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Methadone disposition in oral fluid during pharmacotherapy for opioid-dependence

Teresa R. Gray^a, Riet Dams^{a,1}, Robin E. Choo^{a,2}, Hendree E. Jones^b, and Marilyn A. Huestis^a

Teresa R. Gray: teresa.r.gray@gmail.com; Riet Dams: riet.2.dams@gsk.com; Robin E. Choo: rec27@pitt.edu; Hendree E. Jones: hejones@jhmi.edu; Marilyn A. Huestis: mhuestis@intra.nida.nih.gov

^aChemistry and Drug Metabolism, National Institute on Drug Abuse, National Institutes of Health, 251 Bayview Blvd. Suite 200 Room 05A721, Baltimore, MD, 21224

^bJohns Hopkins School of Medicine, 4940 Eastern Avenue, Mason F. Lord Building, D-East 361 Baltimore, MD 21224

Abstract

Introduction—Oral fluid testing is widely used for detecting drug exposure, but data describing methadone and metabolites in oral fluid during pharmacotherapy for opioid-dependence are relatively limited.

Method—414 oral fluid specimens from 16 opioid-dependent pregnant women receiving daily methadone were analyzed for methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), and methadol by liquid chromatography-mass spectrometry.

Results—All oral fluid specimens contained methadone greater than 1 ng/mL; 88% were positive for EDDP and 12% for methadol. Over 95% of oral fluid specimens exceeded the 20 ng/mL methadone cutoff set by the European Driving Under the Influence of Drugs, Alcohol and Medicines (DRUID) study. Methadone and EDDP oral fluid concentrations were highly variable within and between participants, did not predict methadone dose, but were negatively correlated with pH.

Conclusion—Methadone was readily identified in oral fluid at concentrations greater than 20 ng/mL following daily 30–110 mg/day methadone pharmacotherapy. As no specimens contained only EDDP or methadol, there was no advantage to including these analytes for identification of methadone exposure. As nearly all oral fluid specimens from methadone-maintained patients exceeded the DRUID guideline, the 20 ng/mL cutoff appears to be sensitive enough to detect daily methadone exposure; however, additional indicators of behavioral and/or motor impairment would be necessary to provide evidence of driving impairment.

Keywords

methadone; oral fluid; opioid dependence; saliva

Corresponding Author: Marilyn A. Huestis, PhD, Chief, Chemistry and Drug Metabolism, Intramural Research Program, National Institute on Drug Abuse, NIH, 251 Bayview Blvd. Suite 200 Room 05A721, Baltimore, MD 21224 USA, (443) 740-2524, Fax: (443) 740-2528, mhuestis@intra.nida.nih.gov.

¹Present address: GlaxoSmithKline, Via A. Fleming 4, 37135 Verona, Italy

²University of Pittsburgh at Titusville, Division of Natural Sciences, 504 Main St., Titusville, PA, USA

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Introduction

Methadone is a well-established pharmacotherapy for opioid-dependence [1]. A small proportion of those receiving methadone for chronic pain or opioid-dependence may divert their medication [2]. Others may consume more than the prescribed dose, obtaining additional methadone from unknown sources, and some abuse methadone for its euphoric effects or when heroin is unavailable. Urine methadone and metabolite testing is routine for patients receiving methadone treatment for opioid-dependence and pain management, in workplace drug testing programs and in some jurisdictions to identify driving under the influence of drugs. Urine testing offers several advantages for monitoring including detection windows of several days, sufficient specimen volume, relatively high drug concentrations, and relatively low cost. However, adulteration or simple dilution of urine test results through increased fluid intake occurs frequently, necessitating measurement of urine creatinine and/or specific gravity and adulteration chemicals [3]. A positive urine test documents drug exposure, but not performance impairment.

Oral fluid is suggested as an alternative matrix for drug monitoring for several reasons. Compared to urine, collection is simple, fast, non-invasive, less embarrassing to provide and can be directly observed without needing specialized restroom facilities and a collector of the same gender. Oral fluid drug concentrations and windows of drug detection may be similar to those in plasma and may be useful for therapeutic drug monitoring [4]. However, methadone oral fluid/plasma ratios were shown to vary widely [5,6], with investigators concluding that oral fluid was not an effective matrix for therapeutic drug monitoring.

Oral fluid testing also may be useful in driving under the influence of drugs (DUID) investigations. DUID investigations, particularly in Europe, rapidly increased interest in oral fluid testing. A group of international experts met in Tailliores, France to propose guidelines for drugged driving research [7] that also were adopted by the European DRiving under the Influence of Drugs, Alcohol and Medicines (DRUID) project [8]. A 20 ng/mL methadone oral fluid cutoff was recommended to evaluate the prevalence of drivers consuming methadone [8]. With this criterion, Gjerde et al. identified 0.03% of randomly selected Norwegian drivers as methadone-positive [9]; whether these individuals were maintained on methadone or obtained the drug illicitly was not determined. It is not clear from the literature what methadone dose would generate 20 ng/mL methadone in oral fluid, as data describing dose-concentration relationships for methadone in oral fluid are limited [10,11]. To our knowledge, no studies have evaluated intra-individual concentration differences in multiple collections over time, and whether oral fluid concentrations vary within an individual after different methadone doses.

The aims of this study were to characterize methadone and metabolites 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and methadol concentrations in oral fluid, including inter- and intra-individual differences, dose-concentration relationships, and pH dependence, in opioid-dependent pregnant women receiving methadone pharmacotherapy.

Materials and Methods

Participants

Pregnant women were recruited from the Center for Addiction and Pregnancy (CAP) at Johns Hopkins Bayview Medical Center (JHBMC), a drug treatment facility specializing in obstetric and post-partum patients [12]. The JHBMC and National Institute on Drug Abuse's Institutional Review Boards approved the protocol and participants provided written informed consent. Women were included if they (1) had a current DSM-IV diagnosis of opioid dependence, (2) received daily outpatient methadone pharmacotherapy at CAP, (3)

were at least 18 years old, and (4) were between 8 and 28 weeks gestational age. Study participants resided on the Assisted Living Unit (ALU) during the first seven days of treatment for methadone induction and stabilization. After ALU discharge, participants attended daily group counseling and weekly individual treatment sessions, and received prenatal care [12,13]. Methadone (Methadose™, Mallinckrodt Inc., St. Louis, MO) dosing occurred daily under observed conditions; some women required a split-dose, necessitating methadone pharmacotherapy in the early morning and late afternoon. Dosing data were extracted from CAP records.

Specimen collection

Supervised oral fluid collection occurred three times per week, and specimens remained frozen until analysis. Unstimulated oral fluid specimens were collected using the Salivette® device (Sarstedt, Newton, NC, USA). The neutral cotton pad was chewed for 1 min, placed in a sealed polypropylene tube and centrifuged for 5 min at $1000 \times g$ to separate oral fluid from the cotton roll. The number of oral fluid specimens collected during study enrollment depended upon the length of study enrollment, clinic attendance and subject compliance.

Analytical procedures

Oral fluid methadone, EDDP, and methadol concentrations were determined by a validated liquid chromatography-mass spectrometric (LC-MS) method described previously [14]. Specimen pretreatment included acetonitrile precipitation before injection on the LC-MS [14]. Limits of quantification were 1 ng/mL for methadone and EDDP and 5 ng/mL for methadol. An Orion Model 370 pH meter (Orion Research, Inc., [now Thermo Scientific] Boston, MA) measured oral fluid pH in fully thawed and mixed specimens immediately prior to analysis.

Statistical evaluations

One-sample Kolmogorov-Smirnov test assessed concentration distribution normality. Non-parametric Spearman correlations were utilized for oral fluid concentration and pH evaluations.

Results

Methadone and EDDP disposition in oral fluid

Sixteen opioid-dependent pregnant women participated in the study, providing 414 oral fluid specimens for methadone, EDDP and methadol analysis. The mean $\pm$ SD number of oral fluid specimens obtained per participant was 25.9 ± 11.4 (range 8–50). Table 1 presents the methadone dose at study enrollment and delivery as well as oral fluid methadone, EDDP, and methadol concentration ranges and ratios for each participant. Methadone was found in all oral fluid specimens, while EDDP and methadol were present in 88% and 12%, respectively; methadone concentrations always exceeded those of its metabolites.

Methadone and EDDP concentrations in all oral fluid specimens at different methadone doses (Figure 1) and in two individuals, participants A and M, receiving escalating methadone doses (Figure 2) are shown. No apparent dose-concentration relationships were observed. In addition, methadone and EDDP concentrations in oral fluid displayed high inter- and intra-subject variability. As representative examples, Figure 3 demonstrates methadone and EDDP concentration variability in two participants, C and E, receiving daily 60 mg methadone for several weeks (steady state concentrations).

Oral fluid pH profoundly affected methadone and EDDP concentrations. As oral fluid pH increased, methadone and EDDP concentrations significantly decreased (methadone – $r=-$

0.54, $p < 0.0001$; EDDP – $r = -0.41$, $p < 0.0001$; Figure 4), with non-measurable EDDP if oral fluid pH exceeded 8.1.

Nearly all oral fluid specimens (95.7%) contained >20 ng/mL methadone, suggesting that this cutoff concentration is sufficiently low to identify those receiving daily methadone maintenance. Oral fluid specimens with methadone concentrations <20 ng/mL had a mean $\pm$ SD pH of 7.4 ± 1.1 , significantly higher than the 6.1 ± 0.8 pH of specimens exceeding the DRUID cutoff ($p < 0.0001$). Methadone concentrations <20 ng/mL were not associated with recent initiation of methadone therapy; one oral fluid specimen below 20 ng/mL was collected 24 weeks after the initiation of methadone maintenance.

Discussion

We characterized methadone and EDDP disposition in oral fluid collected from 16 opioid-dependent pregnant women. This population was closely monitored, attending the clinic daily for extensive outpatient treatment and methadone administration. We found methadone in all oral fluid specimens and EDDP in most (88.9%). Previous investigations report varying prevalence of EDDP (range 0–100%) [5,15–17], possibly influenced by analytical procedures, quantification limits (range 0.5–18 ng/mL), and oral fluid pH. To our knowledge, this is the first report of methadol in oral fluid, which was only identified in 12% of specimens. It is not clear why EDDP and methadol were not observed in all oral fluid specimens. It is likely a function of several factors, including pharmacokinetics. Analytical considerations, however, may explain most of the undetected samples. For methadol, most of the positive specimens were slightly above the analytical limit of quantification (5 ng/mL). Likely, had we been able to achieve greater analytical sensitivity, we would have identified a larger proportion of methadol-positive specimens. For EDDP, concentrations were approximately one order of magnitude less than methadone. In most cases where methadone but not EDDP was found, methadone concentrations were relatively low. Again, it is likely that the EDDP was present, but at concentrations too low to be detected in this analytical procedure. Because as the metabolites were always present in conjunction with methadone, it may not be necessary to monitor EDDP or methadol for detecting methadone exposure.

Methadone and EDDP oral fluid concentrations were highly variable within and between participants, but methadone concentrations always exceeded EDDP. Methadone concentrations also did not correlate to dose, contrary to the report from Wolff et al [10]. Concentration variability and lack of a dose-concentration correlation noted in this research could have been influenced by timing of specimen collections after methadone dosing. Oral fluid specimens were not collected at a specified time after dosing, thus high methadone concentrations could be caused by residual contamination of the oral mucosa from the liquid methadone dose. Alternatively, low concentrations may correspond to trough methadone concentrations. The likely variability in time between dosing and oral fluid collection reflects “real world” situations, as would be found in methadone maintenance clinics and DUID investigations.

The chosen population in this study, namely pregnant women, also may explain why no dose-concentration effect was observed. During pregnancy, methadone clearance is increased, possibly due to induced hepatic metabolism or decreased plasma protein concentrations [18]. As a result, plasma methadone concentrations are significantly reduced compared to post-partum levels, and higher methadone doses are necessary to maintain efficacy, as was observed for all women in this study. Thus, despite increasing methadone doses, plasma and consequently, oral fluid methadone concentrations could be relatively unaffected.

Methadone and EDDP concentrations also were influenced by oral fluid pH. As previously shown for other basic drugs [19,20], decreased pH was associated with increased methadone and EDDP concentrations. This phenomenon, known as ion trapping, occurs when the pH of oral fluid is less than blood. As basic analytes diffuse into oral fluid, the molecule becomes ionized and unable to diffuse back into blood as the concentration gradient shifts [21].

Additional variability in concentration could be attributed to the oral fluid collection procedure. The Salivette device employed was shown in our laboratory (unpublished data) and others' [5] to have methadone and EDDP losses of 40 and 30%, respectively, due to drug adsorption to the device. These data highlight that drug adsorption should be characterized when evaluating an oral fluid collection device, as the extent of adsorption likely varies by analyte and collection device [22,23].

Nearly all methadone oral fluid concentrations were ≥ 20 ng/mL, the concentration recommended for future drugged driving research [7]. Based on our data, this cutoff is suitable for identifying individuals maintained on 30 – 100 mg methadone daily. However, a positive oral fluid methadone result alone does not necessarily indicate performance impairment. While methadone recipients routinely perform less well on neuropsychological and cognitive motor tasks than non-drug using controls [24–29], we are aware of only one simulated driving evaluation of methadone-maintained patients in a controlled research setting. Lenne et al. found no performance deficits on a simulated driving course among methadone-maintained individuals [30]. Furthermore, only one retrospective study, to our knowledge, documents driver impairment during methadone dosing by toxicological testing and a standardized clinical assessment consisting of a short interview and a battery of observations [31]. Of approximately 25,000 suspected impaired drivers in Norway over a 5.5 year period, 635 (~2.5%) were identified as methadone-positive by blood analysis; however, polydrug use was prevalent, with over 90% also positive for benzodiazepines and approximately 30% positive for amphetamines or cannabis. In eight cases where methadone was the only psychoactive substance present, five drivers were found to be impaired following clinical evaluation. However, it is not clear how experienced these drivers were with methadone (i.e. stabilized, recently initiated maintenance, etc.) Blood methadone concentrations did not predict whether the driver was or was not impaired. This study highlights several important points. First, methadone-maintained individuals often may be taking other drugs, potentially additional pharmacotherapies (i.e. benzodiazepines) or illicit drugs, either of which may improve or worsen driving performance. Second, it reinforces the need for actual or simulated driving research among methadone maintained individuals to properly assess driving performance and to determine what concentrations in specific biological matrices might be indicative of impairment.

In conclusion, oral fluid is an effective alternative matrix for identifying methadone consumption in pain management, maintenance therapy and DUID investigations. However, more research is necessary to understand and correct for wide inter- and intra-subject variability.

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Figure 1. Methadone and EDDP concentrations in 414 oral fluid specimens with the corresponding methadone dose at collection. Methadone and EDDP concentrations are displayed on a logarithmic scale because of the large range of concentrations.

Figure 3. Methadone and EDDP oral fluid concentrations in Participants C and E receiving 60 mg methadone daily over 20 and 16 weeks, respectively, demonstrating high intra- and inter-individual variability. Methadone and EDDP concentrations are displayed on a logarithmic scale because of the large range of concentrations.

Figure 4. pH effect on methadone and EDDP concentrations in oral fluid. Methadone and EDDP concentrations are displayed on a logarithmic scale because of the large range of concentrations.

Methadone dose and methadone and 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) concentrations in oral fluid specimens from 16 opioid-dependent pregnant women.

Table 1

Subject	Methadone dose		N _{total}	Methadone Range (ng/mL)	% >20 ng/mL Methadone	N _{EDDP} (%)	EDDP Range (ng/mL)	EDDP/Methadone Ratio	N _{Methadone} (%)	Methadone Range (ng/mL)	Methadone/Methadone Ratio
	Enrollment	Delivery									
A	30	65	19	12.4 – 430	89.5	16 (84.2)	1.3 – 12.2	0.020 – 0.041	6 (31.6)	6.0 – 6.4	0.018 – 0.445
B	40	110	9	21.5 – 78225	100	8 (88.9)	2.3 – 1791	0.014 – 0.052	5 (55.6)	5.9 – 6.1	0.006 – 0.284
C	30	60	50	10.7 – 3996	90.0	44 (88.0)	1.2 – 57.7	0.014 – 0.225	5 (10.0)	5.1 – 7.3	0.050 – 0.193
D	40	85	42	16.8 – 879	97.6	39 (92.0)	1.4 – 27.5	0.014 – 0.034	6 (14.3)	6.0 – 6.3	<0.000 – 0.248
E	40	60	34	20.8 – 1899	100	34 (100.0)	1.5 – 52.8	0.019 – 0.071	0 (0.0)		
F	30	45	26	20.8 – 429	100	25 (96.2)	2.2 – 9.9	0.023 – 0.106	5 (19.2)	5.0 – 5.3	0.012 – 0.068
G	50	60	19	6.7 – 324	94.7	16 (84.2)	1.0 – 6.7	0.011 – 0.026	4 (21.1)	5.0 – 5.2	0.020 – 0.076
H	50	80	25	63.9 – 4886	100	25 (100.0)	2.8 – 186	0.024 – 0.070	0 (0.0)		
I	40	45	28	20.5 – 868	100	27 (96.4)	1.8 – 46.5	0.026 – 0.063	7 (25.0)	5.0 – 5.8	0.007 – 0.283
J	30	75	26	47.3 – 416	100	25 (96.2)	1.4 – 14.4	0.022 – 0.058	0 (0.0)		
K	30	70	22	32.5 – 1911	100	21 (95.5)	1.5 – 43.3	0.022 – 0.067	0 (0.0)		
L	90	110	31	31.3 – 734	100	26 (83.9)	2.9 – 33.5	0.016 – 0.059	0 (0.0)		
M	30	90	27	14.5 – 479	88.9	17 (63.0)	1.5 – 18.1	0.012 – 0.043	0 (0.0)		
N	30	65	36	7.1 – 330	86.1	26 (72.2)	1.1 – 302	0.024 – 0.915	8 (22.2)	5.0 – 281.4	0.045 – 0.852
O	Not available		12	5.2 – 449	91.7	11 (91.7)	4.8 – 14.7	0.018 – 0.083	2 (16.7)	6.2 – 6.2	0.030 – 0.030
P	40	70	8	508.0 – 15255	100	8 (100.0)	18.2 – 112	0.007 – 0.036	2 (25.0)	5.2 – 5.3	<0.000 – 0.003
Total			414	5.2 – 78225	95.7	368 (88.9)	1.0 – 1791	0.007 – 0.915	50 (12.1)	5.0 – 281	<0.000 – 0.852