Contribution of the activities of CYP3A, CYP2D6, CYP1A2 and other potential covariates to the disposition of methadone in patients undergoing methadone maintenance treatment

Mohammad-Reza Shiran,* Martin S. Lennard,
Mohammad-Zafar Iqbal,¹ Oldwale Lagundoye,¹
Nicholas Seivewright,¹ Geoffrey T. Tucker & Amin Rostami-Hodjegan

Academic Unit of Clinical Pharmacology, University of Sheffield and ¹Sheffield Care Trust, Substance Misuse Services, Sheffield, UK

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Several cytochromes P450 (CYPs) have been implicated in the metabolism of methadone, but there is no consensus on their relative contributions to overall disposition and hence variability in response.

WHAT THIS STUDY ADDS

- Variability in CYP3A4 activity has statistically significant but nonetheless modest influence on the oral clearance of methadone and its enantiomers.
- However, CYPs 1A2 and 2D6 appear to have no impact at all.

Correspondence

Professor Amin Rostami-Hodjegan,
Academic Unit of Clinical Pharmacology,
Floor M, Royal Hallamshire Hospital,
Sheffield S10 2JF, UK.
Tel: + 44 0114 271 2156
Fax: + 44 114 271 1863
E-mail: a.rostami@Sheffield.ac.uk

*Present address: Department of
Physiology and Pharmacology, School of
Medicine, Mazandaran University of
Medical Sciences, Mazandaran, Iran.

Keywords

CYP1A2, CYP2D6, CYP3A4, methadone,
midazolam

Received

17 February 2008

Accepted

13 September 2008

AIMS

To investigate the influence of different cytochrome P450 (CYP) activities and other potential covariates on the disposition of methadone in patients on methadone maintenance therapy (MMT).

METHODS

Eighty-eight patients (58 male; 21–55 years; 84 White) on MMT were studied. CYP2D6 activity [3 h plasma metabolic ratio of dextromethorphan (DEX) to dextrorphan (DOR)] was determined in 44 patients (29 male; 24–55 years), CYP1A2 activity (salivary caffeine elimination half-life) in 44 patients (21 male; 24–55 years) and CYP3A activity (oral clearance of midazolam) in 49 patients (33 male; 23–55 years). Data on all three CYPs were obtained from 32 subjects. Total plasma concentrations of (*RS*)-methadone and total and unbound plasma concentrations of both enantiomers were measured by LC/MS. Population pharmacokinetics and subsequent multiple regression analysis were used to calculate methadone oral clearance and to identify its covariates.

RESULTS

Between 61 and 68% of the overall variation in total plasma trough concentrations of (*RS*)-, (*R*)- and (*S*)-methadone was explained by methadone dose, duration of addiction before starting MMT, CYP3A activity and illicit morphine use. CYP3A activity explained 22, 16, 15 and 23% of the variation in unbound (*R*)-, unbound (*S*)-, total (*RS*)- and total (*S*)-methadone clearances, respectively. Neither CYP2D6 nor CYP1A2 activity was related to methadone disposition.

CONCLUSIONS

CYP3A activity has a modest influence on methadone disposition. Inhibitors and inducers of this enzyme should be monitored in patients taking methadone.

Introduction

Methadone continues to play a significant role in maintenance treatment of opioid addiction. Many [1–9] but not all [10, 11] controlled clinical trials have demonstrated a relationship between the daily dose of methadone and the outcome of treatment. Given this relationship, a similar, if not stronger correlation between plasma methadone concentration and effect might be expected. A relationship between methadone dose and trough plasma concentration has been established by meta-analysis [12]. However, because of the large interindividual variability in the metabolism of methadone, dose may explain only a small proportion (<50%) of the variation in its plasma concentration [12].

Methadone is eliminated mainly by hepatic metabolic clearance, followed by renal and faecal excretion of its metabolites [13]. The parent compound also undergoes urine pH-dependent renal excretion, and although at typical urinary pH renal clearance constitutes <1% of the overall clearance, at lower urinary pH renal excretion of unchanged methadone increases substantially [14–16]. As a drug with a low extraction ratio [17], the hepatic clearance of methadone following intravenous (i.v.) administration is dependent on its unbound fraction and intrinsic clearance [13]. These factors also determine the oral clearance of methadone, with additional roles for gut metabolism and, potentially, efflux transporters.

In vitro and/or *in vivo* studies have suggested that CYPs 3A4 [17–41], 2D6 [18, 19, 21, 23, 41–43], 1A2 [44, 45], 2B6 [41, 46] and 2C19 [21] are involved in the metabolism of methadone. Although there is no consensus on the relative contributions of each enzyme to the overall disposition of methadone [21, 47], CYP3A4 appears to be the most important. Hepatic CYP3A4 displays up to 30-fold variability in its activity and its abundance in the gut varies 11-fold [48].

The aim of this study was to define the contribution of the activities of different CYPs, urine pH, current drug abuse, duration of addiction before starting methadone maintenance therapy (MMT) and duration of MMT on the disposition of *rac*-methadone and its enantiomers during chronic dosing under real-world administration conditions.

Methods

Subjects

Subjects were recruited to the study over a period of 17 months during routine visits to the MMT clinic (Sheffield Care Trust Substance Misuse Services). All gave written informed consent to participate. There were no restrictions on age, sex, weight and ethnic background. However, patients who were pregnant or HIV seropositive were excluded. A total of 88 patients (58 male; 21–55 years; 84

White) were studied. The patients in the study took methadone with other probe drugs [midazolam, caffeine and dextromethorphan (DEX)] simultaneously. All subjects participated in each of the arms of the study. However, due to problems such as poor venous access (as a result of chronic i.v. drug use) and dry mouth, not all intended blood, urine and saliva samples could be obtained from all subjects. The activities of CYP2D6, CYP1A2 and CYP3A4 were determined in 44 (29 male; 23–55 years), 44 (28 male; 23–55 years, all smokers) and 49 (33 male; 21–55 years) patients, respectively. All three CYP activities were determined in 32 patients. The daily dose of methadone was variable (15–130 mg) and had been determined based on clinical needs as identified by the prescribers. Thus, the study had no intervention with respect to regular doses that patients were receiving. Duration of addiction before starting MMT varied from 48 to 108 months, and duration of MMT was 28–60 months. All the methadone dose intakes were monitored on the study day; however, prior intake of methadone dose had not been witnessed by the dispensing pharmacist for all of the subjects. The study was approved by the Research Ethics Committee of North Sheffield.

Chemicals and drugs

Five litres of methadone solution (1 mg ml⁻¹) identified by Batch Number was supplied by Boots the Chemist Pharmacy, Sheffield, U.K. Midazolam hydrobromide capsules (7.5 mg), dextromethorphan hydrobromide capsules (15 mg immediate release) and coffee (Nescafe Gold®; Nestle) were obtained from commercial UK suppliers. *Rac*-methadone and its enantiomers were a gift from the National Institute on Drug Abuse (Research Triangle Institute, Bethesda, MD, USA). Midazolam HCl was obtained from Antigen Pharmaceuticals Products Ltd. (Roscrea, Co Tipperary, Ireland). Dextromethorphan hydrobromide, dextrophan tartrate, the assay internal standards (laudanosine base and prazepam) and caffeine were of analytical grade from Sigma Chemical Company (Poole, UK), as was β -glucuronidase type H-1 (from *Helix pomatia*). Other chemicals were of high-performance liquid chromatography or analytical grade and were purchased from commercial UK suppliers.

Assessment of CYP activities

The study was conducted on the day that the subjects received their weekly prescription for methadone. Although the assessment of CYP1A2 activity is not affected by residual caffeine present in saliva (see below), patients were asked to abstain from caffeine-containing food and beverages for 24 h prior to the study. Predose samples of blood and urine were collected. Subjects then took their dose of methadone, capsules containing 7.5 mg of midazolam and 15 mg of immediate-release DEX, drank a cup of coffee (approximately 57 mg of caffeine) and rinsed their mouth with 200 ml of tap water. During the following 5 h,

three saliva samples (approximately at 2, 4 and 5 h) were collected in sterile propylene tubes and three venous blood samples (approximately at 1.9, 3 and 5.3 h) were collected in heparinized Monovette® tubes. The exact time was noted in all cases for pharmacokinetic analysis. After centrifugation of the blood for 5 min (1000 g), the plasma was transferred to sterile propylene tubes. All samples were incubated for 30 min in a 58°C preheated water bath to deactivate any HIV. They were then stored at –25°C pending assay.

Plasma concentrations of midazolam were assayed by liquid chromatography/mass spectrometry (LC/MS) [49]. The limit of determination was 0.65 ng ml⁻¹, and the intra-day assay coefficients of variations were ≤8%. The oral clearance of midazolam was used as an index of the combined activity of gut and liver CYP3A activity [50, 51].

DEX and dextrorphan (DOR) were assayed by the method of Chen *et al.* [52] with minor modifications. The limits of determination were 0.3 ng ml⁻¹ and 0.1 ng ml⁻¹ for DEX and DOR, respectively, and intra- and interday assay coefficients of variation were ≤15%. The ratio of DEX/DOR in plasma at 3 h after dosing was used as the index of CYP2D6 activity [53–56].

Caffeine was assayed by the method of O'Connell *et al.* [57]. The limit of determination was 0.06 µg ml⁻¹, and intra- and interday assay coefficients of variation were ≤15%. The elimination half-life of caffeine in saliva (corrected for residual caffeine [58]) was used as the index of CYP1A2 activity.

Detection of drugs of abuse

As part of routine monitoring, urinalyses were carried out for common drugs of abuse in the Department of Clinical Chemistry, Royal Hallamshire Hospital, Sheffield, UK. The procedure involves an initial screen using an enzyme-multiplied immunoassay technique and then confirmation using gas chromatography – mass spectrometry (GC – MS) techniques.

Measurement of urine pH

Urine was collected over 3 h after dosage in sterile propylene tubes and its pH measured immediately using a portable HI-8424 pH meter (Hanna Instrument, Scientific Laboratory Supplies Ltd, Nottingham, UK). The coefficient of variation of repeated measurement of the pH of the same sample was <3%.

Analysis of (RS)-, (R)- and (S)-methadone

Total plasma concentrations of (*rac*)-methadone and its enantiomers were determined by LC-MS [59]. Unbound plasma concentrations of (R)- and (S)- methadone were determined after ultrafiltration as described previously [59]. The limits of determination of (RS)-methadone in plasma, (R)-methadone in plasma ultrafiltrate, (S)-methadone in plasma ultrafiltrate, and total (free + bound) (R)-methadone and (S)-methadone were 0.08, 0.04, 0.20,

0.27 and 0.10 ng ml⁻¹, respectively. Intra- and interday assay coefficients of variation ranged from 0.8 to 18%.

Pharmacokinetic analysis

A population pharmacokinetic analysis was done using P-Pharm software (version 1.5; InnaPhase, Ceretil, France). Various models were fitted to each dataset, and selection of the final model was based on the lowest value of the Akaike Information Criteria and visual inspection of residuals for systematic error. Initial estimates of the pharmacokinetics parameters were derived from values reported in the literature or by using a simplex method within P-Pharm.

Midazolam A two-compartment model was found to be suitable in which oral clearance (CL/F), apparent volume of distribution (V/F), the absorption rate constant (*k_a*), and the transfer rate constants (*k₁₂* and *k₂₁*) were the primary parameters. Log normal distributions were assumed for all parameters.

Caffeine The elimination half-life (*t_{1/2}*) of caffeine was estimated on the basis of a one-compartment user-defined model with first-order input that accounted for an unknown initial concentration in each individual. Since many predose samples contained residual caffeine, correction was required for these initial concentrations. These are described in the Appendix.

Methadone Various population pharmacokinetic models with first-order input and one or two compartmental disposition kinetics were examined under two assumptions:

- 1 Full compliance with the timing of administration (classical steady-state equations for one and two compartmental kinetics with first-order absorption).
- 2 No prior information regarding compliance and only accounting residual concentrations from an unknown previous dose under the assumption that at the time of methadone dose intake the concentration of methadone in plasma is at the post-distribution phase of the kinetics of the previous dose. Both one and two compartmental kinetics models were developed as user-defined models and implemented in P-Pharm software package (version 1.5; InnaPhase, Ceretil, France). All initial estimates of the parameter values were obtained from the literature [60]. A heteroscedastic (1/*y*²) error model was used and parameter distribution was assumed to be log-normal. See Appendix.

Statistical analysis

Statistical testing were carried out using SPSS for Windows (v.12; SPSS Inc., Chicago, IL, USA) and included multiple linear regression to reveal trends in the population data, ANOVA and paired sample *t*-tests and two-tailed Wilcoxon matched pairs signed-rank tests for comparing mean values. *P* < 0.05 was considered to indicate statistical significance.

Figure 1 Frequency distributions and probit plots of markers of CYP enzyme activities in methadone maintenance therapy patients. (a) CYP2D6 activity as reflected by the 3-h plasma dextromethorphan/dextrorphan (DEX/DOR) metabolic ratio. (b) CYP1A2 activity as reflected by caffeine half-life in saliva. (c) CYP3A activity as reflected by the oral clearance of midazolam. [The frequency distributions of the data in (b) and (c) were fitted by normal distribution plots (continuous lines)]

Table 1 Results of regression analysis to determine the contributions of covariates to variability in trough total plasma concentrations of (R)-, (R)- and (S)-methadone in methadone maintenance therapy patients

Covariate	(R)-	(R)-	(S)-
Dose of methadone	29%	29%	25%
Duration of addiction	25%	24%	20%
CYP 3A activity	7%	8%	8%
Continued abuse of morphine	7%	–	8%
Total contribution (%)	68%	61%	61%
P-value	<0.001	<0.001	<0.001

Table 2 Regression equations describing relationships between trough total plasma concentrations (TPC) of (R)-, (R)- and (S)-methadone and significant covariates

TPC of (R)-methadone = 540.5 + (2.9 × dose) + (–1.5 × DA) + (–4.7 × CYP3AA) + (–85.05 × AM)
TPC of (R)-methadone = 238.53 + (1.79 × dose) + (–0.81 × DA) + (–2.41 × CYP3AA)
TPC of (S)-methadone = 263.11 + (1.26 × dose) + (–0.65 × DA) + (–2.06 × CYP3AA –53.38 × AM)

Dose, dose of methadone (mg); DA, duration of addiction before starting methadone maintenance therapy (month); CYP3AA, CYP3A activity [as measured by midazolam oral clearance (l h^{–1})]; AM, abuse of morphine (positive = 1, negative = 0).

Results

Urinalysis was positive for trazodone, dihydrocodeine, 6-monoacetylmorphine, codeine, morphine, benzodiazepines, cannabinoids, cocaine, amphetamines and barbiturates, in four (4%), two (2%), nine (10%), 37 (41%), 57 (63%), 60 (67%), 34 (38%), 29 (32%), three (3%) and three (3%) patients, respectively.

Mean (±SD) values of the plasma DEX/DOR ratio (Figure 1a), the salivary *t*_{1/2} of caffeine (Figure 1b) and the oral clearance of midazolam (Figure 1c) were 0.90 (±2.35), 1.75 (±0.27) h^{–1}, and 47.8 (±8.3) l h^{–1}, respectively. When corrected for body weight, midazolam clearance was higher in women than in men (*P* = 0.04).

Multiple regression analysis revealed that 61–68% of the variation in total trough plasma concentrations of (R)-, (R)- and (S)-methadone was explained by dose, duration of addiction, and CYP3A activity, with dose explaining the largest proportion (Table 1). CYP3A activity accounted for 7, 8 and 8% of the variation in total trough plasma concentrations of (R)-, (R)- and (S)-methadone, respectively

(Table 1). Trough plasma concentrations of methadone increased with increasing dosage, and decreased with increasing CYP3A activity and duration of opiate addiction (Table 2). Trough plasma concentrations of (R)- and (S)-methadone were lower in MMT patients who abused morphine, accounting for the effects of all other covariates within the multiple regression (*P* ≥ 0.001).

There was no significant contribution from other potential covariates, specifically CYP2D6 activity, CYP1A2 activity, weight, age, sex, duration of addiction, duration of MMT and use of other abused drugs.

Multiple regression analysis revealed that 15–36% of the variation in the oral clearances of (R)-, (R)- and (S)-methadone was explained by two variables, namely CYP3A4 activity and the illegal use of morphine (Table 3). CYP3A activity accounted for 22, 16, 15 and 23% of the variation in the clearances of unbound (R)-, unbound (S)-, total (R)-methadone and total (S)-methadone, respectively. The use of morphine explained 14 and 10% of the variation in the clearances of unbound (R)- and unbound

Table 3

Results of regression analysis to determine the contributions of CYP3A activity and use of abused morphine to the oral clearance of (RS)-, (R)- and (S)-methadone

	(RS-) Total	(R-) Unbound	(S-) Total	Unbound
CYP 3A activity	15%	22%	23%	16%
Abused morphine	–	14%	–	10%
Total	15%	36%	23%	26%
P-value	0.033	0.001	0.005	0.009

Table 4

Regression equations describing relationships between oral clearance of methadone and its enantiomers and significant covariates

CL/F of total (RS)-methadone = $4.53 + (0.04 \times \text{CYP3AA})$
CL/F of unbound (R)-methadone = $-141.3 + (6.3 \times \text{CYP3AA}) + (86.5 \times \text{AM})$
CL/F of total (S)-methadone = $3.8 + (0.05 \times \text{CYP3AA})$
CL/F of unbound (S)-methadone = $136.6 + (1.7 \times \text{AM})$

CYP3AA, CYP3A activity [as measured by oral midazolam clearance (l h^{-1})]; AM, abuse of morphine (positive = 1, negative = 0).

(S)-methadone, respectively. There was no significant contribution from other potential covariates, namely methadone dose, weight, age, sex, CYP2D6 activity, CYP1A2 activity, urine pH, duration of addiction, duration of MMT and use of other abused drugs, to methadone clearance. The oral clearance of methadone increased with increasing CYP3A activity, which contributed more to the metabolism of unbound (R)-methadone than to that of unbound (S)-methadone (Table 4). The oral clearance of unbound (R)-methadone was greater in MMT patients who abused morphine.

Discussion

Consistent with previous reports [61–66], our findings indicate statistically significant relationships between dose and trough concentrations of (RS)-methadone, (R)-methadone and (S)-methadone. Eap *et al.* [62] have shown that dose explains <50% of the variability in concentration of (R)-methadone, even in patients not receiving co-medication. In the present study, 25–29% of the variation in total trough plasma concentration of (RS)-, (R)- and (S)-methadone was explained by dose. A factor that might explain the lower correlation coefficients found in the present study might be compliance. Although all the dose intakes for the purpose of this study (at the time taking the other probe substrates) were monitored, prior intake of methadone dose had not been witnessed by the dispensing pharmacist in 51% of our subjects.

Many reports suggest that CYP3A is mainly responsible for the metabolism of methadone [17–41], some indicate that both CYP3A4 and CYP2B6 have major influence, and CYP2D6 is considered to make a minor contribution [41, 46]. CYP3A exhibits marked interindividual variability in catalytic activity [48]. Therefore, it has been suggested that the determination of CYP3A activity prior to methadone administration could help to predict optimal doses under steady-state conditions [31, 67]. The results of the present study question this suggestion, in that a rather modest contribution of CYP3A to methadone disposition was observed. This is in agreement with the observation of a low correlation between the 1'-hydroxy midazolam/midazolam metabolic ratio and (R)-, (S)- and (RS)-methadone plasma concentrations [41]. Shinderman *et al.* [31] have also shown a significant but weak correlation between CYP3A activity and steady-state plasma trough concentrations of (RS)-methadone, also using the 1'-hydroxy midazolam/midazolam ratio. Chalier *et al.* [67] failed to find a significant correlation between the *in vivo* activity of CYP3A indicated by the urinary 6-hydroxycortisol/cortisol ratio and dose-corrected steady-state plasma trough concentration of (RS)-methadone. However, the urinary 6-hydroxycortisol/cortisol ratio is not considered to be a robust index of interindividual variability in CYP3A activity, despite its potential usefulness in monitoring CYP3A activity within individuals [50, 68–70]. The present study has also shown that CYP3A activity contributed more to the clearance of unbound than total methadone. This may reflect additional variability imposed on total concentrations by differences in plasma binding. In agreement with previous studies [18, 19, 21, 23, 41], the results of present study indicate minor roles for CYP2D6 and CYP1A2 in the disposition of methadone. Although ultrarapid metabolizers with respect to CYP2D6 have been shown to have lower trough (RS)-methadone plasma concentrations compared with extensive or intermediate metabolizers, poor metabolizer status had no influence [41]. We demonstrated higher CYP1A2 activity in MMT patients, even after accounting for the effort of smoking. This could be due to enzyme induction by methadone or other drugs that MMT patients receive or to differences in smoking habits (heavier smoking in addicts). Previously, we also reported [49] a high proportion of the poor metabolizer CYP2D6 phenotype in MMT patients, explained by phenocopying due to inhibition of CYP2D6 activity by methadone and/or co-medications. CYP2B6 genotype has been found to influence trough plasma concentrations of both (R)- and (S)-methadone, whereas CYP2C9 and CYP2C19 genotypes were without effect [46]. Polymorphisms in the gene for ABCB1 transporter appear to make a small contribution to interindividual variability in methadone kinetics [41].

A greater clearance of unbound (R)-methadone and lower trough plasma concentrations of (RS)- and (S)-methadone in MMT patients still abusing morphine is dif-

difficult to explain on the basis of any direct influence of morphine on the metabolism of methadone, since morphine is not known to be an inducer of cytochromes P450. An indirect influence mediated by upregulation of P-glycoprotein by morphine [71, 72] modulating transporter–enzyme interplay may account for the observation.

The finding that total plasma trough concentrations of (R)-, (R)- and (S)-methadone, decreased with increase in the duration of heroin abuse, reflects a progressive decrease in oral bioavailability, increase in systemic clearance, or decrease in volume of distribution or all three.

In conclusion, CYP3A activity was shown to have a statistically significant but, on average, relatively modest influence on unbound methadone disposition in MMT patients, whereas CYP2D6 and CYP1A2 played no significant role. The use of CYP3A inhibitors and inducers should be monitored in patients taking methadone, since in some individuals the contribution of this enzyme to net methadone elimination could dominate.

M.R.S. was supported by a PhD studentship funded by the Ministry of Health and Medical Education of the Islamic Republic of Iran.

Appendix

For determining pharmacokinetic parameters of caffeine the elimination rate constant (k), absorption rate constant (k_a) and a mixed function parameter 'A' were considered as primary parameters. A simplex algorithm method was used to estimate initial parameter values, and log normal distributions were assumed for all population pharmacokinetic parameters. Salivary caffeine concentrations C_{saliva} were described by Equation 1:

$$C_{saliva} = A \times (e^{-k(t-t_{lag})} - e^{-k_a(t-t_{lag})}) + Base \times e^{-k(t-t_{lag})} \quad (1)$$

where 'A' is a constant defining dose and bioavailability, 'Base' is the model estimate of the baseline concentration of caffeine in saliva taken prior to the probe dose of caffeine, 't' is the time of caffeine intake, 't_{lag}' is the lag time, 'k' is the elimination rate constant and 'k_a' is the absorption rate constant. The last two parameters were considered as primary parameters. The caffeine salivary half-life was directly estimated from the elimination rate constant ($t_{1/2} = 0.693/k$). The correction procedure used in this study has been recently described by Gupta and co-workers [73].

For methadone kinetic analysis, we used first-order absorption and one- or two-compartment disposition model with similar corrections for residual concentration at the time of intake. Two compartmental disposition was found to be more suitable than the corresponding one-compartment model to describe the steady-state plasma concentrations of methadone and its enantiomers (C_{ss})

(Equation 2). Since the model accounted for residual methadone concentration from previous doses, no assumptions on dosage itself or the degree of compliance were required:

$$C_{ss} = \frac{ka \times D}{V/F} \times \left(\frac{k_{21} - ka}{(\beta - ka) \times (\alpha - ka)} \times e^{-ka(t-t_{lag})} + \frac{k_{21} - \alpha}{(ka - \alpha) \times (\beta - \alpha)} \times e^{-\alpha(t-t_{lag})} + \frac{k_{21} - \beta}{(ka - \beta) \times (\alpha - \beta)} \times e^{-\beta(t-t_{lag})} \right) + Base \times e^{-\beta(t-t_{lag})} \quad (2)$$

$$\text{where } \alpha = \frac{k_{21} \times CL/V}{\beta}$$

$$\text{and } \beta = \frac{1}{2} \times \left[(k_{12} + k_{21} + CL/V) - \sqrt{(k_{12} + k_{21} + CL/V)^2 - 4k_{21} \times CL/V} \right]$$

where 'D' is the dose, 't' is the time since dosing, 'k' is the elimination rate defined as CL/V and 'Base' is the model estimate of the trough concentration of methadone from the previous dose.

REFERENCES

- De Vos JW, Geerlings PJ, Van den Brink W, Ufkes JGR. Pharmacokinetics of methadone and its primary metabolite in 20 opiate addicts. *Eur J Clin Pharmacol* 1995; 48: 361.
- Strain EC, Stitzer ML, Liebson IA, Bigelow GE. Dose–response effects of methadone in the treatment of opioid dependence. *Ann Intern Med* 1993; 119: 23–7.
- Strain EC, Bigelow GE, Liebson IA, Stitzer ML. Moderate- vs high-dose methadone in the treatment of opioid dependence: a randomized trial. *JAMA* 1999; 281: 1000–5.
- Ling W, Wesson DR, Charuvastra C, Klett CJ. A controlled trial comparing buprenorphine and methadone maintenance in opioid dependence. *Arch Gen Psychiatry* 1996; 53: 401–7.
- Hiltunen AJ, Beck O, Hjelm Dahl P, Liljeberg P, Almstrom U, Brodin K, von Wachenfeldt J, Borg S. Rated well-being in relation to plasma concentrations of l- and d-methadone in satisfied and dissatisfied patients on methadone maintenance treatment. *Psychopharmacology (Berl)* 1999; 143: 385–93.
- Havassy BE, Tschann JM. Chronic heroin use during methadone treatment: a test of the efficacy of high maintenance doses. *Addict Behav* 1984; 9: 57–65.
- Farre M, Mas A, Torrens M, Moreno V, Cami J. Retention rate and illicit opioid use during methadone maintenance interventions: a meta-analysis. *Drug Alcohol Depend* 2002; 65: 283–90.
- D'Ippoliti D, Davoli M, Perucci CA, Pasqualini F, Bargagli AM. Retention in treatment of heroin users in Italy: the role of

- treatment type and of methadone maintenance dosage. *Drug Alcohol Depend* 1998; 52: 167–71.
- 9 Dickinson GL, Rostami-Hodjegan A, Lagundoye O, Seivewright N, Pratt P, Lennard MS. A six-year evaluation of methadone prescribing practices at a substance misuse treatment centre in the UK. *J Clin Pharm Ther* 2006; 31: 477–84.
  - 10 Maddux JF, Esquivel M, Vogtsberger KN, Desmond DP. Methadone dose and urine morphine. *J Subst Abuse Treat* 1991; 8: 195–201.
  - 11 Spinzi GC, Bierti L, Bortoli A, Colombo E, Fertitta AM, Lanzi GL, Venturelli R, Minoli G. Comparison of omeprazole and lansoprazole in short-term triple therapy for *Helicobacter pylori* infection. *Aliment Pharmacol Ther* 1998; 12: 433–8.
  - 12 Rostami-Hodjegan A, Foster DJR, Charlier C, Eap CB, Tucker GT. Meta-analysis of the dose–concentration relationship for methadone and a nomogram to assess compliance and metabolic variability [abstract]. *J Psychopharmacol* 2001; 15: A35.
  - 13 Meresaar U, Nilsson MI, Holmstrand J, Anggard E. Single dose pharmacokinetics and bioavailability of methadone in man studied with a stable isotope method. *Eur J Clin Pharmacol* 1981; 20: 473–8.
  - 14 Bellward GD, Warren PM, Howald W, Axelson JE, Abbott FS. Methadone maintenance: effect of urinary pH on renal clearance in chronic high and low doses. *Clin Pharmacol Ther* 1977; 22: 92–9.
  - 15 Inturrisi CE, Verebely K. The levels of methadone in the plasma in methadone maintenance. *Clin Pharmacol Ther* 1972; 13: 633–7.
  - 16 Inturrisi CE, Verebely K. Disposition of methadone in man after a single oral dose. *Clin Pharmacol Ther* 1972; 13: 923–30.
  - 17 Eap CB, Buclin T, Baumann P. Interindividual variability of the clinical pharmacokinetics of methadone: implications for the treatment of opioid dependence. *Clin Pharmacokinet* 2002; 41: 1153–93.
  - 18 Iribarne C, Berthou F, Baird S, Dreano Y, Picart D, Bail JP, Beaune P, Menez JF. Involvement of cytochrome P450 3A4 enzyme in the N-demethylation of methadone in human liver microsomes. *Chem Res Toxicol* 1996; 9: 365–73.
  - 19 Moody DE, Alburges ME, Parker RJ, Collins JM, Strong JM. The involvement of cytochrome P450 3A4 in the N-demethylation of L-alpha-acetylmethadol (LAAM), norLAAM and methadone. *Drug Metab Dispos* 1997; 25: 1347–53.
  - 20 Foster DJR, Somogyi AA, Bochner F. Methadone N-demethylation in human liver microsomes: lack of stereoselectivity and involvement of CYP3A4. *Br J Clin Pharmacol* 1999; 47: 403–12.
  - 21 Gerber JG, Rhodes RJ, Gal J. Stereoselective metabolism of methadone N-demethylation by cytochrome P4502B6 and 2C19. *Chirality* 2004; 16: 36–44.
  - 22 Oda Y, Kharasch ED. Metabolism of methadone and levo-alpha-acetylmethadol (LAAM) by human intestinal cytochrome P450 3A4 (CYP3A4): potential contribution of intestinal metabolism to presystemic clearance and bioactivation. *J Pharmacol Exp Ther* 2001; 298: 1021–32.
  - 23 Wang JS, DeVane CL. Involvement of CYP3A4, CYP2C8, and CYP2D6 in the metabolism of (R)- and (S)-methadone *in vitro*. *Drug Metab Dispos* 2003; 31: 742–7.
  - 24 Rizzo N, Hispard E, Dolbeault S, Dally S, Leverge R, Girre C. Impact of long-term ethanol consumption on CYP1A2 activity. *Clin Pharmacol Ther* 1997; 62: 505–9.
  - 25 Eich-Hochli D, Oppliger R, Golay KP, Baumann P, Eap CB. Methadone maintenance treatment and St. John's Wort – a case report. *Pharmacopsychiatry* 2003; 36: 35–7.
  - 26 de Maat MMR, Ekhardt GC, Huitema ADR, Koks CHW, Mulder JW, Beijnen JH. Drug interactions between antiretroviral drugs and comedicated agents. *Clin Pharmacokinetics* 2003; 42: 223–82.
  - 27 Beauverie P, Taburet AM, Dessalles MC, Furlan V, Touzeau D. Therapeutic drug monitoring of methadone in HIV-infected patients receiving protease inhibitors. *AIDS* 1998; 12: 2510–1.
  - 28 Beauverie P, Furlan V, Edel YA. Slow metabolism and long half life of methadone in a patient with lung cancer and cirrhosis. *Ann Med Interne (Paris)* 2001; 152 (Suppl. 7): 50–2.
  - 29 Boulton DW, Arnaud P, DeVane CL. Pharmacokinetics and pharmacodynamics of methadone enantiomers after a single oral dose of racemate. *Clin Pharmacol Ther* 2001; 70: 48–57.
  - 30 Cobb MN, Desai J, Brown LS Jr, Zannikos PN, Rainey PM. The effect of fluconazole on the clinical pharmacokinetics of methadone. *Clin Pharmacol Ther* 1998; 63: 655–62.
  - 31 Shinderman M, Maxwell S, Brawand-Amey M, Golay KP, Baumann P, Eap CB. Cytochrome P4503A4 metabolic activity, methadone blood concentrations, and methadone doses. *Drug Alcohol Depend* 2003; 69: 205–11.
  - 32 Altice FL, Friedland GH, Cooney EL. Nevirapine induced opiate withdrawal among injection drug users with HIV infection receiving methadone. *AIDS* 1999; 13: 957–62.
  - 33 Heelon MW, Meade LB. Methadone withdrawal when starting an antiretroviral regimen including nevirapine. *Pharmacotherapy* 1999; 19: 471–2.
  - 34 Iribarne C, Berthou F, Carlhant D, Dreano Y, Picart D, Lohezic F, Riche C. Inhibition of methadone and buprenorphine N-dealkylations by three HIV-1 protease inhibitors. *Drug Metab Dispos* 1998; 26: 257–60.
  - 35 Clarke SM, Mulcahy FM, Tjia J, Reynolds HE, Gibbons SE, Barry MG, Back DJ. The pharmacokinetics of methadone in HIV-positive patients receiving the non-nucleoside reverse transcriptase inhibitor efavirenz. *Br J Clin Pharmacol* 2001; 51: 213–7.
  - 36 Clarke SM, Mulcahy FM, Tjia J, Reynolds HE, Gibbons SE, Barry MG, Back DJ. Pharmacokinetic interactions of nevirapine and methadone and guidelines for use of nevirapine to treat injection drug users. *Clin Infect Dis* 2001; 33: 1595–7.
  - 37 Marzolini C, Troillet N, Telenti A, Baumann P, Decosterd LA, Eap CB. Efavirenz decreases methadone blood concentrations. *AIDS* 2000; 14: 1291.

- 38 Gourevitch MN, Friedland GH. Interactions between methadone and medications used to treat HIV infection: a review. *Mt Sinai J Med* 2000; 67: 429–36.
- 39 Bart PA, Rizzardi PG, Gallant S, Golay KP, Baumann P, Pantaleo G, Eap CB. Short communication: methadone blood concentrations are decreased by the administration of abacavir plus amprenavir. *Ther Drug Monit* 2001; 23: 553–5.
- 40 Pinzani V, Faucherre V, Peyriere H, Blayac JP. Methadone withdrawal symptoms with nevirapine and efavirenz. *Ann Pharmacother* 2000; 34: 405–7.
- 41 Crettol S, Deglon JJ, Besson J, Croquette-Krokar M, Hammig R, Gothuey I, Monnat M, Eap CB. ABCB1 and cytochrome P450 genotypes and phenotypes: influence on methadone plasma levels and response to treatment. *Clin Pharmacol Ther* 2006; 80: 668–81.
- 42 Maany I, Dhopes V, Arndt IO, Burke W, Woody G, O'Brien CP. Increase in desipramine serum levels associated with methadone treatment. *Am J Psychiatry* 1989; 146: 1611–3.
- 43 Kosten TR, Gawin FH, Morgan C, Nelson JC, Jatlow P. Desipramine and its 2-hydroxy metabolite in patients taking or not taking methadone. *Am J Psychiatry* 1990; 147: 1379–80.
- 44 Eap CB, Bertschy G, Powell K, Baumann P. Fluvoxamine and fluoxetine do not interact in the same way with the metabolism of the enantiomers of methadone. *J Clin Psychopharmacol* 1997; 17: 113–7.
- 45 Herrlin K. Methadone, ciprofloxacin, and adverse drug reactions. *Lancet* 2000; 356 (9247): 2069–70.
- 46 Crettol S, Deglon JJ, Besson J, Croquette-Krokar M, Gothuey I, Hammig R, Monnat M, Huttemann H, Baumann P, Eap CB. Methadone enantiomer plasma levels, CYP2B6, CYP2C19, and CYP2C9 genotypes, and response to treatment. *Clin Pharmacol Ther* 2005; 78: 593–604.
- 47 Whittington D, Sheffels P, Kharasch ED. Stereoselective determination of methadone and the primary metabolite EDDP in human plasma by automated on-line extraction and liquid chromatography mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 2004; 809: 313–21.
- 48 Paine MF, Khalighi M, Fisher JM, Shen DD, Kunze KL, Marsh CL, Perkins JD, Thummel KE. Characterization of interintestinal and intrainestinal variations in human CYP3A-dependent metabolism. *J Pharmacol Exp Ther* 1997; 283: 1552–62.
- 49 Shiran MR, Chowdry J, Rostami-Hodjegan A, Ellis SW, Lennard MS, Iqbal MZ, Lagundoye O, Seivewright N, Tucker GT. A discordance between cytochrome P450 2D6 genotype and phenotype in patients undergoing methadone maintenance treatment. *Br J Clin Pharmacol* 2003; 56: 220–4.
- 50 Kinirons MT, O'Shea D, Kim RB, Groopman JD, Thummel KE, Wood AJ, Wilkinson GR. Failure of erythromycin breath test to correlate with midazolam clearance as a probe of cytochrome P4503A. *Clin Pharmacol Ther* 1999; 66: 224–31.
- 51 Thummel KE, O'Shea D, Paine MF, Shen DD, Kunze KL, Perkins JD, Wilkinson GR. Oral first-pass elimination of midazolam involves both gastrointestinal and hepatic CYP3A-mediated metabolism. *Clin Pharmacol Ther* 1996; 59: 491–502.
- 52 Chen ZR, Somogyi AA, Bochner F. Simultaneous determination of dextromethorphan and three metabolites in plasma and urine using high-performance liquid chromatography with application to their disposition in man. *Ther Drug Monit* 1990; 12: 97–104.
- 53 Mortimer O, Lindstrom B, Laurell H, Bergman U, Rane A. Dextromethorphan: polymorphic serum pattern of the O-demethylated and didemethylated metabolites in man. *Br J Clin Pharmacol* 1989; 27: 223–7.
- 54 Kohler D, Hartter S, Fuchs K, Sieghart W, Hiemke C. CYP2D6 genotype and phenotyping by determination of dextromethorphan and metabolites in serum of healthy controls and of patients under psychotropic medication. *Pharmacogenetics* 1997; 7: 453–61.
- 55 Hu OY, Tang HS, Lane HY, Chang WH, Hu TM. Novel single-point plasma or saliva dextromethorphan method for determining CYP2D6 activity. *J Pharmacol Exp Ther* 1998; 285: 955–60.
- 56 Shiran MR, Rostami-Hodjegan A, Abdul-Manap R, Lennard MS, Tucker GT. Optimal number of subjects and sampling time to assess CYP2D6 activity using the plasma dextromethorphan/dextrorphan metabolic ratio. *Br J Clin Pharmacol* 2003; 55: 439–40.
- 57 O'Connell SE, Zurzola FJ. Rapid quantitative liquid chromatographic determination of caffeine levels in plasma after oral dosing. *J Pharm Sci* 1984; 73: 1009–11.
- 58 Rostami-Hodjegan A, Rezaee S, Shiran MR, Newbold SG, Lennard MS, Grünwald RA, Tucker GT. Assessment of CYP1A2 activity from caffeine (CA) half-life using saliva samples and POPPK. In: 15th International Symposium on Microsomes and Drug Oxidations: Chemical Biology in the Postgenomic Era New Approaches and Applications, 2004. Mainz (Germany), 2004.
- 59 Shiran MR, Hassanzadeh-Khayyat M, Iqbal MZ, Lagundoye O, Seivewright N, Lennard MS, Tucker GT, Rostami-Hodjegan A. Can saliva replace plasma for the monitoring of methadone? *Ther Drug Monit* 2005; 27: 580–6.
- 60 Foster DJ, Somogyi AA, White JM, Bochner F. Population pharmacokinetics of (R)-, (S)- and rac-methadone in methadone maintenance patients. *Br J Clin Pharmacol* 2004; 57: 742–55.
- 61 Foster DJ, Somogyi AA, Dyer KR, White JM, Bochner F. Steady-state pharmacokinetics of (R)- and (S)-methadone in methadone maintenance patients. *Br J Clin Pharmacol* 2000; 50: 427–40.
- 62 Eap CB, Bourquin M, Martin J, Spagnoli J, Livoti S, Powell K, Baumann P, Deglon J. Plasma concentrations of the enantiomers of methadone and therapeutic response in methadone maintenance treatment. *Drug Alcohol Depend* 2000; 61: 47–54.
- 63 Wolff K, Hay A. Methadone concentrations in plasma and their relationship to drug dosage. *Clin Chem* 1992; 38: 438–9.

- 64** Eap CB, Bertschy G, Baumann P, Finkbeiner T, Gastpar M, Scherbaum N. High interindividual variability of methadone enantiomer blood levels to dose ratios. *Arch Gen Psychiatry* 1998; 55: 89–90.
- 65** Wolff K, Strang J. Therapeutic drug monitoring for methadone: scanning the horizon. *Eur Addict Res* 1999; 5: 36–42.
- 66** Wolff K, Hay A, Raistrick D, Calvert R, Feely M. Measuring compliance in methadone maintenance patients: use of a pharmacologic indicator to 'estimate' methadone plasma levels. *Clin Pharmacol Ther* 1991; 50: 199–207.
- 67** Charlier C, Dessalles MC, Plomteux G. Methadone maintenance treatment: is it possible to adapt the daily doses to the metabolic activity of the patient? *Ther Drug Monit* 2001; 23: 1–3.
- 68** Kinirons MT, O'Shea D, Downing TE, Fitzwilliam AT, Joellenbeck L, Groopman JD, Wilkinson GR, Wood AJ. Absence of correlations among three putative *in vivo* probes of human cytochrome P4503A activity in young healthy men. *Clin Pharmacol Ther* 1993; 54: 621–9.
- 69** Watkins PB, Turgeon DK, Saenger P, Lown KS, Kolars JC, Hamilton T, Fishman K, Guzelian PS, Voorhees JJ. Comparison of urinary 6-beta-cortisol and the erythromycin breath test as measures of hepatic P450III_A (CYP3A) activity. *Clin Pharmacol Ther* 1992; 52: 265–73.
- 70** Hunt CM, Watkins PB, Saenger P, Stave GM, Barlascini N, Watlington CO, Wright JT Jr, Guzelian PS. Heterogeneity of CYP3A isoforms metabolizing erythromycin and cortisol. *Clin Pharmacol Ther* 1992; 51: 18–23.
- 71** Bouer R, Barthe L, Philibert C, Tournaire C, Woodley J, Houin G. The roles of P-glycoprotein and intracellular metabolism in the intestinal absorption of methadone: *in vitro* studies using the rat everted intestinal sac. *Fundam Clin Pharmacol* 1999; 13: 494–500.
- 72** Aquilante CL, Letrent SP, Pollack GM, Brouwer KL. Increased brain P-glycoprotein in morphine tolerant rats. *Life Sci* 2000; 66: PL47–51.
- 73** Gupta P, Hutmacher NN, Frame B, Miller R. An alternative method for population pharmacokinetic data analysis under noncompliance. *J Pharmacokin Pharmacodyn* 2008; 35: 219–33.