Assessment of Driving Capability Through the Use of Clinical and Psychomotor Tests in Relation to Blood Cannabinoids Levels Following Oral Administration of 20 mg Dronabinol or of a Cannabis Decoction Made with 20 or 60 mg Δ^9-THC*

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Abstract

Δ^9-Tetrahydrocannabinol (THC) is frequently found in the blood of drivers suspected of driving under the influence of cannabis or involved in traffic crashes. The present study used a double-blind crossover design to compare the effects of medium (16.5 mg THC) and high doses (45.7 mg THC) of hemp milk decoctions or of a medium dose of dronabinol (20 mg synthetic THC, Marinol®) on several skills required for safe driving. Forensic interpretation of cannabinoids blood concentrations were attempted using the models proposed by Daldrup (cannabis influencing factor or CIF) and Huestis and coworkers. First, the time concentration-profiles of THC, 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC) (active metabolite of THC), and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THCCOOH) in whole blood were determined by gas chromatography–mass spectrometry-negative ion chemical ionization. Compared to smoking studies, relatively low concentrations were measured in blood. The highest mean THC concentration (8.4 ng/mL) was achieved 1 h after ingestion of the strongest decoction. Mean maximum 11-OH-THC level (12.3 ng/mL) slightly exceeded that of THC. THCCOOH reached its highest mean concentration (66.2 ng/mL) 2.5–5.5 h after intake. Individual blood levels showed considerable intersubject variability. The willingness to drive was influenced by the importance of the requested task. Under significant cannabinoids influence, the participants refused to drive when they were asked whether they would agree to accomplish several unimportant tasks, (e.g., driving a friend to a party). Most of the participants reported a significant feeling of intoxication and did not appreciate the effects, notably those felt after drinking the strongest decoction. Road sign and tracking testing revealed obvious and statistically significant differences between placebo and treatments. A marked impairment was detected after ingestion of the strongest decoction. A CIF value, which relies on the molar ratio of main active to inactive cannabinoids, greater than 10 was found to correlate with a strong feeling of intoxication. It also matched with a significant decrease in the willingness to drive, and it matched also with a significant impairment in tracking performances. The mathematic model II proposed by Huestis et al. (1992) provided at best a rough estimate of the time of oral administration with 27% of actual values being out of range of the 95% confidence interval. The sum of THC and 11-OH-THC blood concentrations provided a better estimate of impairment than THC alone. This controlled clinical study points out the negative influence on fitness to drive after medium or high dose oral THC or dronabinol.

Introduction

Cannabis is the most frequently used illicit drug in the Western world. The recreational use and abuse of cannabis have increased considerably during the past few years in Switzerland (1) as well as in other European nations (2). Furthermore, cannabis extracts and marijuana may soon be introduced in the Swiss Pharmacopeia. The therapeutic potential of cannabis is also under investigation in many Western countries (3–5). Synthetic Δ^9-tetrahydrocannabinol (THC) is available on prescription in the U.S., Canada, and several other countries as Marinol®. Moreover, hemp is an ingredient of many alternative foods and beverages (6–9). Because of its high prevalence, cannabinoids are the most frequently detected drugs in blood specimen taken from people suspected of driving under the influence of drugs or involved

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in road accidents (10–12). In experimental studies using driving simulators and on-the-road driving tests, cannabis impairs cognition, psychomotor function, and actual driving performances (11,13,14). However, the simultaneous measurement of blood cannabinoids concentrations, of psychomotor performances, and of driving capability, especially after oral ingestion, has rarely been determined. Furthermore, most of these studies have been performed with low to medium doses of THC. Drummer et al. (15) reported that increment in crash responsibility rates were most prominent at high concentrations of THC (> 5 ng/mL blood), suggesting that drivers are more at risk of being involved in car accidents after exposure to high doses of THC.

The availability of hemp food products, increase in cannabis-based therapeutics, unabated recreational use of oral cannabis, and high reported prevalence of drivers under the influence of cannabis have prompted the need to carry out controlled clinical investigations on oral cannabis to assess its effects on driving performances. Our objective was to evaluate the effects of an acute oral administration of medium and high doses of cannabis extract or of a medium dose of dronabinol (Marinol) on the fitness to drive. Before oral administration, Δ^9-tetrahydrocannabinolic acid A (THC-A), the main cannabinoid of hemp, was decarboxylated by heating to yield active THC. Then, the kinetic profiles of the major cannabinoids [THC, 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC), and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THCCOOH)] in blood were determined and compared to the drug effects. First, THC is metabolized into active 11-OH-THC which undergoes further oxidation to inactive THCCOOH. The oral route was selected because absorption is slow, resulting in delayed maximal plasma concentrations (usually 60–120 min after ingestion) (16–18). Effects also occur at later times and last longer than those observed after smoking. This slow and long-lasting process presented more favorable conditions for investigation of drug effects through the use of a battery of psychomotor tests. It also made the search of a possible correlation between drug effects and cannabinoids levels easier. Finally, we used data obtained from this study to evaluate two models aimed at evaluation of the time since cannabis exposure from cannabinoids blood levels (19,20). The fitness to drive was also assessed through different approaches based on the direct interpretation of cannabinoids blood concentrations (15,21). These models constitute the main strategies for the forensic evaluation of the effects of cannabis on driving capability (22).

Materials and Methods

Preparation of milk hemp decoctions

The cannabis tea used as placebo was commercially available and was found to contain 0.1% THC and 0.4% cannabidiol. Hemp plant fragments containing 1.5% THC and 4.4% THC-A were provided by Hiscia institute in Arlesheim, Switzerland. The hemp fragments were heated under argon for half an hour in an oil bath at 140°C in order to decarboxylate THC-A into THC. The resulting THC concentration was 4.9%. Then THC from 0.41- or 1.22-g hemp fragments containing a total amount of 20 or 60 mg THC, respectively, was extracted in 200 mL hot whole milk for 20 min. The placebo decoction was prepared with 0.8-g hemp fragments containing a total amount of 0.8 mg THC. After filtration, the milk was poured into a thermos flask, and the THC content was determined by high-performance liquid chromatography (HPLC) with diode-array (DAD) and fluorescence detection. The average recovery of the decoction-making process was about 80%, yielding hemp milk decoctions containing 16.5 ± 0.9 and 45.7 ± 0.7 mg THC in 200 mL of whole milk. Marinol, soft gelatin capsules containing 5 mg dronabinol in sesame oil, was provided by Mathias Markert (Thun, Switzerland). Cannabinoid standards were purchased from the Swiss Federal Office of Public Health (THC-A), Lipomed (Arlesheim, Switzerland), Cambridge Isotope Laboratories (Innerberg, Switzerland), or ElSohly Laboratories (Oxford, MS).

Design and participants

The ethics committee of the Department of Internal Medicine of the University of Lausanne approved this double-blind crossover study that was carried out to compare the effects of 20 mg dronabinol and of 2 hemp milk decoctions containing either a medium or a high dose of THC with matched placebos. Eight male subjects aged 22 to 30 years, all occasional cannabis smokers, were enrolled. Volunteers who used any other psychotropic drug or had any psychiatric history were excluded from the study. Their mean body weight was 72.8 ± 5.2 kg. They were required to abstain from any drug or alcohol consumption for one week preceding and during the study. Prior to study participation, volunteers provided detailed medical history, had a medical examination, and gave written informed consent. Cannabis and placebo were identical in appearance and taste for all treatments. Subjects, caregivers, and investigators were blinded to treatment assignment until the end of the trial.

Four gelatin capsules, each containing 5 mg dronabinol in sesame oil (total = 20 mg dronabinol), or a matched placebo were given to each volunteer. In the same session, the volunteers received 200 mL of a milk decoction containing a trace amount of cannabinoids (placebo decoction) or a medium or high dose of THC. The total amount of cannabinoids received by the volunteers was therefore (4 possible treatments): traces (placebo), 16.5 or 45.7 mg THC, or 20 mg dronabinol.

The subjects were tested on 4 different occasions and had a 2-week washout period between treatments. The order of administration was balanced (Latin square) and participants were randomly allocated to treatment order. About 1 h before administration, the subjects were tested for the presence of major psychoactive drugs (amphetamines, opiates, cocaine, cannabis, and benzodiazepines) in urine and for alcohol consumption using a breathalyzer. Before and after treatment, blood was taken at regular intervals (0.0, 1.0, 2.5, 4.0, 5.5, 7.0, 10.0, and 24 h after intake), rapidly frozen, and stored in S-Monovette® tubes at –20°C. Gas chromatography–mass spectrometry-negative ion chemical ionization (GC–MS-NCI) was used for cannabinoids determination. Clinical observations and two psychomotor tests (roadsign and tracking testings) were also

carried out. Furthermore, the subjects were asked to report their willingness to drive under various circumstances and the subjective effects were measured on a visual analog scale (VAS) on a continuous scale from 0 to 10 cm. Overall, 240 observations with blood samplings were undertaken.

Extraction and determination of cannabinoids by HPLC–DAD-fluorescence in hemp powder and hemp milk decoction

Hemp powder. Ten milliliters of dichloromethane/methanol (1:9 v/v) was added to 200 mg hemp powder. After 15 min in an ultrasonic bath, the extract was centrifuged for 10 min at 2500 rpm. Twenty microliters was collected and diluted with 980 µL methanol. After vortex mixing, 10 µL was analyzed by HPLC–DAD-fluorescence detection.

Milk extraction. From 1 mL of filtrated milk, 100 µL was taken and added to 900 µL methanol in a 1.5-mL Eppendorf tube. After vortex mixing and 10 min centrifuging in a Eppendorf 5417R microcentrifuge at maximum speed (14,000 rpm), the supernatant was transferred to another Eppendorf tube and 10 µL were injected into the HPLC.

HPLC–DAD-fluorescence analysis of cannabinoids. An Agilent 1100 HPLC was used for the quantification of cannabinoids in plant and milk extracts. The liquid chromatography system consisted of a vacuum degasser, quaternary pump, autosampler, and thermostatted column compartment. Ten-microliter injections were made onto an XTerra MS C18 column (Waters, 150- × 2.1-mm i.d., 3.5 µm) that was held at 25°C. The mobile phase A consisted of 95:5 (v/v) of 10mM KH_2PO_4 adjusted to pH 7.5 with KOH and acetonitrile. Mobile phase B was 100% acetonitrile. The cannabinoids were separated at a flow-rate of 250 µL/min using a mobile phase gradient. After a 1-min hold, the B percentage was increased from 40% to 80% by 19 min. The final composition was held for another 7 min and then returned to starting composition in 1 min. An Agilent 1100 (DAD) and an Agilent fluorescence detector were used for tentative identification and quantification. Monitoring with DAD was performed at 210 nm (5 nm bandwidth). The excitation wavelength for both THC and THC-A was set at 222 nm. THC-A and THC fluorescence were monitored at 403 and 313 nm, respectively. When monitored at 210 nm, the retention times for Δ^9-THC-A was 11.97 min and those for Δ^9-THC and Δ^8-THC of 24.74 and 24.96 min, respectively. Quantitation was performed by the external standard method, measuring the peak areas.

Quantification of THC, 11-OH-THC, and THCCOOH in whole blood by GC–MS operating in the NCI mode

The method used for the determination of cannabinoids in whole blood was adapted from Giroud et al. (23) for the extraction part and from Huang and co-workers (24) for the GC–MS-NCI part. To 1 mL of whole blood, THC-d_3, 11-OH-THC-d_3, and THCCOOH-d_9 (Lipomed, CIL) at a concentration of 20 ng/mL were added. After protein precipitation with acetonitrile, ultrasonic treatment, and centrifugation, the cannabinoids were extracted through C18AR SPEC™ (Varian) 30-mg extraction columns. After elution from the SPEC column and evaporation under a stream of nitrogen, the dried residue was derivatized for 10 min at 70°C in the presence of 150 µL of chloroform, 150 µL trifluoroacetic anhydride, and 50 µL hexafluoroisopropanol. After evaporation, the derivatized residue was reconstituted with 50 µL of heptane, and 2 µL was splitless injected into the GC. Analyses were performed on an Agilent 6890N GC interfaced with an Agilent 7683 autosampler and an Agilent 5973N MS. The GC column was an HP 1 MS column (12 m × 0.2 mm × 0.33 µm). Helium at a rate of 1.2 mL/min was used as carrier gas. The initial oven temperature was set at 150°C for 1 min, and then increased to 232°C at a rate of 25°C/min, to 240°C at a rate of 4°C/min and to 300°C at a rate of 25°C/min. This final temperature was maintained for 1 min. Temperatures of the injector port, interface, and source were 260, 280, and 150°C, respectively. Methane at a flow of 40% of total flow (5 mL/min) was used as reagent gas. The MS was operated in the SIM mode, the following ions were monitored: *m/z* 410 (THC), *m/z* 413 (THC-d_3), *m/z* 408 (11-OH-THC), *m/z* 411 (11-OH-THC-d_3), *m/z* 590 (THCCOOH), and *m/z* 599 (THCCOOH-d_9). The ion at *m/z* 408 is obtained through loss of the trifluoroacetic group (CF_3COOH) from the di-trifluoroacetyl derivative. Linearity was determined with whole blood samples spiked with increasing concentrations of cannabinoids ranging from 0.3 to 100 ng/mL and from 0.8 to 50 ng/mL for THC and 11-OH-THC, respectively and from 0.1 to 100 ng/mL for THCCOOH. The correlation coefficients (r^2) were found to be higher than 0.999. Coefficient of variation (CV) for intra- and interassay precisions were calculated at three concentrations (2.0, 10.0, and 50.0 ng/mL) for each cannabinoid (triplicate determination). Overall, intra- and interassay CVs were below 11.2% and 6.8%, respectively. The method was accurate at all tested concentrations to within 10% of the target concentration. The limits of quantification were below 1.0 ng/mL for all three cannabinoids (THC: 0.3 ng/mL, 11-OH-THC: 0.8 ng/mL, and THCCOOH: 0.1 ng/mL). The extraction recovery was found to be higher than 50%. Each batch included a blank blood sample and a blood sample fortified with the internal standards only to evaluate the selectivity of the method. No significant interferences could be detected. The cannabinoids were found to be stable for at least two months when the blood samples were stored at –20°C and preserved with 1.2 mg EDTA and 1.0 mg fluoride/mL blood in S-Monovette tubes (Sarstedt, Sevelen, Switzerland).

Clinical observations

Subjects were observed for objective signs of drug influence. Conjunctival reddening was visually assessed, and graded from 1 to 4. Pulse rate and arterial pressure were also recorded.

Subjective effects rating scale

The feeling of intoxication (i.e., the intensity of "high") was reported by the participants on a VAS scale extending from 0 (no effect) to 10 cm (maximum effect experienced in the past while smoking marijuana). The respondents indicated their answer to the question "do you feel intoxicated by cannabis?" by drawing an intersecting line through the 10-cm line. The appreciation of drug effect (i.e., "drug liking") was also reported on a VAS scale extending from 0 (unpleasant), to 5 (unnoticeable), to 10 (pleasant).

Willingness to drive and feeling of inability to drive

The willingness to drive is often used to assess the self-reported deterioration in psychomotor performance following drug or alcohol exposure (25). The willingness to drive was evaluated by asking several questions of great or minor importance to the participants. The agreement to drive a passenger under various emotional and rewarding circumstances was the main focus of these tests. The following questions were asked: (a) do you agree to immediately drive an ill child to the hospital? (b) do you agree to drive a moderately sick friend home? (c) do you agree to drive a friend to a party? and (d) do you assess your driving capability to be significantly decreased? The volunteers reported the results on a continuous 0–10 cm VAS.

Psychomotor and driving simulator testing

These tests are commonly used by the Swiss Society of Traffic Psychology (http://www.vfv-spc.ch/vfv_franz/index.htm) (26). The usefulness of driving simulators in clinical practice has been reviewed recently (27).

Roadsign testing. Twenty pairs of roadsigns were shown in a random order on a screen. A blinking arrow pointed to a single roadsign and the subject was asked to find out the corresponding partner of the pair. Below the second roadsign was a number. This number was also found on a touch-screen in front of the participant. The last task for the subject consisted in pressing the key with the corresponding number. The total time to detect all pairs of roadsigns was measured. This test mainly consists of a visual search task. The speed of visual processing and short term memory as well as accurate perception play a decisive role in this test.

Tracking test. The tracking test consisted of two subtasks. First, the subject was asked to keep a symbolic vehicle with the help of a steering wheel on the main track. Secondly, the subject was asked to press the left or right foot pedal when specific signals appeared to the left or the right of the track. Disturbing signals and dead-end roads appeared also. The percentage of time in the track as well as the number of errors were recorded. The following parameters were assessed: continuous dynamic steering, anticipating perception and pertinent reaction to relevant and disturbing signals, psychomotor coordination, and level of vigilance.

Statistical interpretation

Significance of the results of psychomotor tests and of the driving simulator testing were evaluated using the Statistical Package for the Social Sciences (SPSS® 11.0 for Windows). The nonparametric test of Kruskal-Wallis was used to evaluate the results of roadsign testing, driving simulator, and of the subjective effects and willingness to drive. Pair-wise tests (Tukey test) were used to detect differences between treatments. Kinetic profiles were processed with the Winstat software (Statistics Add-In for Microsoft® Excel). The pharmacokinetic parameters were assessed with the PK Analyst software (PK Analyst for Windows: Pharmacokinetic data analysis version 1.1 for Microsoft Windows, MicroMath Scientific Software). PKmodel # 12 and 14 were selected for the evaluations of the THC, 11-OH-THC, and THCCOOH kinetics.

Interpretation of the results

Forensic interpretation of results. The cannabis influencing factor (CIF) has been proposed by Daldrup's group (21). The CIF is the molar ratio between the sum of THC and 11-OH-THC concentrations and THCCOOH level multiplied by 100. Anyone with a CIF value over 10 is presumably as unfit to drive as one with a blood-alcohol concentration (BAC) value of 1.1 g/kg. CIF parameter can be used on the condition that the blood is drawn between 0.5 and 1.5 h after the event. Considering a specific time-period (1994) and specific area in Germany (Düsseldorf), about 3/4 of the drivers involved in car accidents or having committed serious driving errors (e.g., getting off the roadway) under the influence of alcohol or cannabis were found to have a BAC value higher than 1.1 g/kg or a CIF value higher than 10.

Assessment of the time since exposure. Two mathematical models have been proposed that estimate the time of marijuana exposure from a single plasma measurement of THC alone or of both THC and THCCOOH and provide accompanying 95% confidence interval (16,20). Model I is based on THC concentrations, and model II relies on THCCOOH/THC ratios. Equations are shown in Huestis et al. (20).

Interpretation of blood levels. Various pharmacodynamic models have been proposed to estimate pharmacological effects. The majority provide concentrations estimated in the range of 5–29 ng THC/mL necessary for a significant subjective "high" effects or driving impairment. For instance, a significant linear correlation was found between tracking errors under divided attention and THC plasma levels over 5–25 ng/mL for approximately 2 h after smoking (28). This approach is discussed in several reviews (14,16).

Results and Discussion

Selection of whole milk as a vehicle for THC administration and efficiency of the hemp decoction making process

Because of its poor water solubility, THC was administered in a fat-rich matrix. Dronabinol was solubilized in sesame oil and plant cannabinoids in whole milk containing 2.7% of milk fat. In Pakistan and India, "bhang" is a beverage that is made from an infusion of cannabis leaves and flowering tops combined with milk and nuts (29). Consumption of this milk decoction is quite common; its preparation is relatively similar to the beverage that was used in this study. The recipes for making hemp milk decoctions can also be found in many underground publications (30) or on internet dedicated web sites (e.g., http://cannabisculture.com/backissues/cc00/cooking.html).

Fresh cannabis plant material contains mainly THC-A as the major cannabinoid, typically about 80%. Before oral administration, THC-A must be decarboxylated into active THC. This process is accomplished by heating through several methods (6,31). Heating a "space cake", brownies, or cookies in an oven at 180°C results in the almost complete transformation of THC-A to THC. Drinking a decoction made with cannabis will produce more or less effect depending on the

fraction of THC present at the end of the heating process. The thermal pretreatment of the cannabis plant fragments made easier the preparation of a hemp decoction containing a well-defined dose of THC.

Because only partial conversion of THC-A into THC was accomplished by heating hemp plant fragments in milk for 1 h at 93°C, we decided to heat the hemp powder under argon at 140°C for half an hour. After this thermal pretreatment, THC only, but neither THC-A nor cannabinol, could be detected in the powder by HPLC. This powder was used to prepare a milk decoction. The analysis of the hemp decoction revealed the presence of THC with a minor amount of cannabidiol. About 80% of the THC dose was recovered from the filtrated decoction. Part of the THC was very likely lost during the heating of the milk or the filtration step carried out to remove most of the hemp herb fragments.

Criteria for the selection of the dose

In previous studies, we have reported the effects and the cannabinoids kinetics of the orally administered hemp water and milk decoctions (18). Moderate subjective clinical effects (about 50% of the maximum effect felt in previous experiences) were reported after drinking the hemp milk decoction. The maximum concentrations of THC and 11-OH-THC were found to be lower than 5.0 ng/mL. The ingested dose was 23.2 mg THC. A review of the controlled administration studies has shown that the dose administered through the oral route varies considerably, from 2.5 mg up to about 60 mg THC. The daily dose also varies considerably, reaching a maximum amount of about 210 mg administered orally as capsules containing 30 mg of THC in sesame oil, with 60 mg given prior to sleep (32). Generally, the therapeutic dose is relatively low to minimize the behavioral effects characteristic to marijuana and the risks of unwanted side effects (5,33). Recreational use of oral cannabis may involve very high doses with unpredictable effects because accurate estimation of the ingested dose is almost impossible and also because the psychotropic effects are delayed (30,34). Fifty to about 400 mg of THC per day represent the typical dose of chronic heavy cannabis smokers. The oral doses administered in this study can therefore be considered as a medium and a high single dose of THC.

Adverse events

Unwanted psychiatric side-effects. Two of the 8 subjects were withdrawn from the study after administration of dronabinol or of the hemp milk decoction containing the medium dose of THC. The first participant experienced strong anxiety with paranoid feelings after drinking a milk decoction containing 16.5 mg THC, while the second one experienced anxiety with altered perception of reality after ingestion of 20 mg of dronabinol. Fortunately, all unpleasant effects resolved spontaneously within one day. Cannabis psychosis following bhang ingestion has been already reported (29). The symptoms included grandiosity, excitement, hostility, uncooperativeness, disorientation, hallucinatory behavior, and unusual thought content. Dysphoric reactions to cannabis are not uncommon, especially in naïve subjects (4,35).

Gastrointestinal side-effects. Nausea was often reported. Vomiting was also observed, especially after ingestion of the milk decoction containing the high dose of THC. These effects were more pronounced when the active cannabinoids reached their highest concentrations. Cannabis and synthetic THC (dronabinol) or Nabilone have been advocated for the prevention of nausea and vomiting caused by anticancer drugs (3). Low doses of THC are generally prescribed to induce antiemetic effects. However, the opposite effects were observed in our study with larger doses. High doses or chronic use can indeed induce pro-emetic effects (3,36). Nausea and vomiting were also observed after intravenous injection of marijuana (37). Vomiting is likely to be the consequence of peripheral and central actions. THC alters gastric emptying of solid food in humans, inhibits gastric acid secretion, decreases gastrointestinal motility, and selectively acts on CB1 receptors in specific regions of dorsal vagal complex (38–40). All these effects may inhibit or stimulate emesis depending on the ingested dose and of the presence of co-ingested food and beverages. Simulated driving and watching a moving vehicle on a screen could also enhance the nausea experienced by the volunteers. In our study, this was obviously a triggering or aggravating factor for several subjects. These adverse effects may also happen in "real life" conditions while driving on a sinuous road under the influence of cannabis.

Urine screens, breath ethanol levels, and blood cannabinoids concentrations at the beginning of each session

Screens carried out with immunoassays and breathalyzer showed no detectable levels of major psychoactive drugs in urine or breath ethanol. No cannabinoids were found in blood of the participants before treatments.

Blood kinetics of THC, 11-OH-THC, and THCCOOH

Mean blood levels of THC, 11-OH-THC, and THCCOOH for all 8 subjects (6 participants for the high dose) following ingestion of 20 mg dronabinol, or 16.5 ± 0.9 mg THC or 45.7 ± 0.7 mg THC as a milk decoction are shown in Figure 1. Table I lists the mean maximum cannabinoids levels, average concentrations, and concentration ranges for the one-day time period following dronabinol and decoction ingestions. No cannabinoids could be detected in blood following administration of the placebo gelules and decoction (results not shown). The time-concentration curves in Figure 1A demonstrate that THC was rapidly detectable in whole blood and present for several hours, with average peak concentrations occurring already 1 h after ingestion. Maximum mean THC concentrations were in the same range (2.8 and 3.8 ng/mL) when similar doses were administered, regardless of the type of vehicle used (milk decoction or capsule filled with sesame oil) (Table I). A 2.2- to 3.0-fold increase in peak concentration was observed after drinking the 45.7 mg THC-decoction. Ingestion of the decoctions resulted in mean THC that decreased rapidly after the peak. The mean THC levels remained in the same range for a longer time (1–5.5 h) after intake of dronabinol. The individual results show there was a considerable intersubject variability. This high variability is illustrated by the individual THC kinetics determined after ingestion of the milk

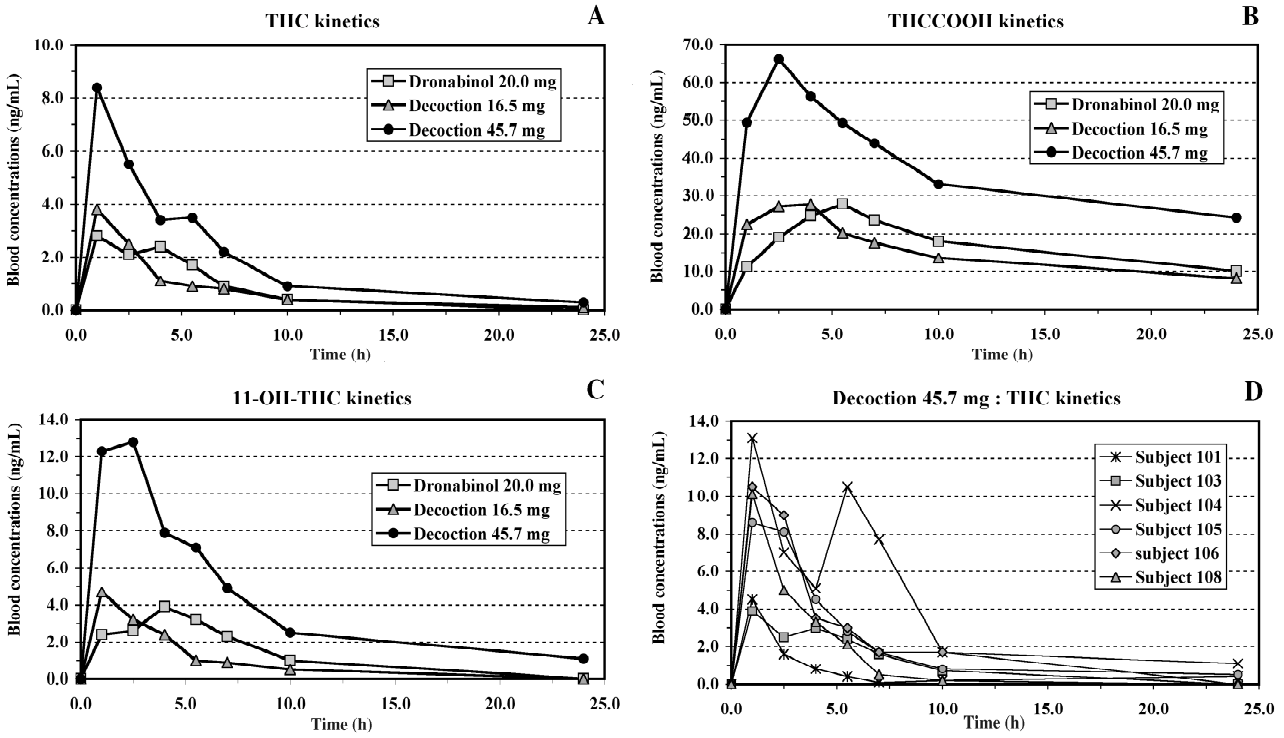

Figure 1. Whole blood levels of mean THC, 11-OH-THC, and THCCOOH and individual THC levels for 8 subjects (6 particpants for the decoction containing 45.7 mg THC) after ingestion of 20 mg dronabinol or a milk hemp decoction containing a mean dose of 16.5 or 45.7 mg THC.

Table I. Mean Whole Blood Concentrations of THC, 11-OH-THC, and THCCOOH and Concentrations Ranges After Administration of 20 mg Dronabinol and of 2 dL of Hemp Milk Decoction Containing Either 16.5 or 45.7 mg THC*

		1 h	2.5 h	4 h	5.5 h	7 h	10 h	24 h
THC µg/L								
Dronabinol	Mean	**2.8**	2.1	2.4	1.7	0.9	0.4	nd
20 mg	Range	nd–5.6	nd–5.0	nd–6.3	nd–3.7	nd–1.7	nd–1.4	nd–0.3
Decoction	Mean	**3.8**	2.5	1.1	0.9	0.8	0.4	< LOQ
16.5 mg THC	Range	1.5–8.3	0.6–6.2	nd–3.6	nd–2.7	nd–2.3	nd–1.7	nd–0.9
Decoction	Mean	**8.4**	5.5	3.4	3.5	2.2	0.9	0.3
45.7 mg THC	Range	3.9–13.1	1.6–9.0	0.8–5.1	0.4–10.5	< LOQ–7.7	< LOQ–1.7	nd–1.1
11-OH-THC µg/L								
Dronabinol	Mean	2.4	2.6	**3.9**	3.2	2.3	1.0	nd
20 mg	Range	nd–6.3	nd–5.2	1.4–8.5	< LOQ–8.4	< LOQ–6.0	nd–2.2	nd
Decoction	Mean	**4.7**	3.2	2.4	1.0	0.9	< LOQ	nd
16.5 mg THC	Range	2.9–7.0	< LOQ–5.6	nd–4.3	nd–2.7	nd–2.7	nd–1.5	nd
Decoction	Mean	12.3	**12.8**	7.9	7.1	4.9	2.5	1.1
45.7 mg THC	Range	4.6–23.8	3.4–24.7	1.7–15.1	1.6–21.0	1.1–17.0	< LOQ–8.2	nd–5.0
THCCOOH µg/L								
Dronabinol	Mean	11.2	19.0	24.7	**27.8**	23.6	18.0	10.1
20 mg	Range	2.5–25.0	2.8–35.5	8.5–47.5	5.4–55.4	3.7–46.4	2.5–35.8	2.8–21.5
Decoction	Mean	22.4	27.2	**27.8**	20.2	17.6	13.6	8.2
16.5 mg THC	Range	13.3–31.4	7.7–41.0	14.1–42.4	4.5–39.7	4.3–35.3	3.2–27.2	2.3–15.5
Decoction	Mean	49.4	**66.2**	56.2	49.3	43.9	33.1	24.3
45.7 mg THC	Range	24.8–85.3	29.0–99.9	31.1–90.6	20.5–85.4	19.9–86.8	13.6–66.6	6.8–64.5

* Maximal concentrations are bold-typed and underscored. < LOQ = lower than the limit of quantification and nd = lower than the limit of detection.

decoction containing the average dose of 45.7 mg of THC (6 individual curves shown in the Figure 1D). Maximum individual levels ranged from 3.9 to 13.1 ng/mL (Table I) and THC remained detectable in whole blood for a time period of 10–24 hours. The area under the curve for the mean data from 0 to 24 h did not show significant differences between dronabinol and the milk decoction containing the medium dose. However, a two-fold increase was noticed after drinking the decoction containing the strongest dose (results not shown).

The active metabolite 11-OH-THC, was detectable in higher

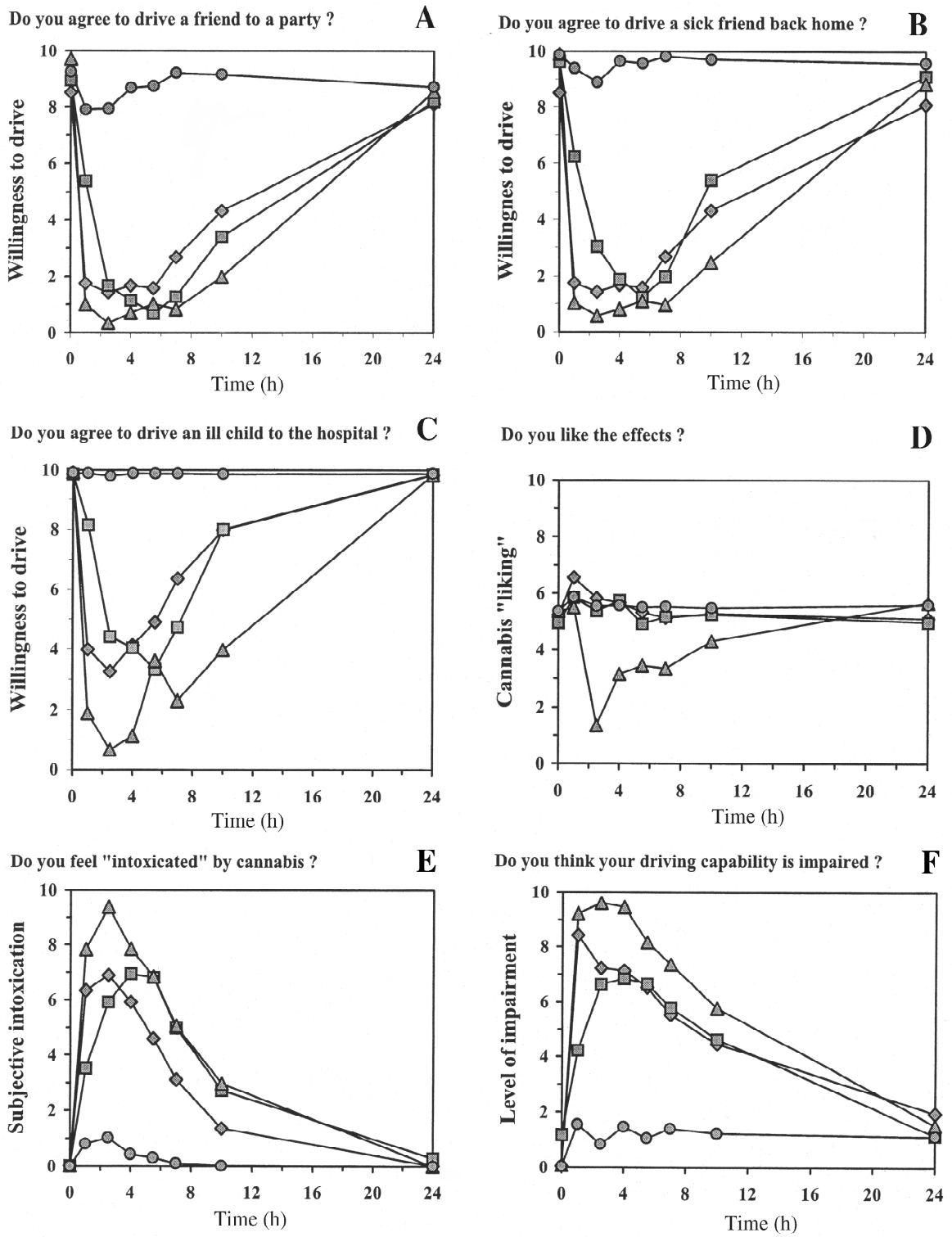

Figure 2. Mean subjective effects and willingness to drive after ingestion of the placebo (•), 20 mg dronabinol (■), 16.5 mg THC (♦), or 45.7 mg THC (▲) in hemp milk decoctions. Differences between treatments and placebo were statistically significant (Kruskal-Wallis test, $p < 0.0001$). Pair-wise comparisons were also significant for each treatment versus placebo (Tukey test, $p < 0.05$).

mean concentrations than THC for all treatments. For instance, THC and 11-OH-THC levels reached a maximum mean concentration of 8.4 and 12.3 ng/mL, respectively (Figures 1A and 1C). Taking into account a plasma/whole blood distribution ratio of 1.6 (23), a 11-OH-THC plasma concentration of 19.7 ng/mL could be calculated. The maximum individual concentration was 24.7 ng/mL whole blood (i.e., 39.5 ng/mL plasma). This maximum plasma concentration is significantly higher than those (3.8–16.0 ng/mL) which were determined after smoking a marijuana cigarette containing 3.55% THC (19). In our study, mean peak levels were noted 1–4 h post-ingestion. 11-OH-THC remained detectable in whole blood for 10–24 h depending on the dose, which was ingested (low versus high dose). It is interesting to note that the highest mean and individual sum of THC + 11-OH-THC levels, the two main active cannabinoids, were 20.7 and 36.9 ng/mL whole blood, corresponding to maximum plasma levels of about 32.1 and 57.2 ng/mL. These concentrations are significantly less than active cannabinoids levels which are typically measured after smoking a marijuana cigarette containing a medium amount of THC (> 100 ng/mL of plasma). The mean [11-OH-THC]/[THC] ratios ranged from 0.86 to 2.56 for the whole range of treatments. A similar range of values can be calculated in the late elimination phase when THC and 11-OH-THC reach low plasma levels after cannabis smoking (< 3 ng/mL plasma) (19).

Mean THCCOOH concentrations in whole blood reached their maximal value later on, between 2.5 and 5.5 h after drug ingestion. The levels were also much higher than those determined for THC and 11-OH-THC with mean maximum values of 27.8, 27.8, and 66.2 ng/mL, after ingestion of 20 mg dronabinol, of 16.5 and 45.7 mg THC, respectively. THCCOOH was still present in significant levels 24 h following ingestion (Figure 1B and Table I) with one participant showing a maximum concentration of 64.5 ng/mL. The mean maximum concentration calculated after 1 day post-ingestion was in the range 10.1–24.3 ng/mL. In contrast with what is generally observed after cannabis smoking, the THCCOOH concentrations remained significantly higher at all times than those of THC and 11-OH-THC.

Objective effects

A slight to moderate conjunctival reddening was consistently observed. The reddening was more intense after drinking the 45.7-mg decoction. The extent of reddening reached its highest level (mean score = 2.2) after 1.0–2.5 h and then decreased continuously to reach baseline levels after one day. Similar effects have already been observed following various routes of administration (41,42). A slight to moderate tachycardia was noted after hemp milk decoction administration as well as dronabinol ingestion. For instance, the pulse increased from a mean value of 58 to 85 bpm 1 h after ingestion of the 45.7-mg decoction.

Subjective effects and willingness to drive

The volunteers reported the subjective effects and willingness to drive on an visual analog scale (0 to 10 cm). When compared to placebo, obvious cannabis influence was observed under almost all treatments (Figure 2). These differences were also statistically significant (Kruskal-Wallis test, $p < 0.0001$). On the whole, pair-wise comparisons were also statistically significant for each treatment versus placebo (Tukey test, $p < 0.05$). However, most pair-wise comparisons between treatments were not significant. We found a moderate degree of acceptance when an absolutely vital demand was addressed to the participants [e.g., do you agree to drive an ill child to the hospital? (Figure 2C)]. On the other hand, we found a strong refusal when the subjects were asked a question of less importance [e.g., do you agree to drive a friend to a party? (Figure 2A)]. Robbe (25) has previously reported that the willingness to drive decreased with increasing doses of cannabis. He also found that the willingness to drive was greatest for urgent trips and increased with time. These results suggest that the subjects were able to balance the importance of the trip against the risk of having an accident. The participants were aware of the effects of the drug and reported a strong feeling of "high". The self-reported intoxication was more intense after ingestion of the highest dose (Figure 2E). Liguori et al. (43,44) have also shown that self-report ratings of "high" and "drug potency" as well as the feeling of impairment increased with the

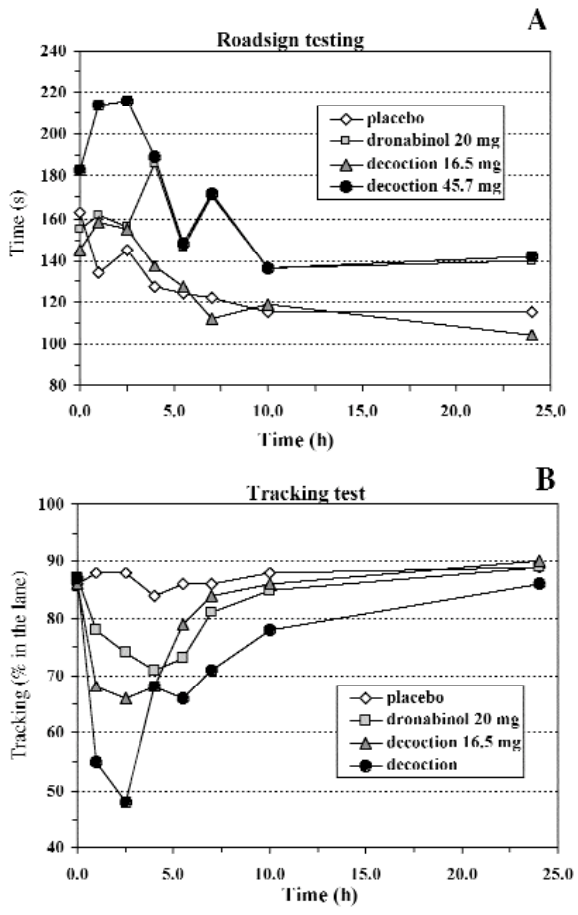

Figure 3. Mean results of roadsign and tracking testings. Differences between treatments and placebo were found to be statistically significant (Kruskal-Wallis test, $p < 0.0001$). Pair-wise comparisons were also statistically significant for each treatment versus placebo (Tukey test, $p < 0.05$). Pair-wise comparisons between treatments were not significant.

smoking dose. However, our subjects did not appreciate the effects, especially after drinking the decoction containing the highest dose of THC. The ingestion of capsules containing 7.5 or 15 mg THC to assess subjective and cognitive effects has an opposite effect producing increased ratings of liking (45). The volunteers also had the feeling that their driving capability was deeply impaired (Figure 2F). Dysphoric effects and nausea, which are often felt after ingestion of medium to high doses of THC and possibly triggered or enhanced by the simulated driving task, may explain why the volunteers did not like the effects. Lack of tolerance may be a contributing factor. Calhoun et al. (46) have shown that dronabinol given orally does not provide the effects that are considered desirable in a drug of abuse. The onset of action is slow and gradual, it is at most only weakly reinforcing, and the overwhelming majority of reports of users indicate that its effects are dysphoric and unappealing. The large differences in effects between smoked and ingested cannabis have several explanations. First, the kinetics are different, secondly, more metabolites are produced through first-pass metabolism after oral intake. 11-OH-THC, could be more dysphoric than THC. However, 1 mg of 11-OH-THC administered intravenously produces psychological and pharmacological effects that mimic those of THC (47,48). In addition, THC and 11-OH-THC were equipotent when infused IV with 25% human serum albumin in 2–3 mg doses (49). Other active metabolites could be also involved. Thirdly, oral ingestion potentially produces more adverse effects on the gastrointestinal tract. Adverse reactions, such as abdominal pain, nausea, and vomiting with an incidence of 3–10% were indeed reported by Unimed Pharmaceuticals, the company marketing Marinol®. Finally, the route of administration could influence the body and brain distribution of cannabinoids (50). In a cocaine fatality associated with coingestion of marijuana, THC, and 11-OH-THC were found to be present in higher concentrations in

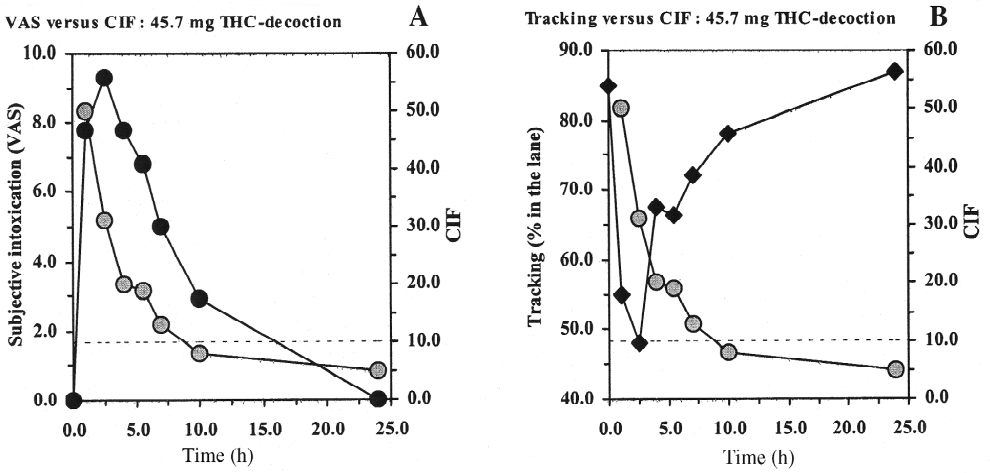

Figure 4. Comparison of subjective rating of intoxication (VAS •) to cannabis influencing factor (CIF ○) as proposed by Daldrup and Meininger (21) (A) and comparison of Tracking results (♦) to CIF values (B).

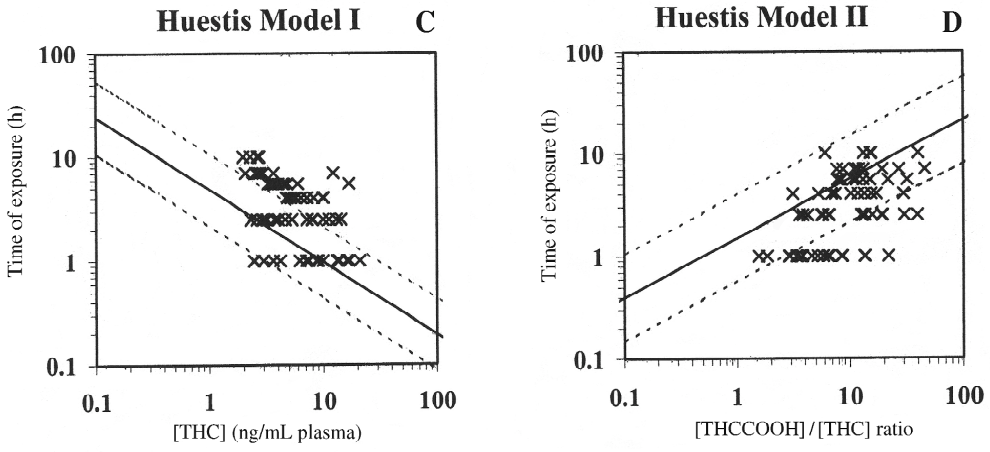

Figure 5. Mathematical models for the prediction of time of marijuana exposure according to Huestis et al. (20) and actual values obtained in this study. Model I is based on THC concentrations (A), and Model II is based on plasma [THCCOOH]/[THC] ratios (B). Dotted lines represent 95% confidence intervals. Plasma concentrations were calculated from whole blood levels determined in this study after ingestion of 20 mg dronabinol, 16.5 mg or 45.7 mg THC in 2 dL hemp milk decoctions. Calculated plasma levels (x) were fitted in both models.

brain cortex than in whole blood (51). Their overall effect could depend on their respective concentrations and molar ratios in target brain structures.

Roadsign and driving simulator testing

Figure 3 shows the mean results of the roadsign testing. The differences between placebo and oral THC were statistically significant ($p < 0.0001$). The total time to achieve the pairing of the 40 road signals was deeply increased after drinking the decoction containing 45.7 mg THC. The impairment was especially noticed during the time period ranging from 1.0 to 5.5 h post ingestion. More obvious effects were detected with the driving simulator. When considering the results of the tracking test, the performances of the participants were strongly impaired. All treatments differ statistically from the placebo (16.5 mg THC-decoction: $p < 0.003$; 20 mg dronabinol: $p < 0.001$; 45.7 mg THC-decoction: $p < 0.0001$). However, nonparametric statistical tests did not reveal differences between vehicles (milk or sesame oil containing gelules) and doses (16.5 or 20 mg versus 45.7 mg) probably because of the small sample size. Nevertheless, the maximum decrease in tracking efficiency was noticed after taking the highest dose. Irrelevant errors (e.g., pressing the pedal when a disturbing signal appears) as well as reaction time were less affected. A moderate increase in reaction time was detected after oral ingestion of dronabinol or THC. This effect was not statistically significant. Therefore, keeping a symbolic vehicle on the track was the most difficult task for the participants under the influence of cannabis. In agreement with these results, Robbe and coworkers (52,53) have shown that standard deviation of lateral position in the road-tracking test was the most sensitive measure for revealing THC's adverse effects.

Forensic interpretation of results

CIF. CIF (21) was calculated from the actual concentrations of THC and its two main metabolites. Figure 4 indicates that mean CIF values higher than 10 matched the mean intoxication level rated by the volunteers as well as the mean decrease in tracking efficiency. Similar relationships were noticed with the 3 active treatments. However, the kinetics were very different with the CIF showing an almost continuous decrease while the subjective effects and the tracking performance records showed a more bell-shaped curve. The CIF reached its maximum before the strongest feeling of intoxication and the maximum tracking impairment. During the first hours following ingestion, the CIF value was decreasing while the rating of intoxication and the impairment level were increasing. These results suggest that the absolute value of the CIF must not be used to assess the severity of intoxication or impairment. Nevertheless, these results suggest that the cut-off of 10 could be roughly used to discriminate between unfit and capable drivers.

Calculation of the time of ingestion. Huestis et al. (19,20) proposed two mathematical models aimed at the prediction of the time of cannabis smoking. These models have also been suggested to assess the time of oral intake. Model II was claimed to be more accurate after marijuana ingestion. Our results show that 56% and 27% of the values were out of the range of the confidence intervals of Models I and II, respectively (Figure 5). Model I tended to underestimate the actual time of ingestion, whereas Model II tended to overestimate the time of intake. Model II should be preferably used because it was more accurate, but also because in forensic practice, the interpretation of results must be in favor of the suspected driver.

Blood concentrations. Drummer et al. (15) recently showed that 58 drivers killed in road traffic crashes with measurable THC concentrations in their blood had a significantly higher likelihood of being culpable than drug-free drivers. The odds ratio was 6.6 for drivers with blood THC concentrations greater or equal to 5 ng/mL. In our study, we found that 20, 36, and 61 out of 154 cannabinoids determinations showed respective THC, 11-OH-THC, and THC + 11-OH-THC blood concentrations greater than 4.6 ng/mL. When considering the mean values, THC levels were below 5.0 ng/mL, although several tests were indicative of significant impairment. A better relationship was found when considering the sum of THC and 11-OH-THC. Without taking into account a slight difference in molecular weight, the sum of THC and 11-OH-THC remained higher than 4.6 ng/mL for 7 h after ingestion of the decoction containing 45.7 mg THC. This time period matched with a significant impairment in tracking performances (compare Figures 1 and 3).

Conclusions

Our study shows that although large doses of THC were ingested and obvious psychoactive effects observed and performance impairments monitored, blood levels of THC and of 11-OH-THC remained lower than 13.1 and 24.7 ng/mL, respectively. A two- to threefold increase in cannabinoid blood concentrations was achieved following ingestion of the milk hemp decoction containing the highest dose (45.7 mg). The willingness to drive was significantly hampered after all treatments. Important effects were also noticed with the roadsign testing and the tracking test. Altogether, the results indicate that oral ingestion of cannabis or dronabinol in medium and high doses can severely impair several performance skills required for safe driving. Finally, three strategies (the first aimed at the estimation of the time of cannabis exposure, the second with the objective to assess the fitness to drive, and the third based on the interpretation of blood levels) were evaluated under our specific experimental setting (i.e., oral intake of a medium or high dose of THC). Our results show that Model II proposed by Huestis et al (20) should be preferred over Model I because it gives a better approximation of time of cannabis or dronabinol ingestion. The CIF yields a rough estimation of the fitness to drive. As far as oral intake is concerned, the sum of THC and 11-OH-THC provides a better estimate than THC alone of cannabis- or dronabinol-associated impairment.

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