Urinary excretion profiles of 11-nor-9-carboxy-$\Delta^9$-tetrahydrocannabinol Study III. A $\Delta^9$-THC-COOH to creatinine ratio study

Albert D. Fraser a,b,*, David Worth a

aDepartment of Pathology & Laboratory Medicine, Queen Elizabeth II Health Sciences Centre, 1278 Tower Road, Halifax, Nova Scotia, Canada B3H 2Y9
bDalhousie University, Halifax, Nova Scotia, Canada

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Abstract

Huestis and Cone reported in [J. Anal. Toxicol. 22 (1998) 445] that serial monitoring of $\Delta^9$-THC-COOH/creatinine ratios in paired urine specimens collected at least 24 h apart could differentiate new drug use from residual $\Delta^9$-THC-COOH excretion following acute marijuana use in a controlled setting. The best accuracy (85.4%) for predicting new marijuana use was for a $\Delta^9$-THC-COOH/creatinine ratio $\div C^{21}0.5$ (dividing the $\Delta^9$-THC-COOH/creatinine ratio of specimen no. 2 by the specimen no. 1 ratio).

In previous studies in this laboratory [J. Anal. Toxicol. 23 (1999) 531 and Forensic Sci. Int. 133 (2003) 26], urine specimens were collected from chronic marijuana users $\div C^{21}24$ h or $\div C^{21}48$ h apart in an uncontrolled setting. Subjects with a history of chronic marijuana use were screened for cannabinoids with the EMIT II Plus cannabinoids assay (cut-off 50 ng/ml) followed by confirmation for $\Delta^9$-THC-COOH by GC–MS (cut-off 15 ng/ml). Creatinine was analyzed as an index of dilution. The objective of the present study was to evaluate whether creatinine corrected specimens could differentiate new marijuana or hashish use from the excretion of residual $\Delta^9$-THC-COOH in chronic marijuana users based on the Huestis 0.5 ratio. Urine specimens ($N = 376$) were collected from 29 individuals $\div C^{96}$ h between urine collections. The mean urinary $\Delta^9$-THC-COOH concentration was 464.4 ng/ml, mean $\Delta^9$-THC-COOH/creatinine ratio (ng/(ml $\Delta^9$-THC-COOH mmol l creatinine)) was 36.8 and the overall mean $\Delta^9$-THC-COOH/creatinine ratio of specimen 2/mean $\Delta^9$-THC-COOH/creatinine ratio of specimen 1 was 1.37. The Huestis ratio calculation indicated new drug use in 83% of all sequentially paired urine specimens. The data were sub-divided into three groups (Groups A–C) based on mean $\Delta^9$-THC-COOH/creatinine values. Interindividual mean $\Delta^9$-THC-COOH/creatinine values ranged from 4.7 to 13.4 in Group A where 80% of paired specimens indicated new drug use ($N = 10$) and 20.4–39.6 in Group B where 83.6% of paired specimens indicated new drug use ($N = 7$). Individual mean $\Delta^9$-THC-COOH/creatinine values ranged from 44.2 to 120.2 in Group C where 84.5% of paired urine specimens indicated new marijuana use ($N = 12$). Correcting $\Delta^9$-THC-COOH excretion for urinary dilution and comparing $\Delta^9$-THC-COOH/creatinine concentration ratios of sequentially paired specimens (collected $\geq 96$ h apart) may provide an objective indicator of ongoing marijuana or hashish use in this population.

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1. Introduction

Individuals with a chronic history of substance abuse are required to submit to random urine drug testing when ordered by the family court. This program is part of an assessment program for child welfare agencies in the
province of Nova Scotia, Canada [1,2]. The average number of urine specimens collected from each client is 25–30 specimens collected 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] is submitted for confirmation testing for 11-nor-9-carboxy-Δ⁹-tetrahydrocannabinol by GC–MS in the selected ion-monitoring (SIM) mode (confirmation cut-off value of 15 ng/ml). All urine specimens are 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 drug-testing program were consistently positive for cannabinoids over a period of 3–6 months or longer. Program staff wanted to determine whether these positive cannabinoid findings were due to frequent drug use or due to cannabinoid metabolite excretion (carry-over) from marijuana use [6] several days or weeks earlier. In the clinical context of substance abuse monitoring and treatment, it is also important to evaluate whether positive cannabinoid results by urine drug testing are due to new drug use or residual metabolite excretion. Individuals given short-term approved absences from psychiatric units may have access to drugs of abuse while outside the centre.

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. In these studies, urine specimens were screened by a commercially available immunoassay at a screening cut-off value of 50 ng/ml followed by confirmation by GC–MS for the major cannabinoid metabolite in urine (Δ⁹-THC-COOH) with a confirmation cut-off 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, individuals remained positive for cannabinoids in urine for 7–10 days after last drug use in the study. 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 Δ⁹-THC-COOH analysis by GC–MS in single urine specimens.

Huestis reported on urinary Δ⁹-THC-COOH to creatinine ratio as a means of predicting new marijuana use [9]. They studied marijuana metabolite excretion profiles in six male subjects who had smoked marijuana cigarettes of known strength in a controlled clinical setting. In urine specimens collected at least 24 h apart, different Δ⁹-THC-COOH to creatinine ratios (Δ⁹-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. They concluded that the best accuracy for prediction of new marijuana use was achieved with a normalized THC-COOH/creatinine ratio ≥0.5 compared to the previous specimen ratio. For testing to be consistent with new marijuana use, the second Δ⁹-THC-COOH to creatinine ratio must be >50% of the previous Δ⁹-THC-COOH/creatinine ratio when collected at least 24 h later.

Manno et al. [10] predicted new marijuana use by using a much higher ratio (≥1.5 or 150%) of the previous total cannabinoids ratio by EMIT® II Plus screening/creatinine ratio. Huestis and Cone [9] reported that the Manno ratio was 74.2% accurate with a 24% false negative rate and a 0.1% false positive rate.

Fraser and Worth [11,12] applied the Huestis and Cone formula [9] and Manno et al. [10] formula to predict new use of marijuana. They recommended that the Huestis ratio is best for clinical settings because of the lower false negative rate. The Manno formula was recommended for forensic analyses because it has a lower false positive rate (0.1%) as first reported by Huestis and Cone in 1998 [9]. The Manno formula conducted in the early 1980’s was based on immunoassay rates only and did not include quantitative analysis of the major urinary cannabinoid metabolite (Δ⁹-THC-COOH).

Manno et al. reported [13] on marijuana use by analysis in plasma and urine of the active drug in marijuana (Δ⁹-tetrahydrocannabinol) and several metabolites including 11-hydroxy-Δ⁹-tetrahydrocannabinol and 11-nor-Δ⁹-tetrahydrocannabinol-9-carboxylic acid. These cannabinoid metabolites were analyzed in plasma and urine in human volunteers. The 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 Δ⁹-tetrahydrocannabinol (the psychoactive THC compound) >1.5 ng/ml suggested marijuana use within an 8 h time window. They indicated that 11-hydroxy-Δ⁹-tetrahydrocannabinol declined more gradually than Δ⁹-tetrahydrocannabinol. Manno concluded that quantitation of 11-nor-Δ⁹-tetrahydrocannabinol-9-carboxylic acid cannot accurately predict time of last marijuana use or suggest any relationship between urine drug concentrations and psychomotor performance.

The objective of this study was to apply the Huestis criteria for new marijuana use when monitoring a series of positive cannabinoids results in 29 chronic marijuana or hashish users in an uncontrolled setting. The analysis was based on the determination of Δ⁹-THC-COOH to creatinine ratios by GC–MS and determining drug metabolite/creatinine ratios for each specimen. The minimum time period between each urine specimen included in the study was ≥96 h (neither ≥24 nor 48 h) as used in earlier studies [11,12] in this centre.

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 in the selected ionization-monitoring mode using the deuterated internal standard 11-nor-9-carboxy-Δ⁹-tetrahydrocannabinol-D₃. The confirmation cut-off value was 15 ng/ml [4]. Creatinine was also 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 serially positive cannabinoid results per individual was 10 confirmed positive results for Δ⁹-THC-COOH over a minimum 30 day period; (2) the minimum time interval between specimen collections was ≥96 h; (3) all administratively defined dilute urine specimens (creatinine <2.2 mmol/l) were included; and (4) all Δ⁹-THC-COOH positive specimens by GC-MS following a negative immunoassay cannabinoid result were considered positive irrespective of the drug metabolite to creatinine ratio. This was because one cannot calculate a ratio when one specimen is negative. The data were divided into three groups (Groups A–C) based on the average cannabinoid metabolite (Δ⁹-THC-COOH) concentration to creatinine ratio for the entire series of specimens for each individual. Interindividual Δ⁹-THC-COOH/creatinine mean values were <15 in Group A (range: 4.7–13.4). In Group B, mean Δ⁹-THC-COOH/creatinine values from 15.1 to 40 were included (actual mean values ranged from 20.4 to 39.6). In Group C, each individual mean Δ⁹-THC-COOH/creatinine values were all >40.0 where the actual mean ratios ranged from 44.2 to 120.2. Urine specimen data points negative for cannabinoids in the screening assay are plotted as zero ratios in figures.

3. Results

In Fig. 1, mean Δ⁹-THC-COOH/creatinine ratios found for all individuals in Groups A–C are presented. There were 10 subjects (91 paired urine specimens) in Group A with mean Δ⁹-THC-COOH/creatinine ratios <15 ng/(ml mmol l). The Group A mean urinary Δ⁹-THC-COOH concentration was 114.5 ng/ml and the mean Δ⁹-THC-COOH/creatinine ratio was 8.7. The Huestis and Cone ratio [9] indicated new marijuana use in 80% of the paired specimens (range: 57–100%). An example of serial Δ⁹-THC-COOH/creatinine ratios from Group A is found in Fig. 2 and a graph of Δ⁹-THC-COOH/creatinine ratio specimen no. 2/Δ⁹-THC-COOH/creatinine ratio specimen no. 1 is presented as shown in Fig. 3. The line connecting each point in Fig. 3 is broken after the three negative results (specimens 2, 12 and 32), since one requires two values greater than zero to calculate metabolite/creatinine of specimen no. 2/specimen no. 1.

For Group B, there were seven subjects (109 paired urine specimens) that met the inclusion criteria (mean Δ⁹-THC-COOH/creatinine ratio: 15.1–40.0 ng/(ml mmol l)). The Group B mean urinary Δ⁹-THC-COOH concentration was 555.5 ng/ml and mean Δ⁹-THC-COOH/creatinine ratio was 27.8. The Huestis and Cone ratio [9] indicated new marijuana use in 83.6% of the paired specimens (range: 73–100%). A representative example from Group B is found in Fig. 4.
There were 12 subjects (177 paired urine specimens) that met the inclusion criteria (mean $\Delta^9$-THC-COOH/creatinine ratio >40.0 ng/(ml mmol l)) in Group C. The Group C mean urinary $\Delta^9$-THC-COOH concentration was 798.3 ng/ml. The mean $\Delta^9$-THC-COOH/creatinine ratio was 65.4. The Huestis and Cone ratio [9] indicated new marijuana use in 84.5% of the paired specimens (range: 66–100%). A representative example of serial $\Delta^9$-THC-COOH/creatinine ratios

Fig. 2. Group A example.

Fig. 3. $\Delta^9$-THC-COOH/creatinine specimen no. 2/$\Delta^9$-THC-COOH/creatinine specimen no. 1 (Group A example).
Fig. 4. Group B example.

Fig. 5. Group C example.
Fig. 6. $\Delta^9$-THC-COOH/creatinine specimen no. 2/$\Delta^9$-THC-COOH/creatinine specimen no. 1 (Group C example). In each figure, the smaller triangles indicate new drug use, the bigger triangles indicate no new drug use, and the solid circles indicate a cannabinoid screening negative specimen.

Table 1
Overall summary of sequentially paired urine specimens in $\Delta^9$-THC-COOH to creatinine ratio study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of paired urine specimens</td>
<td>376</td>
</tr>
<tr>
<td>Mean $\Delta^9$-THC-COOH (ng/ml)</td>
<td>464.4</td>
</tr>
<tr>
<td>Mean $\Delta^9$-THC-COOH/creatinine ratio (ng/ml $\Delta^9$-THC-COOH mmol l creatinine)</td>
<td>36.8</td>
</tr>
<tr>
<td>Mean $\Delta^9$-THC-COOH/creatinine ratio specimen no. 2/specimen no. 1</td>
<td>1.37</td>
</tr>
<tr>
<td>Mean Huestis ratio indicating new marijuana use (%)</td>
<td>83</td>
</tr>
<tr>
<td>Number of specimen pairs collected ≥96 h apart (%)</td>
<td>100</td>
</tr>
<tr>
<td>Number of subjects</td>
<td>29</td>
</tr>
</tbody>
</table>

4. Discussion

Huestis and Cone [9] reported on the use of urinary cannabinoids ($\Delta^9$-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 ≥1.5 or 150% of the previous total cannabinoids ratio total immunoassay response EMIT® dau screening/creatinine ratio. Huestis and Cone [9] evaluated the 150% ratio and determined that the Manno ratio was 74.2% accurate with a 0.1% false positive rate and 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. report in 1984 [10], 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.

The current study required urine pairs to be collected ≥96 h apart compared to >24 h in the Huestis and Cone study [9] or ≥24 or 48 h in earlier studies by Fraser and
Worth [11,12]. The time period between specimen collections (from 24 or 48 to 96 h) may lower the false positive rate. The false positive rate for this study could not be established because it was an uncontrolled study. In Figs. 3 and 6, graphs of \( \Delta^9 \)-THC-COOH/creatinine ratio specimen no. 2/\( \Delta^9 \)-THC-COOH/creatinine ratio specimen no. 1 show the distribution of ratios over time in two individuals. In Fig. 3 (Group A example), the ratio specimen no. 2/specimen no. 1 ranged from 0.2 to 2.63 with a mean value of 1.1. In Fig. 6 (Group C example) the ratio specimen no. 2/specimen no. 1 ranged from 0.2 to 3.45 with a mean value of 1.37. In Fig. 3, of 39 values, 30/39 (77%) had a ratio >0.5 but only 9/39 (23%) had a specimen no. 2/specimen no. 1 ratio >1.5. In Fig. 6 from Group C, of 35 specimen pairs, 30/35 (86%) had a specimen no. 2/specimen no. 1 ratio >0.5, whereas only 7/35 (20%) had a specimen no. 2/specimen no. 1 ratio greater than 1.5 or 150%. All ratio of specimen no. 2/specimen no. 1 values >0.5 are positive for new marijuana use by the Huestis criteria whereas only those with ratio of specimen no. 2/specimen no. 1 values >1.5 or 150% are positive for new marijuana use by the Manno criteria.

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? Calculating the \( \Delta^9 \)-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. The ratio calculation also allows one to discuss the time to a negative urine test for cannabinoids using current screening methods with GC–MS confirmation [7,8]. The ratio calculation may provide helpful information compared to merely stating that the results are consistent with marijuana use sometime in the past. This approach was most helpful when assessing individuals whose urine specimens virtually always confirmed positive for cannabinoids. The limitations of using the \( \Delta^9 \)-THC-COOH/creatinine ratios are that one may reporting a higher incidence of new marijuana use with the Huestis criteria. Secondly, use of their approach requires several consecutive urine collections in a short time period when assessing the possibility of new marijuana use. At the present time, the Huestis ratio calculation based on screening results only is not recommended for forensic purposes without confirmatory analysis of cannabinoids by an alternate method such as GC–MS. One would expect a lower false positive rate with the 0.5 ratio in this study since all urine specimens were collected ≥96 h apart. The possibility of false positives limits the application of the Huestis criteria in forensic settings but not in most clinical situations.

Further studies are needed to determine the false positive rate for new drug use when the time interval between urine collections is 96 h or longer. One approach to answering this question is including quantitative analysis of other cannabinoid metabolites in urine with shorter elimination excretion times than \( \Delta^9 \)-THC-COOH.

References