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Forensic aspects of the metabolism and excretion of cannabinoids following oral ingestion of cannabis resin

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Following oral ingestion of cannabis resin, Δ^9-THC-11-oic acid and its *O*-ester glucuronide were detected using RIA and combined hplc/RIA and shown to be major plasma metabolites of Δ^9-THC. Δ^9-THC-11-oic acid was not excreted in the urine in significant concentrations, the glucuronide conjugate being the major urinary metabolite detected. Δ^9-THC metabolites were detected in blood for up to 5 days and in urine for up to 12 days following a single oral dose of Δ^9-THC (20 mg). Estimates for the half life of Δ^9-THC-11-oic acid and its glucuronide in plasma, and total metabolites in urine have been obtained. Interpretation of blood or urine total cannabinoid levels is most difficult, however, drug/metabolite ratios and metabolite/metabolite ratios may have potential for indicating recent cannabis use.

The development of analytical methods for cannabinoid detection and the interpretation of subsequent findings must be based on a firm understanding of the metabolism, distribution and other pharmacokinetic parameters. This is of particular importance with Δ^9-tetrahydrocannabinol (Δ^9-THC) which is rapidly and extensively metabolized and not excreted in urine (see Wall & Perez-Reyes 1981; Halldin et al 1982a, b). Although a wide variety of methods have been developed for the detection and quantification of Δ^9-THC and its metabolites (Agurell et al 1973; Nordqvist et al 1977; Williams et al 1978), an incomplete understanding of Δ^9-THC metabolism in man has limited the development of the ideal technique for forensic use. Similarly, the lack of pharmacokinetic data on the major metabolites has made interpretation of an analytical results difficult. This is most apparent in relation to the persistence of cannabinoids in blood and urine of man following cannabis use, since few data are available. Wall & Perez-Reyes (1981) have reported levels of Δ^9-THC and metabolites in plasma for up to 72 h after cannabis consumption but it is probable that significant levels of cannabinoids persist in blood and urine for much longer, possibly up to several weeks. Although this persistence is clinically insignificant, it is highly pertinent to the forensic toxicologist. Workers in this field are frequently required to interpret such findings, especially in relation to, time since ingestion, and ultimately give an indication of the psychological status of the subject. In many instances, body fluid samples are obtained many hours or days after an alleged offence was committed.

To our knowledge, this is the first report on long term measurements (>3 days) of concentrations of cannabinoids in blood or urine. Using a previously described high-performance liquid chromatography/radioimmunoassay (hplc/RIA) technique (Law et al 1984a, b), the elimination of both Δ^9-THC-11-oic acid and its *O*-ester glucuronide in plasma and urine has been studied. There are no previous reports on pharmacokinetic data for a cannabinoid conjugate. Although Δ^9-THC-11-oic acid, and presumably its glucuronide, are pharmacologically inactive, the glucuronide is important in the forensic context since it has been shown to constitute the major proportion of RIA-detectable metabolites in human urine (Williams & Moffat 1980), it is also present in blood in significant quantities (Law et al 1984b), and may well be the major Δ^9-THC metabolite in man.

Oral administration was chosen for this initial study, as experiments could be carried out easily on reliable, non-cannabis users who were willing to provide blood and urine samples over a prolonged period. Unlike the absorption of Δ^9-THC following cannabis smoking, its absorption after oral ingestion would not be seriously affected by the naive status of the subjects.

Administration of cannabis resin rather than pure Δ^9-THC was selected to mimic the conditions normally encountered in forensic cases.

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MATERIALS AND METHODS

Reagents

Cannabis resin typical of the type produced in Pakistan was obtained from the Laboratory of the Government Chemist. This had been shown by analysis (Baker et al 1980) to contain Δ^9-THC (6·1%), Δ^9-THC acids (5·4%), cannabinol (0·7%) and cannabidiol (5·6%).

Δ^9-[G-^{3}H]THC (specific activity 230 GBq mmol^{-1}) was obtained from Amersham International Ltd, Amersham, Bucks, UK. Scintillation counting was carried out in an Intertechnique SL30 using a standard triton/toluene scintillant.

Cannabinoid antiserum for Δ^9-THC analysis (Lot No 133Y/22/5) was obtained from Guildhay Antisera, University of Surrey, Guildford, Surrey, UK. Δ^9-THC standards, 0, 0·25, 0·5, 1, 2, 4 and 8 ng ml^{-1} were prepared in 82·5% v/v aqueous methanol.

Volunteers

These were males aged 27–34, average weight 71 kg, who were shown to be in good physical and mental health and who had no previous experience of cannabis use. None of the subjects were taking other drugs and they abstained from alcohol use for at least 24 h before and after the experiment. The experimental protocol was approved by the Committee on the Safety of Human Experiments, Chemical Defence Establishment, Porton Down, Wilts.

Dose and administration of cannabis resin

The experiment was divided into two separate trials 26 days apart. The first trial involved a low cannabis dose and was designed to assess the reaction of the subjects to cannabis exposure. The second trial involved a typical cannabis dose i.e. 20 mg Δ^9-THC.

Trial 1

Each of five subjects took Δ^9-THC (5·0–5·2 mg) in cannabis resin (ca 84 mg). The resin was cut into small pieces (ca 8 mm^3) and mixed with the filling in a meat sandwich. The subjects were monitored for adverse reactions. Pulse rate and conjunctival suffusion were measured at regular intervals for up to 4·5 h after ingestion. Urine samples were collected at intervals during the first 12 h following ingestion and daily thereafter for up to 8 days.

Trial 2

Each of 4 subjects took Δ^9-THC (20 mg) in cannabis resin (ca 330 mg) as in trial 1.

The subjects were continuously monitored by electrocardiography and pulse rate and blood pressure were measured at regular intervals. Conjunctival suffusion and euphoric state were independently assessed.

Venous blood samples were obtained at regular intervals for up to 8 h, and venous and capillary blood samples were taken daily for up to 5 days after ingestion. Samples were taken into heparinized containers, venous blood samples were separated immediately to obtain plasma, capillary samples were stored at 4 °C before separation.

Urine samples were taken regularly for up to 12 h after consumption and daily thereafter for up to 14 days.

All samples were stored at −18 °C before and between analyses.

Analyses

RIA analysis of urine and plasma samples for total cannabinoids was carried out as described by Law et al (1984a), using an iodinated radiotracer. Δ^9-THC-11-oic acid was used as the RIA standard and all levels quoted with the exception of Δ^9-THC are relative to this compound.

With the exception of the following modifications to allow analysis of Δ^9-THC in plasma, hplc/RIA analysis was also as previously described (Law et al 1984b). Before extraction, plasma samples were spiked with the internal standard Δ^9-[G-^{3}H]THC (15 pg, 400 counts min^{-1}) in ethanol (10 µl) and incubated at room temperature (20 °C) for 15 min. Following hplc separation, 45 × 0·5 ml fractions of eluate were collected. An aliquot (0·25 ml) from each of the final 10 fractions collected was analysed in a scintillation counter to determine the retention volume and recovery of Δ^9-THC. Those fractions shown to contain the internal standard were analysed by the following RIA procedure. Standard solution (50 µl) or hplc eluate (50 µl) was mixed with synthetic urine (50 µl). A solution of the iodinated radiotracer (100 µl, 10 000 counts min^{-1}) was added followed by Guildhay antiserum (100 µl), diluted 1/3200. Both radiotracer and antiserum were prepared in assay buffer as used previously (Law et al 1984a). The mixture was incubated at room temperature for 2 h before the addition of aqueous polyetheylene glycol 6000 (0·5 ml, 27·5% w/v) and processed as for the previously described RIA (Law et al 1984a). The sensitivity of the method was 0·4 ng ml^{-1} Δ^9-THC, using 1 ml plasma. Urine samples were also analysed for creatinine content using a commercial kit (Instrumentation Laboratory S.p.A., 20037 Paderno Dugnano, Italy).

Stability of cannabinoids in urine

To determine the stability of the urinary metabolites, a single urine sample was divided and stored at 4 °C and room temperature (12–24 °C) with and without the addition of dilute ammonium hydroxide. The base-treated samples attained a pH of 8·5, untreated samples were pH 5·7.

RESULTS

Clinical signs

In the first trial, none of the subjects reported any psychological effects or demonstrated any reaction associated with cannabis. One subject demonstrated physiological effects attributable to cannabis. These included faintness and pallor and the subject was excluded from further study.

In the second trial, all subjects exhibited physiological signs of cannabis intoxication; tachycardia and conjunctival suffusion. Three of the subjects reported feeling 'high', with typical psychological effects. The fourth subject reported a moderate adverse reaction. These effects commenced at approximately 2 h, and lasted for up to 8 h after consumption of cannabis.

Plasma analysis

The plasma cannabinoid elimination curves for the four subjects measured by direct RIA are given in Fig. 1. After reaching a peak, at ca 4–6 h after ingestion, the level of plasma cannabinoids in each of the four subjects showed a relatively smooth decline over the next 5 days. The assay could clearly distinguish between low levels ca 3 ng ml^{-1} cannabinoid metabolites and cannabinoid-free plasma.

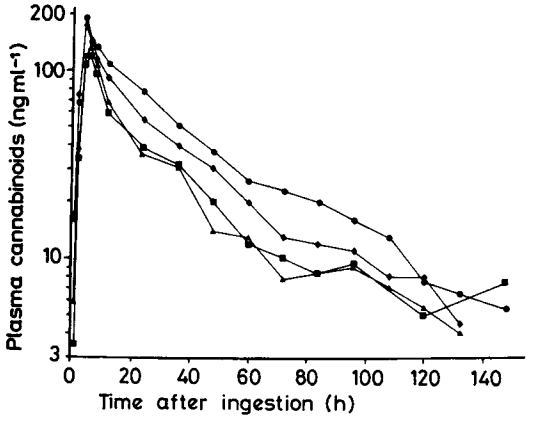

FIG. 1. Plasma cannabinoid concentrations determined by direct RIA analysis for four subjects who ate 20 mg Δ^9-THC in cannabis resin. Concns measured relative to Δ^8-THC-11-oic acid standards.

Analysis by hplc/RIA enabled quantification of parent Δ^9-THC, as well as Δ^9-THC-11-oic acid and its glucuronide conjugate. Table 1 gives the mean concentrations following analyses of the venous plasma samples taken up to 3 days after consumption of cannabis. The low levels of Δ^9-THC detected (<10 ng ml^{-1}) and the marked inter-individual variation are similar to that reported by Ohlsson et al (1980) following oral ingestion of Δ^9-THC. In those samples taken within 2 h of consumption, 11-hydroxy-Δ^9-THC was detected at concentrations comparable to Δ^9-THC itself. Accurate quantification of this metabolite was not possible owing to incomplete separation from the more significant Δ^9-THC-11-oic acid metabolite. Quantification of the latter metabolite however, was not seriously affected. The major proportion of cannabinoid activity in the plasma was due to Δ^9-THC-11-oic acid glucuronide and to a lesser extent the free acid itself.

Table 1. Mean concentrations of Δ^9-THC and metabolites in the plasma of four subjects who ate 20 mg Δ^9-THC in cannabis resin.

	Concentration ng ml^{-1}*			
Time after ingestion (h)	Total†	Δ^9-THC-11-† oic acid glucuronide conjugate	Δ^9-THC-11-† oic acid	Δ^9-THC
1	11 ± 3·5	3·4 ± 1·1	6·6 ± 2·1	1·5 ± 0·2
2	54 ± 10	26 ± 3·4	23 ± 6·3	3·9 ± 1·1
4	146 ± 20	107 ± 16	38 ± 10	6·9 ± 1·4
6	132 ± 5·0	99 ± 8·3	29 ± 4·7	3·0 ± 0·9
8	111 ± 7·9	80 ± 10	24 ± 5·6	1·8 ± 0·4
24	52 ± 9·4	31 ± 1·3	14 ± 2·5	0·5 ± 0·1
48	25 ± 5·1	17 ± 3·9	6 ± 2·0	0·2 ± 0·1
72	13 ± 3·4	9·1 ± 2·7	3·3 ± 0·9	0·1 ± 0·1

* Mean value ± s.e.m., n = 4.
† Concns are measured relative to Δ^8-THC-11-oic acid standards.

These two compounds show similar cross-reactivity with the antiserum used. It is highly significant that around 75% of the total cannabinoid material detected in the plasma was in conjugated form, and in one subject this was as high as 85% during the latter part of the experiment. Linearization of the concentration data over the period 8–72 h gave distribution half lives for Δ^9-THC-11-oic acid and its glucuronide of 22 ± 2 h and 21 ± 2 h respectively (mean ± s.e.m., n = 4). To our knowledge this is the first report of such data.

Urine analyses

Following dosing with 5 mg Δ^9-THC (Trial 1), maximum levels or urinary cannabinoids were attained between 8–10 h after ingestion, these ranged from 112–210 ng ml^{-1} cross-reacting canna-

binoids. Significant positive results were obtained for a further 7 days after consumption.

Following the oral dose of 20 mg Δ^9-THC, urine levels rose to between 185–1063 ng ml^{-1} cross-reacting cannabinoids 6 h after ingestion, with positive results being obtained for at least a further 12 days.

Hplc/RIA analyses of a number of the samples from the four subjects produced a surprising result. In contrast to plasma, Δ^9-THC-11-oic acid was found to be a minor metabolite comprising less than 4% of the total urinary cannabinoid activity. The major proportion of the urine metabolites (ca 65%) was due to the glucuronide conjugate, the remainder being more polar material. Fig. 2 shows a typical urine elimination curve of total metabolites for one of the four subjects. Determination of creatinine concentrations allowed adjustment of the cannabinoid levels for variations in urine flow rate. From the straight line portion of the adjusted elimination curves, a urinary half-life for total metabolites has been calculated as 25 ± 1 h (mean ± s.e.m., n = 4).

Stability of cannabinoids in urine

A urine sample from one of the volunteers, shown on initial analysis to contain the glucuronide but to be free of Δ^9-THC-11-oic acid, was found to contain a significant quantity of this metabolite after storage at room temperature for 12 days. When stored at pH 8·5 the hydrolysis of the glucuronide was more pronounced, with 13% of the total activity being due to Δ^9-THC-11-oic after a 4 day storage period at the same temperature. A similar though less marked change was observed with the alkaline sample stored at 4 °C.

A second, and more pronounced effect due to prolonged storage (90 days at room temperature), resulted in the loss of ca 70% of the RIA cross-reactivity in both the samples (pH 5·7 and 8·5). At 4 °C, the untreated sample (pH 5·75) showed no loss of activity, whilst that at pH 8·5 showed ca 50% loss.

DISCUSSION

Metabolism

Although a number of metabolites have been characterized (Nordqvist et al 1977; Wall & Perez-Reyes 1981; Williams et al 1978) and quantified following cannabis use, this would appear to be the first report on concentrations of a metabolite conjugate following controlled consumption of cannabis.

The concentration of the glucuronide in plasma, although initially exceeded by the concentration of the free acid, rose gradually until it constituted approximately 75% of the total plasma cannabinoid content measured by the RIA. This has important consequences for analytical method design, as those techniques directed towards the unconjugated material alone (Nordqvist et al 1977; Cook et al 1982; Foltz & Hidy 1982) are seriously underestimating plasma metabolite levels, and the sensitivity of such methods is therefore limited.

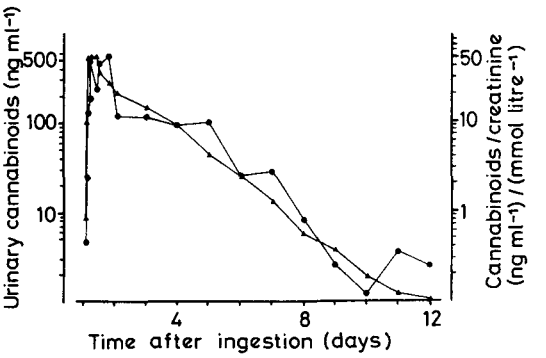

FIG. 2. Urinary cannabinoid concentrations for a subject who ate 20 mg Δ^9-THC in cannabis resin; concentration of cannabinoids (●); ratio of concentrations of cannabinoids to creatinine (▲). Concns measured as in Fig. 1.

The metabolite pattern observed in urine samples was markedly different to that seen in plasma. Although the glucuronide conjugate was the major urinary metabolite, the free acid was of minor importance being present in only low concentrations in fresh samples. It is possible, however, that even these low levels could be artifacts, given the susceptibility of the glucuronide to hydrolysis. The present experiments would also explain the high concentrations of free acid in those samples submitted for forensic analysis and the total absence of glucuronide in those samples with high pH (Law et al 1984b).

The use of a broadly specific assay capable of detecting glucuronide conjugates, or hydrolysis of the sample before the analysis of free Δ^9-THC-11-oic acid, is therefore recommended for forensic work. The failure to adopt such procedures accounts for the poor correlation between broadly specific immunoassay methods and gas chromatography in recent reports on cannabinoid analysis (O'Connor & Rejent 1981; Peat et al 1982).

Stability of urine metabolites

The data reported here extend those previously reported (Law et al 1984a) which showed urine metabolites to be relatively stable at 4 °C and −18 °C. These experiments suggest that storage of

unsterile urine at room temperature or 4 °C is responsible for the loss of urinary cannabinoid cross-reactivity, especially if the urine has a high pH. The loss in cross-reactivity is also accompanied by hydrolysis of the glucuronide conjugate which is particularly unstable when stored in urine at room temperature under alkaline conditions.

Interpretation

Following a single, moderate, oral dose of cannabis, (20 mg Δ^9-THC) significant concentrations of cannabinoid metabolites were detected in plasma samples for up to 5 days and in urine samples for at least 12 days; long after pharmacological action was detectable. The results for urine are in general agreement with a recent report which showed that chronic cannabis use can lead to detectable urinary cannabinoid levels up to 36 days after termination of use (Dackis et al 1982). In that study, analyses were carried out using a commercial enzyme immunoassay (Emit). Also of significance is the fact that a small oral dose of cannabis (5 mg Δ^9-THC) can give relatively high urine cannabinoid levels (up to 210 ng ml^{-1} cross-reacting cannabinoids) without the subject having experienced any pharmacological effect.

These results have important implications for forensic toxicologists who are requested to interpret such findings in cases of drug-related crime. Estimates of 'time since ingestion' derived from a single analytical result, especially from urine analyses, will probably be so broad as to be of little evidential value. Furthermore, it is difficult to state whether the donor of the specimen achieved a state of 'high', let alone to indicate whether the subject was under the influence of cannabis at the time of any alleged offence or accident.

The major active principle of cannabis, viz. Δ^9-THC, persisted in detectable concentrations in plasma for at least 24 h after consumption. This result is in accord with recent work (Ohlsson et al 1980; Cochetto et al 1981; Hollister et al 1981; Perez-Reyes et al 1982) which indicated a poor correlation between the period of pharmacological action and Δ^9-THC plasma levels. From the above results it appears that there is no simple parameter which can be used to interpret plasma cannabinoid levels or give an indication of their likely psychopharmacological effects. We therefore examined other analytical parameters which might be more highly correlated with the period of pharmacological action.

Urine drug/metabolite and metabolite/metabolite ratios have been shown to be useful in determining the 'time since ingestion' of diazepam (McBurney 1981). This form of data manipulation was applied to cannabis consumption, initially by examining the plasma glucuronide/free acid ratio. (The glucuronide is stable in plasma unlike in urine.) The value for this parameter rose during the first 6–10 h after consumption, then remained approximately constant. Although all four subjects gave an initial glucuronide/free acid ratio of <1, there was marked inter-individual variation 9 h after ingestion when the value of the ratio ranged from 2–7. In those rare instances where two blood samples have been obtained from a subject, this ratio could be useful since a higher glucuronide/free acid ratio in the second sample could suggest recent consumption of cannabis, i.e. 1–10 h previously, a period that closely matches the period of pharmacological action.

A more useful function was found in the total metabolite/Δ^9-THC ratio. As expected, the numerical value of this parameter increased throughout the experimental period. However, it was relatively low and constant during the first 4 h after consumption (Fig. 3), overlapping the period of most pronounced pharmacological action, and rose rapidly thereafter. A total metabolite/Δ^9-THC the ratio of <20 may therefore indicate recent oral consumption of cannabis.

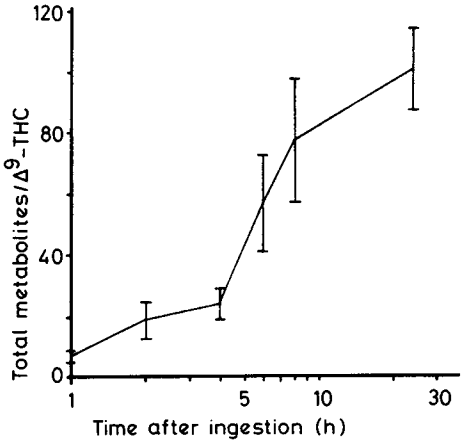

FIG. 3. Variation in the [total metabolite]/[Δ^9-THC] ratio with time after oral ingestion, mean ± s.e.m. for four subjects. Concns measured as in Fig. 1.

Further work is obviously necessary to validate this type of approach, especially by extending the number of subjects used and examining cannabis smoking where the pharmacokinetics may be significantly different. If subsequently proved valid, it may

still be limited by the effects of multiple dosing with cannabis which will tend to produce a steady state total metabolite/Δ^9-THC ratio >20. A similar form of data manipulation using the Δ^9-THC-11-oic acid/Δ^9-THC ratio has been recently shown to give similar results, following administration by smoking (Hanson et al 1983).

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