Drug and Alcohol Dependence 105 (2009) 24–32

Contents lists available at ScienceDirect

Drug and Alcohol Dependence

journal homepage: www.elsevier.com/locate/drugalcdep

Extended urinary Δ9-tetrahydrocannabinol excretion in chronic cannabis users precludes use as a biomarker of new drug exposure

Ross H. Lowe [a], Tsadik T. Abraham [a], William D. Darwin [a], Ronald Herning [b], Jean Lud Cadet [b], Marilyn A. Huestis [a,*]

[a] Chemistry and Drug Metabolism, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Biomedical Research Center, 251 Bayview Boulevard, Suite 05A721, Baltimore, MD 21224 USA
[b] Molecular Neuropsychiatry, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Biomedical Research Center, 251 Bayview Boulevard, Suite 07A721A, Baltimore, MD 21224 USA

ARTICLE INFO

Article history:
Received 29 December 2008
Received in revised form 22 May 2009
Accepted 27 May 2009
Available online 23 July 2009

Keywords:
Tetrahydrocannabinol
Urine
Cannabinoids
Cannabis
THC
Chronic cannabis use

ABSTRACT

Background: Generally, urinary 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THCCOOH) after alkaline hydrolysis is monitored to detect cannabis exposure, although last use may have been weeks prior in chronic cannabis users. Δ9-Tetrahydrocannabinol (THC) and 11-hydroxy-THC (11-OH-THC) concentrations in urine following *Escherichia coli* β-glucuronidase hydrolysis were proposed as biomarkers of recent (within 8 h) cannabis use.
Objective: To test the validity of THC and 11-OH-THC in urine as indicators of recent cannabis use.
Methods: Monitor urinary cannabinoid excretion in 33 chronic cannabis smokers who resided on a secure research unit under 24 h continuous medical surveillance. All urine specimens were collected individually ad libidum for up to 30 days, were hydrolyzed with a tandem *E. coli* β-glucuronidase/base procedure, and analyzed for THC, 11-OH-THC and THCCOOH by one- and two-dimensional-cryotrap gas chromatography mass spectrometry (2D-GCMS) with limits of quantification of 2.5 ng/mL.
Results: Extended excretion of THC and 11-OH-THC in chronic cannabis users' urine was observed during monitored abstinence; 14 of 33 participants had measurable THC in specimens collected at least 24 h after abstinence initiation. Seven subjects had measurable THC in urine for 3, 3, 4, 7, 7, 12, and 24 days after cannabis cessation. 11-OH-THC and THCCOOH were detectable in urine specimens from one heavy, chronic cannabis user for at least 24 days.
Conclusion: For the first time, extended urinary excretion of THC and 11-OH-THC is documented for at least 24 days, negating their effectiveness as biomarkers of recent cannabis exposure, and substantiating long terminal elimination times for urinary cannabinoids following chronic cannabis smoking.
Published by Elsevier Ireland Ltd.

1. Introduction

Cannabinoid detection times in urine depend upon pharmacological factors (e.g., drug dose, route of administration, duration and frequency of use, smoking topography and individual rates of absorption, metabolism and excretion), and methodological issues including analytes evaluated, matrix, type of hydrolysis, cut-off or threshold used, and sensitivity of the method. The primary psychoactive constituent of cannabis, Δ9-tetrahydrocannabinol (THC), is rapidly absorbed during smoking, and due to its high lipophilicity is widely distributed to adipose tissue, liver, lung, and spleen. Body stores of THC increase with increasing frequency and chronicity of cannabis use. THC is rapidly metabolized to the equally psychoactive 11-hydroxy-THC (11-OH-THC), and to the inactive 11-nor-9-carboxy-THC (THCCOOH) metabolite and its glucuronide and sulfate conjugates (Huestis and Smith, 2005). The slow release of THC from fat back into blood was demonstrated to be the rate-limiting step in cannabinoid elimination from the body (Hunt and Jones, 1980). Thus, THC was shown to have a long terminal elimination half-life in blood from chronic cannabis users (Johansson, 1989).

Urine testing remains the most common means of drug monitoring in the United States. The highest numbers of positive urine drug tests in workplace drug testing are for cannabinoids, achieved by immunoassay screening and THCCOOH quantification in urine after alkaline hydrolysis. Urinary THCCOOH excretion patterns have been extensively studied (Smith-Kielland et al., 1999; Huestis et al., 1995; Johansson et al., 1990; Musshoff and Madea, 2006). After occasional use, Huestis et al. reported THCCOOH concentrations above 15 ng/mL by gas chromatography mass spectrometry (GCMS)

* Corresponding author. Tel.: +1 443 740 2524; fax: +1 443 740 2823.
E-mail address: mhuestis@intra.nida.nih.gov (M.A. Huestis).

0376-8716/$ – see front matter. Published by Elsevier Ireland Ltd.
doi:10.1016/j.drugalcdep.2009.05.027

after alkaline hydrolysis for up to 4 days (Huestis et al., 1996; Huestis and Cone, 1998a). Urinary THCCOOH window of detection ranges from several days in infrequent users (Huestis and Cone, 1998b) to months in frequent users (Ellis et al., 1985; Peat, 1989; Kelly and Jones, 1992; Fraser and Worth, 2003; Johansson and Halldin, 1989). Thus, identification of THCCOOH in urine may not indicate recent cannabis exposure (Dackis et al., 1982; McBurney et al., 1986; Fraser and Worth, 2004).

Huestis and Cone developed and validated a model to differentiate new cannabis use from residual drug excretion in occasional cannabis users based on urine THCCOOH concentrations (Huestis and Cone, 1998b). However, this model may not be accurate in chronic cannabis users during the terminal elimination phase, when THCCOOH concentrations are low, below 20–50 ng/mL. In a search for new biomarkers of recent cannabis use, Kemp et al. showed that THC and 11-OH-THC could be detected in urine after cannabis smoking if *Escherichia coli* (*E. coli*) β-glucuronidase hydrolysis was employed to break ether glucuronide bonds (Kemp et al., 1995a). They conducted a controlled cannabis smoking study quantifying THC and 11-OH-THC concentrations in urine specimens collected up to 8 h after cannabis smoking (Kemp et al., 1995b). The presence of THC or 11-OH-THC in urine was stated to indicate cannabis use within 8 h. In attempting to replicate this finding in our cannabinoid smoking studies, we quickly found that 11-OH-THC could be measured for many days after last smoked THC cigarette. Manno et al. later revised their hypothesis to solely rely on the presence of THC in urine (>1.5 ng/mL) to suggest cannabis use in the previous 8 h (Manno et al., 2001). We believed that this hypothesis required evaluation over a much longer timeframe and also, in individuals who were chronic cannabis users.

Cannabis, the most commonly abused drug world wide, is included in workplace, drug treatment, clinical, military and criminal justice drug testing programs. New drug use may have important consequences for employment, child custody, military status, and imprisonment. In treatment programs, urine drug testing is a deterrent to drug use, and is an effective and objective tool in contingency management programs and for evaluating new behavioral and pharmacotherapy treatments. The ability to differentiate new drug use from residual drug excretion would be valuable for clinicians, toxicologists, employee assistance programs, drug treatment providers, clinical trials of cannabis dependence treatment, anti-doping athletic programs, parole officers, attorneys and judges. The interpretation of cannabinoid urine tests would be greatly improved if new cannabis use could be identified in both occasional and chronic cannabis users.

Recent advances in analytical methods have improved sensitivity, specificity and recovery of cannabinoids in biological fluids and tissues. THC and 11-OH-THC are present in urine as glucuronide conjugates that are only effectively recovered following *E. coli* β-glucuronidase hydrolysis (Kemp et al., 1995a). We showed that to ensure comprehensive hydrolysis of all three cannabinoid conjugates, an initial hydrolysis with *E. coli* β-glucuronidase followed by a second hydrolysis with strong base (tandem hydrolysis), obtained better recovery of THCCOOH (Abraham et al., 2007). In addition, two-dimensional gas chromatography mass spectrometry (2D-GCMS) is a new technique (Lowe et al., 2007) that reduces background matrix interference allowing superior resolution and specificity, and achieving more reliable cannabinoid quantification at low concentrations. 2D-GCMS was utilized in this study to verify cannabinoid detection times.

The objective of the current research was to examine the time course of THC, 11-OH-THC, and THCCOOH elimination in urine in subjects with a history of chronic, heavy cannabis use, and to test the efficacy of THC and 11-OH-THC as urinary biomarkers of recent cannabis exposure. Participants resided on a secure clinical research unit, under 24 h medical surveillance, while participating in two studies of neurocognitive impairment in abstinent chronic heavy cannabis users. Subjects with the longest histories of cannabis smoking and highest THCCOOH concentrations on admission participated in the present cannabinoid urinary excretion study. Each urine specimen was individually collected throughout an abstinence period of up to 30 days and THC, 11-OH-THC, and THCCOOH concentrations were simultaneously quantified by one-dimensional GCMS. Because of the importance and controversial nature of the data, and availability of new analytical technology, THC, 11-OH-THC, and THCCOOH concentrations and times of last detection were verified by repeat analysis by 2D-GCMS, with low 2.5 ng/mL limits of quantification (LOQ) for each analyte. These are the first data on extended urinary THC, 11-OH-THC and THCCOOH excretion and detection times in chronic, heavy cannabis users, and the first to test the hypothesis that THC and 11-OH-THC in urine are biomarkers of recent cannabis use.

2. Methods

2.1. Participants

Two clinical research studies at the Intramural Research Program, National Institute on Drug Abuse investigated neurocognitive impairment and effects of cannabis withdrawal in chronic cannabis users during 30 days of continuously monitored abstinence. Neurocognitive performance, fMRI and cerebral blood flow data from these studies have been previously reported (Bolla et al., 2002, 2005, 2008; Eldreth et al., 2004; Herning et al., 2003, 2005; Matochik et al., 2005). We recruited individuals with the most frequent and chronic self-reported cannabis use from January 2002 until July 2004 from these studies to participate in this secondary urinary cannabinoid excretion study. Males and females between 18 and 50 years of age, with cannabis dependence or abuse by DSM-IIIR criteria, and minimum cannabis use for the last two years were recruited. Participants were sequestered on a secure clinical research unit throughout to prevent access to cannabis and other drugs, and to enable collection of all urine voids. This study was approved by the Institutional Review Board (IRB) of the Intramural Research Program of the National Institute on Drug Abuse and participants provided written informed consent. There was no drug administration at any time during participation in the primary or secondary studies.

2.2. Specimens

Every urine void was individually collected in a polypropylene bottle ad libidum from admittance to discharge from the clinical unit (up to 30 days) and immediately refrigerated. Total volume and specific gravity of each urine void was measured and aliquots of urine specimens were stored in 3.5 mL polypropylene screw-cap tubes and 30 mL polypropylene bottles at −20 °C prior to analysis. Urine specimens were analyzed by 1D-GCMS within three years of frozen storage. Due to the surprising extended excretion of THC and 11-OH-THC for multiple days, and the low concentrations quantified, we reanalyzed some urine specimens by 2D-GCMS two to three years later in 2007–2008, when the technology became available in our laboratory. We utilized this new method to verify concentration and detection time data.

2.3. Specimen preparation

Tandem hydrolysis and extraction of urinary THC, 11-OH-THC and THCCOOH were performed according to a previously published procedure (Abraham et al., 2007). To ensure complete hydrolysis of conjugates and capture total analyte content, urine specimens (2 mL) were hydrolyzed by two methods in series. The initial 16 h hydrolysis with *E. coli* β-glucuronidase (Type IX-A) was followed by alkaline hydrolysis with 10N NaOH. Buffered hydrolysates (pH 4.0) were centrifuged and applied to preconditioned solid phase extraction (SPE) columns (Clean Screen® ZSTHC020 extraction columns, United Chemical Technologies). Concentrated extracts were derivatized with *N,O*-bis (trimethylsilyl) trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS).

2.4. GCMS

Trimethylsilyl derivatives of THC, 11-OH-THC, and THCCOOH were initially quantified by 1D-GCMS on an Agilent 6890 GC/5973 MSD system. Capillary separation was achieved on a DB-35MS column (Agilent Technologies). The mass selective detector was operated in electron impact-selected ion monitoring (SIM) mode. Three ions for each analyte and two for each internal standard were acquired. Limit of quantification of the 1D-GCMS method was 2.5 ng/mL for all analytes. Decreasing concentrations of analytes were used to empirically determine limit of detection (LOD) and LOQ. LOD was defined as the lowest concentration with a signal to noise ratio ≥3:1 for target and qualifier ions, ion ratios within 20% of average qualifier ion ratios of calibrators, retention time within ±2% and Gaussian peak shape. At the LOQ, all LOD criteria must be met, signal-to-noise ratio of the target ion is 10:1, and the