# Randomized, Double-Blind, Placebo-Controlled Study About the Effects of Cannabidiol (CBD) on the Pharmacokinetics of $\Delta^9$-Tetrahydrocannabinol (THC) After Oral Application of THC Verses Standardized Cannabis Extract

*Thomas Nadulski,* Fritz Pragst,* Gordon Weinberg,† Patrik Roser,† Martin Schnelle,‡ Eva-Maria Fronk,‡ and Andreas Michael Stadelmann†*

**Abstract:** Cannabidiol (CBD) is known to modify the effects of $\Delta^9$-tetrahydrocannabinol (THC) by decreasing anxiety and antagonizing other THC-effects. As a reason, pharmacodynamic as well as pharmacokinetic mechanisms were suggested. In context of the use of cannabis-based medicine extracts for therapeutic purposes, a study was performed in a double-blind and placebo-controlled cross-over design in which each of 24 volunteers (12 male and 12 female, age 18-45 years) obtained soft-gelatin capsules with 10 mg THC (THC-set), cannabis extract containing 10 mg THC +5.4 mg CBD (CAN-set) or placebo in weekly intervals. Blood samples were taken 30 minutes before and 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, 9 hours and 24 hours after the intake. The concentrations of THC, of its metabolites 11-OH-THC, THC-COOH and of CBD in the plasma samples were determined by automatic solid phase extraction, derivatization with N,O-bis(trimethylsilyl)triflouroacetamide and gas chromatography–mass spectrometry. The concentration versus time curves (maximum concentrations $C_{max}$, corresponding time $t_{max}$ and areas under the curves AUC) were evaluated by statistical methods with respect to equivalence or differences between the CAN-set and the THC-set. Furthermore, the intra-individual ratios of $C_{max}$ and AUC for 11-OH-THC/THC, THC-COOH/THC and THC-COOH/11-OH-THC were compared between the THC-set and the CAN-set. Despite the large variation of the data, evidence emerged from the total of the results that CBD partially inhibits the CYP 2C catalyzed hydroxylation of THC to 11-OH-THC. The probability for this inhibition is particularly high for oral intake because THC and CBD attain relatively high concentrations in the liver and because of the high first-pass metabolism of THC. However, the effect of CBD is small in comparison to the variability caused by other factors. Therefore, a pharmacokinetic reason for the differences determined between pure THC and cannabis extract is improbable at the doses chosen in this study. Significantly higher AUC and $C_{max}$ and shorter $t_{max}$ were found for females as compared with males.

**Key Words:** Cannabidiol effect on THC metabolism, Gender effect on THC metabolism, Pharmacokinetics of cannabinoids, $\Delta^9$-tetrahydrocannabinol oral intake

(*Ther Drug Monit* 2005;27:799–810)

Cannabinoids are increasingly evaluated for the symptomatic treatment of various diseases.[1–6] There is a considerable interest to investigate standardized cannabis extracts[7] instead of isolated THC (Marinol® or dronabinol) as other constituents of the plant, first of all the second main cannabinoid cannabidiol (CBD), may contribute to the therapeutic effects of THC or may alleviate THC-induced side effects as will be detailed below. The CBD/THC ratio depends on the genetic background of the individual plant.[8–10] With respect to the cannabinoid composition the cannabis plants can be divided into a chemotype with a high THC and a low CBD content, an intermediate chemotype with a prevalent CBD content and THC present at various concentrations, and a chemotype with a particularly low THC content. In cannabis-based medicine development, extracts with a high THC content, with a THC/CBD 1:1 content and with high CBD ratios are tested in clinical studies.[6,7]

The effects of CBD under these conditions are not yet fully understood. On one hand, CBD has been reported to have its own anticonvulsive, sedative, anxiolytic, antipsychotic, antinausea and anti-inflammatory effects.[11,12] On the other hand, it has been described that CBD modifies the effect of THC by pharmacodynamic and/or by pharmacokinetic mechanisms.[13–36]

Karniol et al observed in a double-blind procedure with oral application that 15 to 60 mg CBD were efficient in blocking most of the effects of 30 mg THC and decreased the anxiety component of THC in such a way that the subjects reported more pleasurable effects.[19] According to Hollister and Gillespie, the oral combination of 20 mg THC and 40 mg CBD tended to delay the onset and to prolong the effects of

Received for publication February 1, 2005; accepted June 23, 2005.
From the *Institute of Legal Medicine; †Department of Psychiatry, University Hospital Charité, Berlin, Germany; and ‡Institute for Clinical Research, Berlin, Germany.
This study was supported by the Institute for Clinical Research, Berlin, Germany.
Reprints: Prof. Dr. Fritz Pragst, Institute of Legal Medicine, University Hospital Charité, Hittorfstraße, 18 D-14195, Berlin, Germany (e-mail: fritz.pragst@charite.de).

THC whereas making them somewhat more intense.[23] It was verified by Zuardi et al that CBD blocks the anxiety provoked by THC, however this effect also extended to other THC related effects.[26] This antagonism did not appear to be caused by a general block of THC effects, because no change was detected in the pulse-rate measurements.

Dalton et al found in a double-blind study that CBD significantly attenuated the subjective euphoria of THC when 25 μg/kg of THC and 150 μg/kg of CBD were simultaneously smoked.[21] In contrast, if the same amount of CBD was smoked 30 minutes before the THC, it did not alter the effects of THC. In all these investigations CBD alone had no effect on the parameters studied.

Because CBD binds extremely weakly to cannabinoid receptors in the brain, CBD-mediated modulation of THC activity is probably not pharmacodynamic in nature but due to effects on THC metabolism and/or distribution.[33,36] CBD has been found to be a potent inhibitor of hepatic drug metabolism.[14,16,17,24,28,29,31,32,34] Jones and Pertwee noted that pre-treatment of mice with large amounts of CBD causes an increase of the THC level in brain by the factor 1.4.[14] This was confirmed by Reid, Bornheim et al who found that CBD pre-treatment increases the concentrations of THC and its oxidative metabolites in brain of mice to a much higher extent than would be expected from the blood levels.[32,35] Watanabe et al as well as Bornheim and Grillo concluded from in-vitro experiments that the formation of a metabolite CBD-hydroxyquinone might be the penultimate step involved in a CBD-mediated modification and inactivation of P450 3A.[28,34] However, until now CBD-hydroxyquinone was not identified as a metabolite of CBD.

On the other hand, Levy and McCallum found that co-administration of 1 mg THC and 1 mg CBD to male rats had no effect on the rate of disappearance of THC from blood.[20] In a later study with 12 human volunteers Agurell et al did also not detect a significant effect of 40 mg CBD on the THC plasma levels measured at 11 different times after simultaneous oral administration of 20 mg THC in chocolate cookies.[25] Metabolites of THC were not included in these studies and no further investigations were carried out in the last twenty years to clear the discrepancy between the obvious interaction of CBD with the THC metabolism in in vitro and animal experiments and the lack of changes in human THC plasma concentrations after co-administration of CBD and THC. Therefore, in the present randomized, double-blind, placebo-controlled study with 24 volunteers the concentration versus time curves of THC and its main metabolites 11-hydroxy-$\Delta^9$-tetrahydrocannabinol (11-OH-THC) and 11-nor-9-carboxy-$\Delta^9$-tetrahydrocannabinol (THC-COOH) as well as of CBD were measured after oral administration of 10 mg THC alone and in cannabis extract containing 5.4 mg CBD. For evaluation, the course of the concentration versus time curves and the areas under the curves (AUC) were compared and the metabolites were included. In a further part of the study with 12 of the volunteers the effect of food on the concentration versus time curves was tested. Within the study neuro-physiological and cognitive tests were carried out at different times after the intake. The results will be published in another paper.

## MATERIALS AND METHODS

### Volunteers

For the study 28 volunteers were screened from which 27 were randomised (intention to treat set). 24 volunteers (12 male and 12 female, age 18-45 years) finished the study according to the protocol. All volunteers had an occasional cannabis consumption in the past, but, according to their statement in the questionnaire, had been completely drug free for at least one month before the onset of the study. They had no addictive or other psychiatric disease and did not take any medicals during the study.

This investigation was approved by the local Ethics Committee of the University Hospital Charité and written informed consent was obtained from each volunteer.

### Drug Capsules

Liquid extract from *cannabis sativa* (solvent 96% ethanol) and the plant-isolated THC were prepared by the Society for Cancer Research, Arlesheim, Switzerland. The soft-gelatin capsules containing 2.5 mg THC, or cannabis extract with 2.5 mg THC and 1.35 mg CBD or placebo (a mixture of mono-, di- and triglycerids and glycerol) were produced by Scherer GmbH & Co. KG, Eberbach, Germany.

### Design and Performance of the Study

The study was performed in a double-blind and placebo-controlled cross-over design. On three consecutive weeks, each volunteer obtained four capsules with either THC (total dose 10 mg), cannabis extract (total dose 10 mg THC +5.4 mg CBD) or placebo together with 200 mL water. They did not eat any food at least 8 hours before and 4 hours after the administration of the capsules and only 200 mL water was allowed during the time until capsule intake. No alcohol and caffeine was allowed the day before the test until the end of the test (last blood sampling). 1 hour before administration of the capsules a urine sample was collected and analyzed by enzyme immunoassay (EMIT II plus Assay, Dade Behring Limited, Milton Keynes, Atterbury, UK) for amphetamines and ecstasy, benzodiazepines, cannabinoids, cocaine, methadone and opiates. Blood samples (each 10 mL) were taken through an indwelling cannula from the forearm 30 minutes before and 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, 9 hours and 24 hours after the capsule intake. The samples were centrifuged immediately after collection at 1500 g for 15 minutes, the plasma was separated and stored in polypropylene vials at −30°C until analysis. A standard meal (one and two rolls for the female and the male participants respectively with cheese and sausage, yoghurt and 1 apple) was eaten four hours after the capsule administration. The wash-out time of one week between the administrations proofed to be sufficient as it was seen from the negative results of all plasma samples collected 30 minutes before capsule intake.

In an additional non-blind part of the study (DFI = drug-food-interaction study) 12 of the volunteers obtained another four capsules with the cannabis extract. In this case the volunteers did also not eat any food during at least 8 hours before drug administration and obtained a standardized breakfast (100 g cereal/fruit muesli, 200 mL milk, 2 rolls with butter

and cheese, sausage or marmalade) exactly one h after the intake of the capsules. All other conditions were the same as described above.

## Analysis of the Plasma Samples for THC, 11-OH-THC, THC-COOH and CBD

The development and validation of the method for analytical determination of THC, its main metabolites 11-OH-THC and THC-COOH and of CBD were described in detail in another paper.[37] Briefly, 1 mL plasma was analyzed by automatic solid phase extraction on Chromabond C18 ec columns (Macherey-Nagel, Düren, Germany, 200 mg extraction material, 3 mL volume) with an extractor RapidTrace (Zymark, Rüsselsheim, Germany). The extract was derivatized with N,O-bis(trimethylsilyl)triflouroacetamide (BSTFA, containing 1% trimethylchlorosilane) and analyzed by gas chromatography-mass spectrometry (GC-MS) using a gas chromatograph 6890, a mass selective detector 5973 and an autosampler 7673 (Agilent Technologies GmbH, Waldbronn, Germany) and deuterated substances $D_3$-THC, $D_3$-11-OH-THC and $D_3$-THC-COOH as internal standards. The limits of detection (LOD) were 0.24 ng/ml for THC, 0.15 ng/ml for 11-THC-OH, 0.26 ng/ml for THC-COOH and 0.29 ng/ml for CBD. Each sample was analyzed twice. The average deviation of the concentrations from the mean of both concentrations was between 1.7 and 3.9%.

## Statistical Methods and Software

The curve regression and determination of the pharmacokinetic parameters $C_{max}$, $t_{max}$ and AUC as well as statistical testing for equivalence/difference was performed with the program SAS for Windows, version 8.2 (SAS Inc., Heidelberg, Germany). $C_{max}$ and $t_{max}$ were estimated by nonlinear regression, AUC was calculated using the trapezoid formula. For the analysis of these parameters and of the ratios between THC, 11-OH-THC and THC-COOH, a mixed linear model was fitted in each case, with treatment period (THC, cannabis extract, DFI) and sex as fixed factors (including

**TABLE 1.** Maximum Concentrations $C_{max}$ of THC, 11-OH-THC, THC-COOH and CBD, Corresponding Time $t_{max}$ After Application, Detection Times After Intake and Areas Under the Curve for the Three Administration Sets*

| | THC | 11-OH-THC | THC-COOH | CBD |
|---|---|---|---|---|
| Administration of THC (THC-set, n = 24) | | | | |
| $C_{max}$, ng/ml (range) | 0.67–8.00 | 1.12–11.14 | 12.03–57.63 | — |
| $C_{max}$, ng/ml (mean) | 3.19 | 4.48 | 33.07 | — |
| σ of $C_{max}$ | 1.66 | 2.16 | 11.09 | — |
| $t_{max}$, min (range) | 30–183 | 30–190 | 67–235 | — |
| $t_{max}$, min (mean) | 63.6 | 90 | 124 | — |
| σ of $t_{max}$, min | 35.2 | 33.7 | 41.8 | — |
| Detection time after intake, min | 240–360 | 360–540 | >1440 | — |
| AUC, μg·min/L (range) | 66–1013 | 333–2353 | 5065–31187 | — |
| AUC, μg·min/L (mean) | 358 | 928 | 14596 | — |
| σ of AUC, μg·min/L | 196 | 442 | 6278 | — |
| Administration of cannabis extract (CAN-set, n = 24) | | | | |
| $C_{max}$, ng/ml (range) | 1.2–10.3 | 1.8–12.3 | 19.2–70.6 | 0.0–2.6 |
| $C_{max}$, ng/ml (mean) | 4.05 | 4.89 | 35.46 | 0.93 |
| σ of $C_{max}$ | 2.33 | 2.55 | 12.48 | 0.54 |
| $t_{max}$, min (range) | 33–125 | 37–130 | 65–230 | 30–120 |
| $t_{max}$, min (mean) | 56 | 81.9 | 115 | 59.6 |
| σ of $t_{max}$ | 18 | 26 | 36 | 20 |
| Detection time after intake, min | 240–360 | 360–540 | >1440 | 180–240 |
| AUC, μg·min/L (range) | 129–1131 | 383–2021 | 6260–34899 | 2.7–5.6 |
| AUC, μg·min/L (mean) | 450.16 | 1002.55 | 14983.16 | 4.35 |
| σ of AUC, μg·min/L | 251 | 454 | 6678 | 0.91 |
| Drug food interaction (DFI-set, n = 12) | | | | |
| $C_{max}$, ng/mL (range) | 2.4–8.2 | 3.0–12.1 | 15.9–67.2 | 0.39–1.9 |
| $C_{max}$, ng/mL (mean) | 5.29 | 5.75 | 30.45 | 1.13 |
| σ of $C_{max}$, ng/mL | 2.20 | 3.03 | 13.93 | 0.54 |
| $t_{max}$, min (range) | 34–120 | 35–123 | 65–130 | 30–120 |
| $t_{max}$, min (mean) | 62 | 82 | 95 | 64 |
| σ of $t_{max}$ | 28 | 26 | 23 | 31 |
| Detection time after intake, min | 240–360 | 360–540 | >1440 | 180–240 |
| AUC, μg·min/L (range) | 174–1058 | 547–2176 | 4684–23384 | 2.5–5.3 |
| AUC, μg·min/L (mean) | 564 | 1118 | 11652 | 4.40 |
| σ of AUC, μg·min/L | 269 | 556 | 5440 | 0.95 |

*$C_{max}$: highest measured concentrations. $t_{max}$: estimated from the curves obtained by spline fitting to the measured points. AUC was calculated by the trapezium formula.

interaction between treatment and sex), and the study subjects as random factor. Within each model the influence of each parameter was estimated and tested by *t* test. The values of $C_{max}$, AUC and the ratios had to be log-transformed before analysis to obtain normally distributed data. Tests for bioequivalence were performed using a critical threshold for equivalence of $\delta = 0.8$ (corresponding to $\delta' = -\ln(0.8) = 0.223$ in the actual analysis using the log-transformed data). Tests were two-sided with an error level of $\alpha = 5\%$. With regard to adjusting for multiple testing, the tests for equivalence had an *a priori* ordering of hypotheses such that AUCs of THC, 11-OH-THC and THC-COOH and afterward the corresponding $C_{max}$ values were tested in that order. *P* values smaller than 0.05 were only interpreted as significant in a confirmatory way if all other previous tests had also revealed significant differences. No further adjustments for multiple testing was done regarding other comparisons.

## RESULTS

The concentrations of THC, 11-OH-THC, THC-COOH and CBD were determined in the plasma samples collected immediately before and at eight times between 0.5 and 24 hours after oral administration of the capsules with THC, cannabis extract or placebo as described in the experimental part. In none of the placebo samples any of the compounds were detected. The concentration versus time profiles of the four substances were separately evaluated for the application of capsules with 10 mg THC alone (THC-set, 24 volunteers), cannabis extract containing 10 mg THC +5.4 mg CBD without food (CAN-set., same 24 volunteers) and the same cannabis extract with a standardised breakfast one hour after drug administration (Drug Food Interaction = DFI-set, 12 of the same volunteers). The maximum concentrations $C_{max}$, the corresponding maximum times $t_{max}$ and the area under the curves AUC (mean, range and standard deviation) are given in Table 1 for the four substances in the three administration sets. $C_{max}$ is the highest measured concentrations, $t_{max}$ was estimated from the curves obtained by spline fitting to the measured points and AUC was calculated by the trapezium formula.

Although the capsules were taken always under exactly the same conditions, a large variety in the maximum concentrations as well as in the course of the concentration versus time curves was found. Two typical examples are shown in Figure 1 for the CAN-set. In the majority of the cases high concentrations were already found after 30 minutes with a THC maximum between 30 and 60 minutes (Fig. 1a). But there are also a few cases with a delayed resorption and still very low concentrations in the 30 minutes and 1 hour sample. In some other cases an intermittent resorption with a shoulder or a second maximum between 3 and 4 hours was observed (Fig. 1b). The data measured in the three administration sets will be separately compared for the four compounds.

### Concentration Versus Time Curves

#### THC

In Figure 2a–c the THC concentrations obtained from all volunteers and the mean curves obtained from these concentrations are shown. Because THC was not detected in the 24 hours samples, the comparison is limited to 540 minutes. It is seen that the concentrations vary strongly at all measurement times for all three kinds of administration. In Figure 2d the mean curves of the three administrations are compared. As it can be seen from the mean curves, in general higher concentrations of THC were attained 30 minutes and 1 hour after intake of the cannabis extract. From 120 minutes to 540 minutes no clear difference between the THC-set and the CAN-set are seen. The highest mean concentrations were found in the DFI-set, where the concentration is above the THC values also 120 minutes after drug administration. However, because the standardised breakfast was ingested only 1 hour after the capsule intake, at least the increased 30 minutes and 60 minutes values in comparison to the

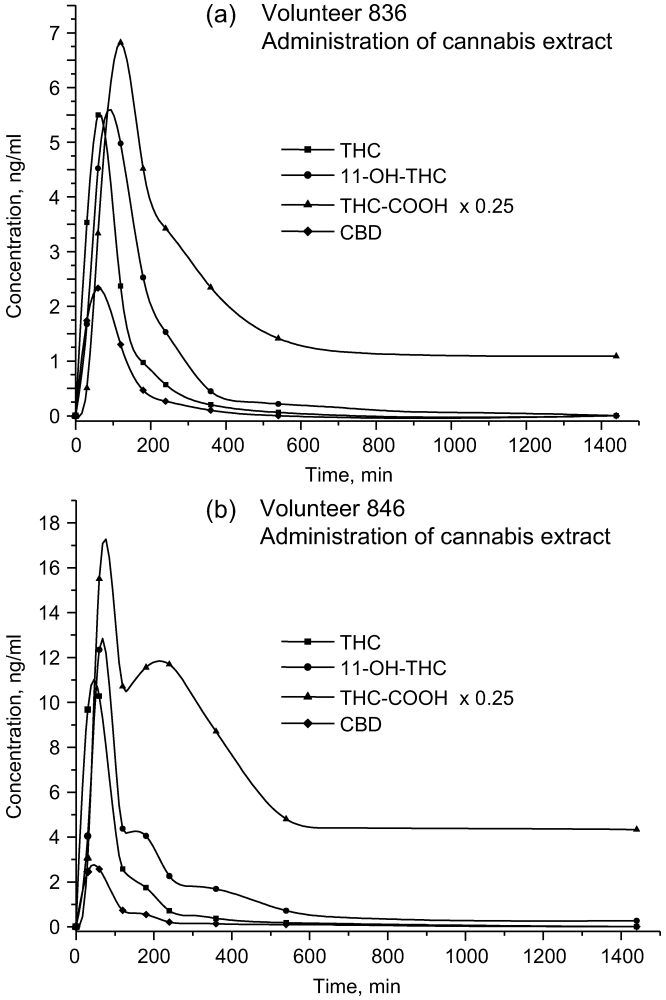


**FIGURE 1.** Concentrations of THC, 11-OH-THC, THC-COOH and CBD in plasma after oral administration of cannabis extract containing 10 mg THC and 5.4 mg CBD (CAN-set). (a) Volunteer 836 (female, age 29), fast resorption and steady decrease of the concentrations. (b) Volunteer 846 (also female, age 29) with partly delayed resorption and irregular decrease of concentrations.

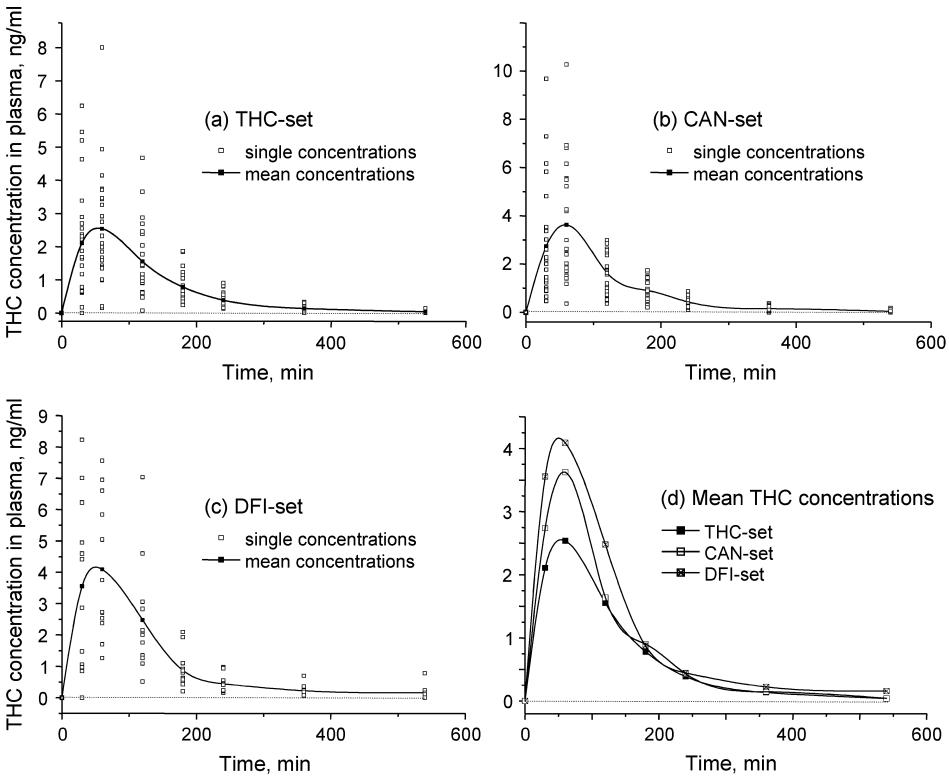


**FIGURE 2.** THC concentrations in plasma measured in the THC-set (a, 24 volunteers), the CAN-set (b, 24 volunteers) and DFI-set (c, 12 volunteers) and comparison of the mean THC concentrations (d) of the three sets.

corresponding CAN-set concentrations seem to be rather accidental. No relevant difference is seen between the concentrations of the three sets from 180 to 540 minutes.

## 11-OH-THC

The primary metabolite 11-OH-THC could also only be detected until 540 minutes after intake of the capsules and its concentrations varied strongly (Fig. 3 a–c). The maximum concentrations of 11-OH-THC are attained by about 25 minutes later than of THC (Table 1). The mean concentration curves of the three administrations (Fig. 3d) increase after one and two hours in the same order as for THC (THC-set < CAN-set < DFI set) but to a smaller degree. From 180 to 540 minutes no clear difference can be seen between the three sets.

## THC-COOH

Because of the higher concentration, THC-COOH was still well detectable after 24 hours. Also the concentration of this metabolite varied strongly between the volunteers (Fig. 4a–c). Comparing the four substances, THC-COOH attains its maximum value at the latest time ($t_{max}$ = 65 to 230 minutes, mean 124 minutes). Only small differences between the three kinds of applications are seen from the mean curves shown in Figure 4d. For this metabolite the curves of the THC-set and CAN-set are very close together with a slightly higher maximum concentrations in the CAN-set. The samples of the DFI-set contained the lowest concentrations for THC-COOH after 120 minutes.

## CBD

This compound was only present in the samples from the CAN-set and the DFI-set. The strongly varying maximum concentrations were only between 0.30 and 2.57 ng/ml at 30–120 minutes (mean 60 and 64 minutes respectively) after administration (Fig. 5a–b). It was only detected up to 540 minutes after administration. No real differences could be seen between both kinds of intake (Fig. 5c). It appears that the absorption had already been nearly half completed at the time of the standardized breakfast. However, for CBD and for THC (Fig. 2d) an increased concentration was found at 120 minutes which could be caused by an enhancement of the absorption by the food.

## Areas Under the Curves (AUC), Maximum Concentrations $C_{max}$ and Maximum Times $t_{max}$

In the next step, it was examined whether significant differences can be found between the THC-set and the CAN-set and between the CAN-set and the DFI-set in the areas under the concentration versus time curves (AUC), the maximum concentrations $C_{max}$ and the times after administration $t_{max}$ at which this concentration is attained for THC, 11-OH-THC, THC-COOH and, as far as involved, for CBD. The AUC were calculated using the trapezium formula. $C_{max}$ was the highest measured concentration whereas $t_{max}$ was estimated from the individual concentration versus time curves obtained by spline fitting to the measured points. AUC and $C_{max}$ were not normally distributed within the groups. However, for the logarithms of AUC and $C_{max}$ normal

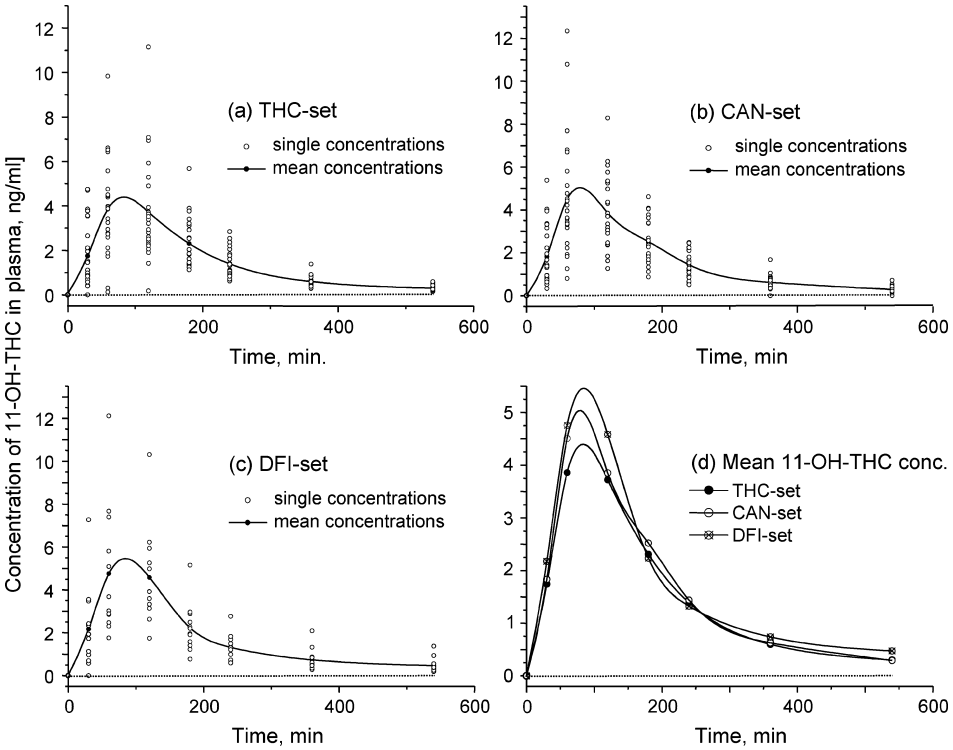


**FIGURE 3.** 11-OH-THC concentrations in plasma measured in the THC-set (a, 24 volunteers), the CAN-set (b, 24 volunteers) and DFI-set (c, 12 volunteers) and comparison of the mean 11-OH-THC concentrations (d) of the three sets.

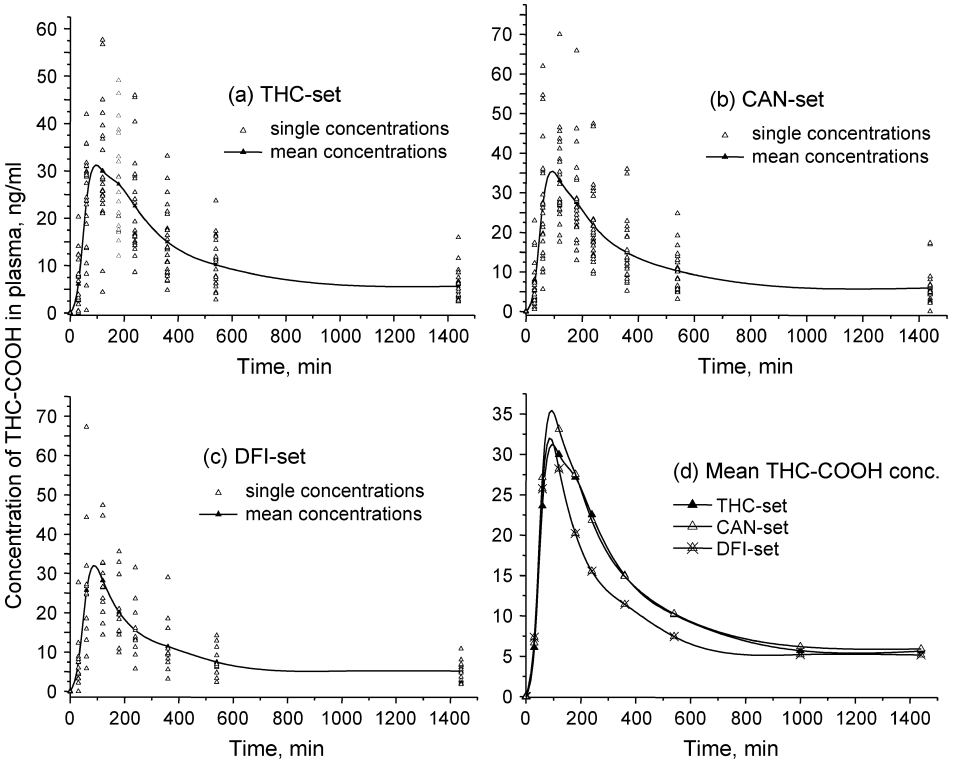


**FIGURE 4.** THC-COOH concentrations in plasma measured in the THC-set (a, 24 volunteers), the CAN-set (b, 24 volunteers) and DFI-set (c, 12 volunteers) and comparison of the mean THC-COOH concentrations (d) of the three sets.

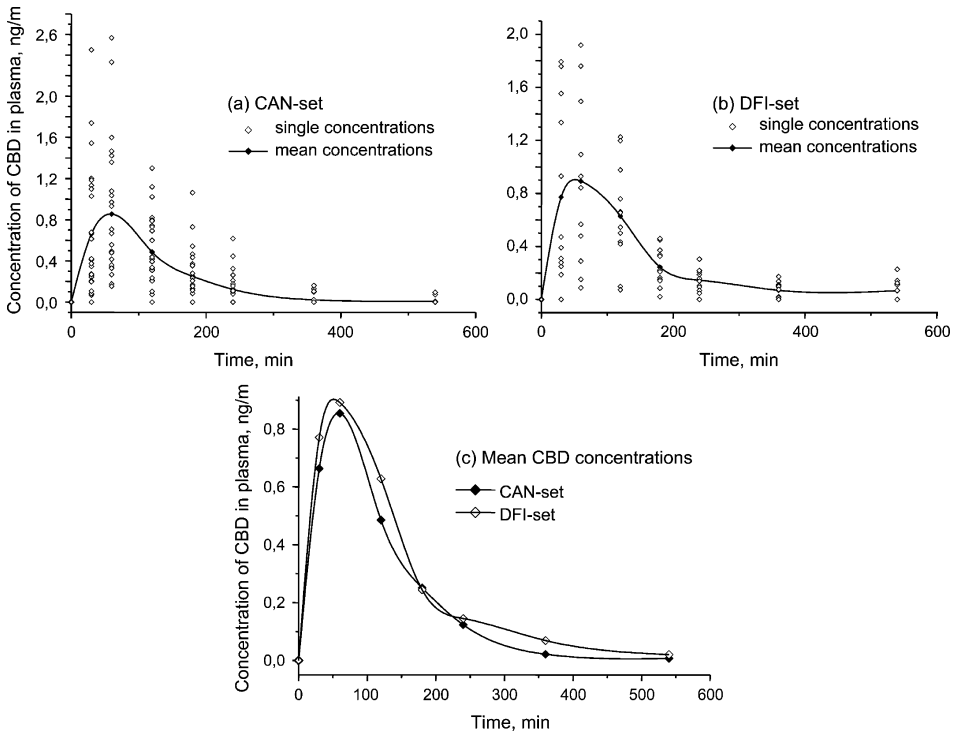


**FIGURE 5.** CBD concentrations in plasma measured in the CAN-set (a, 24 volunteers) and DFI-set (b, 12 volunteers) and comparison of the mean CBD concentrations (c) of both sets.

distribution was obtained. Besides the kind of administration, also the gender of the participants (12 male and 12 female) was modulated. The following results were obtained by this evaluation:

## Effect of Gender and Body Mass Index

- For all four compounds AUC and $C_{max}$ were significantly higher for the female than for the male participants when tested over all three treatment periods except for the AUC of THC-COOH where only a corresponding trend was found ($P = 0.0989$). This is also shown by the box and whiskers plots in Figure 6 for the AUC of the CAN-set. The mean values of the ratio $AUC_{female}/AUC_{male}$ for the different substances in the three sets were 1.26-1.75. This was not only caused by the lower body weight of the female volunteers because the mean ratios decreased only to 1.12-1.52

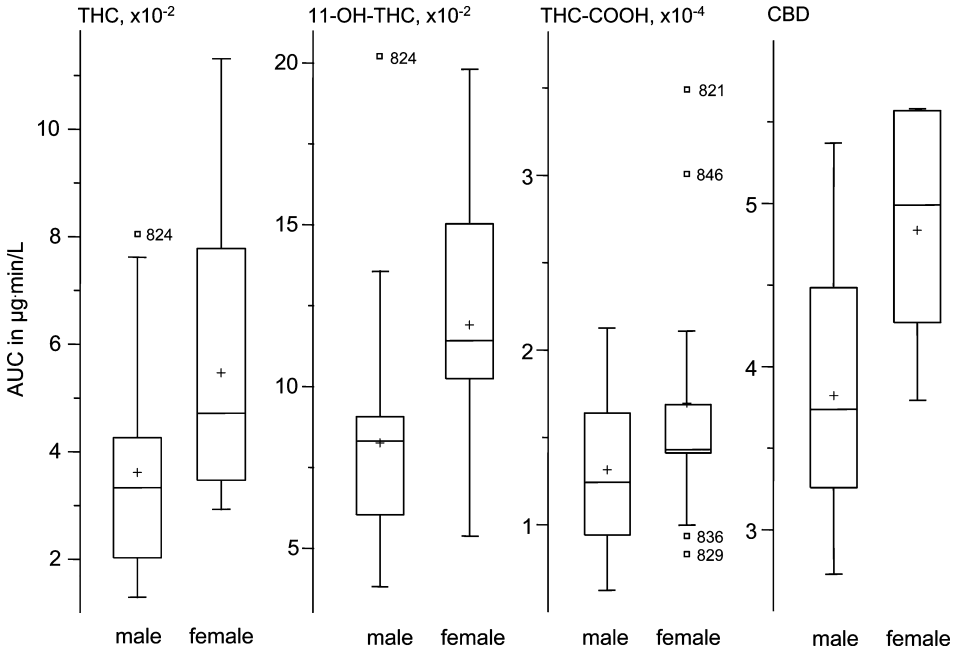


**FIGURE 6.** Box and whiskers plots of the AUC of THC, 11-OH-THC, THC-COOH and CBD. Comparison of male and female volunteers in the CAN-set.

after correction for body weight. Obviously, women have a smaller volume of distribution than man in this part of the THC metabolism.
- $t_{max}$ of THC, 11-OH-THC and CBD were significantly longer for male participants as compared with female participants. No significant difference was found for $t_{max}$ of THC-COOH between the genders.
- There was no significant correlation between AUC or $C_{max}$ and the body mass index of the volunteers.

## Comparison of CAN-set with THC-set

- No significant equivalence was found for AUC and $C_{max}$ of THC which, as a mean, were by about 20% lower for THC administration as compared with cannabis extract administration.
- No significant difference could be found for AUC and $C_{max}$ of 11-OH-THC and THC-COOH
- No significant equivalence was found for $t_{max}$ which as a mean was slightly shorter for all three substances in the CAN-set.

## Comparison DFI-set with CAN-set

- No significant difference was detected for AUC and $C_{max}$ of THC, 11-OH-THC and CBD.
- A significant difference was found for AUC and $C_{max}$ of THC-COOH which were by about 20% lower in the DFI-set.
- No significant equivalence was found for $t_{max}$ which was shorter for THC, 11-OH-THC and THC-COOH and longer for CBD in the DFI-set.

In Figure 7 these results are illustrated by box and whisker plots of ln AUC for the four substances. The plot of the logarithmic data was chosen because the numeric data displayed a skew distribution which was at least in part corrected in the logarithmic plots.

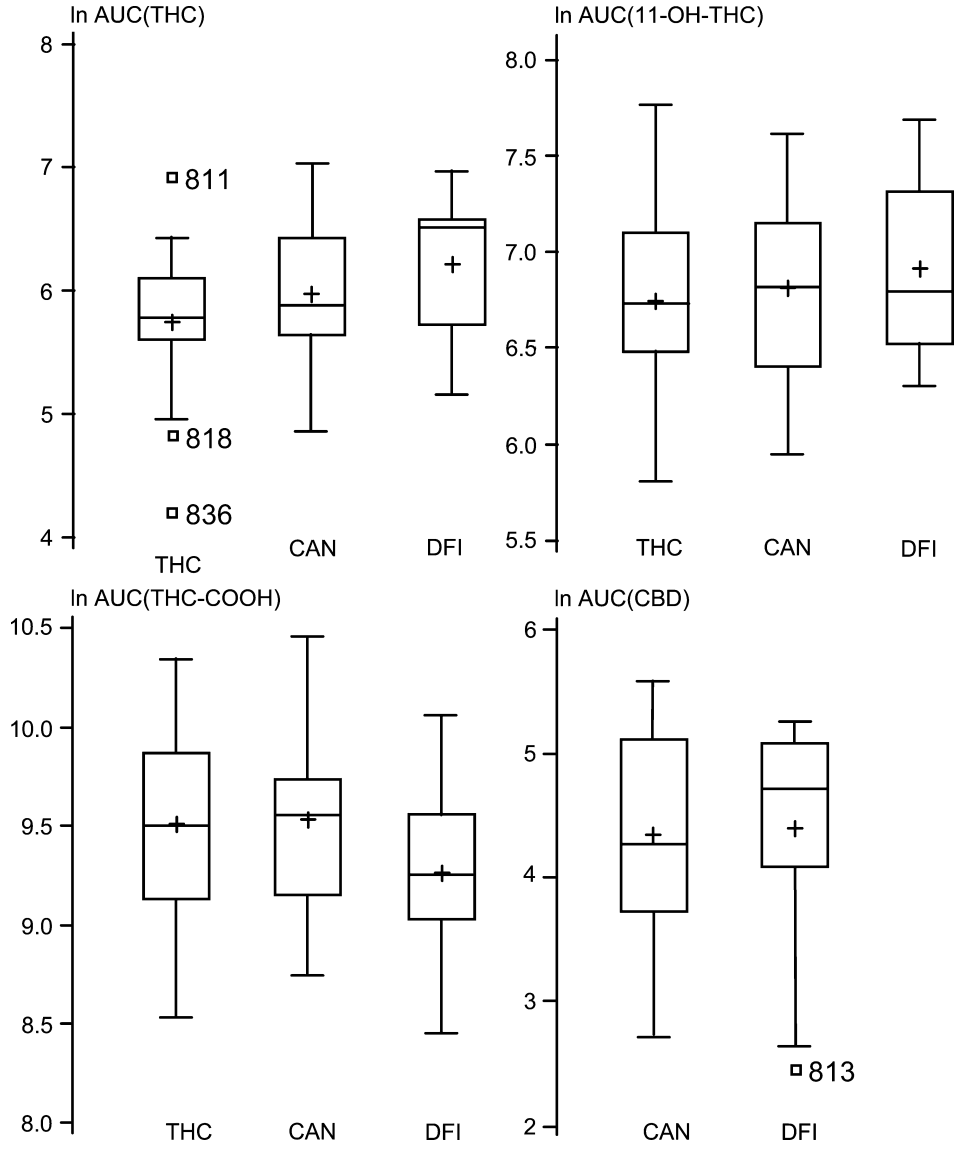


**FIGURE 7.** Box and whiskers plot of the AUC of THC, 11-OH-THC, THC-COOH and CBD. Comparison between THC-set, CAN-set and DFI-set. Because of the skew distribution of the original values, the logarithms of the data were evaluated.

**TABLE 2.** Mean Values of the Relative Intra- and Inter-Individual Differences of AUC, $C_{max}$ and $t_{max}$ between the THC-set, CAN-set and DFI-set

| | Relative Deviation, %* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Intra-individual, Between Set | | | Inter-individual, Within Set | | | Inter-individual, Between Sets | | |
| | AUC | $C_{max}$ | $t_{max}$ | AUC | $C_{max}$ | $t_{max}$ | AUC | $C_{max}$ | $t_{max}$ |
| THC/CAN sets | | | | | | | | | |
| THC | 35.7 | 43.7 | 41.35 | 57.3 | 56.2 | 38.9 | 58.5 | 57.6 | 39.1 |
| 11-OH-THC | 26.1 | 37.0 | 38.8 | 49.8 | 50.6 | 38.2 | 46.5 | 48.5 | 37.3 |
| THC-COOH | 22.3 | 20.2 | 35.4 | 45.2 | 36.2 | 36.5 | 42.9 | 34.5 | 36.3 |
| CAN/DFI sets | | | | | | | | | |
| THC | 44.7 | 30.3 | 39.5 | 57.6 | 51.5 | 48.1 | 63.0 | 51.4 | 30.0 |
| 11-OH-THC | 28.1 | 27.5 | 26.8 | 51.1 | 53.0 | 38.4 | 51.4 | 50.3 | 32.2 |
| THC-COOH | 27.5 | 24.3 | 31.8 | 46.5 | 42.8 | 26.3 | 48.6 | 40.3 | 35.7 |
| CBD | 15.2 | 14.7 | 36.7 | 25.2 | 23.6 | 44.6 | 22.2 | 22.2 | 46.3 |

*The relative intra-individual deviation in % e.g. for AUC and the volunteer M was calculated by equ. (a). Correspondingly, the inter-individual deviation between the volunteers M and N was calculated within set by equ. (b) and between sets by equ. (c). The calculations were in the same way performed for $C_{max}$ and $t_{max}$.

$$200\ |AUC(M)_{CAN\text{-}set} - AUC(M)_{THC\text{-}set}|/[AUC(M)_{CAN\text{-}set} + AUC(M)_{THC\text{-}set}] \quad (a)$$
$$200\ |AUC(M)_{THC\text{-}set} - AUC(N)_{THC\text{-}set}|/[AUC(M)_{THC\text{-}set} + AUC(N)_{THC\text{-}set}] \quad (b)$$
$$200\ |AUC(M)_{CAN\text{-}set} - AUC(N)_{THC\text{-}set}|/[AUC(M)_{CAN\text{-}set} + AUC(N)_{THC\text{-}set}] \quad (c)$$

## Intra-individual Comparison between the CAN-set and the THC-set of the AUC and $C_{max}$ Ratios 11-OH-THC/THC, THC-COOH/THC and THC-COOH/11-OH-THC

Figures 2 to 5 demonstrate that the concentrations vary strongly between the volunteers and that this inter-individual variation aggravates the recognition of pharmacokinetic differences between the administration sets. Therefore, an effect of CBD on the metabolization rate of THC should more sensitively be recognized from an intra-individual change of the metabolite/parent drug ratio of AUC or $C_{max}$ or from an intra-individual shift of the $t_{max}$ difference between metabolite and parent drug than from the statistic comparison of the whole administration sets performed above.

If the large variation of the data was caused to an essential part by differences in the individual pharmacokinetic characteristics of the volunteers, the intra-individual variation between both sets should be clearly smaller than the inter-individual one. Because each volunteer obtained two or three doses of THC, this was examined in the following way: In a matrix, for each volunteer the relative deviation of the parameter (AUC, $C_{max}$ or $t_{max}$) between both sets were calculated. For comparison, the relative deviations of the parameter from that of each other volunteer in the same set

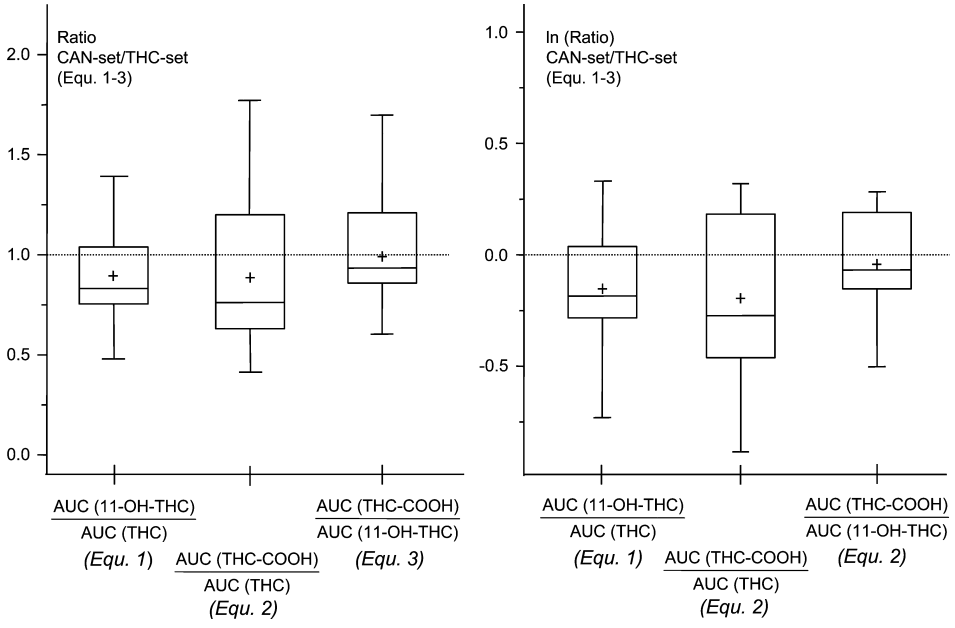


**FIGURE 8.** Intra-individual comparison between CAN-set and THC-set based on the ratios calculated by equ. (1), (2) and (3) by numeric and logarithmic box an whiskers plots. The ratio for AUC(11-OH-THC)/AUC (THC) characterizes the hydroxylation THC → 11-OH-THC, the ratio for AUC(THC-COOH)/AUC(THC) the total transformation THC → THC-COOH and the ratio for AUC (THC-COOH)/AUC(11-OH-THC) the dehydrogenation 11-OH-THC → THC-COOH. A ratio below 1.0 (numeric) or 0.0 (logarithmic) means that the reaction rate was decreased in the CAN-set as compared with the THC-set.

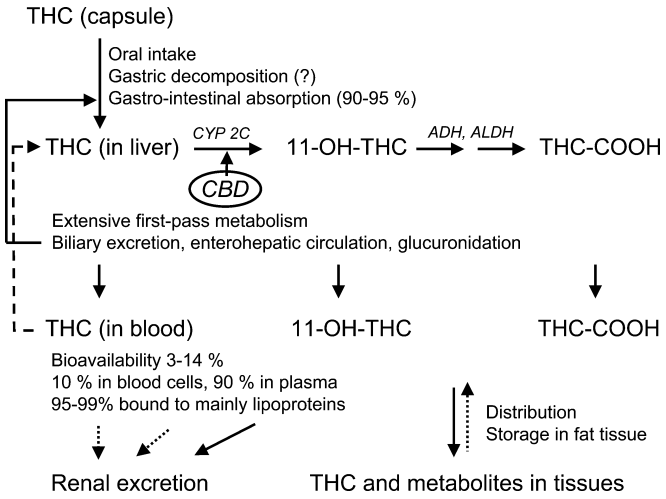


**FIGURE 9.** Main steps and influence factors in the pharmacokinetic of THC after oral administration. An effect of CBD is particularly probable after oral intake in the resorption period because of the extensive first-pass metabolism and because in this time both THC and CBD have high concentrations in the liver.

and in the other set were calculated. The calculation formula and the mean values are given in Table 2. It was found that, for AUC and $C_{max}$, the intra-individual differences between the compared sets were clearly smaller than the inter-individual differences. Therefore, a more specific evaluation can be expected from AUC and $C_{max}$ ratios. For $t_{max}$ no difference between intra-individual and inter-individual data were seen.

For each of the 23 volunteers who participated in both the THC-set and the CAN-set the ratios (1) to (3) were calculated. For instance, AUC(11-OH-$THC_{CAN}$) means the AUC for 11-OH-THC in the CAN-set:

$$[AUC(11\text{-OH-TH}\,C_{CAN}) \cdot AUC(THC_{THC})]/ [AUC(THC_{CAN}) \cdot AUC(11\text{-OH-}THC_{THC})] \quad (1)$$

$$[AUC(THC\text{-}COOH_{CAN}) \cdot AUC(THC_{THC})]/ [AUC(THC_{CAN}) \cdot AUC(THC\text{-}COOH_{THC})] \quad (2)$$

$$[AUC(THC\text{-}COOH_{CAN}) \cdot AUC(11\text{-OH-}THC_{THC})]/ [AUC(11\text{-OH-}THC_{CAN}) \cdot AUC(THC\text{-}COOH_{THC})] \quad (3)$$

The same ratios were calculated also for $C_{max}$ instead of AUC. The ratio (1) compares the hydroxylation of THC to 11-OH-THC between both sets. An inhibiting effect of CBD on this hydroxylation should lead to a ratio below 1.0 whereas in case of no interference by CBD the ratio should be 1.0. In the same way the ratios (2) and (3) should indicate an effect of CBD on the total reaction from THC to THC-COOH and on the dehydrogenation from 11-OH-THC to THC-COOH respectively. In Figure 8 the box and whiskers plots of the AUC ratios are shown. Because of the skew distribution of the data, again the logarithmic ratios were also evaluated. It is seen that the ratio (1) is clearly below 1.0 (mean 0.89, median 0.83; $P = 0.0153$). This is also the case for the ratio (2) (mean 0.89, median 0.76). Although the variation of the data is much higher, this is partly adjusted for by the log-transformation, yielding also a statistically significant difference ($P = 0.0274$). For ratio (3), the mean value as well as the median are much closer to 1.0 (mean 0.99, median 0.93). The ratios for $C_{max}$ in tendency behave in the same way, but none of these reach a statistically significant difference.

Altogether, these results of the statistic evaluation suggest that, in the CAN-set, the hydroxylation of THC to 11-OH-THC is partially inhibited by CBD. This can still be seen in the ratio between THC and THC-COOH, whereas the dehydrogenation steps from 11-OH-THC to THC-COOH remain unaffected.

## DISCUSSION

The processes which have an effect on the concentrations of THC and its main metabolites in plasma are shown in Figure 9. The concentrations depend on many factors and conditions such as yield of the gastrointestinal absorption, extent of the first pass metabolism and biliary excretion, rate and extent of distribution to tissues, clearance and metabolism from systemic blood. Furthermore, the polymorphic nature of the gene encoding the CYP 2C enzyme family should contribute to the variability of the THC metabolism. The courses of the concentration versus time curves of THC and its metabolites found in this study (Figs. 1–4) are typical for oral intake of the drug.[27,38–40] In contrast to smoking or injection, the concentration of THC-COOH was from the beginning higher than that of THC (examples see Figure 1, note that the concentrations of THC-COOH are plotted in the scale 0.25). As a rule, the concentration of 11-OH-THC was also higher than that of THC and this primary metabolite could be detected for a longer time than THC. It is known from literature,[2] and it could also be seen in the present investigation from a semi-logarithmic concentration versus time plot (not shown) that the elimination of THC follows at least a two phase kinetics (distribution and terminal). This is still more complicated by irregularities in the slow absorption during the first hours after intake. Therefore, a complete evaluation of the curves with respect to the pharmacokinetic parameters was not possible and the interpretation was restricted to AUC, $C_{max}$ and $t_{max}$.

Despite the large variation between the concentrations of the volunteers, strong indications were obtained from the statistical evaluation that CBD under the experimental conditions of this study partially inhibits the cytochrome P450 catalyzed formation of 11-OH-THC from THC: Increase of the mean THC concentrations in the CAN-set during the first hours after intake (Fig. 2 days), increase of AUC and $C_{max}$ of THC (Fig. 7) and decrease of the $t_{max}$ for all substances in the CAN-set. Particularly the smaller intra-individual AUC and $C_{max}$ ratios 11-OH-THC/THC in the CAN-set in comparison to the THC-set can be interpreted as a clear indication for the decreased rate of this metabolic step under the effect of CBD. However, the subsequent dehydrogenation of 11-OH-THC to THC-COOH is not affected. The enzymes responsible for the oxidation of 11-OH-THC to the intermediate aldehyde

(11-oxo-THC) is not yet known. According to Watanabe et al, the transformation of 11-oxo-THC to THC-COOH is catalyzed by a microsomal aldehyde oxygenase (MALDO), a member of the CYP2C subfamily.[41]

CBD has similar pharmacokinetic properties as THC.[25,27,42] The probability for its inhibiting action is particularly high during the first hours after oral intake of THC and CBD because, at this time, both compounds attain relatively high concentrations in the liver and because THC is to a high extend already metabolised during the first liver passage. With an oral bioavailability of only 3–14% which is mainly caused by the first-pass metabolism,[2] a small decrease of the metabolic activity can lead to a measurable rise of the THC concentration in plasma and to an earlier THC peak concentration.

## Conclusions

In this study experimental evidence was obtained for the first time, that CBD probably inhibits the metabolic hydroxylation of THC also in human volunteers. However, this effect of CBD is small in comparison to the variability caused by all other factors (Fig. 9). Therefore, a pharmacokinetic reason for the differences described in literature between the effects of pure THC and of cannabis extract is improbable at the doses chosen in this study. This should generally be true for recreational cannabis consumption where, as a rule, cannabis plants with a high THC and a low CBD content are used. However, a modifying pharmacokinetic influence of CBD on the THC effects cannot be excluded if cannabis-based medicine with much higher CBD doses are administered. Further studies are necessary to confirm such influence of CBD for higher doses and chronic use.

## ACKNOWLEDGMENT

*Study medication was provided and monitoring, data management and statistical analyses were supported by the Institute for Clinical Research, Berlin, Germany.*

## REFERENCES

1. Kleiber D, Kovar K-A. Auswirkungen des Cannabiskonsums. Stuttgart: Wiss. Verl.-Ges.; 1998: 226–237.
2. Grotenhermen F. Pharmacokinetics and pharmacodynamics of cannabinoids. *Clin Pharmacokinet*. 2003;42:327–360.
3. Croxford JL. Therapeutic potential of cannabinoids in CNS disease. *CNS Drugs*. 2003;17:179–202.
4. Baker D. Therapeutic potential of cannabis and cannabinoids in experimental models of multiple sclerosis. In: Guy GW, Whittle BA, Robson PJ, eds. *The medical uses of cannabis and cannabinoids*. London, Chicago: Pharmaceutical press; 2004:141–163.
5. Robson PJ, Guy W. Clinical studies of cannabis-based medicines. In: Guy GW, Whittle BA, Robson PJ, eds. *The medical uses of cannabis and cannabinoids*. London, Chicago; Pharmaceutical press; 2004:228–269.
6. Notcutt W. Cannabis in the treatment of chronic pain. In: Guy GW, Whittle BA, Robson PJ, eds. *The medical uses of cannabis and cannabinoids*. London, Chicago: Pharmaceutical press; 2004:272–299.
7. Cannabis-based medicines-GW pharmaceuticals: high CBD, high THC, medicinal cannabis-GW pharmaceuticals, THC:CBD. *Drugs R D.* 2003;4: 306–309.
8. Potter D. Growth and morphology of medicinal cannabis. In: Guy GW, Whittle BA, Robson PJ, eds. *The medical uses of cannabis and cannabinoids*. London, Chicago: Pharmaceutical press; 2004:17–54.
9. De Meijer EP, Bagatta M, Carboni A, et al. The inheritance of chemical phenotype in Cannabis sativa L. *Genetics*. 2003;163:335–346.
10. De Meijer E. The breeding of cannabis cultivars for pharmaceutical end uses. In: Guy GW, Whittle BA, Robson PJ, eds. *The medical uses of cannabis and cannabinoids*. London, Chicago: Pharmaceutical press; 2004:55–69.
11. Mechoulam R, Parker LA, Gallily R. Cannabidiol: an overview of some pharmacological aspects. *J Clin Pharmacol*. 2002;42:11S–19S.
12. Mechoulam R, Hanus L. Cannabidiol: an overview of some chemical and pharmacological aspects. Part I: chemical aspects. *Chem Phys Lipids*. 2002;121:35–43.
13. Paton WD, Pertwee RG. Effect of cannabis and certain of its constituents on pentobarbitone sleeping time and phenazone metabolism. *Br J Pharmacol*. 1972;44:250–261.
14. Jones G, Pertwee RG. A metabolic interaction in vivo between cannabidiol and 1-tetrahydrocannabinol. *Br J Pharmacol*. 1972;45: 375–377.
15. Karniol IG, Carlini EA. Pharmacological interaction between cannabidiol and delta 9-tetrahydrocannabinol. *Psychopharmacologia*. 1973;33: 53–70.
16. Fernandes M, Warning N, Christ W, et al. Interactions of several cannabinoids with the hepatic drug metabolizing system. *Biochem Pharmacol*. 1973;22:2981–2987.
17. Bailey K, Toft P. Difference spectra of rat hepatic microsomes induced by cannabinoids and related compounds. *Biochem Pharmacol*. 1973;22: 2780–2783.
18. Fernandes M, Schabarek A, Coper H, et al. Modification of delta9-THC-actions by cannabinol and cannabidiol in the rat. *Psychopharmacologia*. 1974;38:329–338.
19. Karniol IG, Shirakawa I, Kasinski N, et al. Cannabidiol interferes with the effects of delta 9-tetrahydrocannabinol in man. *Eur J Pharmacol*. 1974; 28:172–177.
20. Levy S, McCallum NK. Cannabidiol and its pharmacokinetic interaction with delta1-tetrahydrocannabinol. *Experientia*. 1975;31:1268–1269.
21. Dalton WS, Martz R, Lemberger L, et al. Influence of cannabidiol on delta-9-tetrahydrocannabinol effects. *Clin Pharmacol Ther.* 1976;19:300–309.
22. Lemberger L, Dalton B, Martz R, et al. Clinical studies on the interaction of psychopharmacologic agents with marihuana. *Ann N Y Acad Sci*. 1976; 281:219–228.
23. Hollister LE, Gillespie H. Interactions in man of delta-9-tetrahydrocannabinol. II. Cannabinol and cannabidiol. *Clin Pharmacol Ther.* 1975;18: 80–83.
24. Borys HK, Karler R. Cannabidiol and delta 9-tetrahydrocannabinol metabolism. In vitro comparison of mouse and rat liver crude microsome preparations. *Biochem Pharmacol*. 1979;28:1553–1559.
25. Agurell S, Carlsson S, Lindgren JE, et al. Interactions of delta 1-tetrahydrocannabinol with cannabinol and cannabidiol following oral administration in man. Assay of cannabinol and cannabidiol by mass fragmentography. *Experientia*. 1981;37:1090–1092.
26. Zuardi AW, Shirakawa I, Finkelfarb E, et al. Action of cannabidiol on the anxiety and other effects produced by delta 9-THC in normal subjects. *Psychopharmacology (Berl)*. 1982;76:245–250.
27. Agurell S, Halldin M, Lindgren JE, et al. Pharmacokinetics and metabolism of delta 1-tetrahydrocannabinol and other cannabinoids with emphasis on man. *Pharmacol Rev.* 1986;38:21–43.
28. Watanabe K, Arai M, Narimatsu S, et al. Self-catalyzed inactivation of cytochrome P-450 during microsomal metabolism of cannabidiol. *Biochem Pharmacol*. 1987;36:3371–3377.
29. Watanabe K, Usami N, Yamamoto I, et al. Inhibitory effect of cannabidiol hydroxy-quinone, an oxidative product of cannabidiol, on the hepatic microsomal drug-metabolizing enzymes of mice. *J Pharmacobiodyn*. 1991;14:421–427.
30. Zuardi AW, Morais SL, Guimaraes FS, et al. Antipsychotic effect of cannabidiol. *J Clin Psychiatry.* 1995;56:485–486.
31. Yamamoto I, Watanabe K, Narimatsu S, et al. Recent advances in the metabolism of cannabinoids. *Int J Biochem Cell Biol*. 1995;27:741–746.
32. Bornheim LM, Kim KY, Li J, et al. Effect of cannabidiol pretreatment on the kinetics of tetrahydrocannabinol metabolites in mouse brain. *Drug Metab Dispos*. 1995;23:825–831.
33. Showalter VM, Compton DR, Martin BR, et al. Evaluation of binding in a transfected cell line expressing a peripheral cannabinoid receptor (CB2): identification of cannabinoid receptor subtype selective ligands. *J Pharmacol Exp Ther.* 1996;278:989–999.

 

34. Bornheim LM, Grillo MP. Characterization of cytochrome P450 3A inactivation by cannabidiol: possible involvement of cannabidiol-hydroxyquinone as a P450 inactivator. *Chem Res Toxicol*. 1998;11:1209–1216.
35. Reid MJ, Bornheim LM. Cannabinoid-induced alterations in brain disposition of drugs of abuse. *Biochem Pharmacol*. 2001;61:1357–1367.
36. Bisogno T, Hanus L, De Petrocellis L, et al. Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. *Br J Pharmacol*. 2001;134:845–852.
37. Nadulski T, Sporkert F, Schnelle M, et al. Simultaneous and sensitive analysis of THC, 11-OH-THC, THC-COOH, CBD and CBN by GC-MS in plasma after oral application of small doses of THC and cannabis extract. *J Anal Toxicol*. 2005 (in press).
38. Ohlsson A, Lindgren JE, Wahlen AS, et al. Plasma delta-9 tetrahydrocannabinol concentrations and clinical effects after oral and intravenous administration and smoking. *Clin Pharmacol Ther*. 1980;28:409–416.
39. Wall ME, Sadler BM, Brine D, et al. Metabolism, disposition, and kinetics of delta-9-tetrahydrocannabinol in men and women. *Clin Pharmacol Ther*. 1983;34:352–363.
40. Frytak S, Moertel CG, Rubin J. Metabolic studies of delta-9-tetrahydrocannabinol in cancer patients. *Cancer Treat Rep*. 1984;68:1427–1431.
41. Watanabe K, Matsunaga T, Yamamoto I, et al. Involvement of CYP2C in the metabolism of cannabinoids by human hepatic microsomes from an old woman. *Biol Pharm Bull*. 1995;18:1138–1141.
42. Ohlsson A, Lindgren JE, Andersson S, et al. Single-dose kinetics of deuterium-labelled cannabidiol in man after smoking and intravenous administration. *Biomed Environ Mass Spectrom*. 1986;13:77–83.

## ERRATUM

"Quantification of Imatinib in Human Plasma by High-Performance Liquid Chromatography-Tandem Mass Spectrometry"

Karine Titier, Stéphane Picard, Dominique Ducint, Emmanuelle Teilhet, Nicholas Moore, Patrice Berthaud, François-Xavier Mahon, Mathieu Molimard

*Ther Drug Monit* 2005;27:634–640

Two abbreviations in the above referenced article that ran in the October 2005 issue were misprinted in error.

Liquid chromatrography mass spectrometry is LC-MS/MS, not LC-NS-NS.

Electrospray ionization liquid chromatography mass spectometry is LC-ESI-MS/MS, not LC-ESS-NS-NS.

We regret the errors.