Cannabinoid Concentrations in Spot Serum Samples 24–48 Hours After Discontinuation of Cannabis Smoking

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Abstract

A blood concentration of tetrahydrocannabinol (THC) in the low nanograms-per-milliliter range is often claimed to result from drug use more than 24–48 h previously. The present investigation determined concentrations of cannabinoids in blood collected at least 24 h from smoking in an in-patient setting. During sampling, distinctive effects due to drug use could not be observed. The randomly collected samples from heavy (n = 16, > 1 joint/day), moderate (n = 15, ≤ 1 joint/day), and light (n = 6, < 1 joint/week) users of cannabis were analyzed for THC, 11-hydroxy-THC (OH-THC), and free 11-nor-9-carboxy-THC (THCCOOH) by gas chromatography–mass spectrometry as well as for glucuronidated THCCOOH by liquid chromatography–tandem mass spectrometry. THC was detectable in 9, 6, and 1 samples from heavy, moderate, and light users, respectively. Although cannabinoid concentrations were overlapping between groups, there was a trend towards higher concentrations of both conjugated and free THCCOOH in regular users compared to occasional users. The present findings appear to indicate that low levels of THC, or of THC along with OH-THC, may not unequivocally prove a very recent use of cannabis.

Introduction

Toxicologists are frequently confronted with the question of how long a drug can be detected in blood. Although cannbis is the most prevalently used illicit drug throughout the world, there is limited information on the detection time of cannabinoids in humans (1,2). This also applies to blood, where a positive finding can generally be interpreted with greater confidence as being applicable to very recent drug use (1,3–5).

The major psychoactive compound in the complex mixture of cannabinoids present in hashish or marijuana is Δ^9-tetrahydrocannabinol (THC). Smoking remains the most efficient and preferred route of drug administration. Peak concentrations of THC in blood are reached within a few minutes, then quickly decrease (6). The initial decline is due to THC rapidly penetrating the tissues and, to a lesser extent, the metabolism of the drug (1,5).

The major metabolic route involves hydroxylation of the allylic methyl group followed by further oxidation to 11-nor-9-carboxy-THC (THCCOOH), which also forms an acyl glucuronide (THCCOOglu) (7). The 11-hydroxy-THC (OH-THC) which is initially formed in a low concentration relative to that of THC has a psychoactive activity similar to that of the parent drug (6).

With prolonged exposure, THC concentrates in fatty tissue, which has been identified as the major long-term storage site (8,9). Small blood levels and a long terminal half-life result from the release of THC from human fat. Estimates of the drug's half-life range from 20–29 h (10), to 56 h, and up to 4.3 d (8). However, the terminal half-life of THC in humans is still not known with certainty, which may be due to study periods that have not been long enough to establish the true elimination half-life or to assays with inadequate accuracy or sensitivity (1,3,8).

Generally, concentrations of THC dropped below 1.0 ng/mL within a few hours after exposure to cannabis (11–13). For example, Huestis et al. (12) reported detection times of THC in plasma ranging from 3 to 12 h. There is consensus from driving studies that most behavioral and physiological effects also return to baseline within this time period (14). In forensic cases, a low concentration of THC in blood is often claimed to result from drug use more than 24–48 h ago and indicate that impairment is rather unlikely.

The present investigation determined concentrations of cannabinoids in blood collected at least 24 h from smoking in an in-patient setting.

Materials and Methods

Study design

The study protocol was approved by the Ethics Committee of

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the University Heidelberg, and written informed consent was obtained from each subject (31 male and 6 female subjects ranging in age from 21 to 36 years). On admission to the closed detoxification ward, individuals were checked for drugs, and they were under constant supervision for the duration of the study. There was also a ban on visitors and letters. The body mass index (BMI) was estimated from subjects' weight and height. Participants were diagnosed with opiate abuse or dependence and reported a history of concomitant cannabis use. Information was given on the frequency and last administration of the drug, which was exclusively via inhalation. Subjects smoking more than 1 joint/day were classified as heavy, regular users (n = 16, group 1). Consumption of up to 1 joint/day or week was attributed to a moderate, regular (n = 15, group 2), or light use (n = 6, group 3), respectively. Twenty-nine blood samples were collected 24–48 h after abstaining from cannabis use; four blood samples were obtained at least 48 h apart from smoking from heavy and moderate users, respectively.

Signs indicating impairment, such as conjunctival injection and an increased pulse rate, and subjective rating of effects due to cannabis consumption were recorded at the time of blood sampling. After collection, blood was centrifuged; serum was removed and stored at –20°C until analysis. The concentrations of THC, OH-THC, and free THCCOOH were determined by a routinely applied gas chromatography–mass spectrometry (GC–MS) assay (15), and quantitative analysis of THCCOOglu was performed by liquid chromatography–tandem mass spectrometry (LC–MS–MS) as previously described (16,17).

Chemicals

Deuterated and nondeuterated THC, OH-THC, and THCCOOH were purchased from Cerilliant (Wesel, Germany), and deuterated and nondeuterated THCCOOglu was supplied by Alltech Associates (State College, PA). Acetonitrile, methanol, acetone, acetic acid, ammonium hydroxide, ammonium acetate, n-hexane, and ethyl acetate were purchased from Roth (Karlsruhe, Germany), and double-distilled water was from Braun (Melsungen, Germany). N,O-Bis-(trimethylsilyl)-trifluoroacetamide containing 1% trimethylchlorosilane was used as the reagent for silylation (Sigma-Aldrich, Steinheim, Germany).

Analysis of THC, OH-THC, and THCCOOH by GC–MS

Internal standards (10 ng THC-d_3 and OH-THC-d_3 and 25 ng THCCOOH-d_3) were added to 1 mL of each specimen, calibrator, and control sample, respectively. Each sample was treated with 1 mL 0.25M acetic acid and 1 mL of n-hexane/ethyl acetate (9:1, v/v). The organic layer was removed, taken to dryness, and derivatized with 20 µL of the reagent for silylation (30 min, 60°C). One microliter of the derivatized extract was injected into the GC–MS system (column: CP-Sil 5 CB, Chrompack, Middleburgh, The Netherlands; GC HP 6890, mass selective detector 5973, autosampler 7673, Agilent, Waldbronn, Germany). Details regarding the analysis have been published by Mauden et al. (15). The LOD was 0.3 ng/mL for THC and OH-THC and 1.0 ng/mL for THCCOOH for a 1-mL extracted specimen.

Analysis of THCCOOglu by LC–MS–MS

Fifty nanograms of THCCOOglu-d_3 and 500 µL of water were added to 500 µL of serum, calibrator, and control. Determination of THCCOOglu involved a solid-phase extraction on HF Bond Elut Certify extraction columns (300 mg, Varian, Darmstadt, Germany) that were prewashed with methanol and water. After a washing (500 µL of water) and a drying step (40 µL of acetone at reduced pressure), the extraction column was eluted with 2 × 750 µL of methanol. The residue was reconstituted in 50 µL of the mobile phase (acetonitrile/methanol/20mM ammonium acetate buffer, pH 4.0, 41:41:18, v/v), and 10 µL was injected into the LC–MS–MS system (column: Zorbax Eclipse XDB C8 column, Agilent, Waldbronn, Germany; HPLC pump equipped with an autosampler, series 200, Perkin Elmer, Überlingen, Germany; API 365 MS, Applied Biosystems, Toronto, ON, Canada). Details of the assay have previously been published (16). The LOD was 1.1 ng THCCOOglu/mL for a 0.5-mL extracted specimen.

Results

Impairment could not be assessed by clinical signs or subjective rating in any subject at the time of blood sampling. The analytical results are summarized in Tables I–III according to the classification as heavy, moderate, and light users.

Eight specimens of group 1 (n = 16, Table I) were tested positive for THC (range: 1.2–6.4 ng THC/mL serum) 24–48 h after cessation of drug use, including 5 OH-THC positive samples. A single specimen (subject 16, BMI 30.7, Table I) contained THC and OH-THC even 120 h apart from smoking. THCCOOH was detected in all samples with one exception. This specimen, which was collected 35 h after smoking, contained only THCCOOglu (subject 5, BMI 17.9, Table I). During the 24–48 h period, samples had THCCOOglu concentrations in the range of 9–1048 ng/mL.

With concentrations ranging from 1.0 to 2.6 ng/mL serum, THC was detectable in six samples of group 2 (n = 15, Table II). Both THC and OH-THC could be determined from two samples obtained 24–48 h apart, but in none of the specimens collected > 48 h after smoking. Levels of THCCOOH and THCCOOglu ranged from 7 to 101 ng/mL and 6 to 368 ng/mL, respectively. Cannabinoids could not be detected in sample 30.

All samples (n = 6) in group 3, with one exception (sample 36, BMI 27.1, Table III), were tested negative for THC and OH-THC. Specimens had THCCOOH and THCCOOglu concentrations in the range of > 1.0–17 ng/mL and 10–204 ng/mL, respectively.

Discussion

There are far more data on the detection time of cannabinoids in urine than in blood (1). The few data available on the disposition of cannabinoids in blood were collected during pharmacokinetic studies mostly involving administration of a single

dose to subjects which had been carefully selected. Further, few studies have included OH-THC or THCCOOglu measurements. The present empirical approach could be considered typical of what daily casework might reflect. It may appear as an alternative when approval of a controlled study cannot be granted (11). At least, such an approach may complement data obtained from controlled, therapeutic regimes.

There may be problems regarding the accuracy of self-report measures (19). In this study, participation was voluntary, and there were no consequences.

The detection time of illicit drugs is generally dependent on many variables (e.g., the dosage, the frequency or route of administration, concomitant drug use, sex, body mass, health, and genetic factors as well as on the history of the specimen and the sensitivity of the analytical assay). In the present study, consumption was exclusively by inhalation. Blood samples were stored immediately at 4°C; serum was removed within 1–3 h after collection and stored frozen until analysis, thus ensuring stability of the analyte pattern (17). It should be considered that the present data were derived from serum and not from whole blood. A blood-to-plasma ratio of 0.55 has been reported for THC (18). Essentially identical ratios of 0.65 ± 0.05 were determined for THCCOOH and its glucuronide, which did not differ at levels of 100 ng/mL and 500 ng/mL, respectively (17). The applied analytical methods have been proven with particular emphasis on a low limit of detection, an excellent precision for all analytes, and a reliable extraction of the labile glucuronide (15,16).

To the authors' knowledge, a single case study investigating elimination of THCCOOH from patients in a detoxification ward has recently been published (11). Although samples were collected on each of the first seven days after admission, THC was not present in a single serum specimen. In the present investigation, THC along with OH-THC could be determined in some heavy users more than 24 h after abstaining from cannabis smoking. The positive THC findings in regular

Table I. Serum Analysis for THC, OH-THC, Free, and Glucuronidated THCCOOH (ng/mL) in Heavy, Regular Users of Cannabis (group 1) 24–48 h and More Than 48 h After Discontinuation of Drug Use*

Subject	Sex	BMI	THC (ng/mL)	OH-THC (ng/mL)	THCCOOH (ng/mL)	THCCOOglu (ng/mL)
24–48 h after last drug use						
1	m	26.3	2.2	nd	25	141
2	f	19.2	1.3	positive	52	160
3	m	28.9	nd	nd	14	163
4	m	21.8	1.2	nd	42	153
5	f	17.9	nd	nd	nd	9
6	m	24.7	2.3	1.1	43	211
7	m	23.7	nd	nd	6	45
8	m	26.6	nd	nd	11	118
9	m	21.4	1.9	positive	281	539
10	m	17.9	4.6	1.4	297	1048
11	m	28.7	6.4	2.4	92	356
12	m	22.1	1.0	nd	6	128
> 48 h after last drug use						
13	m	21.6	nd	nd	50	150
14	m	21.5	nd	nd	17	40
15	m	21.2	nd	nd	nd	7
16	m	30.7	2.0	positive	63	268

* BMI: body mass index; nd: not detectable (LOD: 0.3 ng THC or OH-THC/mL serum, 1.0 ng THCCOOH/mL serum); positive: finding above 0.3 ng THC or OH-THC/mL serum, and below the LOQ (1.0 ng THC or OH-THC/mL serum).

Table II. Serum Analysis for THC, OH-THC, Free, and Glucuronidated THCCOOH (ng/mL) in Moderate, Regular Users of Cannabis (group 2) 24–48 h and More Than 48 h After Discontinuation of Drug Use*

Subject	Sex	BMI	THC (ng/mL)	OH-THC (ng/mL)	THCCOOH (ng/mL)	THCCOOglu (ng/mL)
24–48 h after last drug use						
17	m	31.2	nd	nd	31	35
18	m	22.7	positive	positive	8	70
19	m	20.7	1.0	nd	24	39
20	f	23.1	nd	nd	7	66
21	m	23.0	nd	15	87	
22	f	21.7	1.8	1.2	30	116
23	m	28.7	0.7	nd	42	111
24	m	28.3	positive	nd	13	71
25	m	26.5	2.6	nd	101	368
26	f	37.6	nd	nd	10	70
27	m	21.8	nd	nd	18	53
> 48 h after last drug use						
28	m	26.6	nd	nd	nd	6
29	m	22.0	nd	nd	nd	positive
30	m	20.5	nd	nd	nd	nd
31	f	20.6	nd	nd	26	36

* BMI: body mass index; nd: not detectable (LOD: 0.3 ng THC or OH-THC/mL serum, 1.0 ng THCCOOH/mL serum, 1.1 ng THCCOOglu/mL serum); positive: finding above 0.3 ng THC or OH-THC/mL serum, 1.0 ng THCCOOH/mL serum or 1.1 ng THCCOOglu/mL serum and below the corresponding LOQ (1.0 ng THC or OH-THC/mL serum, 2.4 ng THCCOOH/mL serum, 4.5 ng THCCOOglu/mL serum).

Table III. Serum Analysis for THC, OH-THC, Free, and Glucuronidated THCCOOH (ng/mL) in Light Users of Cannabis (group 3) 24–48 h and More Than 48 h After Discontinuation of Drug Use*

Subject	Sex	BMI	THC (ng/mL)	OH-THC (ng/mL)	THCCOOH (ng/mL)	THCCOOglu (ng/mL)
24–48 h after last drug use						
32	m	29.4	nd	nd	positive	34
33	m	20.6	nd	nd	positive	20
34	m	23.2	nd	nd	5	10
35	m	22.3	nd	nd	positive	43
36	m	27.1	1.4	nd	17	204
37	m	31.9	nd	nd	5	57

* BMI: body mass index; nd: not detectable (LOD: 0.3 ng THC or OH-THC/mL serum, 1.0 ng THCCOOH/mL serum, 1.1 ng THCCOOglu/mL serum); positive: finding above 0.3 ng THC or OH-THC/mL serum, 1.0 ng THCCOOH/mL serum or 1.1 ng THCCOOglu/mL serum and below the corresponding LOQ (1.0 ng THC or OH-THC/mL serum, 2.4 ng THCCOOH/mL serum, 4.5 ng THCCOOglu/mL serum).

users may result from release of the drug from fatty tissue into blood. OH-THC is formed from THC in low quantities. After inhalation, the maximal OH-THC concentration was reported to represent only 5–10% of the THC peak concentration (1). Accordingly, OH-THC may either have been formed from THC or may have directly been released from fatty tissue. The latter seems a likely supposition in that Leighty et al. (9) identified fatty acid conjugates of hydroxylated cannabinoids as long retained metabolites.

THC along with OH-THC was not detectable in any specimen collected in group 3. A positive finding of THC and OH-THC in occasional users may therefore result from recent drug administration rather than from cannabis use 1 or 2 days prior to blood sampling. Vice versa, prolonged detection times of THC and OH-THC can be observed in regular users (20).

Nevertheless, differentiation of groups 1–3 based on individual results does not seem possible. Levels of 50–150 ng THCCOOH/mL serum have been discussed as a help in reaching a decision to differentiate between occasional and regular users (21,22). The observed levels of THCCOOH and THCCOOglu are not likely to support a "cut-off" value, for a wide variation was noticed within and between groups. Interestingly, smaller amounts of THCCOOH were present in those samples where OH-THC or THC was no longer detectable. This is in line with previously published results on the urinary disposition of THCCOOH which had been exclusively administered to individuals to avoid any influence arising from a body burden of THC (23).

In any case, the glucuronide's concentration was higher than that of the corresponding acid. There was a trend towards a higher level of both free and glucuronidated THCCOOH in regular users compared to occasional users. Figures also point to the time elapsed since last drug use as the major determinant influencing the pattern of cannabinoids in blood. Huestis et al. (24) previously published models for estimating time of last cannabis use. Testing the present data in those models revealed that all values, with one exception (subject 9, 95% CIs: 4.4–31.6 h, model II), predicted that last administration occurred less than 24 h previously.

Conclusions

Although the authors are aware of the small study number, the present findings appear to indicate that low levels of THC along with OH-THC may not unequivocally prove a recent use of hashish or marijuana. Depending on the frequency and intensity of drug use, detection of psychoactive cannabinoids seems possible over a time period of more than 24–48 h after abstaining from cannabis smoking.

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