A single dose of methadone inhibits cytochrome P-4503A activity in healthy volunteers as assessed by the urinary cortisol ratio

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Aims To examine the effect of a single oral dose of methadone on cytochrome P450 (CYP) 3A activity using the urinary 6 β -hydroxycortisol to cortisol ratio (UCR) as a marker of CYP3A activity.

Methods A single oral dose (0.2 mg kg⁻¹) of *rac*-methadone was administered to eight healthy female volunteers. Frequent blood samples and all urine over seven time periods was collected for 96 h following dosing. The UCR and the concentration of the major CYP3A metabolite of methadone, EDDP, were measured in urine. Methadone enantiomer concentrations were determined in plasma and urine. All quantifications were performed by validated high performance liquid chromatography assays.

Results In all volunteers a significant decline of the UCR from immediately predose values was observed at the 4–8 and 8–12 h collection periods ($P < 0.05$, 95% CI for the differences: 0.4,16 and 0.6,16, respectively) with a return to immediately predose values after 2–3 days, suggesting methadone was an inhibitor of CYP3A. The UCR was found to be significantly correlated with the amount of EDDP excreted in the urine and with the area under the plasma concentration *vs* time profile for total (R + S) methadone, supporting *in vitro* data that CYP3A is primarily responsible for EDDP formation and has a significant influence on methadone disposition.

Conclusions Methadone appears to be a CYP3A inhibitor *in vivo* following a single oral dose and measurements of the urinary cortisol ratio appear to be a useful index to follow this inhibition.

Keywords: cortisol ratio, CYP3A, drug interactions, methadone

Introduction

Methadone is a synthetic μ -opioid receptor antagonist widely used in the prevention of opiate abstinence syndrome and as an analgesic in patients with severe pain [1, 2]. Methadone shows considerable interindividual variability in its pharmacokinetics and pharmacodynamics, and, as a consequence, prescribers empirically titrate the dose of methadone against clinical response [1, 3]. The major metabolic pathway for methadone is *N*-demethylation

to an inactive metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), which is catalysed by cytochrome P450 (CYP) 3A4, although CYP2C9, CYP2C19 and CYP1A2 also play minor roles [4–9]. Up to fivefold interindividual differences in the amount of EDDP excreted in the urine have been reported [10, 11], which probably results from the 5–20-fold range of CYP3A4 activity observed in the general population [12]. Additionally, methadone metabolism by CYP3A4 is possibly induced at steady-state, as greater amounts of EDDP are recovered in the urine upon chronic dosing compared with single doses (2.45 ± 1.00 *vs* $16.7 \pm 9.9\%$ of methadone dose administered, respectively) [11]. In contrast, *in vitro* microsomal experiments show that methadone is an inhibitor of CYP3A4 [6]. However, both of these properties may act together to produce a composite effect on CYP3A4 activity at steady-state.

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CYP3A4, with a small contribution by CYP3A5, is primarily responsible for the 6 β -hydroxylation of the endogenous steroid cortisol [13]. The ratio of 6 β -hydroxycortisol to cortisol (the urinary cortisol ratio, UCR) in urine has been employed as an endogenous measure of CYP3A4/5 (CYP3A) activity [14–16]. The aim of this study was to examine CYP3A activity following a single oral dose of methadone to healthy female volunteers using the UCR as a marker.

Methods

Subjects, dosing and sample collection

Eight female subjects, six Caucasian and two African American, were enrolled in the study and were judged as being healthy following a physical examination, ECG and standard blood biochemistry tests. All were nonsmokers and were not taking any medications within 1 week of drug administration. Subjects were admitted to a research ward the night prior to methadone administration and fasted overnight and until 4 h postdose. A 0.2 mg kg⁻¹ dose of *rac*-methadone was administered orally as a liquid (Roxane Laboratories, Columbus, OH, USA). A urine sample was collected immediately predose (morning spot collection immediately prior to dosing at 08.00 h), and all urine was collected cumulatively from 0–4, 4–8, 8–12, 12–24, 24–48, 48–72, 72–96 h postdose. The volume of each collection was recorded and an aliquot (20 ml) frozen immediately. Plasma samples were collected frequently over the 96 h postdose and then frozen immediately. The study was approved by the Medical University of South Carolina Institutional Review Board and written informed consent was obtained from the subjects prior to entry into the study.

Urinary cortisol ratio determination

A liquid–liquid extraction procedure was used to recover 6 β -hydroxycortisol and cortisol from urine ($\approx$ 1.5 ml) [14]. Recovery of 6 β -hydroxycortisol, cortisol and the internal standard, dexamethasone, was 55 ± 5 , 83 ± 2 and $75 \pm 2\%$ (all 1 μ g in 1 ml of urine, $n=3$), respectively. 6 β -Hydroxycortisol and cortisol concentrations were determined by a reversed-phase gradient h.p.l.c. method [15]. Standard curves were linear ($r^2 > 0.98$) from 1 to 1000 ng ml⁻¹ ($n=15$) and their y-intercepts were not significantly different from zero. Interday ($n=9$) and intraday ($n=3$) coefficients of variation were $< 8\%$ for all analytes. The limit of quantification (lowest standard) was 1 ng ml⁻¹ for both analytes. Neither methadone nor EDDP interfered with the analytes of interest.

Methadone concentration determination

Methadone enantiomer and total EDDP concentrations in urine and plasma were determined by a previously reported validated chiral h.p.l.c. method developed in our laboratory [17].

Data analysis

Repeated measures ANOVA with Dunnett's multiple comparisons test *post hoc* was employed to examine for significant changes in the UCR over the first 24 h intensive urine collection period (InStat[®] 3.01, GraphPad Software Inc., San Diego, CA, USA). Plasma pharmacokinetic parameters were calculated by standard methods [18]. Relationships between UCRs and the amount of EDDP and methadone pharmacokinetic parameters were examined by linear regression (InStat[®]). The level of significance was set at $P=0.05$. Data are expressed as mean $\pm$ s.d. All changes in UCR are expressed as changes from their corresponding immediately predose (morning spot urine) values.

Results

All subjects completed the study and all reported one or more adverse effects associated with methadone administration, including nausea, headache, drowsiness, visual hallucinations, photophobia and lightheadedness.

UCR, EDDP and methadone excretion and plasma area under the concentration *vs* time profile (AUC) data are shown in Table 1. In all individuals, a decrease was observed in the UCR over the first 24 h following administration of methadone and almost all of the volunteers had UCR values near their immediately predose values in their 72–96 h samples (see Figure 1). The mean decrease in UCR over the first 24 h was $33 \pm 33\%$ ($n=32$) and $19 \pm 33\%$ ($n=64$) over the entire 96 h postdose period. The mean maximal decrease in UCR was $70 \pm 19\%$ ($n=8$), occurring either in the 8–12 or the 12–24 h samples (median: 8–12 h) with both of these collection periods having significantly lower UCR values (95% CI for the differences: 0.4,16 and 0.6,16, respectively, both $n=8$).

Correlation analyses for the UCR *vs* EDDP and total methadone excretion *vs* plasma AUC are shown in Table 1. The UCR and EDDP (as percentage of dose) from pooled 96 h collection and from each individual collection period showed significant correlations ($r^2=0.7804$, $P=0.0053$, $n=8$ and $r^2=0.4045$, $P=0.002$, $n=64$, respectively). Plasma methadone displayed enantioselective disposition in most individuals with the active (R)-enantiomer predominating (see Table 1). There was a significant relationship for UCR

Table 1 Urinary cortisol ratio (UCR), EDDP and methadone urinary excretion and plasma area under the curve (AUC) for total methadone data following oral administration of 0.2 mg kg⁻¹ *rac*-methadone to eight healthy female volunteers.

Volunteer	Pre dose UCR	Collection interval with minimum UCR (h)	Minimum UCR	Mean UCR# (0–96 h)	EDDP excreted (0–96 h) (% of dose)	Total methadone excreted (0–96 h) (% of dose)	Total methadone plasma AUC(0,96 h) (ng ml ⁻¹ h)
1	12.2	8–12	2.0	6.8	1.0	6.9	678
2	46.1	12–24	20.3	28.8	10.3	5.9	1727
3	26.1	8–12	1.6	23.3	13.0	5.8	1675
4*	6.3	8–12	1.4	5.6	5.2	17.5	373
5	7.0	8–12	1.6	5.5	2.1	8.6	442
6	12.1	8–12	5.1	11.3	4.3	16.0	1630
7	15.9	12–24	10.4	14.9	4.8	5.1	1872
8*	12.6	8–12	2.5	9.5	3.8	8.4	634
Mean ± s.d.	17.3 ± 13.2	8–12 (median)	5.6 ± 6.7	13.2 ± 8.6	5.6 ± 4.1	9.3 ± 4.8	1129 ± 649
Correlation with mean UCR (0–96 h):				<i>P</i> value	0.005	0.20	0.02
				<i>r</i> ²	0.78	0.25	0.60

*African-American, all others were Caucasian, #calculated from the total 96 h urinary recovery of the analytes.

and total (the sum of R + S) methadone plasma AUC(0,96 h) ($P=0.02$, $r^2=0.60$, $n=8$). No significant correlations with individual enantiomer plasma or urinary excretion data and the 0–96 h or individual urine sample UCRs were observed.

Discussion

This study observed transient decreases in UCR values in all volunteers after taking a single oral dose of methadone, suggesting methadone acts as an inhibitor of CYP3A. The UCR has been utilized previously to examine CYP3A induction by drugs such as rifampicin and phenytoin [19, 20], while other studies have measured decreases in 24 h 6 β -hydroxycortisol excretion caused by CYP3A4 inhibitors such as nelfinavir [21]. The study reported here appears to be the first in which the UCR has been applied to examine CYP3A inhibition. However, there is some controversy regarding the utility of the UCR in measuring CYP3A activity.

Two studies have reported that the UCR shows an almost threefold increase in the afternoon and evening compared to morning values [16, 22], whereas another study has found no circadian variation in the ratio [14]. If present, a diurnal rise in the UCR would result in an underestimation of the degree of CYP3A inhibition by methadone, particularly in the late afternoon and evening. However, due to our finding of a decrease in UCR over at least the first 24 h postmethadone dosing, UCR diurnal variation does not alter our finding of inhibition *per se* but may affect the absolute values of the UCR over the first 24 h intensive collection period.

Figure 1 Collection mid-point urinary 6 β -hydroxycortisol:cortisol ratios in eight healthy female volunteers following oral administration of 0.2 mg kg⁻¹ *rac*-methadone. ‘*’ indicates significantly different mean UCR values from immediately predose ($P<0.05$, RM ANOVA with Dunnett multiple comparisons test *post hoc*, 95% CI for the differences: 0.4,16 and 0.6,16% for the 4–8 and 8–12 h collection intervals, respectively).

The UCR has been reported to have poor correlations with the other common probes of CYP3A4 activity, specifically the erythromycin breath test [23–26] and oral midazolam clearance [23, 26]. However, the failure of the erythromycin breath test and midazolam clearance to also

show a significant correlation in many cases [23, 24, 26], suggests that an ideal and specific CYP3A4 probe has yet to be identified. Nevertheless, all of these methods, including the UCR, are likely to be of some value in examining relative changes in CYP3A activity in the same individual.

Some clinical studies examining methadone disposition have reported small to moderate increases (<20%) in methadone steady-state plasma concentrations compared with those predicted by single or early doses [27–30]. Increases in the amount of EDDP excreted at steady-state point to CYP3A4 induction as one possible contributor to this phenomenon [11]. In contrast, *in vitro* studies using human liver microsomes show methadone is a 'mixed' or 'noncompetitive' reversible inhibitor of CYP3A4 (K_i of 100 μM) [6]. In addition, it is clear that methadone disposition is affected by a number of CYP3A4 substrates, inhibitors or inducers and that methadone also alters the disposition of other CYP3A4 substrates [31–36].

Concentrations of methadone in tissues and plasma are likely to be influenced by the rate of formation of EDDP from methadone by CYP3A. This is supported by the significant relationship between plasma total methadone AUC(0,96 h) and the UCR (Table 1). However, our results and data from other studies show that methadone displays enantioselective disposition in humans (see AUC data in Table 1) [29, 37]. The main factors involved in this enantioselective disposition are currently poorly understood and may be due to numerous factors including enantioselective protein binding [38]. However, *in vitro* evidence showed that the formation of EDDP from methadone by human liver microsome CYP3A4 is not enantioselective [8]. Non-enantioselective metabolism by CYP3A4 may be a contributing factor as to why the relationships between the UCR and total methadone were significant but the relationships for the individual enantiomers were not, as both enantiomers were metabolized, but not eliminated, at similar rates.

In vitro data indicating CYP3A4 inhibition by methadone are supported by our study as the inhibition of CYP3A appears reversible as a return to immediately predose activity was observed as plasma concentrations and urinary excretion rates of methadone decline over time (Figure 1). However, a limitation of our study is that it only examines the UCR following a single oral dose of methadone. It would be unlikely that our study design would detect CYP3A induction owing to the lag-time associated with enzyme synthesis and the single dose exposure of the subjects to methadone. Thus, our data do not rule out the possibility of a combined induction and inhibition of CYP3A by methadone at steady-state which may result in an overall composite increase in CYP3A activity. Some individuals returned to immediately

predose UCRs after 24 h (see Figure 1) while plasma methadone concentrations were still relatively high. This may have been due to the onset of CYP3A induction or due to the relatively high capacity of CYP3A4. Clearly, more work is required to examine changes in UCR with prolonged exposure to methadone.

This study has shown that the UCR may be useful as a marker of CYP3A inhibition. It has been shown through the UCR that a single oral dose of methadone causes significant but transient inhibition of CYP3A activity in healthy volunteers and that some caution may be necessary when coadministering certain drugs with methadone. However, additional work is required to examine the consequences of chronic methadone dosing on CYP3A activity.

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