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Urinary excretion profiles of 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol and 11-hydroxy-Δ^9-THC: cannabinoid metabolites to creatinine ratio study IV

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Abstract

The objective of this study was to compare urinary excretion patterns of two cannabinoid metabolites in subjects with a history of chronic marijuana use. The first metabolite analyzed was nor-9-carboxy-Δ^9-tetrahydrocannabinol (Δ^9-THC-COOH), the major urinary cannabinoid metabolite that is pharmacologically inactive. The second metabolite 11-OH-Δ^9-THC is an active cannabinoid metabolite and is not routinely measured. Urine specimens were collected from four subjects on 12–20 occasions $\geq$96 h apart in an uncontrolled clinical setting. Creatinine was analyzed in each urine specimen by the colorimetric modified Jaffé reaction on a SYVA 30R biochemical analyzer. All urine specimens analyzed for 11-OH-Δ^9-THC had screened positive for cannabinoids with the EMIT II Plus cannabinoids assay (cut-off 50 ng/mL) on a SYVA 30R analyzer and submitted for Δ^9-THC-COOH confirmation by GC–MS (cut-off concentration 15 ng/mL). Eleven-OH-Δ^9-THC was measured by GC–MS with a cut-off concentration of 3 ng/mL. Both GC–MS methods for cannabinoid metabolites used deuterated internal standards for quantitative analysis. The mean (range) of urinary Δ^9-THC-COOH concentration was 1153 ng/mL (78.7–2634) with a cut-off of 15 ng/mL. The mean (range) of Δ^9-THC-COOH/creatinine ratios (ng/mL Δ^9-THC-COOH/mmol/L creatinine) was 84.1 (8.1–122.1). The mean (range) urinary of 11-OH-Δ^9-THC concentration was 387.6 ng/mL (11.9–783) with a cut-off of 3 ng/mL, and the mean (range) of 11-OH-Δ^9-THC/creatinine ratio (ng/mL 11-OH-Δ^9-THC/mmol/L creatinine) was 29.7 (1.2–40.7). Of the 63 urine specimens submitted for Δ^9-THC-COOH confirmation by GC–MS, 59/63 urine specimens (94%) were positive for Δ^9-THC-COOH and 51/63 (81%) were positive for 11-OH-Δ^9-THC. Overall, the concentrations of 11-OH-Δ^9-THC in urine specimens collected $\geq$96 h apart were lower than Δ^9-THC-COOH concentrations in 50/51 of the urine specimens in this population. Further urinary cannabinoid excretion studies are needed to assess whether 11-OH-Δ^9-THC analyses have a role when assessing previous marijuana or hashish use in chronic users whose urine specimens remain positive for Δ^9-THC-COOH for an extended period of time after last drug use.

Keywords: Drugs of abuse; Cannabinoid metabolites; Last drug use; GC–MS

1. Introduction

Individuals with a chronic history of substance abuse are required to submit to random urine drug testing in an assessment program for child welfare agencies in the province of Nova Scotia, Canada [1,2]. The average number of urine specimens collected from these individuals is 25–30 over several months. All urine specimens are screened for cannabinoids with the EMIT® II Plus assay with a cut-off value of 50 ng/mL. Each presumptive positive cannabinoid specimen [3,4] was submitted for confirmation testing for 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol

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(Δ^9-THC-COOH) by GC–MS in the selected ion-monitoring (SIM) mode (confirmation cut-off value of 15 ng/mL). All urine specimens were analyzed for creatinine as an index of urine dilution or potential adulteration by dilution [5] with a cut-off concentration of 2.2 mmol/L.

Many individuals in this program consistently tested positive for cannabinoids over a period of 3–6 months. Program staff wanted to determine whether positive cannabinoid findings were due to ongoing drug use or due to cannabinoid metabolite excretion (carry-over) from marijuana use [6] several days or weeks earlier. In the forensic and clinical context of substance abuse monitoring and treatment, it is often important to evaluate whether positive cannabinoid results are due to new drug use or residual metabolite excretion.

Recent studies on cannabinoid metabolite excretion times [7,8] in human volunteers in controlled settings have been reported where access to marijuana or other drugs outside the study setting is impossible. Urine specimens were screened by a commercially available immunoassay at a screening cut-off value of 50 ng/mL and a confirmation cut-off value of 15 ng/mL. These studies demonstrated that occasional users of marijuana had positive urine specimens for 72–96 h after receiving a standard dose of marijuana. In heavy users of marijuana, urine specimens remained positive for cannabinoids for 7–10 days after last drug use. Therefore, it is very difficult to predict when an individual was last exposed to marijuana or hashish based on the urinary excretion of total cannabinoids using an immunoassay screening test or quantitative Δ^9-THC-COOH analysis by GC–MS in single urine specimens.

Huestis and Cone [9] reported on urinary Δ^9-THC-COOH to creatinine ratio as a means of predicting new marijuana use. They studied marijuana metabolite excretion profiles in six male subjects who had smoked marijuana cigarettes in a controlled clinical setting. In urine specimens collected >24 h apart, Δ^9-THC-COOH to creatinine ratios (Δ^9-THC-COOH to creatinine) were assessed to determine optimal criteria for differentiating new marijuana use from residual metabolite excretion still being excreted in the urine from earlier marijuana use. The best accuracy for prediction of new marijuana use was achieved with a normalized THC-COOH/creatinine ratio $\geq$0.5 compared to the previous specimen ratio.

Manno et al. [10], Fraser and Worth [11,12] applied the Huestis and Cone [9] formula to predict new use of marijuana in chronic marijuana/hashish users. They recommended that the Huestis ratio is best for clinical settings because of the lower false negative rate. In their experience, serial analyses for Δ^9-THC-COOH in urine specimens of chronic marijuana users (even when urine specimens were collected >48–96 h apart) did not allow one to objectively differentiate residual cannabinoid metabolite excretion from new drug use.

Manno et al. [13] recently reported on marijuana use by analysis in plasma and urine of the active drug in marijuana (Δ^9-tetrahydrocannabinol) and several metabolites including Δ^9-THC-COOH and 11-OH-Δ^9-THC in human volunteers. These investigators wanted to determine if one could establish a time of last drug use based on the absolute or relative amounts of these metabolites in biological fluids. They concluded that urinary concentrations of Δ^9-tetrahydrocannabinol (the psychoactive THC compound) >1.5 ng/mL suggested marijuana use within an 8-h time window. They indicated that 11-OH-Δ^9-THC declined more gradually than the parent substance Δ^9-tetrahydrocannabinol. These investigators concluded that quantitation of the major metabolite (Δ^9-THC-COOH) cannot accurately predict the time of last marijuana use or suggest any relationship between urine drug concentrations and psychomotor performance.

The objective of this study was to compare urinary excretion patterns of both Δ^9-THC-COOH and 11-OH-Δ^9-THC in four subjects with a history of chronic marijuana use over time. Urine specimens were collected on 12–20 occasions from each subject in an uncontrolled clinical setting. The evaluation was based on the analysis of Δ^9-THC-COOH and 11-OH-Δ^9-THC by GC–MS and determining drug metabolite/creatinine ratios for each urine specimen. The minimum time period between each urine specimen included in the study was $\geq$96 h.

2. Materials and methods

Urine specimens were screened for cannabinoids using the Dade Behring EMIT II Plus assay (Dade Behring Canada, Mississauga, ON, Canada) on a SYVA 30R clinical analyzer with a screening cut-off value of 50 ng/mL. All presumptive positive specimens were confirmed by GC–MS for Δ^9-THC-COOH (cut-off value was 15 ng/mL) [4] in the selected ionization monitoring mode using the deuterated internal standard 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol-D_3. The confirmation of 11-OH-Δ^9-THC was also measured quantitatively by GC–MS using the deuterated internal standard 11-OH-Δ^9-THC-D_3 as reported by Manno et al. [13].

Drug standards and deuterated internal standards were purchased from Cerilliant, Round Rock, TX, USA. Creatinine was analyzed on the SYVA 30R clinical analyzer by the modified Jaffé colorimetric reaction using reagents purchased from Beckman Canada, Mississauga, ON, Canada. Criteria for inclusion of subjects and results were as follows: (1) the minimum number of positive cannabinoid results per individual was 12 results by immunoassay over a minimum 30-day period; (2) the minimum time interval between specimen collections was $\geq$96 h; (3) all dilute urine specimens (creatinine cut-off value of 2.2 mmol/L) were included; and (4) all screening positive specimens were submitted for GC–MS analysis of Δ^9-THC-COOH and 11-OH-Δ^9-THC with confirmation cut-off values of 15 and 3 ng/mL, respectively.

3. Results

A representative example of serial cannabinoid metabolites to creatinine ratios is found in Case 1 (Fig. 1). In 20 urine specimens from Case 1, the mean Δ^9-THC-COOH concentration was 797 ng/mL and the mean Δ^9-THC-COOH/creatinine ratio was 89.6 ng/mL per mmol/L of creatinine. The mean 11-OH-Δ^9-THC concentration was 421 ng/mL and the mean 11-OH-Δ^9-THC/creatinine ratio was 40.7 ng/mL per mmol/L of creatinine. All 20 specimens confirmed positive for Δ^9-THC-COOH above 15 ng/mL and 11-OH-Δ^9-THC concentrations above 3 ng/mL. In Case 2 (Fig. 2), there were 12 serial urine collections where the mean Δ^9-THC-COOH concentration was 79 ng/mL and the mean 11-OH-Δ^9-THC concentration was 14.9 ng/mL. In this individual, the mean Δ^9-THC-COOH/creatinine ratio was 8.1 ng/mL per mmol/L of creatinine and the mean 11-OH-Δ^9-THC/creatinine ratio was 1.2 ng/mL per mmol/L of creatinine. Ten of the 12 urine specimens were positive for Δ^9-THC-COOH above 15 ng/mL, and 7 of the 12 specimens had 11-OH-Δ^9-THC concentrations above the 3-ng/mL cut-off value. Cases 3 and 4 are presented in Fig. 3, and also had the highest mean concentrations of Δ^9-THC-COOH. There were 15 urine specimens collected from Case 3 and 16 specimens from Case 4. The mean Δ^9-THC-COOH concentration was 2634 ng/mL and the mean Δ^9-THC-COOH/creatinine ratio was 122.1 ng/mL per mmol/L of creatinine (Case 3). The mean 11-OH-Δ^9-THC concentration was 783 ng/mL and the mean 11-OH-Δ^9-THC/creatinine ratio was 36.4 ng/mL per mmol/L of creatinine. In this individual, 14 of the 15 urine specimens were positive for Δ^9-THC-COOH above 15 ng/mL and 9 of the 15 were positive for 11-OH-Δ^9-THC concentration above 3 ng/mL.

The mean Δ^9-THC-COOH concentration was 1103 ng/mL and the mean Δ^9-THC-COOH/creatinine ratio was 116.7 ng/mL per mmol/L of creatinine (Case 4). The mean 11-OH-Δ^9-THC concentration was 334.4 ng/mL and the mean 11-OH-Δ^9-THC/creatinine ratio was 40.6 ng/mL per mmol/L of creatinine. In this individual, 15 of the 16 urine specimens were positive for Δ^9-THC-COOH above 15 ng/mL and 15 of the 16 specimens were positive for 11-OH-Δ^9-THC concentrations above 3 ng/mL.

A composite summary of all the study results is found in Table 1.

4. Discussion

Huestis and Cone [9] reported using urinary cannabinoid (Δ^9-THC-COOH) to creatinine ratios as one approach to help predict new marijuana or hashish use in a series of human volunteers in a controlled clinical research setting. In >1800 urine pairs, the overall prediction accuracy was 85.4% with a 5.6% false positive rate and 7.4% false negative rate when using the 0.5 ratio. In an earlier study by Manno et al. [10], the recommended ratio for predicting new marijuana use was $\geq$1.5 or 150% of the previous immunoassay response (EMIT dau cannabinoids screen-

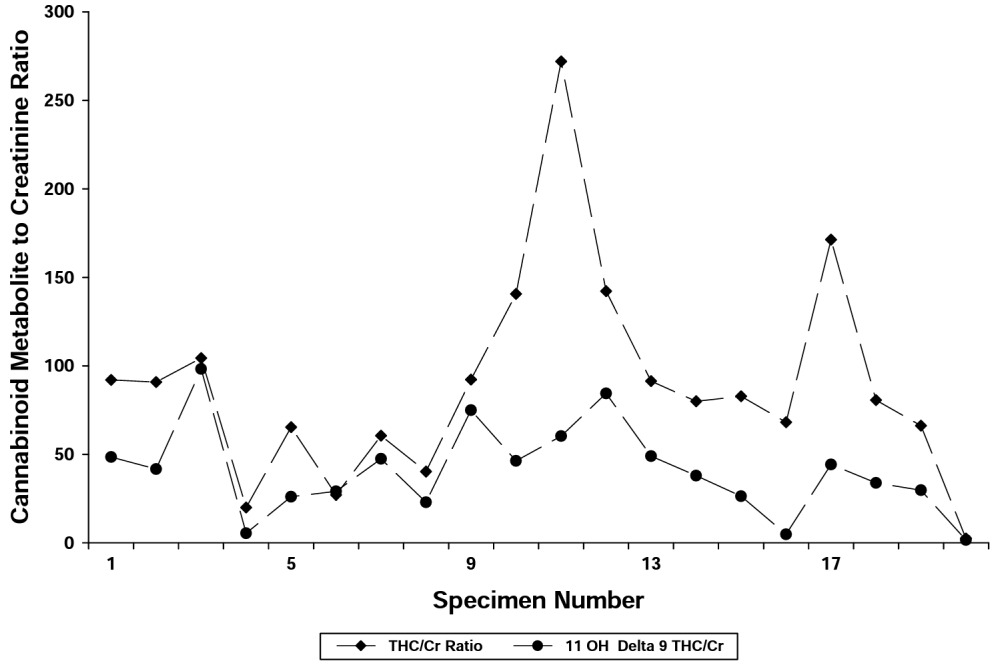

Fig. 1. Cannabinoid metabolites series: case 1.

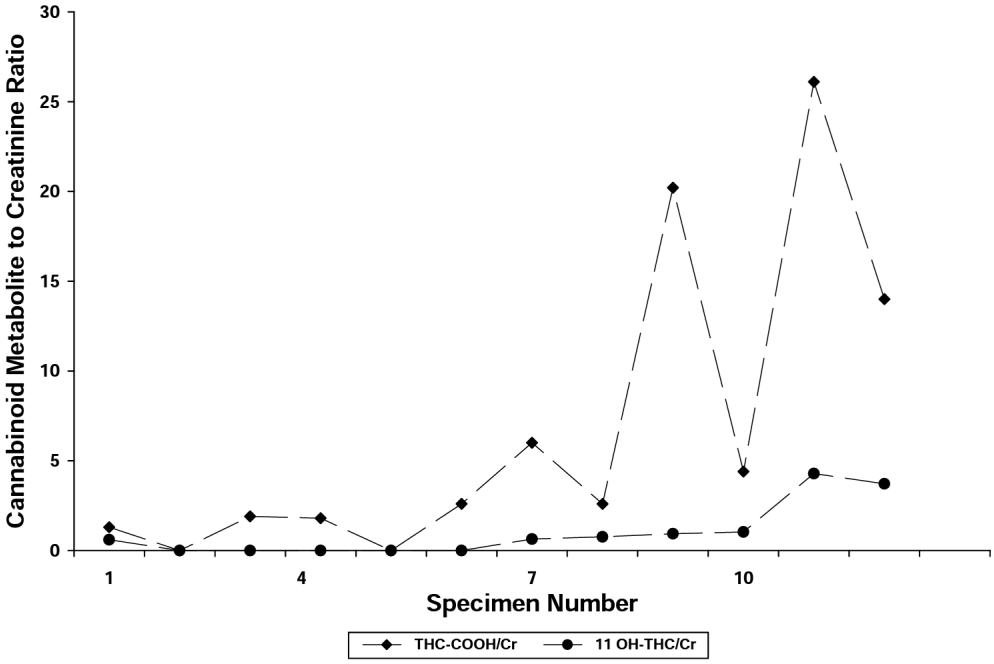

Fig. 2. Cannabinoid metabolites series: case 2.

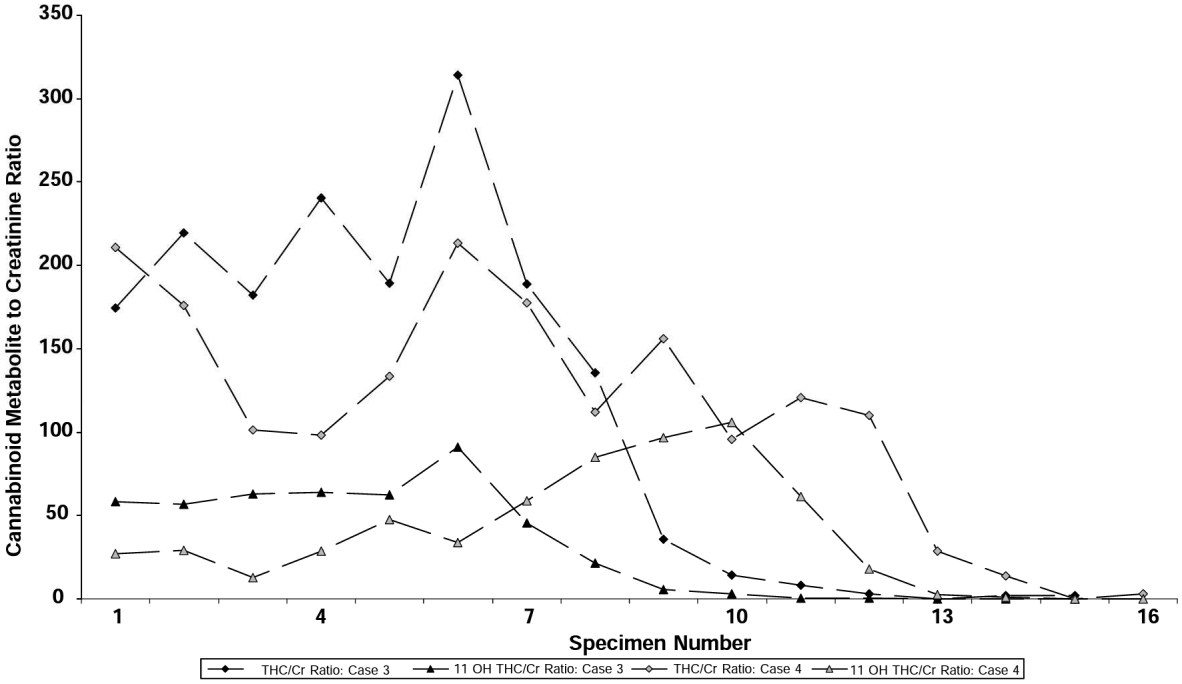

Fig. 3. Cannabinoid metabolites series: cases 3 and 4.

Table 1
Overall summary of sequentially paired urine specimens: cannabinoid metabolites to creatinine ratio study

Number of subjects	4
Number of urine specimens (≥96 h between collections)	63
Mean Δ^9-THC-COOH (range)	1153 ng/mL (78–2634 ng/mL)
Mean Δ^9-THC-COOH/creatinine ratio (range)	84.1 ng/mL Δ^9-THC-COOH /mmol/L creatinine (8.1–122.1 ng/mL per mmol/L creatinine)
Mean 11-OH-Δ^9-THC (range)	387 ng/mL (11.9–783 ng/mL)
Mean 11-OH- Δ^9-THC/creatinine ratio (range)	29.7 ng/mL per mmol/L creatinine (1.2–40.7 ng/mL per mmo/L creatinine)
Δ^9-THC-COOH positive rate	59/63 (94%)
11-OH-Δ^9-THC positive rate	51/63 (81%)

ing/creatinine ratio). Huestis and Cone [9] evaluated the 150% ratio and determined that the Manno ratio was 74.2% accurate with a 24% false negative rate and a 0.1% false positive rate. It is difficult to make a direct comparison between the Manno et al. [10] and Huestis and Cone[9] studies, since the Manno data is based on immunoassay cross-reactivity towards all cannabinoid metabolites in urine whereas Huestis measured specifically the major urinary metabolite of marijuana or hashish. Since the Manno et al. [10] report in 1984, many forensic toxicologists use the 150% or 1.5 ratio to help determine the frequency of new marijuana use. The low false positive rate of the Manno ratio (0.1%), as reported by Huestis and Cone [9] makes this ratio more acceptable in the legal setting compared to the 5.6% false positive rate when using the 0.5 ratio.

In the subjects monitored by social service agencies in this study [1,2], caseworkers and legal counsel often wanted to assess whether individual clients were occasional or regular marijuana users. For example, when an individual had four positive cannabinoid results by GC–MS within 10–12 days, were these findings due to drug use before the testing started or did they indicate on-going marijuana use during the testing period? Calculating the Δ^9-THC-COOH to creatinine ratio and plotting the results in a graph allows one to consider the possibility of new drug use while testing is ongoing. This approach was most helpful when assessing individuals whose urine specimens virtually always confirmed positive for cannabinoids. The limitations of using the Δ^9-THC-COOH/creatinine ratios include the fact that Δ^9-THC-COOH is a slowly excreting metabolite and several confirmed positive results are often found after marijuana use had ceased.

Another approach reported by Manno et al. [13] was analysis of other cannabinoid metabolites that have shorter elimination half-lives than Δ^9-THC-COOH. In that study, GC–MS analysis was included for Δ^9-THC-COOH and for the more rapidly eliminated metabolite 11-OH-Δ^9-THC. As shown in Figs. 1–3, the concentrations of Δ^9-THC-COOH were consistently greater than 11-OH-Δ^9-THC values. The overall confirmation positive rates were almost identical in these subjects while using a 3-ng/mL cut-off value for 11-OH-Δ^9-THC. In the Manno study, eight individuals were monitored in urine for 11-OH-Δ^9-THC up to 8 h after smoking a marijuana cigarette (1.77 or 3.58% THC). Eight hours after the single THC cigarette, the mean urinary 11-OH-Δ^9-THC concentrations had lowered to a mean of 9.7 ng/mL (low THC dose) to 13.9 ng/mL (higher THC dose). Manno also stated that 11-OH-Δ^9-THC peaked in plasma 3 h after smoking and declined at a rate of 7.8 and 12.7 ng/mL/h for the low and high THC doses. The results indicate that Case 3 was a heavy user of marijuana or hashish but had reduced THC use over time. In the last six time points (10–15) in the figures, five of the six specimens were positive for 11-OH-Δ^9-THC and four of the six positive for 11-OH-Δ^9-THC for Case 3. Despite faster 11-OH-Δ^9-THC excretion compared to Δ^9-THC-COOH in single dose THC studies [13], one cannot conclude that 11-OH-Δ^9-THC provides any better indication of stopping marijuana use based on our results. One would anticipate that the positive rate for Δ^9-THC-COOH above 15 ng/mL would be higher than the positive rate for 11-OH-Δ^9-THC in situations where last drug use was ≥96 h, previously. Unfortunately, our study was uncontrolled, so one cannot assume that these individuals did not continue consuming marijuana or hashish between collections. Further studies will include analysis of unchanged THC in urine specimens from chronic marijuana/hashish users.

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