Steady-state pharmacokinetics of (R)- and (S)-methadone in methadone maintenance patients

David J. R. Foster,¹ Andrew A. Somogyi,^{1,3} Kyle R. Dyer,^{1,2} Jason M. White¹ & Felix Bochner^{1,3}

¹Department of Clinical and Experimental Pharmacology, University of Adelaide, Adelaide, 5005, Australia. ²Department of Psychology, University of Adelaide, Adelaide 5005, Australia. ³Department of Clinical Pharmacology, Royal Adelaide Hospital, Adelaide 5000, Australia

Aims To investigate the steady-state pharmacokinetics of (R)- and (S)-methadone in a methadone maintenance population.

Methods Eighteen patients recruited from a public methadone maintenance program underwent an interdosing interval pharmacokinetic study. Plasma and urine samples were collected and analysed for methadone and its major metabolite (EDDP) using stereoselective h.p.l.c. Methadone plasma protein binding was examined using ultrafiltration, and plasma α_1 -acid glycoprotein concentrations were quantified by radial immunoassay.

Results (R)-methadone had a significantly ($P < 0.05$) greater unbound fraction (mean 173%) and total renal clearance (182%) compared with (S)-methadone, while maximum measured plasma concentrations (83%) and apparent partial clearance of methadone to EDDP (76%) were significantly ($P < 0.001$) lower. When protein binding was considered (R)-methadone plasma clearance of the unbound fraction (59%) and apparent partial intrinsic clearance to EDDP (44%) were significantly ($P < 0.01$) lower than for (S)-methadone, while AUC_{τ}^{ss} (167%) was significantly ($P < 0.001$) greater. There were no significant ($P > 0.2$) differences between the methadone enantiomers for AUC_{τ}^{ss} , steady-state plasma clearance, trough plasma concentrations and unbound renal clearance. Patients excreted significantly ($P < 0.0001$) more (R)-methadone and (S)-EDDP than the corresponding enantiomers. Considerable interindividual variability was observed for the pharmacokinetic parameters, with coefficients of variation of up to 70%.

Conclusions Steady-state pharmacokinetics of unbound methadone are stereoselective, and there is large interindividual variability consistent with CYP3A4 mediated metabolism to the major metabolite EDDP; the variability did not obscure a significant dose-plasma concentration relationship. Stereoselective differences in the pharmacokinetics of methadone may have important implications for pharmacokinetic-pharmacodynamic modelling but is unlikely to be important for therapeutic drug monitoring of methadone, in the setting of opioid dependence.

Keywords: 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, drug dependence, metabolism, methadone, pharmacokinetics, protein binding, stereoselectivity

Introduction

Since its formal introduction in 1965 [1], methadone has

become the most widely used pharmacological agent for the treatment of opioid dependence [2]. *Rac*-methadone (6-dimethylamino-4,4-diphenyl-3-heptanone) is a chiral molecule of (R)- and (S)-enantiomeric forms in which (R)-methadone has a 10-fold higher affinity at μ and δ opioid receptors [3] and possesses up to 50 times the analgesic activity of (S)-methadone in human and animal models of antinociception [4]. (R)-methadone prevents the occurrence of opioid withdrawal symptoms, while

Correspondence: David J. R. Foster, Department of Clinical and Experimental Pharmacology, University of Adelaide, Adelaide 5005, South Australia. Work Tel.: + 61 88303 5985; Fax: + 61 88224 0685; E-mail: david.foster@adelaide.edu.au

Received 1 February 2000, accepted 20 July 2000.

(S)-methadone is ineffective [5, 6]. Despite this high eudismic ratio, methadone is used as the racemate in most countries.

Methadone is primarily eliminated from the body by metabolism with nine metabolites having been identified in human urine [7–9], although the data have been, in most cases, qualitative rather than quantitative. Mono *N*-demethylation, a primary metabolic pathway, results in the formation of a highly unstable compound which then undergoes spontaneous cyclization and dehydration to form 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP). Other identified metabolites appear to contribute quantitatively little to the overall metabolism of methadone [8, 10], although complete mass balance has not been achieved. After oral dosing, unchanged methadone and EDDP are found in similar amounts in human urine and account for up to 50% of the dose [8, 10, 11].

Few studies have examined the pharmacokinetics of the individual enantiomers after administration of *rac*-methadone. After a single dose for pain control, the two enantiomers differed significantly in their disposition [12], with (R)-methadone having a larger volume of distribution, longer terminal elimination half-life and higher total body clearance.

Examination of the disposition of the methadone enantiomers after chronic dosing of racemic methadone has been limited. Studies that have used a stereoselective assay have confined their investigations to enantiomeric ratios in plasma samples taken at single time points, for example at the end of an interdosing interval, and have reported wide interpatient variability [13–15]. Kreek and coworkers [16, 17] administered an equal amount of each stable isotope labelled methadone enantiomer, used mass-spectrometry to examine stereoselectivity in the pharmacokinetics of methadone in maintenance subjects, and reported similar results to those of Kristensen and coworkers [12].

Compared with the (S)-enantiomer, (R)-methadone has lower plasma protein binding, with α_1 -acid glycoprotein being the predominant binding protein [18, 19]. No study to date has examined the role of variability in protein binding in the pharmacokinetics of total (R)- and (S)-methadone. It is not known whether stereoselectivity in total body clearance is due to metabolism (intrinsic clearance) and/or protein binding.

The aim of the present study was to determine the steady-state pharmacokinetics of (R)- and (S)-methadone in a methadone maintenance population, and to examine factors which might contribute to their variability.

Methods

Patients

Ethical approval was obtained from the Royal Adelaide Hospital Research Ethics Committee. The patients had been enrolled in the South Australian Public Methadone Maintenance Program for at least 6 months (range 6 months to 10 years) and had not had a methadone dose change for at least 2 months. There were 11 males and 7 females; body weights ranged from 60 to 94 kg (mean $\pm$ s.d.; 74 ± 10 kg); ages ranged from 21 to 45 years (35 ± 7 years). The once-daily methadone dose ranged from 7.5 to 130 mg day⁻¹, which corresponded to 0.12–1.9 mg kg⁻¹ (0.88 ± 0.50 mg kg⁻¹). The patients were allowed to take benzodiazepines in therapeutic doses. The majority smoked cigarettes, 7 showed positive urinalysis for benzodiazepines, 10 for cannabinoids, 2 for opioids other than methadone, 1 for barbiturates, 2 for sympathomimetic amines and 4 consumed alcohol regularly in quantities less than 40 g day⁻¹. Patients were excluded from the study if they were pregnant or had positive HIV serology.

Each patient was admitted to the inpatient facility of the maintenance program 1 h before their scheduled daily dose and remained in the unit for the subsequent 24 h. Methadone was administered as a syrup under supervision of the study personnel. An 18 gauge indwelling venous catheter (JelcoTM, Critikon Corp, Tampa, Fla, USA) was inserted into a forearm vein prior to the daily dose and kept patent with a Teflon stylet (JelcoTM). A 5 ml blood sample was collected before the dose and at the following times after dosing: 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 9 and 12 h, and at the end of the interdosing interval (23–24 h). Blood was centrifuged, and the plasma stored at -20°C until analysis. In 10 subjects (9 male, 1 female), a 24 h pooled urine sample was also obtained, volume and pH measured and an aliquot stored at -20°C until analysis. These patients have been described elsewhere [20], in which plasma *rac*-methadone concentration-effect relationships were investigated.

Chemicals

Rac-methadone as the hydrochloride salt, (R)- and (S)-methadone as the free bases, and (R)- and (S)-2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) as the perchlorate salts were obtained from the National Institute on Drug Abuse (Rockville, MD, USA). Racemic-EDDP as the hydroiodide salt was purchased from Alltech-Applied Science Labs (State College, PA, USA). 3-methoxymorphinan as the hydrobromide salt was a kind gift from Roche Products Pty Ltd (Sydney, NSW, Australia). Other materials were obtained from the

following sources: bovine serum albumin (fraction V), and human α_1 -acid glycoprotein (purified from Cohn fraction VI) from Sigma Chemical Company (St Louis, MO, USA). H.p.l.c. grade acetonitrile, methanol and triethylamine were from BDH Laboratory Supplies (Poole, UK). All other reagents and chemicals were obtained from commercial sources and were of analytical grade quality.

Sample analysis

Quantification of (R)- and (S)-methadone in plasma and plasma ultrafiltrate This method was based upon that of Norris & coworkers [21], with major modifications. The h.p.l.c. system comprised a LC-10AT pump (Shimadzu, Kyoto, Japan) at a flow rate of 1 ml min⁻¹, a Sil-10A autoinjector (Shimadzu), a Cyclobond I 2000 RSP column (250 × 4.6 mm, Astec, Whippany, NJ, USA) with a Cyclobond I 2000 RSP precolumn (20 × 4.0 mm, Astec), and a SPD-M10A photo-diode array detector (Shimadzu) set at 210 nm. The system was controlled using Class-LC10 software (version 1, Shimadzu) running under Windows 3.11 (Microsoft Corporation, WA, USA) on a 486 DX IBM compatible computer. Optimal separation of the compounds of interest was achieved with a mobile phase of 9:11:80 (v/v) methanol:acetonitrile:1% triethylamine (v/v) in water with the final pH adjusted to 6.0 with ortho-phosphoric acid. Retention times of (R)-methadone, (S)-methadone and 3-methoxymorphinan (internal standard) were 8.3, 9.6 and 21 min, respectively. Under these conditions all compounds of interest were baseline resolved. Enantioseparation of EDDP was not achieved, and the peak corresponding to EDDP was not resolved from (R)-methadone under these chromatography conditions. Recently Rudaz & Veuthey [22] reported similar inability to resolve EDDP from methadone with the Cyclobond I 2000 RSP column. However, EDDP is not back-extracted into 5 mM HCl and did not interfere with the analysis of (R)- and (S)-methadone in this assay.

Patient samples were quantified with calibration curves consisting of eight standards over the concentration range 15–600 ng ml⁻¹ of (R)- and (S)-methadone prepared in blank plasma. Plasma samples (1 ml) and internal standard (100 µl 5 µg ml⁻¹ 3-methoxymorphinan in water) were aliquoted into 10 ml tapered bottom plastic tubes, alkalinized (0.4 ml 0.1 M Na₂CO₃ pH 10) and extracted with 6 ml of 30:70 (v/v) diethyl ether:hexane for 20 min on a rotary mixer. Samples were then centrifuged (2000 g, 10 min) and the organic phase transferred to a clean 10 ml tapered bottom plastic tube containing 0.25 ml 5 mM HCl and vortexed for 1 min. Samples were then centrifuged (2000 g, 10 min), the organic phase aspirated to waste and 100 µl of the acid phase was injected onto the chromatography system. The analysis of (R)- and (S)-

methadone in plasma ultrafiltrate (see below) was performed as for plasma, with the following modifications: calibration curves consisted of eight standards over the concentration range 12.5–500 ng ml⁻¹ of (R)- and (S)-methadone prepared in isotonic phosphate buffer (67 mM Na₂HPO₄/NaH₂PO₄ in 0.9% NaCl, final pH 7.4); only 0.35 ml plasma ultrafiltrate was assayed; and 50 µl of 5 µg ml⁻¹ 3-methoxymorphinan was used as the internal standard.

Interassay variability was monitored with quality control (QC) samples prepared in duplicate at three concentrations; low (LQC, 54 ng ml⁻¹), medium (MQC, 90 ng ml⁻¹) and high (HQC, 350 ng ml⁻¹) of (R)- and (S)-methadone. Interassay accuracy and precision (mean ± %coefficient of variation (%CV); *n* = 14 assays on separate days) was 106 ± 7% (LQC), 103 ± 4% (MQC) and 100 ± 4% (HQC) for (R)-methadone, and 103 ± 7% (LQC), 105 ± 5% (MQC) and 103 ± 6% (HQC) for (S)-methadone. Similarly, intra-assay accuracy and precision (*n* = 6 replicate samples) was 108 ± 8% (LQC), 106 ± 7% (MQC) and 107 ± 4% (HQC) for (R)-methadone, and 104 ± 7% (LQC), 106 ± 6% (MQC) and 112 ± 5% (HQC) for (S)-methadone. The assay was both precise and accurate at the limit of quantification (15 ng ml⁻¹), with interassay accuracy and precision (*n* = 11 assays on separate days) being 101 ± 4% and 104 ± 4% for (R)- and (S)-methadone, respectively. Calculated from intra-assay replicate plasma QC samples, mean (*n* = 6 replicate samples) extraction efficiencies of (R)- and (S)-methadone were 101 ± 6% and 99 ± 7% (LQC); 101 ± 4% and 100 ± 3% (MQC); 101 ± 3% and 103 ± 2% (HQC), respectively, while extraction efficiency (*n* = 6 replicate samples) of the internal standard was 84 ± 1%. Similar assay validation and extraction efficiency results were obtained for the plasma ultrafiltrate assay.

Quantification of (R)-methadone, (S)-methadone, (R)-EDDP and (S)-EDDP in urine Quantification of the enantiomers of methadone and EDDP was achieved using a previously validated stereoselective h.p.l.c. assay, which has been reported elsewhere [23]. Patient samples were quantified with calibration curves consisting of seven standards over the concentration range 0.13–12.5 µM of (R)- and (S)-methadone (39–3900 ng ml⁻¹) and (R)- and (S)-EDDP (35–3500 ng ml⁻¹) prepared in blank urine. Inter-assay variability was monitored with QC samples prepared in duplicate at three concentrations. Inter-assay inaccuracy (% bias) and precision (*n* = 4 assays on separate days) was <10% for all compounds at all QC concentrations, with the exception of (S)-EDDP for the LQC precision, which was 13%. Similarly, intra-assay inaccuracy (% bias) and precision (*n* = 6, replicate samples) was <12% for all compounds at all QC concentrations. The assay was both precise and accurate at the limit of quantification (39 ng ml⁻¹ for (R)- and (S)-methadone and 35 ng ml⁻¹

for (R)- and (S)-EDDP) with interassay inaccuracy (% bias) and precision ($n=4$ assays on separate days) being $<6\%$ for all compounds.

Quantification of rac-methadone in plasma The method used for the analysis of rac-methadone in plasma has been previously reported [20]. Concentrations of rac-methadone obtained by this assay were used to compare the sum of (R)- and (S)-methadone in plasma obtained with the stereoselective assay. This was performed in order to provide a further validation of the stereoselective assays.

Determination of plasma (R)- and (S)-methadone unbound fraction Determination of the unbound (nonprotein bound) (R)- and (S)-methadone concentration in plasma samples was performed using an ultrafiltration method modified from Wilkins and coworkers [24]. Initial experiments indicated very low unbound methadone concentrations in the patient samples. In order to quantify the unbound methadone concentrations it was necessary to increase the concentration of total (bound plus unbound) rac-methadone in plasma. This was achieved by the addition of 10–15 μl of a concentrated rac-methadone solution in isotonic phosphate buffer to a pooled 1.2 ml plasma sample obtained from each subjects' set of plasma samples. The volume of methadone solution was chosen to yield a rac-methadone concentration of 1500 ng ml^{-1} , taking into consideration the original methadone concentration present in the sample. Preliminary investigations demonstrated that (R)- and (S)-methadone plasma protein binding (bound concentration *vs* bound/unbound concentration) was linear up to 5000 ng ml^{-1} of each enantiomer in the plasma of two healthy volunteers (results not shown). A 100 μl aliquot was taken, diluted with 900 μl of blank plasma and assayed for total (bound plus unbound) (R)- and (S)-methadone to accurately determine the concentration of total (bound plus unbound) (R)- and (S)-methadone. The remaining sample was warmed to 37°C in a shaking heated water bath, and a 1 ml aliquot was then transferred to an ultrafiltration device (MPS-1, Amicon, MA, USA) which had been prewarmed to 37°C. Prior to the addition of plasma, each device was passivated overnight with a solution of 60 g l^{-1} bovine serum albumin in isotonic phosphate buffer (pH 7.4), rinsed three times with isotonic phosphate buffer and dried. The passivation procedure was necessary as preliminary experiments indicated up to 30% nonspecific binding to the device. The filtration membrane (YMT 30000 molecular weight cut off, Amicon) did not demonstrate any nonspecific binding ($<1\%$). Samples were then processed according to the manufacturers' directions and the method of Wilkins and coworkers [24], using a Beckman J2–21 centrifuge with both the chamber and the fixed angle rotor (JA-20.1, Beckman) prewarmed to 37°C. Briefly, samples were centrifuged at 1500 g for a maximum of 30 min or until a

maximum of 400 μl of filtrate had been collected. Concentrations of (R)- and (S)-methadone in the resulting plasma ultrafiltrate were quantified using h.p.l.c.

Quantification of plasma α_1 -acid glycoprotein concentration Plasma concentrations of α_1 -acid glycoprotein were determined using radial immunoassay plates (Behring Diagnostics, Marburg, Germany). Measurement of the diameter of the precipitated antibody was performed using a calibrated 10 $\times$ magnification eyepiece. Two mutually perpendicular measurements were taken and the average value was used for calculations. Quality control samples were prepared at three concentrations (LQC: 30.5 mg dl^{-1} , MQC: 61 mg dl^{-1} , HQC: 122 mg dl^{-1}) by diluting a commercially available reference sample (N/T Protein Control SL/H, Behring Diagnostics) in isotonic phosphate buffer. Inter-plate precision ($n=4$ plates) was 2% (LQC), 4% (MQC) and 8% (HQC).

Pharmacokinetic and statistical analysis

(R)- and (S)-methadone unbound fraction (f_u) was calculated as plasma ultrafiltrate concentration divided by total (protein bound and nonprotein bound) plasma concentration, and was expressed as a percentage. Area under the (R)- and (S)-methadone concentration-time curve during the dosing interval at steady-state ($\text{AUC}_{\tau}^{\text{ss}}$) was determined by the linear trapezoidal method. Steady-state concentrations ($C_{\text{av}}^{\text{ss}}$) were calculated by dividing $\text{AUC}_{\tau}^{\text{ss}}$ by the dosing interval and normalized to a 70 mg rac-methadone (35 mg each enantiomer) dose. Time to reach (t_{max}) maximum measured steady-state plasma concentration ($C_{\text{max}}^{\text{ss}}$), minimum plasma concentration prestudy dose ($C_{\text{min}(\text{first})}^{\text{ss}}$) and 24 h postdose ($C_{\text{min}(\text{last})}^{\text{ss}}$) were obtained by direct observation of the data. $C_{\text{max}}^{\text{ss}}$, $C_{\text{min}(\text{first})}^{\text{ss}}$ and $C_{\text{min}(\text{last})}^{\text{ss}}$ were normalized to a 70 mg rac-methadone (35 mg each enantiomer) dose. Apparent plasma clearance at steady-state (CL/F) was calculated as dose/ $\text{AUC}_{\tau}^{\text{ss}}$, renal clearance (CL_R) as amount of methadone recovered in the 0–24 h urine sample divided by methadone $\text{AUC}_{\tau}^{\text{ss}}$, and apparent partial clearance of methadone to EDDP ($\text{CL}_{\text{MD} \rightarrow \text{EDDP}}$) as amount of EDDP recovered in the 0–24 h urine sample divided by methadone $\text{AUC}_{\tau}^{\text{ss}}$, taking into consideration molecular weight differences. Percent dose recovered (f_e) was expressed as the percentage of the dose administered (assuming an equal amount of each enantiomer in the racemic dose) that was recovered in the 0–24 h urine sample, taking into consideration molecular weight differences in the case of EDDP. These pharmacokinetic parameters were also calculated with respect to the unbound enantiomers ($C_{\text{max},u}^{\text{ss}}$, $C_{\text{min}(\text{first}),u}^{\text{ss}}$, $C_{\text{min}(\text{last}),u}^{\text{ss}}$, $\text{AUC}_{\tau,u}^{\text{ss}}$, $C_{\text{av},u}^{\text{ss}}$, CL_{u}/F , $\text{CL}_{R,u}$ and $\text{CL}_{\text{MD} \rightarrow \text{EDDP},u}$), by either multiplying or dividing the corresponding parameters by f_u . Peak to trough plasma concentration ratios

for each patient were calculated by dividing $C_{\max}^{\text{ss}}$ by $C_{\min(\text{last})}^{\text{ss}}$. Ratios of (R)- to (S)-methadone were calculated for $C_{\min(\text{first})}^{\text{ss}}$ and $C_{\min(\text{last})}^{\text{ss}}$, and the resultant parameters corrected for plasma binding, by the division of (R)-methadone by (S)-methadone unbound fraction values. Additionally, ratios of total and unbound (R)- to (S)-methadone concentrations were calculated separately at the $t_{\max}$ of (R)-methadone ($R_{\max}^{\text{ss}}/S_{\max}^{\text{ss}}$, $R_{\max_{\text{u}}}^{\text{ss}}/S_{\max_{\text{u}}}^{\text{ss}}$) and the $t_{\max}$ of (S)-methadone ($R_{\max}^{\text{ss}}/S_{\max}^{\text{ss}}$, $R_{\max_{\text{u}}}^{\text{ss}}/S_{\max_{\text{u}}}^{\text{ss}}$) by the division of (R)-methadone $C_{\max}^{\text{ss}}$ by the corresponding (S)-methadone concentration at (R)-methadone $t_{\max}$ and the division of the (R)-methadone concentration at the (S)-methadone $t_{\max}$ by (S)-methadone $C_{\max}^{\text{ss}}$, respectively. Ratios of plasma (R)- to (S)-methadone concentrations were also calculated at each time point.

As a validation procedure, ordinary least products linear regression analysis [25, 26] was used to compare the sum of plasma (R)- and (S)-methadone concentrations in the subjects' samples with the results from the racemic plasma assay using Excel (Excel v7.0a, Microsoft). Statistically significant differences between enantiomers were assessed using paired *t*-tests, while other comparisons were performed using un-paired *t*-tests and linear regression analysis was performed to yield Pearson's *r* values (GraphPad Prism v2.01, GraphPad Software, CA, USA). Mean ratios of (R)- to (S)-methadone concentrations calculated at each time point were compared with a theoretical value of 1 using a 2-tailed 1 sample *t*-test. Differences were considered significant at $P < 0.05$. All data are presented as mean $\pm$ s.d.

Results

Ordinary least products linear regression analysis comparing the sum of (R)- and (S)-methadone plasma concentrations in patient samples ($n = 185$) with the results obtained with the racemic plasma assay yielded a strong and significant relationship ($r^2 = 0.98$, $P < 0.05$) and the 95% confidence intervals of the slope (0.988; 1.052) and intercept (-18.4 ; 1.46) included 1 and 0, respectively.

Figure 1 shows the interdosing interval mean plasma (R)- and (S)-methadone concentration-time profiles of the 18 subjects, normalized to a 70 mg *rac*-methadone dose.

Pre-dose ($C_{\min(\text{first})}^{\text{ss}}$) and 24 h postdose ($C_{\min(\text{last})}^{\text{ss}}$) plasma concentrations were not statistically significantly different (P value, mean difference; 95% CI) for (R)-methadone ($P = 0.86$, -1 ng ml^{-1} ; $-11, 9 \text{ ng ml}^{-1}$) or (S)-methadone ($P = 0.31$, 7 ng ml^{-1} ; $-7, 21 \text{ ng ml}^{-1}$). Similarly, $C_{\min(\text{first})}^{\text{ss}}$ ($P = 0.55$; Table 1) and $C_{\min(\text{last})}^{\text{ss}}$ ($P = 0.95$; Table 1) values were not statistically significantly different between the two enantiomers. In contrast (R)-methadone $C_{\max}^{\text{ss}}$ values were on average 83% those of (S)-methadone ($P = 0.0002$; Table 1). When corrected for protein binding, mean (R)-methadone $C_{\min(\text{first})_{\text{u}}}^{\text{ss}}$, $C_{\min(\text{last})_{\text{u}}}^{\text{ss}}$ and $C_{\max_{\text{u}}}^{\text{ss}}$ values were between 170% and 175% those of (S)-methadone ($P < 0.0001$; Table 2). The peak to trough plasma concentration ratio of (R)-methadone was a mean of 79% of that obtained for (S)-methadone ($P = 0.0001$; Table 1). (R)-methadone $t_{\max}$ ($3.1 \pm 1.9 \text{ h}$) values were significantly ($P = 0.0072$, 0.6 h; 0.2, 1.1 h) longer than for (S)-methadone ($2.4 \pm 1.6 \text{ h}$).

Figure 1. Mean plasma (R)-methadone (●) and (S)-methadone (○) concentration-time profiles for 18 methadone maintenance patients. Data are represented as concentration normalized to a 70 mg *rac*-methadone dose. Error bars indicate s.d.

The ratio of plasma (R)- to (S)-methadone concentrations predose ($R_{\min(\text{first})}^{\text{ss}}/S_{\min(\text{first})}^{\text{ss}}$) was not significantly different ($P=0.58$; Table 3) from that obtained at the end of the dosing interval ($R_{\min(\text{last})}^{\text{ss}}/S_{\min(\text{last})}^{\text{ss}}$). Similarly, unbound plasma (R)- to (S)-methadone concentration ratios at predose ($R_{\min(\text{first})_u}^{\text{ss}}/S_{\min(\text{first})_u}^{\text{ss}}$) and at the end of the dosing interval ($R_{\min(\text{last})_u}^{\text{ss}}/S_{\min(\text{last})_u}^{\text{ss}}$) were not significantly different ($P=0.52$; Table 3). In contrast, ratios of (R)- to (S)-methadone calculated separately at the $t_{\max}$ of (R)-methadone ($R_{\max}^{\text{ss}}/S^{\text{ss}}$) and (S)-methadone ($R_u^{\text{ss}}/S_{\max_u}^{\text{ss}}$) were significantly lower compared with $R_{\min(\text{last})}^{\text{ss}}/S_{\min(\text{last})}^{\text{ss}}$ ($P<0.0001$ and $P=0.0002$, respectively; Table 3). Similarly, ratios of unbound (R)- to (S)-methadone calculated separately at the $t_{\max}$ of (R)-methadone ($R_{\max_u}^{\text{ss}}/S_u^{\text{ss}}$) and (S)-methadone ($R_u^{\text{ss}}/S_{\max_u}^{\text{ss}}$) were significantly lower compared with $R_{\min(\text{last})_u}^{\text{ss}}/S_{\min(\text{last})_u}^{\text{ss}}$ ($P=0.0003$ and $P=0.0008$, respectively; Table 3). The ratios of (R)- to (S)-methadone concentrations were significantly lower than unity at 1 ($P>0.0003$, -0.16 ; -0.24 , -0.09), 1.5 ($P<0.0001$, -0.18 ; -0.24 , -0.12), 2 ($P=0.0004$: -0.16 , -0.24 , -0.08), 3 ($P=0.0053$, -0.12 ; -0.20 , -0.04) and 4 ($P=0.0139$, -0.12 ; -0.22 , -0.03) h postdose (Figure 2), but not at other times ($0.94\geq P\geq 0.14$; Figure 2).

There was a highly significant relationship between

plasma AUC_{τ}^{ss} and dose for (R)-methadone ($r^2=0.68$, $P<0.0001$; Figure 3a) and (S)-methadone ($r^2=0.47$, $P=0.002$; Figure 3b). The plasma AUC_{τ}^{ss} of (R)-methadone was not statistically significantly different ($P=0.21$; Table 1) from the value for (S)-methadone. (R)-methadone $C_{\text{av}}^{\text{ss}}$ was an average of 90% that obtained for (S)-methadone ($P=0.0571$; Table 1), but this was only of borderline statistical significance. However, when corrected for protein binding, plasma $AUC_{\tau_u}^{\text{ss}}$ ($P=0.0003$; Table 2) and $C_{\text{av}_u}^{\text{ss}}$ ($P<0.0001$; Table 2) of (R)-methadone were on average 167% and 158% of those obtained for (S)-methadone, respectively.

The plasma unbound fractions of (R)-methadone were 171% those of (S)-methadone ($P<0.0001$; Table 2). There was no significant difference in unbound fractions between males and females for (R)-methadone ($3.62\pm 0.98\%$ vs $3.69\pm 0.65\%$, respectively; $P=0.88$, -0.07% ; -0.89 , 1.02%) or (S)-methadone ($2.15\pm 0.58\%$ vs $2.04\pm 0.42\%$, respectively; $P=0.69$, 0.11% ; -0.69 , 0.47%). There was a weak but significant relationship between plasma α_1 -acid glycoprotein concentration and ratio of bound/unbound methadone concentrations in the patient samples for (R)-methadone ($r^2=0.31$; $P=0.019$; Figure 4a) and (S)-methadone ($r^2=0.30$; $P=0.021$; Figure 4b).

The mean concentration of α_1 -acid glycoprotein in

Table 1 Disposition of total (bound plus unbound) (R)- and (S)-methadone following chronic dosing of between 7.5 mg and 130 mg *rac*-methadone once daily in 18 patients enrolled in a maintenance programme.

Parameter ¹		Mean	Range	%CV	<i>P</i> value ² (mean difference; 95% CI)
$C_{\min(\text{first})}^{\text{ss}}$ (ng ml ⁻¹)	(R)-methadone	147	42–223	30	0.55
	(S)-methadone	153	31–261	43	(-6; -24, 14)
$C_{\min(\text{last})}^{\text{ss}}$ (ng ml ⁻¹)	(R)-methadone	148	40–243	34	0.78
	(S)-methadone	146	31–242	42	(2; -15, 20)
$C_{\max}^{\text{ss}}$ (ng ml ⁻¹)	(R)-methadone	251	120–362	27	0.0002
	(S)-methadone	303	116–447	32	(-52; -74, -29)
$C_{\text{av}}^{\text{ss}}$ (ng ml ⁻¹)	(R)-methadone	168	67–237	29	0.0571
	(S)-methadone	186	52–304	37	(-18; -36, 1)
AUC_{τ}^{ss} (ng ml ⁻¹ h)	(R)-methadone	3484	608–7263	53	0.21
	(S)-methadone	3797	589–7229	55	(-313; -814, 188)
CL/ <i>F</i> (ml min ⁻¹)	(R)-methadone	161	103–363	42	0.86
	(S)-methadone	159	79–465	60	(2; -18, 21)
CL _R (ml min ⁻¹)	(R)-methadone	27.4	11.9–46.6	37	<0.0001
	(S)-methadone	15.1	7.0–26.5	39	(12.4; 9.2, 15.5)
CL _{MD→EDDP} (ml min ⁻¹)	(R)-methadone	21.9	3.6–37.3	48	0.0088
	(S)-methadone	28.8	5.5–58.9	55	(-6.9; -11.6, 2.2)
P/T	(R)-methadone	1.81	1.35–3.58	30	<0.0001
	(S)-methadone	2.30	1.68–4.49	34	(-0.49; -0.68, -0.29)

¹ $C_{\min(\text{first})}^{\text{ss}}$ = plasma concentration, normalized to a 70 mg *rac*-methadone dose, predose; $C_{\min(\text{last})}^{\text{ss}}$ = plasma concentration, normalized to a 70 mg *rac*-methadone dose, 24 h after dosing; $C_{\max}^{\text{ss}}$ = maximum measured plasma concentration, normalized to a 70 mg *rac*-methadone dose; $C_{\text{av}}^{\text{ss}}$ = steady-state plasma concentration, normalized to a 70 mg *rac*-methadone dose; AUC_{τ}^{ss} = area under the plasma concentration-time curve during the inter-dosing interval; CL/*F* = apparent plasma clearance at steady-state; CL_R = renal clearance; CL_{MD→EDDP} = apparent partial clearance of methadone to EDDP; P/T = peak to trough plasma concentration ratio. ²Comparison of (R)-methadone vs (S)-methadone.

Table 2 Disposition of unbound (R)- and (S)-methadone following chronic dosing of between 7.5 mg and 130 mg *rac*-methadone once daily in 18 patients enrolled in a maintenance programme.

Parameter ¹		Mean	Range	%C	P value ² (mean difference; 95% CI)
f _u (%)	(R)-methadone	3.6	1.8–4.8	24	<0.0001
	(S)-methadone	2.1	1.2–2.8	25	(1.5; 1.3, 1.8)
C _{min(first)_u} ^{ss} (ng ml ⁻¹)	(R)-methadone	5.3	2.0–8.0	33	<0.0001
	(S)-methadone	3.1	0.8–5.9	46	(2.2; 1.6, 2.8)
C _{min(last)_u} ^{ss} (ng ml ⁻¹)	(R)-methadone	5.4	1.9–7.8	33	<0.0001
	(S)-methadone	3.1	0.8–5.5	43	(2.3; 1.7, 2.9)
C _{max_u} ^{ss} (ng ml ⁻¹)	(R)-methadone	8.9	4.3–13.4	29	<0.0001
	(S)-methadone	6.2	2.8–9.2	34	(2.7; 2.0, 3.5)
C _{av_u} ^{ss} (ng ml ⁻¹)	(R)-methadone	6.0	2.3–11.0	34	<0.0001
	(S)-methadone	3.8	1.4–6.1	40	(2.3; 1.6, 2.9)
AUC _{τ_u} ^{ss} (ng ml ⁻¹ h)	(R)-methadone	117	28–268	51	0.0003
	(S)-methadone	70	16–126	46	(47; 26, 68)
CL _u /F (ml min ⁻¹)	(R)-methadone	4611	2214–10590	46	0.0001
	(S)-methadone	7845	3994–17750	51	(-3235; -4590, -1879)
CL _{R_u} (ml min ⁻¹)	(R)-methadone	847	256–1832	48	0.63
	(S)-methadone	819	265–1444	44	(28; -100, 155)
CL _{MD→EDDP_u} (ml min ⁻¹)	(R)-methadone	727	77–1890	70	0.0017
	(S)-methadone	1656	207–3506	66	(-930; -1406, -453)

¹f_u = unbound fraction; C_{min(first)_u}^{ss} = unbound plasma concentration, normalized to a 70 mg *rac*-methadone dose, predose; C_{min(last)_u}^{ss} = unbound plasma concentration, normalized to a 70 mg *rac*-methadone dose, 24 h after dosing; C_{max_u}^{ss} = maximum measured unbound plasma concentration, normalized to a 70 mg *rac*-methadone dose; C_{av_u}^{ss} = steady-state plasma concentration of unbound drug, normalized to a 70 mg *rac*-methadone dose; AUC_{τ_u}^{ss} = area under the plasma unbound concentration–time curve during the interdosing interval; CL_u/F = apparent plasma clearance at steady-state of the unbound drug; CL_{R_u} = renal clearance of the unbound drug; CL_{MD→EDDP_u} = apparent partial intrinsic clearance of methadone to EDDP.

²Comparison of (R)-methadone *vs* (S)-methadone.

Table 3 (R)/(S) enantiomeric ratio at peak and trough concentrations following chronic dosing of between 7.5 mg and 130 mg *rac*-methadone once daily in 18 patients enrolled in a maintenance programme.

Parameter ¹	Mean	Range	%CV	P value ² (mean difference; 95% CI)
R _{min(first)} ^{ss} /S _{min(first)} ^{ss}	1.07	0.68–2.28	34	0.57 (-0.02; -0.10, 0.06)
R _{min(last)} ^{ss} /S _{min(last)} ^{ss}	1.10	0.70–1.97	27	
R _{max} ^{ss} /S _{max} ^{ss}	0.88	0.68–1.40	19	<0.0001 (-0.21; -0.30, -0.13)
R _{max} ^{ss} /S _{max} ^{ss}	0.84 ³	0.66–1.07	15	0.0002 (-0.26; -0.38, -0.15)
R _{min(first)_u} ^{ss} /S _{min(first)_u} ^{ss}	1.91	1.20–4.11	38	0.52 (-0.05; -0.20, 0.10)
R _{min(last)_u} ^{ss} /S _{min(last)_u} ^{ss}	1.96	1.24–3.54	33	
R _{max_u} ^{ss} /S _{max_u} ^{ss}	1.55	1.09–2.52	26	0.0003 (-0.41; -0.60, -0.22)
R _u ^{ss} /S _{max_u} ^{ss}	1.45 ⁴	1.09–2.04	20	0.0008 (-0.51; -0.77, -0.25)

¹R_{min(first)}^{ss}/S_{min(first)}^{ss} = ratio of (R)- to (S)-methadone plasma concentrations predose; R_{min(last)}^{ss}/S_{min(last)}^{ss} = ratio of (R)- to (S)-methadone plasma concentrations 24 h after dosing; R_{max}^{ss}/S_{max}^{ss} = ratio of (R)-methadone C_{max}^{ss} and the corresponding (S)-methadone concentration at (R)-methadone t_{max}; R_{max}^{ss}/S_{max}^{ss} = ratio of (R)-methadone concentration at the (S)-methadone t_{max} and (S)-methadone C_{max}^{ss}; R_{min(first)_u}^{ss}/S_{min(first)_u}^{ss} = ratio of (R)- to (S)-methadone plasma concentrations of the unbound drug predose; R_{min(last)_u}^{ss}/S_{min(last)_u}^{ss} = ratio of (R)- to (S)-methadone plasma concentrations of the unbound drug 24 h after dosing; R_{max_u}^{ss}/S_{max_u}^{ss} = ratio of (R)-methadone C_{max_u}^{ss} and the corresponding (S)-methadone concentration of the unbound drug at (R)-methadone t_{max}; R_u^{ss}/S_{max_u}^{ss} = ratio of (R)-methadone concentration of the unbound drug at the (S)-methadone t_{max} and (S)-methadone C_{max_u}^{ss}.

²Comparison within each group *vs* the parameter with a blank cell in the P value column; ³Statistically significant difference (P value, mean difference; 95% CI) compared with R_{max}^{ss}/S_{max}^{ss} (P=0.043, 0.048; 0.002, 0.094); ⁴statistically significant difference compared with R_{max_u}^{ss}/S_u^{ss} (P=0.047, 0.098; 0.002, 0.195).

Figure 2. Mean plasma methadone (R)/(S) concentration ratio *vs* time profiles for 18 methadone maintenance patients. * Statistically significantly different from unity. Error bars indicate s.d. The *y*-axis has been truncated to enlarge the scale.

plasma samples from the patients was 115 ± 24 mg dl⁻¹ (range: 73–155 mg dl⁻¹). These concentrations were not significantly different ($P=0.68$, 5 mg dl⁻¹; -30, 20 mg dl⁻¹) between males (117 ± 25 mg dl⁻¹; range: 79–155 mg dl⁻¹) and females (112 ± 23 mg dl⁻¹; range: 73–150 mg dl⁻¹).

The renal clearance of total (bound plus unbound) (R)-methadone was a mean of 182% that of (S)-methadone ($P<0.0001$; Table 1). After correcting for protein binding, there was no significant difference for CL_{Ru}, between (R)-methadone and (S)-methadone ($P=0.63$; Table 2). The excretion of unchanged (R)-methadone was a mean of 160% that of (S)-methadone ($P<0.0001$; Table 4). Conversely, the excretion of (R)-EDDP was a mean of 68% of that obtained for (S)-EDDP ($P<0.0001$; Table 4). These differences resulted in a significantly ($P=0.0008$, -1.36; -1.99, -0.73) lower urinary ratio of EDDP/methadone for the (R)-enantiomer (0.93 ± 0.50) compared with the (S)-enantiomer (2.29 ± 1.37). However, there was no significant difference in excretion for the sum of methadone and EDDP of the (R)-enantiomers ($17.8 \pm 4.2\%$) compared with the (S)-enantiomers ($18.1 \pm 5.0\%$; $P=0.75$, -0.3%; -1.9, 1.5%), or between the sum of (R)- and (S)-methadone ($15.6 \pm 4.9\%$) and (R)- and (S)-EDDP ($20.3 \pm 8.2\%$; $P=0.17$, -4.8%; -12.0, 2.5%). The mean total urinary recovery of (R)- and (S)-methadone and (R)- and (S)-EDDP was $35.9 \pm 8.9\%$ of the dose.

The apparent plasma clearance with respect to total (bound plus unbound) methadone was not significantly different for (R)-methadone and (S)-methadone

($P=0.86$; Table 1). When corrected for protein binding, (R)-methadone CL_u/F was on average 59% that of (S)-methadone ($P=0.0001$; Table 2). The apparent partial clearance of (R)-methadone to (R)-EDDP was on average 76% of the clearance of (S)-methadone to (S)-EDDP ($P=0.0088$; Table 1). When corrected for protein binding, apparent partial intrinsic clearance of (R)-methadone to (R)-EDDP was on average 44% of the clearance of (S)-methadone to (S)-EDDP ($P=0.0017$; Table 2).

There was no statistically significant effect of gender on any of the pharmacokinetic parameters derived from plasma concentration-time profiles for either (R)-methadone ($0.92 > P > 0.10$) or (S)-methadone ($0.83 > P > 0.10$).

Discussion

To our knowledge, this is the first study to comprehensively describe the disposition of methadone in a large cohort of methadone maintenance patients, with respect to stereoselectivity. The study found substantial stereoselectivity of most pharmacokinetic parameters, and also intersubject variability, which could contribute to altered therapeutic efficacy. The significantly lower CL_u/F for (R)-methadone, which describes the elimination of the unbound pharmacologically active methadone enantiomer, indicates that (R)-methadone has a lower intrinsic clearance when compared with (S)-methadone. Further, there was a significantly greater fraction of the dose excreted in the urine as (S)-EDDP and (R)-methadone,

Figure 3. Relationship between dose and (a) (R)-methadone (●; $r^2=0.68$, $P<0.0001$) and (b) (S)-methadone (○ $r^2=0.47$, $P=0.002$) AUC_{τ}^{ss} . Dashed lines represent 95% confidence intervals.

than the corresponding enantiomers, which resulted in a stereoselective difference in the urinary ratio of EDDP/methadone concentrations, suggesting significantly less (R)-methadone than (S)-methadone was metabolized to EDDP. Stereoselectivity in the renal clearance of methadone is unlikely to explain this phenomenon, as there was no difference in the renal clearance of unbound methadone. There was no significant difference between the enantiomers in the sum of methadone and EDDP, or between the sum of (R)- and (S)-methadone and (R)- and (S)-EDDP. These data show that important characteristics of drug metabolism would fail to be observed using nonchiral analytical techniques. These data are in contrast to the results of *in vitro* metabolism studies with methadone which found no stereoselectivity in the intrinsic clearance of methadone to EDDP [27]. Reasons for this lack of agreement include the possibility that methadone may be eliminated by other metabolic pathways which display

stereoselectivity, and/or that there is stereoselectivity in the binding of methadone to proteins in the *in vitro* liver microsomal fraction, which may mask any stereoselectivity in the metabolism by the enzymes mediating the formation of EDDP. It is possible that there may be stereoselectivity in the renal clearance and/or in the elimination of EDDP via faeces, such that the combined urinary and faecal recovery of (R)- and (S)-EDDP are the same. In support of this, significant amounts of EDDP have been recovered in the faeces of methadone maintenance patients (6% [28] to 18% [29]), while methadone was found to account for less than 1% [28, 29]. *N*-demethylation and hydroxylation metabolites of EDDP have been reported [8, 10]. Although these metabolites appear to contribute very little to the metabolic profile of methadone, it is possible that there is stereoselectivity in the metabolism of EDDP to these other metabolites. We did not quantify EDDP in plasma

Figure 4. Relationship between plasma α_1 -acid glycoprotein concentration and (a) plasma (R)-methadone ($\bullet$; $r^2=0.31$, $P=0.019$) and (b) (S)-methadone ($\circ$; $r^2=0.30$, $P=0.021$) bound/unbound concentration ratio. Dashed lines represent 95% confidence intervals. The x-axis has been truncated to maintain a consistent scale.

or faeces, or other possible EDDP metabolites in urine, so are unable to examine these mechanisms further. We observed stereoselectivity in the apparent partial clearance to EDDP ($CL_{MD \rightarrow EDDP}$), and apparent partial intrinsic clearance to EDDP ($CL_{MD \rightarrow EDDP}$) with (R)-methadone values significantly lower than for (S)-methadone (44% and 76%, respectively). The reference to 'apparent' was used as calculations were based on urinary excretion data only and EDDP is eliminated to some extent in faeces [28, 29], and further metabolized to a limited, albeit poorly defined, extent [8, 10]. If one assumes that methadone is not eliminated by other metabolic pathways which display marked net stereoselectivity, and that there is no stereoselectivity in the microsomal protein binding of methadone, then comparison of these data with *in vitro* data [27] indicates that the net nonrenal elimination (faecal elimination and/or further metabolism) of (R)-EDDP is greater than that of (S)-EDDP.

Recent *in vitro* studies have shown CYP3A4 to be the major CYP isoform mediating EDDP formation from *rac*-methadone [30–32] and the individual enantiomers [27]. We observed a large degree of interindividual variability (4- to 5-fold) in $C_{av,u}^{ss}$, and CL_u/F . These pharmacokinetic parameters are mainly determined by metabolic activity, and the interindividual variability observed in this study is consistent with reported *in vitro* [33–35] and *in vivo* [36–39] estimates of variability in hepatic CYP3A4 expression. Markedly higher interindividual variability was observed for AUC_{τ}^{ss} (≈ 12 -fold), $CL_{MD \rightarrow EDDP}$ (≈ 10 -fold) and $CL_{MD \rightarrow EDDP_u}$ (≈ 20 -fold), however, these are influenced by additional factors which include in some cases dose, plasma binding, and nonrenal elimination of EDDP.

There was no difference in the steady-state plasma clearance (CL/F) between the two enantiomers. This observation is in contrast to Kristensen and coworkers

Table 4 Urinary recovery during an interdosing interval of the enantiomers of methadone and EDDP from 10 methadone maintenance patients following chronic dosing of between 7.5 mg and 130 mg *rac*-methadone once daily.

Patient	Dose (mg kg ⁻¹)	(R)-methadone (%)	(S)-methadone (%)	% Dose recovered		Total dose (%)
				(R)-EDDP (%)	(S)-EDDP (%)	
9	1.91	8.6	5.3	9.8	14.2	37.9
10	0.33	6.1	3.7	10.7	16.6	37.1
11	0.82	10.6	7.8	8.2	11.5	38.1
12	0.30	10.7	6.5	8.9	12.9	39.0
13	0.86	13.3	6.7	10.8	15.2	46.0
14	0.77	12.0	7.2	3.8	5.3	28.3
15	0.12	5.8	3.3	1.9	2.9	13.9
16	1.41	7.3	4.3	11.0	16.6	39.2
17	0.93	13.7	10.1	6.5	8.6	38.9
18	0.68	8.0	4.6	10.6	17.4	40.6
Mean	0.88	9.6	6.0 ¹	8.2	12.1 ²	35.9
%CV	57	30	35	39	41	25

¹Statistically significant difference (*P* value, mean difference; 95% CI) compared with (R)-methadone (*P* < 0.0001, -3.6; -4.6, -2.7); ²statistically significant difference compared with (R)-EDDP (*P* = 0.0001, 3.9; 2.5, 5.3).

[12], who reported that the mean plasma clearance of (R)-methadone (158 ml min⁻¹) was significantly greater than for (S)-methadone (129 ml min⁻¹), although the magnitude of the difference was small. In contrast, bioavailability was not found to be stereoselective [12]. Recent population pharmacokinetic modelling studies have shown that the pharmacokinetics of *rac*-methadone differ between single doses in healthy normal subjects and methadone maintenance patients after a single dose [40], and that there are time-dependent changes in the clearance and volume of distribution of *rac*-methadone in methadone maintenance patients while steady-state is being achieved [41]. Comparison with the results of Kristensen and coworkers [12] is difficult, as our study involved patients at steady-state, whereas Kristensen and coworkers studied acute methadone administration. The subject populations were also markedly different, as Kristensen and coworkers' patients were receiving opioids for chronic pain control, and our patients were in a methadone maintenance programme. Assuming a bioavailability of 90%, our results for methadone plasma clearance are in close agreement with those of Kristensen and coworkers [12], being 149 ml min⁻¹ and 143 ml min⁻¹ for (R)- and (S)-methadone, respectively.

The highly significant relationships between plasma AUC_τ^{ss} and dose for both (R)-methadone and (S)-methadone indicate that the pharmacokinetics (extent of absorption, clearance) of each enantiomer are linear after administration of the racemate over a wide dosage range (7.5–130 mg day⁻¹). This is the first time that this has been demonstrated for the individual methadone enantiomers, and confirms previous reports of the linearity of *rac*-methadone pharmacokinetics [42]. It also should be

noted that this relationship was established in separate individuals taking a range of fixed doses, rather than within single individuals all given a range of dosages. This demonstrates that the extent of interindividual variation in metabolic activity does not prevent a dose-plasma concentration relationship, indeed approximately 68% and 47% of the large interindividual variability in the AUC_τ^{ss} of (R)-methadone and (S)-methadone, respectively, is explained by variation in dose, while the remaining variability is mainly due to differences in clearance.

In contrast to the results for AUC_τ^{ss} and CL/*F*, plasma C_{max}^{ss} and peak to trough plasma concentration ratios were significantly lower for total (bound plus unbound) (R)-methadone compared with (S)-methadone. Additionally, Figure 2 shows that the ratio of (R)- to (S)-methadone plasma concentrations is not stable over an interdosing interval. The ratio decreases as plasma concentrations increase, with values at 1–4 h postdose, significantly lower than unity, while ratios at other time points are not different to unity. Indeed, (R)/(S) ratios calculated predose (R_{min(first)}^{ss}/S_{min(first)}^{ss}) and postdose (R_{min(last)}^{ss}/S_{min(last)}^{ss}) were not significantly different, but were both significantly greater than ratios calculated separately at the *t*_{max} of (R)-methadone (R_{max}^{ss}/S_{max}^{ss}) and (S)-methadone (R_{max}^{ss}/S_{max}^{ss}), with identical results obtained when protein binding was considered (Table 3).

These differences may be explained by volume of distribution differences. As (R)-methadone had a lower protein binding compared to (S)-methadone, it is likely to have a greater volume of distribution, as has been previously reported [12]. This would result in lower C_{max}^{ss} values for (R)-methadone in comparison with (S)-

methadone, and hence a lower peak to trough concentration ratio. Recently, we have shown in methadone maintained patients that withdrawal severity can be related to the rate of change of plasma *rac*-methadone concentrations from the time of peak plasma concentration, and that this relationship has a very high Hill slope factor [20]. The stereoselective difference in the magnitude of fluctuation of plasma methadone concentrations reported here may have important implications for pharmacokinetic-pharmacodynamic modelling of methadone, as measurement of *rac*-methadone may not provide an accurate reflection of plasma concentrations of the active (R)-methadone enantiomer. In contrast, therapeutic drug monitoring for patient compliance is unlikely to require stereoselective measurement of plasma methadone concentrations given the close dose-plasma concentration relationship demonstrated for the individual methadone enantiomers found in this study, and for *rac*-methadone by others [41–43].

The plasma protein binding of methadone was found to be stereoselective, with unbound fractions of (R)-methadone ($3.84 \pm 0.86\%$) significantly greater than for (S)-methadone ($2.11 \pm 0.52\%$) in accord with previous findings [18, 44]. However, in contrast to previous studies [18, 19, 24, 44–46], we observed lower unbound fractions. A possible reason for this difference may be that the pH of plasma samples was not maintained at 7.4. Although the ultrafiltration device maintains a constant sample pH during the filtration process, there is evidence that the pH of plasma samples may increase during storage, and changes in plasma pH may alter unbound fractions of other drugs [47]. Despite this possibility, stereoselectivity in methadone plasma protein binding was observed. A weak relationship was found between plasma α_1 -acid glycoprotein concentration and the ratio of bound/unbound concentrations, confirming earlier reports in methadone maintenance patients [19] and other subject populations [18, 19, 48]. Although α_1 -acid glycoprotein has been reported to be the predominant binding protein, investigators have reported that albumin [19, 48] and lipoproteins [19] may also play a role. However, the individual role of each protein appears to be minor compared with that of α_1 -acid glycoprotein, as plasma albumin [18, 19], cholesterol [18], triglyceride [18] and total protein concentrations [18] have been shown not to contribute significantly to the binding of methadone.

The renal clearance of total (bound plus unbound) (R)- and (S)-methadone accounted for approximately 10–20% of CL/F , consistent with previous reports for *rac*-methadone [49–51]. The renal clearance of *rac*-methadone has previously been shown to be pH dependent [50, 51]. Examination for pH dependency of the renal clearance of the methadone enantiomers was not possible in the present study, due to a very narrow pH range amongst samples collected. Of the 10 samples, only two were below pH 6

and the remainder were within the range of 6.0–6.4 pH units. When corrected for protein binding, the renal clearance for each enantiomer (range: 256–1832 ml min^{-1}) was between 2- and 15-fold greater than glomerular filtration rate, indicating extensive net tubular secretion. After correcting for protein binding there was no significant difference ($P=0.63$) between the enantiomers, indicating that the net secretion and reabsorption of methadone is not a stereoselective process.

Plasma concentrations of methadone were quantified with both a chiral and nonchiral assay, allowing a thorough validation of the results. Ordinary least products linear regression analysis is sensitive to both fixed and proportional bias, unlike conventional linear-regression analysis, as it does not assume that one axis is error-free [25, 26]. Comparisons between the stereoselective and racemic assays of methadone in plasma yielded a very strong and highly significant correlation with the 95% confidence intervals of the slope including 1 (indicating no proportional bias), and the intercept including 0 (indicating no fixed bias). This analysis demonstrated excellent performance of the assays, indicating that it is unlikely that there was any interference from other licit and illicit drugs. Combined with the intra- and interassay precision and accuracy of quality control samples, these data indicate that the assays were selective, precise and accurate. A similar validation procedure was used for the analysis of (R)- and (S)-methadone and (R)- and (S)-EDDP in urine [23].

The data presented in this study highlight the importance of protein binding and stereochemical considerations when drawing conclusions on the pharmacokinetics and metabolism of compounds, and demonstrate that care should be taken when interpreting pharmacokinetic and metabolism data of chiral compounds based only upon results of nonchiral analytical techniques. In conclusion, we have shown that the pharmacokinetics of methadone are stereoselective, and that there is large interindividual variability, consistent with CYP3A4 mediated metabolism to the major metabolite EDDP. However this variability did not obscure a strong dose-plasma concentration relationship. Stereoselective differences in the pharmacokinetics of methadone may have important implications for pharmacokinetic-pharmacodynamic modelling but is unlikely to be important for therapeutic drug monitoring of compliance with methadone in the setting of opioid dependence.

The results were presented in part at the XIIIth International Congress of Pharmacology in 1998 (Munich, Germany). David Foster was supported by a Dawes Scholarship from the Royal Adelaide Hospital and a Dora Lush (Biomedical) Postgraduate Scholarship from the National Health and Medical Research Council of Australia. Kyle Dyer was supported by a National Drug Strategy Postgraduate Research Scholarship. The authors wish to thank the National Institute on Drug Abuse for supplying the drug

compounds, and also the Royal Adelaide Hospital, the University of Adelaide and the National Health and Medical Research Council of Australia for financial support, and the staff at the Warinilia Clinic of the South Australian Methadone Maintenance Programme for their invaluable assistance in this project.

References

- Dole VP, Nyswander M. A medical treatment for diacetylmorphine (heroin) addiction. *JAMA* 1965; **193**: 80–84.
- Farrell M, Ward J, Mattick R, *et al.* Methadone maintenance treatment in opiate dependence: a review. *Br Med J* 1994; **309**: 997–1001.
- Kristensen K, Christensen CB, Christrup LL. The μ_1 , μ_2 , δ , κ opioid receptor binding profiles of methadone stereoisomers and morphine. *Life Sci* 1995; **56**: 45–50.
- Scott CC, Robbins EB, Chen KK. Pharmacologic comparison of the optical isomers of methadone. *J Pharmacol Exp Ther* 1948; **93**: 282–286.
- Scherbaum N, Finkbeiner T, Leifert K, Gastpar M. The efficacy of L-methadone and racemic methadone in substitution treatment for opiate addicts—a double-blind comparison. *Pharmacopsychiatry* 1996; **29**: 212–215.
- Isbell H, Eisenman AJ. The addiction liability of some of the drugs of the methadone series. *Fed Proc* 1948; **7**: 162.
- Sullivan HR, Smits SE, Due SL, Booher RE, McMahon RE. Metabolism of d-methadone: Isolation and identification of analgesically active metabolites. *Life Sci* 1972; **11**: 1093–1104.
- Sullivan HR, Due SL. Urinary metabolites of dl-methadone in maintenance subjects. *J Med Chem* 1973; **16**: 909–913.
- Pond SM, Kreek MJ, Tong TG, Raghunath J, Benowitz NL. Altered methadone pharmacokinetics in methadone-maintained pregnant women. *J Pharmacol Exp Ther* 1985; **233**: 1–6.
- Änggård E, Gunne L-M, Homstrand J, McMahon RE, Sandberg CG, Sullivan HR. Disposition of methadone in methadone maintenance. *Clin Pharmacol Ther* 1975; **17**: 258–266.
- Kreek MJ, Bencsath FA, Field FH. Effects of liver disease on urinary excretion of methadone and metabolites in maintenance patients: quantitation by direct probe chemical ionization mass spectrometry. *Biomed Mass Spectrom* 1980; **7**: 385–395.
- Kristensen K, Blemmer T, Angelo HR, *et al.* Stereoselective pharmacokinetics of methadone in chronic pain patients. *Ther Drug Monit* 1996; **18**: 221–227.
- Pham-Huy C, Chikhichorfi N, Galons H, *et al.* Enantioselective high-performance liquid chromatography determination of methadone enantiomers and its major metabolite in human biological fluids using a new derivatized cyclodextrin-bonded phase. *J Chromatogr B* 1997; **700**: 155–163.
- Beck O, Boreus LO, Lafolie P, Jacobsson G. Chiral analysis of methadone in plasma by high-performance liquid chromatography. *J Chromatogr B* 1991; **570**: 198–202.
- Eap CB, Finkbeiner T, Gastpar M, Scherbaum N, Powell K, Baumann P. Replacement of (R)-methadone by a double dose of (R,S)-methadone in addicts: interindividual variability of the (R)/(S) ratios and evidence of adaptive changes in methadone pharmacokinetics. *Eur J Clin Pharmacol* 1996; **50**: 385–389.
- Kreek MJ, Hachey DL, Klein PD. Stereoselective disposition of methadone in man. *Life Sci* 1979; **24**: 925–932.
- Nakamura K, Hachey DL, Kreek MJ, Irving CS, Klein PD. Quantitation of methadone enantiomers in humans using stable isotope-labeled [$^2\text{H}_3$]-, [$^2\text{H}_5$]-, and [$^2\text{H}_8$]-Methadone. *J Pharm Sci* 1982; **71**: 40–43.
- Eap CB, Cuendet C, Baumann P. Binding of d-methadone, L-methadone, and dl-methadone to proteins in plasma of healthy volunteers: role of the variants of α_1 -acid glycoprotein. *Clin Pharmacol Ther* 1990; **47**: 338–346.
- Romach MK, Piafsky KM, Abel JG, Khouw V, Sellers EM. Methadone binding to orosomucoid (α_1 -acid glycoprotein): determinant of free fraction in plasma. *Clin Pharmacol Ther* 1981; **29**: 211–217.
- Dyer KR, Foster DJ, White JM, Somogyi AA, Menelaou A, Bochner F. Steady-state pharmacokinetics and pharmacodynamics in methadone maintenance patients: comparison of those who do and do not experience withdrawal and concentration-effect relationships. *Clin Pharmacol Ther* 1999; **65**: 685–694.
- Norris RL, Ravenscroft PJ, Pond SM. Sensitive high-performance liquid chromatographic assay with ultraviolet detection of methadone enantiomers in plasma. *J Chromatogr B* 1994; **661**: 346–350.
- Rudaz S, Veuthey JL. Chiral stationary phases in HPLC for the stereoselective determination of methadone. *Chirality* 1999; **11**: 319–325.
- Foster DJR, Somogyi AA, Bochner F. Stereoselective quantification of methadone and its major oxidative metabolite, EDDP, in human urine using stereoselective high-performance liquid chromatography. *J Chromatogr B*, 2000; **744**: 165–176.
- Wilkins JN, Ashofteh A, Setoda D, Wheatley WS, Huigen H, Ling W. Ultrafiltration using the Amicon MPS-1 for assessing methadone plasma protein binding. *Ther Drug Monit* 1997; **19**: 83–87.
- Ludbrook J. Comparing methods of measurements. *Clin Exp Pharmacol Physiol* 1997; **24**: 193–203.
- Brace RA. Fitting straight lines to experimental data. *Am J Physiol* 1977; **233**: R94–R99.
- 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**: 403–412.
- Kreek MJ, Bencsath FA, Fanizza A, Field FH. Effects of liver disease on fecal excretion of methadone and its unconjugated metabolites in maintenance patients. Quantitation by direct probe chemical ionization mass spectrometry. *Biomed Mass Spectrom* 1983; **10**: 544–549.
- Verebely K, Volavka J, Mulé S, Resnick R. Methadone in man: pharmacokinetic and excretion studies in acute and chronic treatment. *Clin Pharmacol Ther* 1975; **18**: 180–190.
- Iribarne C, Berthou F, Baird S, *et al.* Involvement of cytochrome P450 3A4 enzyme in the N-demethylation of methadone in human liver microsomes. *Chem Res Toxicol* 1996; **9**: 365–373.
- Iribarne C, Dreano Y, Bardou LG, Menez JF, Berthou F. Interaction of methadone with substrates of human hepatic cytochrome P450 3A4. *Toxicology* 1997; **117**: 13–23.
- Moody DE, Alburges ME, Parker RJ, Collins JM, Strong JM. The involvement of cytochrome P450 3A4 in the

- N-demethylation of 1- α -acetylmethadol (laam), norlaam, and methadone. *Drug Metab Dispos* 1997; **25**: 1347–1353.
- 33 Forrester LM, Henderson CJ, Glancey MJ, et al. Relative expression of cytochrome P450 isoenzymes in human liver and association with the metabolism of drugs and xenobiotics. *Biochem J* 1992; **281**: 359–368.
- 34 Shimada T, Yamazaki H, Mimura M, Inui Y, Guengerich FP. Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J Pharmacol Exp Ther* 1994; **270**: 414–423.
- 35 Chauret N, Gauthier A, Martin J, Nicollgriffith DA. *In vitro* comparison of cytochrome P450-mediated metabolic activities in human, dog, cat, and horse. *Drug Metab Dispos* 1997; **25**: 1130–1136.
- 36 Thummel KE, Shen DD, Podoll TD, et al. Use of midazolam as a human cytochrome P450 3A probe: I. *In vitro*–*in vivo* correlations in liver transplant patients. *J Pharmacol Exp Ther* 1994; **271**: 549–556.
- 37 Thummel KE, Shen DD, Podoll TD, et al. Use of midazolam as a human cytochrome P4503A probe: II. Characterization of inter- and intraindividual hepatic CYP3A variability after liver transplantation. *J Pharmacol Exp Ther* 1994; **271**: 557–566.
- 38 Lown KS, Thummel KE, Benedict PE, et al. The erythromycin breath test predicts the clearance of midazolam. *Clin Pharmacol Ther* 1995; **57**: 16–24.
- 39 Thummel KE, Shea DO, Paine MF, et al. Oral first-pass elimination of midazolam involves both gastrointestinal and hepatic CYP3A-mediated metabolism. *Clin Pharmacol Ther* 1996; **59**: 491–502.
- 40 Wolff K, Rostami-Hodjegan A, Shires S, et al. The pharmacokinetics of methadone in healthy subjects and opiate users. *Br J Clin Pharmacol* 1997; **44**: 325–334.
- 41 Rostami-Hodjegan A, Wolff K, Hay AW, Raistrick D, Calvert R, Tucker GT. Population pharmacokinetics of methadone in opiate users: characterization of time-dependent changes. *Br J Clin Pharmacol* 1999; **48**: 43–52.
- 42 Wolff K, Sanderson M, Hay AW, Raistrick D. Methadone concentrations in plasma and their relationship to drug dosage. *Clin Chem* 1991; **37**: 205–209.
- 43 Wolff K, Hay AW, Raistrick D, Calvert R. Steady-state pharmacokinetics of methadone in opioid addicts. *Eur J Clin Pharmacol* 1993; **44**: 189–194.
- 44 Eap CB, Cuendet C, Baumann P. Selectivity in the binding of psychotropic drugs to the variants of α_1 -acid glycoprotein. *Naunyn Schmiedebergs Arch Pharmacol* 1988; **337**: 220–224.
- 45 Hervé F, Duché JC, d'Athis P, Marché C, Barré J, Tillement JP. Binding of disopyramide, methadone, dipyrindamole, chlorpromazine, lignocaine and progesterone to the two main genetic variants of human α_1 -acid glycoprotein: evidence for drug-binding differences between the variants and for the presence of two separate drug-binding sites on α_1 -acid glycoprotein. *Pharmacogenetics* 1996; **6**: 403–415.
- 46 Judis J. Binding of codeine, morphine, and methadone to human serum proteins. *J Pharm Sci* 1977; **66**: 802–806.
- 47 Ho Ngoc-Ta Trung A, Sirois G. Influence of plasma pH on quinidine uptake by erythrocytes: estimation of free drug fraction in plasma from blood/ and erythrocyte/plasma concentration ratios. *Pharm Acta Helv* 1987; **62**: 61–64.
- 48 Abramson FP. Methadone plasma protein binding: alterations in cancer and displacement from α_1 -acid glycoprotein. *Clin Pharmacol Ther* 1982; **32**: 652–658.
- 49 Inturrisi CE, Colburn WA, Kaiko RF, Houde RW, Foley KM. Pharmacokinetics and pharmacodynamics of methadone in patients with chronic pain. *Clin Pharmacol Ther* 1987; **41**: 392–401.
- 50 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**: 92–99.
- 51 Nilsson MI, Widerlov E, Meresaar U, Änggård E. Effect of urinary pH on the disposition of methadone in man. *Eur J Clin Pharmacol* 1982; **22**: 337–342.