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RESEARCH PAPER

Comparison of cannabinoid concentrations in oral fluid and whole blood between occasional and regular cannabis smokers prior to and after smoking a cannabis joint

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Abstract A cross-over controlled administration study of smoked cannabis was carried out on occasional and heavy smokers. The participants smoked a joint (11 % Δ9-tetrahydrocannabinol (THC)) or a matching placebo on two different occasions. Whole blood (WB) and oral fluid (OF) samples were collected before and up to 3.5 h after smoking the joints. Pharmacokinetic analyses were obtained from these data. Questionnaires assessing the subjective effects were administered to the subjects during each session before and after the smoking time period. THC, 11-hydroxy-THC (11-OH-THC) and 11-nor-9-carboxy-THC (THCCOOH) were analyzed in the blood by gas chromatography or liquid chromatography (LC)-tandem mass spectrometry (MS/MS). The determination of THC, THCCOOH, cannabinol (CBN), and Δ9-tetrahydrocannabinolic acid A (THC-A) was carried out on OF only using LC-MS/MS. In line with the widely accepted assumption that cannabis smoking results in a strong contamination of the oral cavity, we found that THC, and also THC-A, shows a sharp, high concentration peak just after smoking, with a rapid decrease in these levels within 3 h. No obvious differences were found between both groups concerning THC median maximum concentrations measured either in blood or in OF; these levels were equal to 1,338 and 1,041 μg/L in OF and to 82 and 94 μg/L in WB for occasional and heavy smokers, respectively. The initial WB THCCOOH concentration was much higher in regular smokers than in occasional users. Compared with the occasional smokers, the sensation of confusion felt by the regular smokers was much less while the feeling of intoxication remained almost unchanged.

Keywords Cannabis · Pharmacokinetic · Oral fluid · Whole blood · Heavy use · Occasional use

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Introduction

Cannabis is the most widely used illegal drug in the world, and Δ9-tetrahydrocannabinol (THC) is frequently detected in the blood or oral fluid (OF) of impaired drivers arrested for erratic driving or involved in road accidents [1, 2]. Accurate measurements and proper interpretation of cannabinoid concentrations

are therefore important in order to reconstruct the accident scene, to evaluate the exact time of consumption, and to assess the level of driving impairment. In this regard, the ability (short-term) and fitness (long-term) to drive can be influenced by many parameters. One of them is the frequency of cannabis use. Several studies suggest that heavy cannabis smokers are less likely to feel the desired effects, as well as the adverse symptoms, of acute cannabis smoking when compared with light users [3]. One key issue is therefore to find identification criteria that allow the distinction between these two groups of consumers. In this respect, questionnaires and brief medical examinations have a limited reliability. In general, it is preferable to rely on objective data which complement the aforementioned subjective observations. Chronic use can be assessed by measuring cannabinoid concentrations in hair. However, this matrix is not always available, or can be contaminated by cannabis smoke [4] or by contact with dirty hands, thus making its interpretation quite challenging. Furthermore, in comparative studies, discrepancies were found between "positive" urine specimens and hair tested as "negative" [5, 6]. Thus, measurement of cannabinoids in biofluids offers another strategy. To this end, Daldrup et al. [7] have shown that, if the serum concentration of THCCOOH is lower than 5 μg/L, the consumption is assumed to be occasional while levels above 75 μg/L are associated with regular use. This criterion could be applied in routine forensic examinations provided serum samples are taken within a prescribed 8-day period following the last cannabis use [6].

To demonstrate that the defendant or the car driver was under the influence of cannabis during a relevant event, one could demonstrate that blood levels of active cannabinoids at the time of the event were compatible with the impairing effects of cannabis. Another possibility is to show that the time lapse between exposure to cannabis and the event was less than a few hours. In this respect, OF and blood determination of cannabinoids can be helpful. Detection of THC in OF has been associated with a strong contamination of the oral cavity during smoking and to a recent cannabis use. On the other hand, THC and its metabolites are only poorly excreted from the blood and tissues into this matrix. In line with these observations, analysis of OF revealed very high concentrations of THC in OF just after cannabis smoking, while 11-hydroxy-THC (11-OH-THC) was not detected and only trace amounts of 11-nor-9-carboxy-THC (THCCOOH) were measured [8]. Studies that used intravenous administration of THC have suggested that the transfer of THC from the blood into OF is limited [9]. Since THCCOOH is not known to be present in cannabis smoke, its detection in OF could only result from active cannabis consumption.

Knowledge of the pharmacokinetic (PK) parameters of cannabinoids in blood and saliva and of their mutual relationship with drug effects should also help to assess the level of impairment of the driver. On the other hand, the determination of cannabinoids in urine can be used to demonstrate a cannabis exposure. In 2001, in a study on the detection of THC in OF, Niedbala et al. [10] showed that, during the elimination phase, THC levels in OF follow a similar decrease to those observed in plasma after cannabis smoking. In 2004, Huestis and Cone [11] obtained the same results using a controlled administration of smoked cannabis. More recently, Laloup et al. studied the correlation of THC concentrations in OF and plasma [12]. Their results indicated a good correlation between OF and plasma concentrations and were in concordance with studies of Toennes et al. [13, 14]. However, although THC is now commonly detected in OF, there is still little information about other cannabinoids and the determination of their PK parameters in simultaneously, or near-simultaneously, collected blood and OF specimens. Recently, Lee et al. investigated the change in the OF/plasma cannabinoid ratios following controlled oral THC and smoked cannabis administration [15]. They suggest that a direct prediction of plasma THC concentrations based on OF levels is not appropriate. On the other hand, OF THCCOOH could estimate plasma THCCOOH levels. Throughout the last decade, several studies have been carried out to determine PK parameters of cannabinoids in blood, plasma, or serum [16–21] in humans. Some other studies were performed with oral fluid only [22] or in tandem with serum [23] or plasma [11, 24] with a limited number of ten participants in each study. Two, three, and more compartment PK and kinetic models have been applied with varying degrees of success to describe the cannabinoid time profiles [25]. Non-compartmental and PK/pharmacodynamic approaches were also used. No general consensus could be reached from any of these studies.

In this study, 23 heavy and 25 occasional cannabis users smoked cannabis or a placebo joint during two separate sessions. Whole blood (WB) and OF samples were collected before and up to 3.5 h after smoking a joint or a placebo. PK parameters of THC, 11-OH-THC, THCCOOH in WB, and of THC, THCCOOH, cannabinol (CBN) and THC acid A (THC-A) in OF were determined. The most salient features that distinguish the kinetic profiles of the two consumer groups are presented. The kinetic profiles are correlated to two typical subjective effects of cannabis. We also outline the usefulness of THC-A as a possible marker of cannabis smoking.

Experimental

Study design

The overall design of the study has been described in a previous publication and its supportive information [26]. The study was expanded in order to include regular cannabis smokers. The methods are briefly described below. Forty-

eight healthy male volunteers between 18 and 30 years old, 23 heavy smokers, and 25 occasional smokers, who completed the sessions successfully, are included in this study. All participants underwent a structured interview conducted by a medical staff (psychiatrist, clinician, and research technician). The mean consumption of cannabis during the 3 months preceding inclusion in the study was set to a minimum of one joint per month and a maximum of one joint per week for occasional smokers, while it was set to a minimum of ten joints per month (i.e., 2.3 joints/week) and possibly less than three joints per day for heavy smokers. These criteria were in accordance with those of Toennes et al. [21], who considered a use higher than four times per week for heavy smokers and a weekly use or less for the occasional cannabis users. Very heavy cannabis smokers (up to several dozen joints per day) were excluded because we considered them unable to refrain from smoking during a full day and, above all, the day they had to smoke the placebo. Volunteers who used any illegal drug (cocaine, amphetamines, and opiates) other than cannabis were also excluded from the study. The urine test for THC metabolites was required to be positive for subjects enrolled in the group of regular smokers, but it could either be positive or negative in occasional users. Subjects recruited for the study participated in two independent cross-over experimental sessions where they smoked either a joint of pure cannabis (Bedrobinol, 11 % total THC (less than 0.5 % free THC [27]), <1 % CBD, obtained from Bedrocan, Veendam, The Netherlands) or a placebo (Santhica variety, no THC, < 0.1 % CBD, provided by the French National Federation of Hemp Growers, FNPC, Le Mans, France). Throughout the day, WB and OF samples were collected. OF samples from occasional smokers were collected a few minutes before inhalation (t = 0 h) and afterwards at 0.65 and 2.75 h. OF samples from heavy smokers were collected a few minutes before inhalation (t = 0 h) and at 0.35, 0.65, 1.9, 2.75, and 3.5 h after inhalation. Specimens were stored for a few hours at room temperature before freezing and storage at −80 °C. WB samples were taken a few minutes before inhalation (t = 0 h) and at 0.2, 0.3, 0.4, 0.65, 1.9, 2.5, and 3.5 h after smoking the joint. Samples were immediately frozen and kept at −80 °C before analysis. For occasional smokers, the last blood sample was omitted because experimental sessions should not last more than 3 h after inhalation. On six occasions during the experimental day, volunteers were also asked to fill out questionnaires on the subjectively experienced effects of smoking a joint. Subjective effects were assessed by asking participants to indicate the intensity of their feeling on a 100-mm visual analog scale (VAS) between 0 (no effect) and 100 (most intense effect ever felt). The questionnaires were administered at regular time intervals before and after smoking the cannabis joint or its matching placebo.

This study was approved by the Cantonal Research Ethics Committee (Vaud). The subjects gave written informed consent and received financial compensation for their participation.

Joint making and inhalation protocol

The cannabis plant material (0.7 g Bedrobinol or 0.8 g Santhica variety) was chopped, and the pure ground-up buds were added to a pre-rolled king-size joint with a roach. The joints were of identical size and were visually indistinguishable. The enrolled subjects smoked the joints according to a fixed paced procedure. Each inhalation cycle was composed of four steps: getting ready and start signal, 3 s; inhalation, 2 s; breath-holding, 5 s; exhalation and rest, 50 s. This sequence was repeated until two thirds of the joint was consumed, up to a line drawn 3 cm above the cardboard filter.

Estimation of the usual and actual smoked amount of cannabis

So as to create the most realistic conditions for the subjects, the volunteers were asked about their smoking habits and ways of preparing joints. To estimate the amount of cannabis used to prepare a self-made joint, a picture showing a joint and increasing amounts of cannabis and tobacco, and a metric scale, was shown to each participant. The self-reported amount of cannabis used to make a joint was divided by the number of participants sharing the same joint in a joint session. The quantity actually smoked during this experiment was determined by weighing the initial amount of cannabis put into the joint and subtracting the residual amount found in the left part and in the butt. In contrast to the usual habits of smokers, the marijuana was not mixed with tobacco in order to eliminate any nicotine effects.

Materials

THC, 11-OH-THC, THCCOOH, CBN, CBD, THC-A, and internal standards THC-d_3, 11-OH-THC-d_3, THCCOOH-d_9 were purchased from Cerilliant Corporation (Round Rock, TX, USA). Oral fluid samples were collected with a Quantisal™ (nal von minden GmbH, Regensburg, Germany) device for heavy smokers and with Salivette® (Sarstedt AG, Sevelen, Switzerland) for occasional users. When the study began with the occasional consumers, the Quantisal™ collection device was still uncommon and the data relating to its use were very incomplete. In recent years, published results have demonstrated the superiority of the Quantisal™ over the Salivette® sampling device [28], which is why it was selected for the heavy smokers.

Methods of analysis

Analyses of OF samples were performed according to a previously published procedure [8]. The collection devices were weighed before and after OF sampling to determine the quantity of OF collected. The concentrations were then expressed in nanograms per milliliter of undiluted OF. OF was extracted from the collection device by centrifugation (Salivette®) or by squeezing the pad onto the walls of the plastic tube (Quantisal™). Subsequently, methanol (1.5 mL) was added to the saliva collector to wash off the residual cannabinoids. The methanolic extracts were evaporated to dryness under N_2 before adding the OF samples. The cannabinoids were then extracted by a LLE with heptane/ethyl acetate (4:1, *v/v*). Chromatographic separation was achieved using a Dionex UltiMate 3000 Rapid Separation LC system equipped with a Kinetex C18 100A column (150×2.1 mm). Tandem mass spectrometry (MS/MS) operated either in negative or positive electrospray ionization mode was carried out on an ABSciex API 5000™ triple quadrupole system. Limits of quantification (LOQs) were 0.5 μg/L for THC, THC-A, CBN, and CBD and 0.08 μg/L for THCCOOH.

The blood samples of the occasional smokers were analyzed according to the procedure described by Thomas et al. [29]. The cannabinoids were extracted from 500 μL of WB by a simple liquid–liquid extraction with hexane/ethyl acetate (9:1, *v/v*) and then derivatized with a mixture of trifluoroacetic anhydride and hexafluoro-2-propanol as fluorinated agents. Mass spectrometric detection of the analytes was performed in the selected reaction-monitoring mode on a Varian 1200 L MS/MS triple quadrupole instrument after negative-ion chemical ionization. The LOQs of the method were 0.5 μg/L for THC and 11-OH-THC and 2.5 μg/L for THCCOOH.

WB samples of the regular smokers were extracted using the same procedure but analyzed by LC-MS/MS without a derivatization step. The ion transitions, MS parameters, and LC-MS/MS equipment were the same as those described in the method used for the analysis of OF samples. The LC-MS/MS method used for the analysis of blood specimens was validated according to the recommendations of the "Société Française des Sciences et Techniques Pharmaceutiques". The same quality control (QC) specimens were used to cross-validate the gas chromatography-MS/MS and the LC-MS/MS methods. The two techniques gave the same results for the analysis of the QCs; no significant difference was therefore observed between both methods. The LC-MS/MS method was linear for THC and 11-OH-THC from 0.5 to 20 μg/L and for THCCOOH from 2.5 to 100 μg/L. The trueness was determined with four QC (0.5, 1, 10, 20 μg/L for THC and 11-OH-THC and 2.5, 10, 50, 100 μg/L for THCCOOH) analyzed four times on the same day. The results ranged between 86 and 115 %. The coefficients of variation (CV) of interday and intermediate precisions were lower than 15 % in all cases, except for 11-OH-THC at a concentration of 0.5 μg/L (25.8 %). LOQ values were determined at the lower QC with trueness higher than 85 % and CVs lower than 20 %. LOQs were 0.5, 1, and 2.5 μg/L for THC, 11-OH-THC, and THCCOOH, respectively.

Evaluation of the data

The concentration–time profiles were analyzed with a non-compartmental PK approach, using Microsoft Excel 2007. The maximum concentrations (Cmax) and their corresponding times (Tmax) were obtained from the kinetics. The elimination half-lives ($T_{1/2}$) were calculated by log-linear regression of the concentration–time curves (from the result of exponential regression of the data). The areas under the curves (AUC) were estimated using the trapezoidal rule.

Results and discussion

Sociodemographic comparison of occasional and heavy cannabis smokers

The sociodemographic characteristics are presented in Table 1. The mean (SD) age was 23.9 (3.0) for occasional and 22.7 (2.4)years for heavy smokers. Paper-wrapped joints filled with a half/half mixture of marijuana and tobacco was the preferred way of consumption as reported in the self-administered questionnaires. On average, the cannabis joints were smoked from 3.5 to 77.9 occasions per month over the past 3 months preceding the study, depending on whether they were occasional or heavy smokers. When taking into account the fact that joints are shared by several smokers, all participants included in the group of occasional users smoked one or less joint per week. Weekly consumption in heavy cannabis smokers was more evenly distributed between 2 and 13 joints per week. One smoker admitted to consuming 25 joints per week. Consequently, all the occasional users met the inclusion criterion set for the frequency of use (between one joint per month and one joint per week). The heavy smokers met the minimum frequency of use considered as mandatory for inclusion in this group (between ten joints per month and two joints per day). The subjects of both groups started to smoke cannabis at a median age of 16.3 years. Their way of smoking differed considerably. The heavy smokers reported inhaling more deeply and preferring higher-dose joints than the occasional subjects. Finally, it is interesting to note that almost all the occasional smokers (22 out of 25) had a driving license, whereas only 14 heavy cannabis users (out of 23) had one.

Table 1 Sociodemographic characteristics and self-rated patterns of cannabis use in occasional and heavy smokers

	Number	Mean	SD	Median	Maximum	Minimum
Occasional smokers						
Age		23.9	3.0	24	29	19
Ethnicity	Caucasian (23), Asian (1), Eurafrican (1)					
Driving license	22 (21 car, 1 motorbike)					
Age at first cannabis use		16.3	2.9	16	23	9
Total years of lifetime cannabis use		7.6	3.2	7	15	4
Preferred forms of cannabis	Marijuana (21), hashish (11), hashish oil (5), pollen (2)					
Preferred methods of consumption	Joint (25), water pipe (bong, bhang) (7), pipe (chillum, sebsi) (10), cigar (blunt) (3), vaporizer (1)					
Assessment of the usual size of a joint (grams)		0.4	0.3	0.3	1.0	0.1
Estimation of the [%] of cannabis in the cannabis/tobacco mix		47	18	50	70	20
Frequency of use (occasions/month, last 3 months)		3.5	2.3	3.5	10	1
Number of people with whom the joint is shared		3.3	1.0	3.5	5	2
Prefer light (L) or strong (S) cannabis	15 L, 9 S					
Usually inhale deeply (yes/no)	7 Y, 20 N					
Heavy smokers						
Age		22.7	2.4	22	28	19
Ethnicity	Caucasian (20), Asian (1), Eurafrican (2)					
Driving license	14					
Age at first cannabis use		16.3	2.3	16	20	12
Total years of lifetime cannabis use		6.3	2.9	6	13	2
Preferred forms of cannabis	Marijuana (23), hashish (14), pollen (4)					
Preferred methods of consumption	Joint (23), water pipe (bong, bhang) (18), pipe (chillum, sebsi) (17), cigar (blunt) (2), vaporizer (2)					
Assessment of the usual size of a joint (grams)		0.4	0.1	0.4	1.0	0.1
Estimation of the [%] of cannabis in the cannabis/tobacco mix		52	15	50	70	15
Frequency of use (occasions/month, last 3 months)		77.9	51.7	62.5	250	20
Number of people with whom the joint is shared		2.5	0.9	2.5	4	0
Prefer light (L) or strong (S) cannabis	8 L, 15 S					
Usually inhale deeply (yes/no)	19 Y, 4 N					

Comparison of the usual and actual smoked amounts of cannabis

The usual amount smoked varied considerably between individuals and frequency of use, with a median quantity of 0.3 g for occasional smokers and 0.4 g for regular users. The actual quantity of cannabis smoked was equal to 0.39 g for both groups, with little variance for heavy smokers and a slightly wider variance for occasional users. Taking into consideration a mean concentration of 11 % THC in the Bedrobinol head tops, the total quantity of THC used during each smoking session was estimated at around 43 mg. By way of comparison, Mariani et al. [30] reported that an amount of 0.66 g is used in making joints in the USA (generally uncut with tobacco) while typical European joints contain 0.33–0.40 g of plant material and 20–50 mg of THC. The typical THC concentration was reported to be around 8 % for marijuana and 10 % for hashish in France in 2010 (OFDT report, 2012); corresponding results reported by the Swiss Society of Forensic Toxicology were 11 % THC for marijuana and 16.8 % for hashish for the second semester of 2012.

Kinetic profiles and pharmacokinetic parameters of THC, 11-OH-THC, and THCCOOH in whole blood

Figure 1 shows the individual time profiles of THC, 11-OH-THC, and THCCOOH for heavy and occasional smokers. Occasional and heavy smokers presented similar kinetics, except for the THC, 11-OH-THC, and THCCOOH C_0. As indicated in Table 2, C_0 were 2.1 and 0.3 μg/L for THC; 0.9 and 0 μg/L for 11-OH-THC; and 20 and 0 μg/L for THCCOOH for heavy and occasional cannabis users, respectively. Heavy smokers' C_0 were significantly higher ($p < 0.0001$) than those of occasional ones for each of the three cannabinoids.

Maximal concentration (Cmax), time to the maximal concentration (Tmax), elimination half-life ($T_{1/2}$), and area under

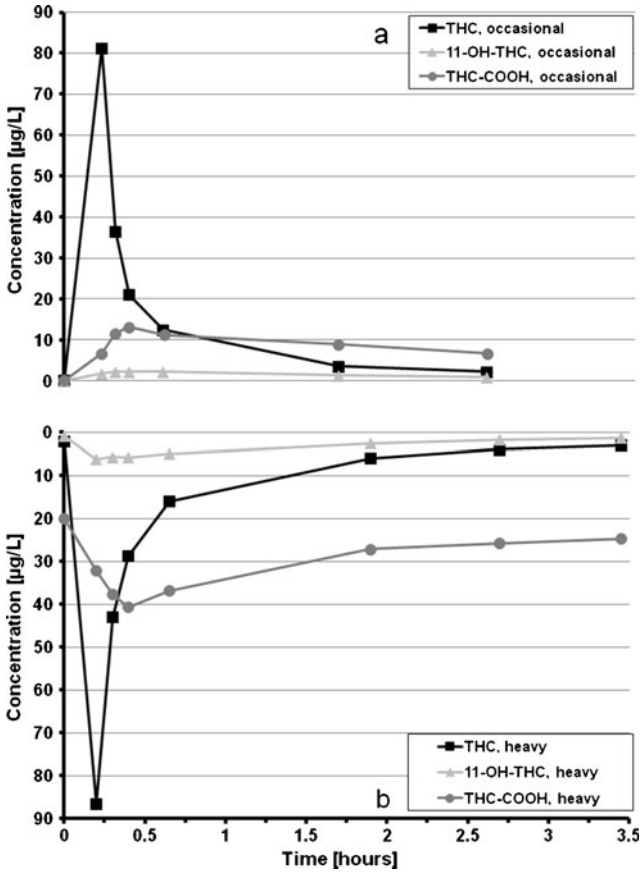

Fig. 1 Time profiles of THC, 11-OH-THC, and THCCOOH in whole blood. *Upper* subfigure **a** occasional smokers; *bottom* subfigure **b** heavy smokers

the curve ($AUC_{2.5h}$ and $AUC_{3.5h}$) are presented in Table 2. $T_{1/2}$ and $AUC_{2.5h}$ were determined by blood samples collected until 2.5 h after inhalation for both occasional and heavy smokers in order to compare their individual PK parameters. As blood samples were collected till 3.5 h after inhalation for heavy smokers, $AUC_{3.5h}$ was also determined for this group. THC, 11-OH-THC, and THCCOOH presented all a median *C*max in blood that was higher for heavy users than for occasional smokers: Median THC *C*max were 87 and 75 μg/L, median 11-OH-THC *C*max were 6.5 and 2.6 μg/L, and median THCCOOH *C*max were 41 and 13 μg/L for heavy and occasional smokers, respectively. Unexpectedly, the difference in THC between the groups was not significant. Before carrying out this study, we suspected that experienced chronic users should have a more efficient inhalation technique resulting in higher blood concentrations than occasional smokers with less experience and a poor puffing method. Indeed, it is known that the bioavailability of THC is variable and is influenced by individual techniques and smoking experience [25, 31]. Since no obvious difference was found, we hypothesized that the inhaled dose was less influenced by frequency and previous experience/use since all smokers inhaled following a fixed paced protocol and also because the experienced cannabis smokers would be more able to titrate the effects than the inexperienced ones. The observation that occasional and heavy smokers consumed approximately the same quantity of cannabis (0.4 g) is in line with this hypothesis. Furthermore, the occasional subjects who smoked without inhaling (i.e., those who "crapote") were excluded. The fact that the regular smokers felt the effects of confusion less than the occasional users (see next paragraph) can be explained by the development of tolerance, but also by a better ability to titrate the effects, especially those perceived as negative (confusion). On the other hand, the differences between both groups were highly significant for 11-OH-THC and THCCOOH ($p < 0.0001$). For these two metabolites, the differences could be explained by the frequency of consumption and their long terminal half-life.

The three cannabinoids remained detectable in all blood specimens until the end of the investigation day. THC, 11-OH-THC, and THCCOOH levels were higher than the LOQ in all the heavy smokers' samples. Median THC concentrations measured 2.5 h after smoking were 4.2 and 2.1 μg/L for the heavy and occasional smokers, respectively, while median THCCOOH concentrations at the same time were 26 and 7.1 μg/L. Thus, THCCOOH concentrations in the heavy smokers were significantly higher than those of the occasional cannabis users ($p < 0.0001$).

As expected and presented in previous studies [11, 21, 23], THC reached its highest concentration first, followed by 11-OH-THC and by THCCOOH. Median THC *T*max were 0.20 and 0.23 h for the heavy and occasional groups, respectively, while median 11-OH-THC Tmax were slightly delayed to 0.28 and 0.32 h and median THCCOOH *T*max even more to 0.38 and 0.40 h. The differences between both groups for the THC and THCCOOH were not significant. It was marginally significant for 11-OH-THC ($p < 0.05$). THCCOOH *T*max values were slightly different from those reported by Kauert et al. [23] (mean of 0.25 h) and by Toennes et al. [21] (0.75 h for heavy and 0.25 h for occasional smokers).

Median THC, 11-OH-THC, and THCCOOH $AUC_{2.5h}$ in blood were higher for the heavy than for the occasional smokers. THC $AUC_{2.5h}$ were 43 and 33 μg.h/L; 11-OH-THC $AUC_{2.5h}$ were 10 and 4.2 μg.h/L, and THCCOOH $AUC_{2.5h}$ were 79 and 23 μg.h/L for the heavy and occasional smokers, respectively. THC $AUC_{2.5h}$ values were marginally different between both groups ($p < 0.05$). This could be explained by the fact that THC *C*max of the heavy smokers was slightly higher than THC *C*max of the occasional smokers (without being significantly different). On the other hand, differences were highly significant for 11-OH-THC and THCCOOH $AUC_{2.5h}$ ($p < 0.0001$). These differences could be explained as follows: AUC measured for the heavy smokers included both past and recent inhalations, while those

Table 2 Pharmacokinetic parameters of THC, 11-OH-THC, and THCCOOH in whole blood for heavy and occasional smokers

	C_0 (μg/L)	*C*max (μg/L)	*T*max (h)	$AUC_{2.5h}$ (μg.h/L)	$AUC_{3.5h}$ (μg.h/L)	$T_{1/2}$ (h)
THC heavy						
Median	2.1	87	0.2	43	45	1.0
Mean	2.4	95	0.2	47	50	1.0
SD	1.6	47	0.1	20	21	0.3
Min	0.8	37	0.1	19	20	0.5
Max	7.1	192	0.4	86	91	1.9
THC occasional						
Median	0.3	75	0.2	33	–	0.8
Mean	0.3	76	0.2	31	–	0.9
SD	0.4	46	0.1	18	–	0.2
Min	0	8.2	0.2	4.1	–	0.7
Max	1.4	168	0.4	68	–	1.4
11-OH-THC heavy						
Median	0.9	6.5	0.3	10	12	1.7
Mean	1.2	6.9	0.3	10	11	1.8
SD	0.8	3.4	0.1	4.1	5.0	0.5
Min	0.4	2.8	0.2	3.7	4.1	1.0
Max	3.0	16	0.4	17	20	3.1
11-OH-THC occasional						
Median	0	2.6	0.3	4.2	–	1.6
Mean	0.1	3.4	0.4	4.7	–	2.7
SD	0.2	3.3	0.3	4.4	–	2.3
Min	0	0.6	0.2	0.7	–	1.0
Max	0.6	18	1.6	23	–	11
THCCOOH heavy						
Median	20	41	0.4	79	99	3.9
Mean	22	45	0.4	90	113	3.8
SD	16	24	0.1	53	70	1.7
Min	2.5	17	0.2	28	35	1.7
Max	51	106	0.7	227	304	9.2
THCCOOH occasional						
Median	0	13	0.4	23	–	3.33
Mean	1.3	15	0.4	27	–	4.45
SD	3.7	8.9	0.1	19	–	3.69
Min	0	2.0	0.2	3.7	–	1.59
Max	17	38	0.7	86	–	17.3

calculated for the occasional users reflected current inhalation only. To better compare the $AUC_{2.5h}$, those of regular smokers were corrected by baseline subtraction. To this end, a trapezoid area was calculated by multiplying the investigated time-period (2.5 h) by the THCCOOH C_0 level. This value was weighted by the terminal half-life of THCCOOH [6]. The median corrected $AUC_{2.5h}$ for heavy smokers was then 16.1 μg.h/L and not significantly different from the THCCOOH $AUC_{2.5h}$ of occasional smokers.

The THC $T_{1/2}$ in blood was shorter than 11-OH-THC $T_{1/2}$ which was also shorter than THCCOOH $T_{1/2}$. Similar observations were drawn by Kauert et al. in a study carried out with recreational cannabis users [23]. In our study, median THC $T_{1/2}$ were 0.8 and 1.0 h for the occasional and heavy smokers, respectively, while the corresponding value reported by Kauert et al. was 1.4±0.1 h. Our calculated 11-OH-THC $T_{1/2}$ were 1.7 h for the heavy and 1.6 h for the occasional smokers versus 1.9 h, as reported by Kauert. The same authors indicated a THCCOOH half-life of 3.0 h while we found respectively 3.3 and 3.9 h for the occasional and heavy cannabis users. These differences can be explained by the longer observation time in the Kauert study (6 vs. 2.5 and 3.5 h in our case). The investigation time period has a great influence on

the determination of the half-life; for instance, Wall et al. [32] calculated a terminal half-life between 25 and 36 h for THC, with a study lasting 72 h.

Pharmacokinetic parameters of THC, THC-A, CBN, and THCCOOH for heavy and occasional cannabis smokers in oral fluid

Median time profiles of THC, THC-A, CBN, and THCCOOH are presented for the heavy smokers using a double *y*-axis plot in Fig. 2. The right axis is used for THCCOOH concentrations with a magnitude extending from 0 to 0.7 μg/L, and the other data subset (THC, THC-A and CBN) used the left axis with a 1,000 times higher range scaled from 0 to 700 μg/L. THC concentrations reached very high values and significantly exceeded those of THC-A and CBN which were quite similar. THCCOOH concentrations were lower with a median peak value below 400 ng/L. THCCOOH levels remained higher than the LOQ value up to 2.5 h after inhalation. In all but one sample of the heavy smokers, THC remained detectable in oral fluid until 3.5 h after smoking. The median THC concentration at the end of the investigation day was 22 μg/L. THC-A was still detectable in 18 out of 23 samples with a median concentration of 1.9 μg/L. As for THC, CBN remained detectable to the end of the investigation period in all but one sample, although its median concentration after 3.5 h was much lower, 2.4 μg/L. Median THC concentrations measured 3.5 h after smoking were higher in OF than in blood (22 vs. 4.2 μg/L). This confirms that THC can be detected slightly longer in oral fluid than in blood, as already reported by Drummer [33] and Verstraete [34]. Consequently, oral fluid could be an interesting matrix, offering a greater likelihood of recent cannabis use detection. The presence of THC-A in OF was likely to be due to its incomplete decarboxylation while the presence of CBN was the consequence of THC oxidation and pyrolysis occurring during the smoking of the joint [35, 36]. As THC-A remained detectable throughout the experiment, its use as a marker of recent exposure is questionable. However, since most THC/CBD-based medicines do not contain THC-A, this molecule could be used as a marker to detect crude cannabis use. The plant cannabinoid THC-A was also detected in serum samples and was recently suggested as a possible marker to differentiate an intake of illegal cannabis products from an administration of therapeutic THC (dronabinol, Marinol™) [37].

Pharmacokinetic parameters, such as C_0, *C*max, C_t, *T*max, AUC, and elimination $T_{1/2}$ are listed in Table 3. The heavy smokers presented C_0 values significantly higher than those measured for the occasional users for THC ($p < 0.0001$), THC-A ($p < 0.001$), and CBN ($p < 0.001$). The median THC C_0 for regular smokers was relatively high (9.7 μg/L). A null median value was found for the initial THCCOOH concentration which was determined prior to smoking. If THCCOOH could be occasionally detected in heavy smokers, it remained undetectable in occasional users.

Among all participants, THC *C*max ranged from 19 to 3,170 μg/L, with median *C*max values of the occasional smokers reaching 1,320 μg/L and exceeding those of the heavy users (636 μg/L). Similar differences were observed with THC-A and CBN *C*max: THC-A *C*max were 130 and 59 μg/L and CBN *C*max were 125 and 81 μg/L for the occasional and heavy smokers, respectively. THC-A peak concentrations differed significantly between both groups ($p < 0.01$). The higher buccal contamination observed among the occasional smokers could be explained by differences in smoking and inhalation techniques. It is known that the bioavailability of these cannabinoids can be influenced by many

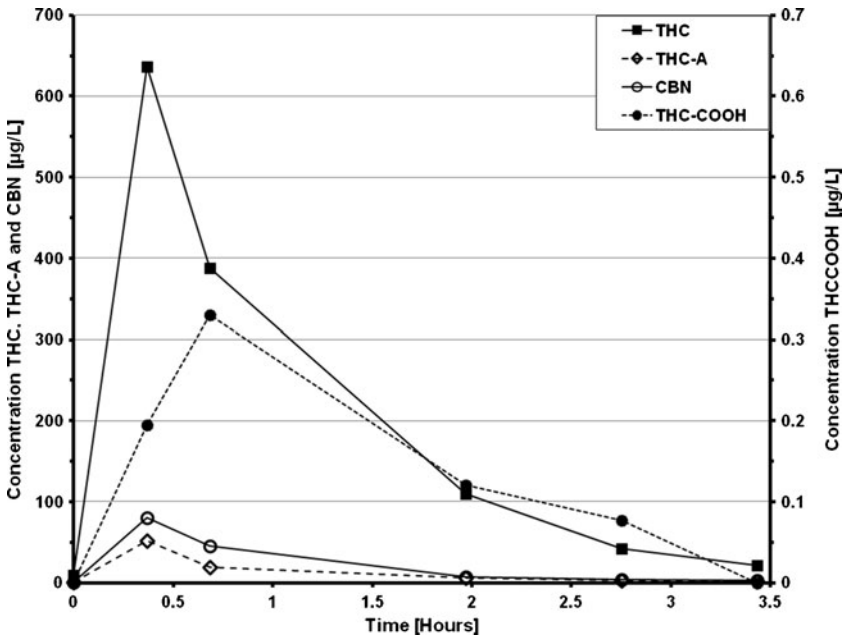

Fig. 2 Time profiles of THC, THC-A, CBN, and THCCOOH in oral fluid for heavy smokers

Table 3 Pharmacokinetic parameters of THC, THC-A, CBN, and THCCOOH in oral fluid for occasional and heavy smokers

	C_0 (μg/L)	*C*max (μg/L)	$C_{2.5h}$ (μg/L)	$C_{3.5h}$ (μg/L)	*T*max (h)	$AUC_{2.5h}$ (μg.h/L)	$T_{1/2}$ (h)
THC heavy							
Median	9.7	636	42	22	0.4	745	0.9
Mean	24	1047	70	60	0.5	1120	1.3
SD	59	967	77	103	0.6	1179	1.0
Min	0	19	1.1	0	0.2	25	0.3
Max	290	3170	232	140	3.5	4226	3.4
THC occasional							
Median	0	1320	216	–	0.3	–	–
Mean	0.6	1388	258	–	0.3	–	–
SD	2.5	782	286	–	0.1	–	–
Min	0	367	9	–	0.2	–	–
Max	12	3110	1400	–	0.6	–	–
THC-A heavy							
Median	1.2	59	2.5	1.9	0.4	48	1.3
Mean	4.1	73	4.3	13	0.5	59	1.5
SD	7.3	59	6.1	50	0.7	41	1.1
Min	0	5.9	0	0	0	7.3	0.3
Max	32	240	29	8.6	3.5	162	4.4
THC-A occasional							
Median	0	130	11	–	0.3	–	–
Mean	0	334	37	–	0.3	–	–
SD	0.1	610	84	–	0.1	–	–
Min	0	4.8	0	–	0.2	–	–
Max	0.5	2910	412	–	0.6	–	–
CBN heavy							
Median	0.5	81	4.0	2.4	0.4	83	1.0
Mean	1.2	107	5.4	3.8	0.4	101	1.1
SD	1.9	87	5.3	4.4	0.1	82	0.6
Min	0	6.0	0	0	0.2	5.5	0.4
Max	6.7	310	25	22	0.5	297	2.7
CBN occasional							
Median	0	125	19	–	0.3	–	–
Mean	0	192	29	–	0.3	–	–
SD	0	198	23	–	0.1	–	–
Min	0	17	0	–	0.2	–	–
Max	0	756	79	–	0.6	–	–
THCCOOH heavy							
Median	0	0.3	0.1	0	0.6	0.6	1.3[a]
Mean	0.1	0.6	0.1	0.1	0.7	0.8	1.9[a]
SD	0.1	0.6	0.1	0.1	0.5	0.8	1.9[a]
Min	0	0.1	0	0	0.3	0.1	0.8[a]
Max	0.5	2.4	0.3	0.4	2.0	3.7	7.2[a]

[a] Parameters determined with ten values only

factors, e.g., how deep the smoke is inhaled in the lungs, the number of puffs and puff volume, the strength of inhalation, the size of smoked particles and the distribution between the gas phase, and the particle phase and the residence time in the mouth [9]. Heavy and occasional users smoked the same quantity of cannabis (0.39 g). However, the cannabinoid concentrations in blood were slightly higher for the heavy than for the occasional smokers, but not significantly different. We suggest that, during inhalation, the occasional smokers kept the cannabis smoke a little longer in their mouths than the heavy users before its inhalation into the lungs. The mouths of the occasional cannabis smokers could have been more contaminated, resulting in higher levels of THC, THC-A, and CBN. Furthermore, extraction efficiency could have been influenced by the type of saliva collector used for each group of smokers, although both extraction methods were cross-validated. Indeed, the interaction of the cotton roll of the Salivette® or of the pad of the Quantisal™ with the oral cavity may have been different. A strong decrease in median concentrations of THC, THC-A, and CBN was observed for both groups of smokers within 2.5 h. The magnitude of the decrease was significantly higher for heavy smokers with *p* values (Mann–Whitney *U* test) lower than 0.05.

Almost no difference was observed for the time period separating the first puff from the observed *C*max. THC, THC-A, and CBN *C*max happened concurrently a short time after inhalation of the cannabis joint (*T*max=0.3–0.4 h). A similar observation has been already reported in several other studies [11, 23, 38, 39]. For instance, the *C*max determined by Kauert et al. [23] for THC was already measured in the first OF sample taken just 0.25 h after starting inhalation. The concentrations were poorly related to the administered dose: 900±589 or 1,041±652 μg/L after smoking a joint containing 250 μg/kg body weight (BW) or 500 μg/kg BW, respectively. Broad variations in the THC concentrations measured in OF were observed between studies. For example, Milman et al. [39] reported a median THC highest concentration of 2,629 μg/L at 0.25 h after smoking. Huestis and Cone [11] indicated an OF THC *C*max of 5,800 μg/L 0.2 h after inhalation. Several parameters could explain these large variations of THC *C*max in OF. First, the devices used for collecting OF differ and may influence the THC levels recovered from the saliva. The expectoration [39] provided undiluted OF. However, it presents some limitations, such as low specimen volume, high viscosity, and decreased drug stability. Expectoration after stimulation with citric acid [11] enhances sample volume, but also changes salivary composition, and could affect cannabinoid concentrations in OF [40]. Collecting OF with Quantisal or Intercept devices [23, 41] results in diluted OF mixed with extracting buffer containing potential analytical interferents (detergent, coloring agent, preservative substances). Langel et al. [28] studied nine different collection devices. Their conclusions were that the extraction buffer of the collection devices may help to increase the recovery of drugs and improve the stability of the samples. Secondly, the type of joint, the concentration of THC in the cannabis plant

material, and the quantity of THC inhaled are also influential. In our study, volunteers smoked half a cannabis joint containing 11 % THC, which corresponds to a median value of 43 mg of THC. Subjects enrolled in the study of Kauert et al. smoked either 13.8–22.3 or 27.5–44.5 mg THC. Huestis and Cone administered cannabis cigarettes with 3.55 % THC, which corresponds to 33.8 mg THC. Milman et al. provided the participants with joints containing 6.8 % THC. Thirdly, the bioavailability of THC after cannabis smoking is variable and influenced by individual techniques of inhalation and previous history of use, as already mentioned [25, 31].

THCCOOH *C*max ranged between 0.3 and 2.4 μg/L in the heavy smokers. This metabolite was not detected in OF samples of the occasional smokers. Since THCCOOH is not found in cannabis plants, neither in joints nor in cannabis smoke, its presence in OF could result only from human metabolism. THCOOH is very likely poorly excreted from the plasma into the saliva as a free molecule [8]. Its concentration in OF is related to that found in plasma. Our study has shown that THCCOOH levels were significantly higher in the blood of the heavy smokers than in the light users. Therefore, a lower concentration was to be suspected and was subsequently found to be below the detection value limit in the OF of the occasional smokers compared with the heavy users. In contrast to THC, THC-A, and CBN peaks, THCCOOH highest concentration level was time-delayed. The median (range) OF THCCOOH *T*max occurred 0.6 (0.3–2.0 h) after inhalation. These values correspond to those of Milman et al. [39]. They determined cannabinoids in oral fluid and obtained a median *T*max for THCCOOH of 1 h (range was between 0.25 and 2.0 h). The observed delay is in line with the assumption that THCCOOH in OF originates from THC metabolism. Oral fluid AUC and $T_{1/2}$ for the occasional smokers could not be determined because only two samples could be collected after inhalation. Median $AUC_{3.5h}$ of THC was much higher in OF than in blood (745 versus 45 μg/L h) because THC concentrations in OF widely exceeded those measured in blood. On the other hand, THCCOOH was found in much lower concentrations in OF than in blood, explaining why the $AUC_{3.5h}$ in OF was inferior to that in blood.

The samples of the ten volunteers who remained positive until the end of the investigation day were used to calculate the THCCOOH $T_{1/2}$. The three final OF samples collected between 1.9 and 3.5 h were used. The median elimination $T_{1/2}$ were respectively 0.9, 1.3, 1.0, and 1.3 h for THC, THC-A, CBN, and THCCOOH. These values were not significantly different from each other. Surprisingly, THC OF $T_{1/2}$ was not significantly different from THC blood $T_{1/2}$. On the other hand, the difference between THCCOOH $T_{1/2}$ in OF and blood was very significant ($p<0.001$). In a study lasting 4 h, Huestis and Cone [11] estimated the OF terminal half-life of THC to be 0.8 h, in line with the value obtained in our study (0.9 h). In a longer study lasting 8 h, Toennes et al. [21] found an identical $T_{1/2}$ value of 1.6 h for occasional and chronic users.

THC-A and CBN OF concentrations were correlated to THC with a significant Spearman correlation coefficient ($\rho=0.71$ and 0.88, $p<0.001$). Elimination half-lives of these two cannabinoids were not significantly different from THC $T_{1/2}$, suggesting that the elimination of these three compounds follows approximately the same course. The molar ratios of THC-A/THC were determined during the day of experiment for heavy and occasional smokers. In occasional smokers, before smoking, THC was detected in only two OF samples and THC-A in only one. Consequently, their molar ratios could not be determined. The median THC-A/THC molar ratios were 0.08, 0.07, and 0.05 at 0.35, 0.65, and 2.75 h after inhalation, respectively. For heavy smokers, these median ratios varied between 0.03 and 0.09 during the day of experiment. For each group, the ratios were not significantly different between each time points. Furthermore, we found no significant differences between occasional and heavy smokers.

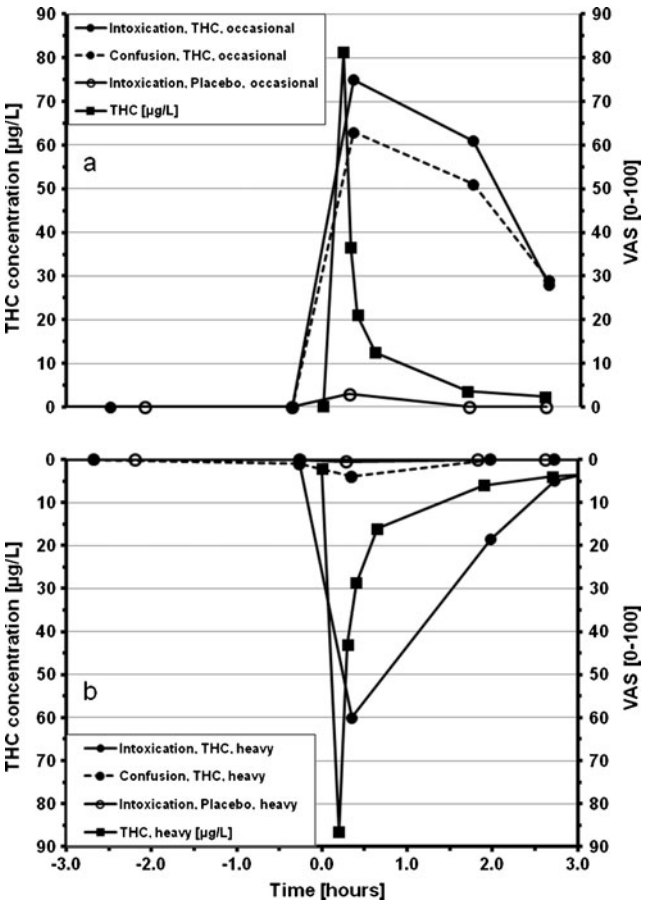

Fig. 3 VAS scores for the feeling of intoxication and confusion and THC time profile in whole blood after smoking a cannabis joint or a matching placebo. *Upper* subfigure **a** occasional smokers; *bottom* subfigure **b** heavy smokers

Assessment of subjective effects with questionnaires

The median results for feelings of intoxication and confusion are presented in Fig. 3a and b. One effect (intoxication) is expected and can be considered as desired and rewarding, while the second (confusion) is an unwanted side effect [42, 43]. The sensations felt by the occasional smokers are plotted in the upper right side subfigure (a), while those experienced by the heavy users are displayed in the lower subfigure (b). The two subfigures are mirrored with inverted *y*-axes. The placebo effects were very low and are only shown for the sensation of intoxication. The feeling of intoxication after placebo smoking was slightly more intense among the occasional than the heavy smokers. The kinetic profiles of THC are shown on the same graphs to correlate the blood concentrations with the subjective effects. It is clear that cannabis inhalation by the occasional smokers (Fig. 3a) induced a strong feeling of intoxication and confusion immediately after smoking, with a VAS score of 75 and 63, respectively. Then, these effects decreased slowly but did not completely disappear after 2.5 h (VAS score of 28 and 29). As to the heavy smokers (Fig. 3b), the feeling of confusion (discontinuous line) remained low (VAS score equal or lower than 4), whereas the feeling of intoxication (straight line) reached significant values immediately after inhalation (VAS 60). This effect decreased rapidly and disappeared almost completely after 2.5 h (VAS score of 2). These results suggest that, in comparison to occasional smokers, heavy smokers remain able to feel the intoxication effects, but with less intensity, while the negative symptoms (confusion) become strongly attenuated. These differences between occasional and heavy smokers could be explained by the specific habits of consumption (frequency and dose) of each group. Heavy smokers are more accustomed to the effects of cannabis than occasional users and could therefore better manage the way they feel intoxicated or confused. The weakened feeling of confusion among heavy smokers reflects a greater tolerance to negative symptoms. These effects as felt by occasional smokers are discussed in depth by Battistella et al. [26]. Similar effects involving a tolerance mechanism have been found by others and are presented in a recent paper of Theunissen et al. [44].

Limitations of the study

For each clinical trial, compromises and choices must be made. In this broad cannabis administration study, two parameters have greatly influenced the time-schedule of the day of experiment. First, we choose to keep the volunteers under close supervision for several hours before administration of the joint. The second parameter was the timing of the fMRI sessions. Finally, the infrastructure of the clinical research center, the ethical committee, as well as the volunteers' availability were not compatible with a multi-day experiment. The drawback was that a long investigation after inhalation was not possible. Because two different OF collecting devices were used, the comparison of the magnitude of cannabinoid concentrations was difficult. However, our opinion is that the concentration ratios and the variations in cannabinoid levels can be profitably compared.

Conclusion

Pharmacokinetic analyses revealed that initial cannabinoid concentrations in whole blood and oral fluid were different between occasional and heavy smokers, especially in the case of THCCOOH. However, occasional and heavy smokers presented a similar median THC maximal concentration in WB. In OF, THC and to a lesser extent THC-A were found in high concentrations for both groups. The molar ratios of THC-A over THC in OF remained the same during the day of experiment and did not significantly differ between both groups of consumers. The presence of THC-A in OF indicated that it is only partially decarboxylated during the smoking of the joint. Since THC-A is not known to be present in THC/CBD-based medicines, it could be used as a marker of cannabis smoking. Compared with occasional smokers, the intensity of the feeling of confusion was much lower in heavy users while the sensation of intoxication was only slightly lower.

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