanced response to repeated d-amphetamine challenge: evidence for behavioral sensitization in humans. *Biol Psychiatry* 40(9): 872-880.
Twa MD, Bailey MD, Hayes J and Bullimore M (2004) Estimation of pupil size by digital photography. *J Cataract Refract Surg* 30(2): 381-389.
de Visser SJ, van der Post J, Pieters MS, Cohen AF and van Gerven JMA (2001) Biomarkers for the effects of antipsychotic drugs in healthy volunteers. *Br J Clin Pharmacol* 51(2): 119-132.
Vollenweider FX, Vollenweider-Scherpenhuyzen MF, Bäbler A, Vogel H and Hell D (1998) Psilocybin induces schizophrenia-like psychosis in humans via a serotonin-2 agonist action. *Neuroreport* 9(17): 3897-3902.
Wong DF, Kuwabara H, Horti AG, Raymont V, Brasic J, Guevara M, et al. (2010) Quantification of cerebral cannabinoid receptor subtype 1 (CB_1) in healthy subjects and schizophrenia by the novel PET radioligand [^{11}C]OMAR. *Neuroimage* 52(4): 1505-1513.
Zuurman L, Roy C, Schoemaker RC, Hazekamp A, den Hartigh J, Bender JC, et al. (2008) Effect of intrapulmonary tetrahydrocannabinol administration in humans. *J Psychopharmacol* 22(7): 707-716.

TABLE 1 Least square means for pharmacodynamic outcome measures.

	placebo	olanzapine alone	THC alone	THC + olanzapine	THC + diphenh.
Positive PANSS	7.4	7.7	8.9	8.4	9.0
Positive PANSS (responders)	7.4	7.6	9.3	8.4	9.1
Negative PANSS	8.1	11.4	8.8	12.6	9.0
General PANSS	17.2	19.9	18.3	21.5	19.1
VAS feeling high (mm)	0.0	0.8	10.7	8.7	11.8
VAS internal perception (mm)	0.0	0.3	0.4	0.7	0.6
VAS external perception (mm)	0.0	0.4	1.7	1.4	1.7
VAS alertness (mm)	51.0	45.5	48.8	42.5	47.4
VAS calmness (mm)	51.2	52.4	52.9	53.4	52.9
VAS mood (mm)	52.1	51.4	53.2	51.1	52.6
Saccadic peak velocity (deg/s)	480	382	481	373	470
Saccadic inaccuracy (%)	6.4	8.7	7.1	8.7	7.4
Saccadic reaction time (ms)	200	228	207	251	209
Smooth pursuit (%)	48.1	38.7	45.7	34.9	46.4
Body sway (mm)	220	486	345	610	347
Pupil / iris - ratio left	0.55	0.27	0.54	0.28	0.54
Serum cortisol (µmol/L)	0.23	0.13	0.28	0.21	0.24
Serum prolactin (µg/L)	9.0	32.0	7.5	28.9	7.7
VVLT - First recall (No correct)	9.5	8.5	8.7	6.1	8.4
VVLT - Second recall (No correct)	14.1	10.5	13.3	9.1	12.8
VVLT - Third recall (No correct)	17.0	13.2	15.6	10.6	15.0
VVLT - Delayed recall (No correct)	13.8	8.7	11.9	5.8	10.8
VVLT - Recognition (No correct)	24.9	22.4	24.2	21.3	22.7
Stroop (No incorrect)	0.1	0.4	0.3	0.8	0.1
Stroop (reaction time, ms)	74	57	72	79	69

TABLE 2 Estimates of difference, 95% confidence intervals and p-values for main contrasts.

	olanzapine alone	THC alone	THC + olanzapine
	vs	vs	vs
	placebo	placebo	THC alone
Positive PANSS	+3.4%	+20.6%	-5.7%
	-3.0 - +10.3%	+13.1 - +28.6%	-11.5 - +0.4%
	0.305	<0.001	0.066
Positive PANSS (responders)	+2.4%	+25.1%	-9.5%
	-4.7 - +9.9%	+16.6 - +34.1%	-15.5 - -3.1%
	0.516	<0.001	0.005
Negative PANSS	+41.0%	+9.5%	+42.5%
	+28.1 - +55.2%	-0.5 - +20.5%	+29.7 - +56.5%
	<0.001	0.063	<0.001
General PANSS	+15.6%	+6.2%	+17.4%
	+9.6 - +22.0%	+0.7 - +12.0%	+11.4 - +23.7%
	<0.001	0.027	<0.001
VAS alertness (mm)	-5.4	-2.2	-6.2
	-7.0 - -3.9	-3.8 - -0.6	-7.8 - -4.7
	<0.001	0.006	<0.001
VAS calmness (mm)	+1.2	+1.7	+0.5
	+0.2 - +2.3	+0.6 - +2.7	-0.6 - +1.6
	0.024	0.003	0.351
VAS mood (mm)	-0.7	+1.1	-2.2
	-1.8 - 0.4	-0.0 - +2.2	-3.3 - -1.1
	0.217	0.054	<0.001
Saccadic peak velocity (deg/s)	-98	+1	-108
	-110 - -85	-11 - +13	-121 - -95
	<0.001	0.884	<0.001
Saccadic inaccuracy (%)	+2.3	+0.7	+1.7
	+1.6 - +3.0	+0.1 - +1.3	+1.0 - +2.4
	<0.001	0.033	<0.001
Saccadic reaction time (ms)	+28	+6	+44
	+19 - +36	-2 - +14	+35 - +52
	<0.001	0.111	<0.001

Smooth pursuit (%)	-9.4	-2.4	-10.8
	-13.1 - -5.7	-6.1 - +1.3	-14.4 - -7.1
	< 0.001	0.208	< 0.001
Body sway (mm)	+121	+56	+77
	+95 - +150	+39 - +76	+54 - +104
	< 0.001	< 0.001	< 0.001
Pupil / iris - ratio left	-0.27	-0.01	-0.27
	-0.29 - -0.25	-0.02 - +0.01	-0.28 - -0.25
	< 0.001	0.558	< 0.001
Serum cortisol (µmol/L)	-45%	+22%	-25%
	-54 - -35	+3 - +44	-36 - -11
	< 0.001	0.024	0.001
Serum prolactin (µg/L)	+255%	-17%	+286%
	+224 - +289	-24 - -9	+253 - +321
	< 0.001	< 0.001	< 0.001
VVLT - First recall (No correct)	-1.0	-0.8	-2.6
	-2.2 - +0.1	-2.0 - +0.3	-3.8 - -1.4
	0.083	0.157	< 0.001
VVLT - Second recall (No correct)	-3.6	-0.7	-4.2
	-5.2 - -2.0	-2.3 - +0.8	-5.8 - -2.6
	< 0.001	0.341	< 0.001
VVLT - Third recall (No correct)	-3.8	-1.4	-5.1
	-5.5 - -2.1	-3.0 - +0.3	-6.8 - -3.4
	< 0.001	0.098	< 0.001
VVLT - Delayed recall (No correct)	-5.2	-2.0	-6.0
	-6.8 - -3.6	-3.6 - -0.4	-7.7 - -4.4
	< 0.001	0.016	< 0.001
VVLT - Recognition (No correct)	-2.5	-0.7	-2.9
	-4.4 - -0.6	-2.6 - +1.2	-4.9 - -0.9
	0.010	0.472	0.004
Stroop (No incorrect)	+0.3	+0.2	+0.5
	-0.0 - +0.7	-0.1 - +0.5	+0.1 - +0.8
	0.076	0.272	0.010
Stroop (reaction time, ms)	-18	-2	+7
	-42 - +6	-26 - +22	-17 - +31
	0.149	0.854	0.568

TABLE 3 Estimated pharmacokinetic parameters (± SE) for THC, olanzapine and diphenhydramine.

	THC	Olanzapine	Diphenhydramine
V_D/F (L)	10.3 (0.8)	7.0 (0.2)	805 (56)
CL/F (L/hr)	149.0 (5.0)	0.3 (0.0)	106 (5)
K_A	-	5.6 (0.8)	18.9 (3.4)
lag time (hr)	-	1.47 (0.06)	0.98 (0.00)
V_2 (L)	34.5 (2.2)	-	-
Q (L/hr)	62.9 (3.5)	-	-

FIGURE 1 Effect on the positive subscale of the PANSS for responders over time (mean ± SD).

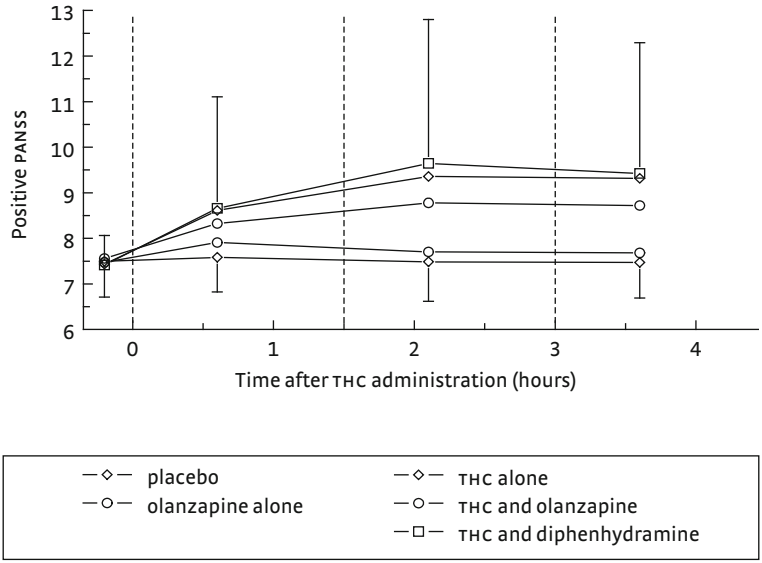

FIGURE 2 Effect on visual analogue scale feeling high over time (mean ± SD).

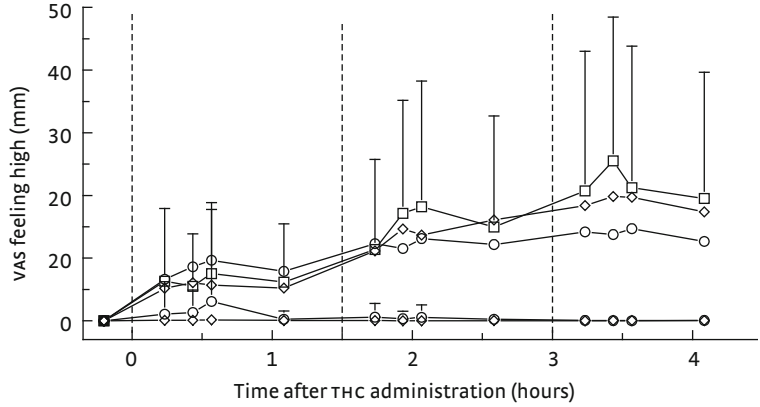

FIGURE 3 Prolactin concentrations in serum (mean ± SD).

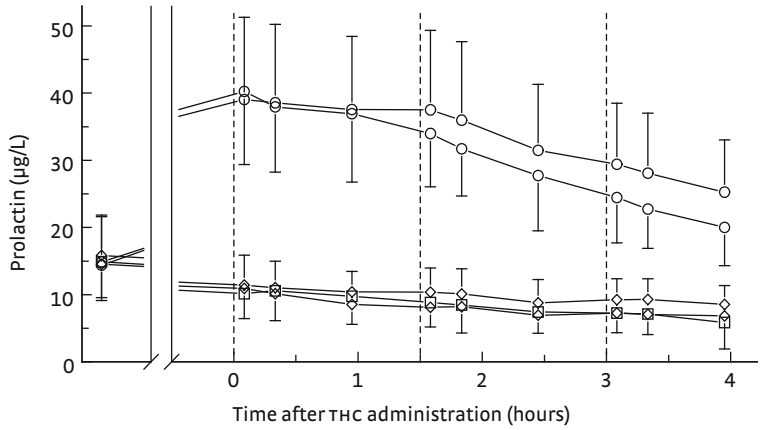

FIGURE 4 Simulated (median, 95%CI) and observed concentrations for THC, olanzapine and diphenhydramine pharmacokinetics.