HEMP TEA VERSUS HEMP MILK: BEHAVIORAL, PHYSIOLOGICAL EFFECTS, BLOOD, URINE, SALIVA AND SWEAT CANNABINOIDS LEVELS FOLLOWING INGESTION BY TWO GROUPS OF SIX HEALTHY VOLUNTEERS

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ABSTRACT: Two clinical studies were carried out: a smoking study and a controlled oral administration study. Hemp was ingested either as a water or a milk decoction. Because of its lipophylic character, much higher levels of THC were extracted into milk compared to water during the tea-making process. The highest mean concentrations of THC, 11-OH-THC and THC-COOH were 4.0, 3.4 and 24.5 ng/ml whole blood after drinking the milk decoction. Trace amounts of THCCOOH only could be measured in the blood after ingestion of the water tea and this without any perceptible effects typical of cannabis use. Unlike the water tea, ingestion of 23.2 mg of THC with the milk decoction resulted in significant psychoactive and clinical effects. The time since exposure was estimated with the mathematical models I and II set up by Huestis et al. and the results compared to the actual time of use. In our set of experimental conditions, model I yielded much better predictions of time of cannabis exposure than model II. Insofar as an oral intake is suspected, e.g. when the THC/11-OH-THC ratio is close to one, it is recommended to use model I exclusively. Alternatively, model II modified to take account of our own set of data could be also used to achieve an acceptable time estimate.

KEY WORDS: Cannabis; THC; Hemp.

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INTRODUCTION

Forensic toxicologists are often requested to interpret cannabinoids levels in whole blood specimens. The two mathematical models developed by Huestis et al. [2, 5] are of valuable help to predict the time of cannabis usage: model I is based on THC plasma concentrations while model II relies on THC ratios. Because these models are based on the plasma cannabinoids levels, the plasma to whole blood distribution ratios of the

major cannabinoids should be determined before using them to evaluate the time of exposure from the whole blood levels.

Two clinical studies were performed with the agreement of the local ethical committee, after receiving the informed consent of all volunteers: a smoking study and a controlled oral administration study. The main goal of the smoking study was the determination of plasma to whole blood concentrations distribution ratios. A second goal was to compare the time of exposure calculated with the Huestis models to the times claimed by the cannabis smokers.

During the oral study, biological samples were taken at regular intervals and the cannabinoids levels determined by GC/MS. Subjective and clinical effects were also measured and their kinetics compared to the cannabinoids concentrations time-curves in whole blood. The elapsed times since hemp decoction ingestion were calculated through models I and II and the results compared to the actual time values.

MATERIALS AND METHODS

Smoking study

Seven blood samples were collected during routine medical examination at the drug addiction unit of Lausanne on 7 volunteers who smoke cannabis on a regular basis. The volunteers were also asked about their drug intake habits and time of last use. The blood samples were split into 2 parts, one was centrifuged in order to get plasma of it while the other one was used to assess cannabinoids levels in whole blood.

Oral administration study scheme

In order to get rid of the likely unreliable time data given by the drug addicts, a controlled study was performed with 6 volunteers. Because only scarce data are available following cannabis ingestion [1, 3, 4], cannabinoids were administered orally as a hemp decoction.

During the first part of the study, 5 grams of cannabis containing 35 mg total THC was administered to half of the volunteers as a filtered water decoction and to the other half as a filtered milk decoction. Two weeks later, the study was repeated with the difference that the groups who were administered the milk or water hemp infusions were permuted.

Because of its lipophilic character, THC was better extracted into milk than into water. About 1.6 mg free THC only and a mean value of 23.2 mg free THC were recovered in total from the 2 dl hemp water and milk decoctions, respectively.

During the study, whole blood, urine, sweat and saliva specimens were collected at regular intervals. The rating degree of “high” and the vigilance level were assessed with a Visual Analog Scale. Pulse rate and conjunctival injection were measured as well.

Only mean urine and whole blood cannabinoids kinetics will be presented here and their results compared to the subjective and objective effects time-curves.

Cannabinoids levels were determined by GC/MS following solid-phase extraction on SPEC C18 columns and methylation with iodomethane. Deuterated internal sandards were used for accurate determinations.

RESULTS AND DISCUSSION

Cannabinoids plasma/whole blood distribution ratios

Cannabinoids concentrations were measured by GC/MS both in whole blood and in plasma, results are shown in table I. The plasma to whole blood distribution ratio was determined and found to be about 1.6 for all 3 cannabinoids. For THC, values range between 1.4 and 1.9, for 11-OH-THC, values extend from 1.5 to 1.8. A weak dispersion of the plasma to whole blood ratios around 1.6 was also observed with THCCOOH. Assuming that the mean hematocrit value is about 0.45, these results indicate that almost 90% of the cannabinoids can be found in the plasma fraction while about 10% are bound to the red blood cells.

The *in vivo* results are in agreement with previous *in vitro* studies of cannabinoids distribution in whole blood which have shown that THC and 11-OH-THC are associated 80–90% with plasma proteins [6]. These results were obtained through equilibrium dialysis and centrifugation. (^{3}H)-radiolabelled cannabinoids were used for this purpose. No data were available for THCCOOH plasma/whole blood distribution.

In order to assess the usefulness of the models under our set of experimental conditions, the predicted times and their respective confidence intervals were calculated through Huestis models I and II. The results were then compared to the actual time of cannabis smoking indicated by the drug addicts (results not shown).

For model I, one time-value only, corresponding to V11, was within the estimated time interval. Model II yielded better results with 3 time-values, V1, V3 and V11, included in their respective confidence intervals.

TABLE I. PLASMA/WHOLE BLOOD CANNABINOIDS CONCENTRATIONS RATIOS

[ng/ml]	THC		THCCOOH		11-OH-THC		Plasma/whole blood distribution ratios		
	Blood	plasma	blood	plasma	blood	plasma	THC	THCCOOH	11-OH-THC
v1	4.8	7.0	118.5	195.0	1.8	3.2	1.5	1.6	1.8
v2	6.1	11.4	43.7	63.4	2.9	3.7	1.9	1.5	1.5
v3	5.3	7.9	75.2	114.1	2.3	2.6	1.5	1.5	1.5
v5	0.7	0.7	8.1	11.7	0.2	0.4	nv	1.4	nv
v8	1.7	2.4	36.7	57.3	0.8	1.0	1.4	1.6	1.6
v9	3.3	4.8	18.7	32.2	0.7	1.1	1.5	1.7	1.7

v11	0.8	1.3	35.8	66.1	0.4	0.7	1.6	1.8	1.8
Mean							1.6	1.6	1.6

"v" – volunteer, "nv" – not valid (cannabinoids levels too low to be used for the calculation of the plasma to whole blood ratio).

The comparison of the actual and predicted time-values indicated that time error seems to increase with time with model I and II tending to underestimate the time of exposure. Model II seems to give better predictions than model I.

Here it is to point out that some of the time of smoking indicated by the drug addicts could be wrong yielding erroneous predictions.

THCCOOH kinetics in urine

A maximum average THCCOOH concentration of about 30 ng/ml urine was measured about 4 hours after ingestion of the water decoction. A similar time-curve was obtained when results were related to the creatinine levels instead of the urine volume (results not shown).

Maximum mean concentration in urine following administration of the milk decoction was about 25 times higher than that measured after drinking the water decoction. After ingestion of the milk decoction, THCCOOH levels remained higher than the NIDA cut-off value of 15 ng/ml urine during the whole study, i.e. about 100 hours and only for a few hours following drinking the water decoction.

Cannabinoids kinetics in whole blood

When hemp was infused with water, only low amounts of THC were ingested yielding trace amounts of THC and 11-OH-THC in whole blood. Only THCCOOH reached significant concentrations with an average maximum level of about 3 ng/ml whole blood. THCCOOH remained detectable for about 10 hours (Figure 1).

A very different pattern of excretion was observed following ingestion of the milk decoction. Both mean maximum concentrations of THC and 11-OH-THC were in the range of 4 ng/ml whole blood while THCCOOH average highest concentration value was in the range of 25 ng/ml. The peak level was observed about 1–3 hours following intake (Figure 2).

Subjective effects

Significant subjective and clinical effects were measured after drinking the milk decoction only. Indeed, the total amount of THC ingested with the water decoction was very likely below the minimum level required to induce noticeable psychoactive effects.

The kinetic of the mean level of high reported by the 6 volunteers paralleled the time-curves of both THC and 11-OH-THC concentrations. The rating degree of high was delayed in onset and its maximum level occurred somewhat later than the THC and

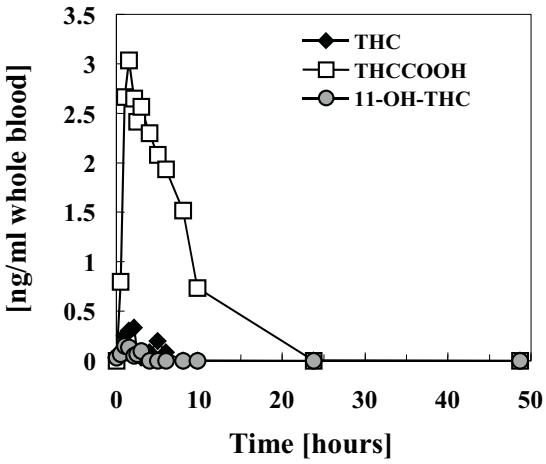

Fig. 1. Average blood cannabinoids levels kinetics following drinking 2 dl of a water hemp decoction containing a mean amount of 1.7 mg THC.

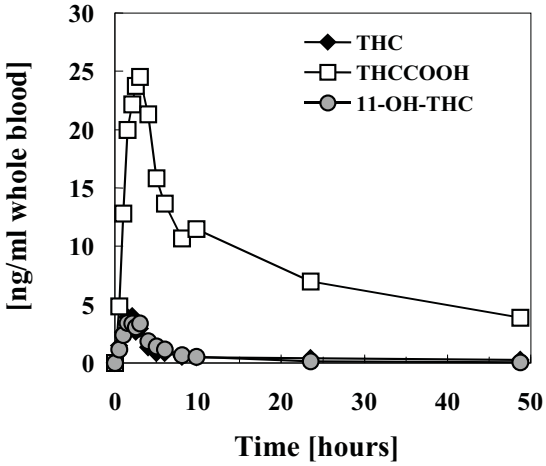

Fig. 2. Average blood cannabinoids levels kinetics following drinking 2 dl of a milk hemp decoction containing a mean amount of 23.1 mg THC.

11-OH-THC peak concentrations (Figure 3). A counterclockwise hysteresis was determined when drug effects were plotted against THC concentrations.

A mirror effect was observed when the time-curves of THC and 11-OH-THC were compared to that of the vigilance degree reported by the volunteers on a VAS scale scored from 0 to 10. The vigilance level reached its minimum value when the THC and 11-OH-THC concentrations were at their top level (results not shown).

Clinical effects

A weak increase in pulse rate was detected following ingestion of the hemp milk decoction. The average peak effect and the mean maximum THC and 11-OH-THC levels occured almost simultaneously (results not shown). A similar picture was

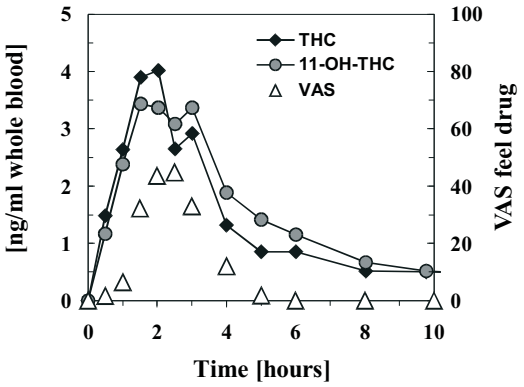

Fig. 3. Kinetics of THC and 11-OH-THC versus "rating level of high" reported by the 6 volunteers on a Visual Analog Scale (VAS) VAS scored from 0 to 100.

obtained when the intensity of eye reddening was compared to the THC and 11-OH-THC levels. Average conjunctival injection topped with a very little delay (results not shown).

The cannabis intoxication factor was also determined and found to reach its average maximum level during the absorption phase. In contrast to the mean VAS "feel drug" which displays a bell-shaped curve, the time-curve of the CIF value showed a continuous decrease during the first 4 hours. The CIF mean value remained above the cutoff of 10 reported by Daldrup to be related to a significant decrease in car driving capacity for about 10 hours (results not shown).

Mathematical models I and II aimed at the prediction of cannabis usage

The plasma to whole blood distribution ratios of THC was used to calculate the plasma concentrations from the whole blood values. The mathematical models of Huestis et al. were then used to predict the time of ingestion and compared to the actual time of use. Thereafter, they were used to determine the mean absolute time error by subtracting the actual elapsed time from the calculated predicted time. Model I yielded better predictions with less time errors than Model II. The mean absolute time error was found to increase with time whatever the model used. Alternatively, the level of accuracy of both models was assessed by comparing the actual time of exposure to the 95% confidence interval of the predicted time of cannabis ingestion. Figure 4 illustrates how the data fit in Huestis model I. The predicted time tended to be underestimated when compared to the actual times of drug exposure.

Model II yielded less accurate predictions with several values being scattered far away of the 95% confidence interval (Figure 5).

The equations of models I and II reported by Huestis [2, 6] and those calculated with the values obtained during the controlled oral administration study are shown in Table II. The author's model and the Huestis model I equations are very similar with a little

difference in the Y intercept value while relatively large differences can be noticed in both the slope and the Y intercept between Huestis model II and our own model.

TABLE II. MATHEMATICAL MODELS I AND II.

Authors	Model I	Model II
Huestis et al. Smoking study	Log T = – 0.698 x log THC + 0.687	Log T = 0.576 x log THCCOOH/THC – 0.176

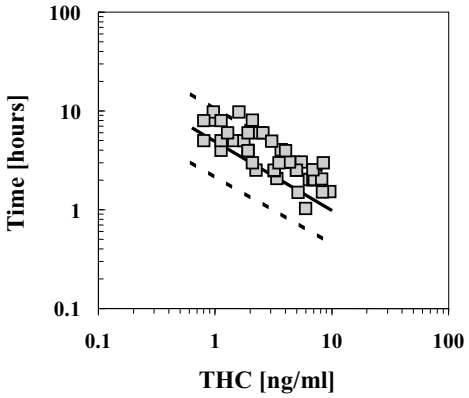

Fig. 4. Accuracy evaluation of model I. Actual times of hemp milk decoction ingestion were compared to the 95% confidence interval of the predicted time of cannabis intake for plasma THC levels (blood levels x 1.6) obtained from one controlled oral ingestion study carried out with 6 volunteers.

This work Oral intake study	Log T = – 0.695 x log THC + 0.841	Log T = 0.973 x log THCCOOH/THC – 0.425

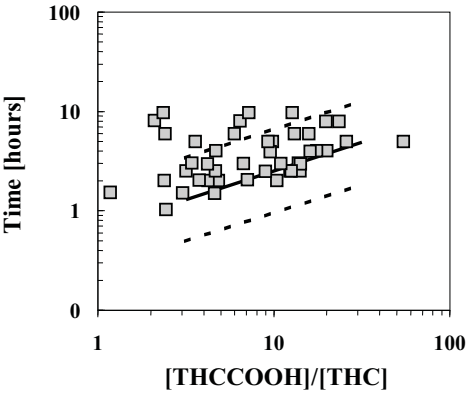

Fig. 5. Accuracy evaluation of model II. Actual times of hemp milk decoction ingestion were compared to the 95% confidence interval of the predicted time of cannabis intake for plasma THC levels (blood levels x 1.6) obtained from one controlled oral ingestion study carried out with 6 volunteers.

Model I is derived from linear regression analysis of plasma THC concentrations and elapsed time after marijuana smoking, model II from THCCOOH/THC ratios versus elapsed time after marijuana smoking. For the oral study, equations were obtained on the same way. Blood levels were multiplied by 1.6 in order to calculate plasma concentrations.

CONCLUSIONS

When cannabinoids levels are determined in whole blood, a plasma to blood concentrations distribution ratio of 1.6 can be used to calculate the plasma concentrations from whole blood values. Following oral intake, THC and 11-OH-THC levels remain close to each other with a THC to 11-OH-THC ratio of about one. Unlike oral administration, smoking cannabis results in much higher levels. However, during the late excretion phase, the ratio lowers to values close to those determined for the oral administration study.

Cannabinoids are better extracted into a lipophilic matrix, i.e. milk than into water explaining why no psychoactive effect could be detected after drinking the water decoction. Conversely, a counterclockwise hysteresis was noticed when cannabinoids effects were reported to their blood levels following ingestion of the milk decoction. A total amount of 1.6 mg THC when taken orally was therefore insufficient to induce psychoactive effects typical of cannabis. Unlike the water tea, ingestion of 23.2 mg of THC in milk resulted in significant psychoactive and clinical effects.

In our set of experimental conditions, that is oral administration, Huestis model I yielded better predictions of time of cannabis exposure than model II.

When oral intake is suspected, that is THC/11-OH-THC ratio is close to one and at least less than 3, it is therefore recommended to use either Huestis model I or our own model II to achieve a good time estimate.

Finally, we would like to stress the point that more controlled studies must be carried out under various circumstances of cannabis usage to assess the accuracy of the mathematical models and if needed, set up new and more accurate models.

Acknowledgments:
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