(R)- and (S)-methadone and buprenorphine concentration ratios in maternal and umbilical cord plasma following chronic maintenance dosing in pregnancy

Andrea L. Gordon,¹ Olga V. Lopatko,¹ Andrew A. Somogyi,¹
David J. R. Foster² & Jason M. White¹

¹Discipline of Pharmacology, University of Adelaide, L5 Medical School North, Frome Road, Adelaide, SA 5005, Australia and ²Sansom Institute and School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, SA 5001, Australia

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

Both methadone and buprenorphine cross the placenta during pregnancy which can result in neonatal abstinence syndrome (NAS). Reduced NAS in buprenorphine-exposed infants may be as a result of relatively less buprenorphine reaching the foetal circulation compared with methadone.

WHAT THIS STUDY ADDS

Under chronic dosing conditions in humans, the transfer of methadone and buprenorphine appears greater than previously found in human placental tissue *in vitro* models. Infants born to buprenorphine maintained women may be exposed to relatively less of the maternal dose compared with infants born to women maintained on methadone during pregnancy. There is stereoselectivity in the transfer of the individual enantiomers of methadone across the placenta to the foetal circulation.

Correspondence

Dr Andrea L. Gordon, Discipline of Pharmacology, Level 5 Medical School North, Frome Road, University of Adelaide, SA 5005, Australia.
Tel.: + 61 4 1525 8104
Fax: + 61 8 8302 2168
E-mail: andrea.gordon@unisa.edu.au

Keywords

buprenorphine, methadone, neonatal abstinence syndrome, placental transfer, plasma concentration

Received

14 December 2009

Accepted

22 June 2010

AIMS

The aim of this study was to compare the transfer of buprenorphine and methadone between maternal and cord blood in women under chronic dosing conditions and to determine if differences exist in the transfer of the two methadone enantiomers.

METHODS

Maternal and cord blood samples were collected at delivery from women maintained on methadone (35, 25–140 mg day⁻¹) (median; range) or buprenorphine (6.00, 2–20 mg day⁻¹) during pregnancy. Plasma concentration ratios are presented as an indicator of foetal exposure relative to the mother.

RESULTS

Methadone was quantified in all samples, with cord : maternal plasma methadone concentration ratios ($n = 15$ mother-infant pairs) being significantly higher ($P < 0.0001$; mean difference (MD) 0.07; 95% confidence interval (CI) 0.048, 0.092) for the active (R)-methadone enantiomer (0.41; 0.19, 0.56) (median; range) compared with (S)-methadone (0.36; 0.15, 0.53). (R)- : (S)-methadone concentration ratios were also significantly higher ($P < 0.0001$; MD 0.24 95% CI 0.300, 0.180) for cord (1.40; 0.95, 1.67) compared with maternal plasma (1.16; 0.81, 1.38). Half the infant buprenorphine samples were below the assay lower limit of quantification (LLOQ) (0.125 ng ml⁻¹). The latter was four-fold lower than the LLOQ for methadone (0.50 ng ml⁻¹). The cord : maternal plasma buprenorphine concentration ratio ($n = 9$ mother-infant pairs) was 0.35; 0.14, 0.47 and for norbuprenorphine 0.49; 0.24, 0.91.

CONCLUSIONS

The transfer of the individual methadone enantiomers to the foetal circulation is stereoselective. Infants born to buprenorphine maintained women are not exposed to a greater proportion of the maternal dose compared with methadone and may be exposed to relatively less of the maternal dose compared with infants born to women maintained on methadone during pregnancy.

Introduction

Methadone maintenance treatment is currently the treatment of choice during pregnancy to promote abstinence from illicit opioids, and has been used in this population of patients since the early 1970s [1]. Although buprenorphine is now also being widely used for opioid dependence, its use during pregnancy is restricted in some countries due to the limited data on its effects on pregnancy progression, the newborn and the development of the offspring. Large case series and smaller randomized controlled trials, however, have observed less severe neonatal abstinence syndrome (NAS) in buprenorphine compared with methadone exposed infants. Fewer buprenorphine exposed infants required treatment to control NAS, less medication was required to control NAS in buprenorphine exposed infants and more protracted withdrawal was observed in methadone exposed infants [2–6].

These differences in withdrawal severity remain unexplained. It is unlikely that half-life affects the severity and onset of infant withdrawal, as data from several studies have not observed any significant differences in the timing of onset of withdrawal when comparing methadone and buprenorphine exposed infants and both buprenorphine/norbuprenorphine and methadone have similar long half-lives [4, 5, 7–15]. Other differences in pharmacological properties between methadone and buprenorphine such as binding affinities, efficacy and agonistic properties, and plasma binding may play a role in differences in withdrawal. However these issues are complex and beyond the scope of the current paper. It has been suggested, however, that the reduced NAS in buprenorphine exposed infants may be the result of reduced placental transfer of buprenorphine [16, 17].

While studies in human placental tissue *in vitro* models have observed similar relative placental transfer of methadone and buprenorphine, as indicated by the percentage of foetal exposure to the maternal dose, there have been limited human studies performed under chronic dosing conditions to observe maternal and foetal plasma concentration ratios of methadone, and none performed to assess buprenorphine concentration ratios.

Experimental models have shown the transfer of methadone between the maternal and foetal circulation to be bidirectional, and in the presence of an inhibitor, to be regulated by P-glycoprotein (P-gp) resulting in less than 10% foetal exposure to the maternal dose [18, 19]. Nanovskaya *et al.* [17], also studied the placental transfer of buprenorphine in a model similar to that used for methadone and observed that less than 10% of the maternal dose of buprenorphine appeared in the foetal circulation.

To date, there are limited data in humans concerning maternal and foetal concentrations of methadone and buprenorphine. Blinick *et al.* [20] observed plasma methadone concentrations of 12 women maintained on methadone (doses ranging between 40 and 100 mg day⁻¹,

calculated mean $\pm$ SD, 72 ± 20 mg day⁻¹) to be between 5 and 38 ng ml⁻¹ while their infants had umbilical cord plasma methadone concentrations of 3–14 ng ml⁻¹. The average ratio of methadone in cord plasma to that in maternal plasma was 0.57. In 21 women maintained on methadone (doses ranging between 20 and 80 mg day⁻¹, mean $\pm$ SD, 47 ± 16 mg day⁻¹), Doberczak *et al.* [21] observed a mean maternal plasma methadone concentration 10 h post delivery of 183 ± 118 ng ml⁻¹, while the corresponding mean infant plasma concentration was 26 ± 8 ng ml⁻¹. There are no reports on concentrations of the individual enantiomers of methadone, in particular the μ opioid active (R)-methadone enantiomer responsible for the effects of methadone.

The only clinical study to measure buprenorphine concentrations in umbilical cord blood was that of Johnson *et al.* [22], who reported a range of cord plasma concentrations from three infants of 101–137 ng l⁻¹, compared with maternal post-delivery plasma samples ranging from 115–798 ng l⁻¹. A blood sample from one infant taken 1 h after delivery had a plasma concentration of buprenorphine of 97 ng l⁻¹.

Given these limited data in humans for methadone and only isolated case studies presented for buprenorphine, the aim of the present study was to determine whether the transfer of buprenorphine and methadone between maternal and cord blood observed in human placental tissue *in vitro* models is also found in humans under chronic dosing conditions. A secondary aim was to examine differences in the transfer of the two enantiomers of methadone as the pharmacokinetics and metabolism of this drug have been shown to be stereoselective [23–25]. Plasma concentration ratios were used to assess foetal exposure to the drug relative to the mother for the individual enantiomers of methadone and both buprenorphine and norbuprenorphine.

Methods

Participants

Samples were collected from women enrolled in a non-randomized open label flexible dosing study assessing the effects of methadone and buprenorphine use during pregnancy between September 2002 and September 2004. Including those women who did not change their dose from the time of study enrolment, methadone maintained women were stable on their dose for 5.50 (median) weeks (range 1–31 weeks) prior to delivery and buprenorphine maintained women for 9.50 weeks (range 1–37 weeks) (not significantly different from methadone). Ethical approval for the study was obtained from the Women's and Children's Hospital (WCH) Research Ethics Committee (REC 1330/6/2005). Subject participation in the study was on a voluntary basis. All subjects provided written informed

consent prior to study commencement. Participants were informed that all information collected as part of the trial would be confidential.

Sample collection

Corresponding 3 ml maternal and umbilical cord blood samples were collected from women maintained on methadone and buprenorphine and their infants. Both samples were collected at the time of delivery and not at a predetermined time following maternal dosing. As such plasma concentration ratios are used as an indicator of foetal exposure to the drug relative to the mother. Delivery occurred 6.25 (median) h (range 1–23 h) following maternal dosing for methadone maintained mothers and 10.25 h (0.25–34 h) following maternal dosing for buprenorphine maintained mothers. Samples were collected in lithium heparin tubes and where possible within 30 min of each other and delivery (methadone: 60% within 30 min, 33% ≤ 2 h and 7% > 2 h; buprenorphine: 56% within 30 min, 33% ≤ 2 hours, 11% > 2 h), by Women's and Children's Hospital (WCH) delivery suite staff and refrigerated (4°C). Once collected by the researcher from the delivery suite they were centrifuged at 3000 rev min⁻¹ for 10 min, the plasma removed and frozen at -20°C until the time of assay.

Sample analysis

Methadone Samples were analyzed for (R)- and (S)-methadone in accordance with methods described by Foster *et al.* [23], with a lower limit of quantification (LLOQ) of 0.50 ng ml⁻¹. Mean accuracy was between 98 and 108% across the entire calibration range for all analytes. Inter- and intra-day precision (% coefficient of variation) were between 0.1 and 4.5% and 0.8–8.1%, respectively, across the entire calibration range for all analytes.

Buprenorphine Buprenorphine and norbuprenorphine sample analysis was based on the previously published method of Jensen *et al.* [26], with adaptations. Calibration curves consisting of eight standards were constructed in blank plasma over the concentration range 0.125–10 ng ml⁻¹ of each analyte. Low (LQC), medium (MQC) and high (HQC) quality control samples were also prepared in duplicate, with final concentrations of 0.35 ng ml⁻¹, 2.5 ng ml⁻¹ and 7 ng ml⁻¹ for each analyte, respectively. Calibration curves for all analytes were linear over the 0.125–10 ng ml⁻¹ concentration range, with r^2 values greater than 0.99 for all assays with no evidence of time related changes in slope values. Inter-assay accuracy and precision (accuracy $\pm$ RSD %) were 101 $\pm$ 5 (HQC), 105 $\pm$ 6 (MQC), 102 $\pm$ 8 (LQC), 101 $\pm$ 1 (LLOQ, 0.125 ng ml⁻¹), for buprenorphine and 100 $\pm$ 7 (HQC), 107 $\pm$ 9 (MQC), 92 $\pm$ 14 (LQC), 101 $\pm$ 5 (LLOQ) for norbuprenorphine. Similarly, intra-assay accuracy and precision (accuracy $\pm$ RSD %) were 103 $\pm$ 2 (HQC), 107 $\pm$ 2 (MQC), 91 $\pm$ 2 (LQC), 101 $\pm$ 3 (LLOQ, 0.125 ng ml⁻¹), for buprenorphine and 97 $\pm$ 1

(HQC), 105 $\pm$ 2 (MQC), 89 $\pm$ 10 (LQC), 104 $\pm$ 12 (LLOQ) for norbuprenorphine. The LLOQ (0.125 ng ml⁻¹) for this method is acceptable in relation to LLOQ's discussed by others who have used this method of quantification [27, 28]. The LLOQ for buprenorphine/norbuprenorphine is four-fold lower than for methadone (0.50 ng ml⁻¹).

Statistical analysis

All data were analyzed using GraphPad Prism v4.03® (GraphPad Software, CA, USA). Data comparing maternal and cord plasma concentration ratios were compared using two-tailed paired and unpaired *t*-tests or Mann-Whitney U-tests where appropriate and results expressed as *P* value. Results are presented as mean of difference (MD) and 95% confidence interval (CI) for the difference between means, median and range. Statistical significance was set at *P* < 0.05.

Results

Maternal and umbilical cord blood samples were collected from 15 methadone mother/infant pairs (maternal methadone dose 35.00, 25–140 mg day⁻¹). Methadone was able to be detected and quantified in all samples collected. There were no cord sample concentrations that exceeded maternal sample concentrations. Maternal methadone dose was significantly correlated with both (R)- and (S)-methadone maternal concentrations (*P* < 0.01, *r* = 0.71 and *P* < 0.01, *r* = 0.69, respectively) and with both (R)- and (S)-methadone cord concentrations (*P* < 0.001, *r* = 0.82 and *P* < 0.01, *r* = 0.72, respectively). Maternal and cord plasma (R)- and (S)-methadone concentrations are displayed in Table 1.

Maternal and cord plasma methadone concentration ratios are presented in Table 2. Significantly higher cord:maternal (R)-methadone plasma, compared with cord:maternal (S)-methadone plasma concentration ratios were observed (*P* < 0.0001; MD 0.070; 95% CI 0.048, 0.092). In addition significantly higher cord (R):(S)-methadone plasma, compared with maternal (R):(S)-methadone plasma concentration ratios were observed (*P* < 0.0001; MD 0.240; 95% CI 0.30, 0.18).

Maternal and umbilical cord plasma samples were collected from 18 buprenorphine mother/infant pairs (maternal dose 6.00, 2–20 mg day⁻¹). Maternal plasma buprenorphine concentrations were able to be quantified in samples from 17 of the mothers (maternal buprenorphine dose 6.00, 2–16 mg day⁻¹). The concentration of buprenorphine in the remaining sample was below the LLOQ (maternal buprenorphine dose 20 mg day⁻¹). Cord plasma buprenorphine concentrations were only able to be determined for half (nine) of the cord samples (maternal buprenorphine dose 87.6, 6–16 mg day⁻¹) as nine were below the LLOQ (maternal buprenorphine dose 6.0, 2–20 mg day⁻¹). There were no cord sample concentrations

Table 1

Maternal and cord plasma (R)- and (S)-methadone concentrations and ratios

Median (range)	Maternal (n = 15)	Cord (n = 15)
Plasma concentration (ng ml ⁻¹) (R)-methadone	62.75 (25.1–159.8)	20.74 (10.6–71.2)
Below LLOQ (0.50 ng ml ⁻¹)	n = 0	n = 0
Plasma concentration ratio Cord : maternal (R)-methadone	0.41**** (0.19–0.56)	
Plasma concentration (ng ml ⁻¹) (S)-methadone	45.66 (20.6–197.7)	15.80 (7.42–51.1)
Below LLOQ (0.50 ng ml ⁻¹)	n = 0	n = 0
Plasma concentration ratio Cord : maternal (S)-methadone	0.36 (0.15–0.53)	
Plasma concentration ratio (R)- : (S)-methadone	1.16 (0.81–1.38)	1.40**** (0.95–1.67)

****P < 0.0001 compared with maternal. ****P < 0.0001 compared with (S)-methadone.

Table 2

Maternal and cord plasma buprenorphine and norbuprenorphine concentrations and ratios

Median (range)	Maternal	Cord
Plasma concentration (ng ml ⁻¹) Buprenorphine above LLOQ (0.125 ng ml ⁻¹)	0.50 (0.14–3.43) n = 17	0.23 (0.13–0.75) n = 9
Below LLOQ (0.125 ng ml ⁻¹)	n = 1	n = 9
Plasma concentration ratio Cord : maternal buprenorphine	0.35 (0.14–0.47), n = 9	
Plasma concentration (ng ml ⁻¹) Norbuprenorphine above LLOQ (0.125 ng ml ⁻¹)	0.71 (0.30–2.75) n = 17	0.43 (0.15–0.87) n = 16
Below LLOQ (0.125 ng ml ⁻¹)	n = 1	n = 2
Plasma concentration ratio Cord : maternal norbuprenorphine	0.49 (0.24–0.91), n = 16	

that exceeded maternal sample concentrations. There was no significant difference in the daily maternal buprenorphine dose of those cord samples that were below the LLOQ compared with those where buprenorphine could be quantified. Maternal plasma norbuprenorphine concentrations were not able to be determined in the single mother's sample that was below the LLOQ for buprenorphine. Cord plasma norbuprenorphine concentrations were not able to be determined for two cord samples (buprenorphine was detected in one of these sample) as these were below the LLOQ. For both buprenorphine and norbuprenorphine, if the maternal sample was below the LLOQ then the corresponding infant sample was also below the LLOQ. Maternal buprenorphine dose was correlated ($P < 0.05$, $r = 0.72$) with norbuprenorphine cord concentrations, but not with maternal or cord buprenorphine concentrations or maternal norbuprenorphine concentrations. Maternal and cord plasma buprenorphine and norbuprenorphine concentrations for those samples that were able to be analyzed are presented in Table 2.

As a result of the maternal and cord samples that were below the LLOQ, only nine cord : maternal plasma concentration ratios were able to be determined for buprenorphine and only 16 were able to be determined for norbuprenorphine. Maternal and cord plasma concentration ratios for the samples are presented in Table 2.

Using only those samples where the buprenorphine concentration was above the LLOQ, cord : maternal plasma concentration ratios were not significantly different between buprenorphine and (R)-methadone (the active enantiomer of methadone).

Discussion

Maternal and umbilical cord blood samples were collected from women participating in methadone and buprenorphine maintenance programmes during pregnancy to assess the transfer of methadone and buprenorphine across the placenta in humans under chronic dosing conditions. Cord : maternal concentration ratios indicated that under chronic dosing conditions in humans, the transfer of methadone and buprenorphine were greater than observed in human placental tissue *in vitro* models. Methadone was quantifiable in all maternal and cord samples collected, whereas buprenorphine concentrations were below the assay LLOQ for half of the cord samples collected, providing indication that the relative transfer of buprenorphine across the placenta is at least no greater, and potentially less, than that of methadone. Maternal methadone dose was correlated with both maternal and cord plasma concentrations, where as

buprenorphine dose was not. A secondary aim of the study observed that significantly more of the active enantiomer of methadone ((R)-methadone), relative to the less active enantiomer ((S)-methadone) was present in the foetal circulation, an outcome that has not previously been investigated.

The cord : maternal buprenorphine concentration ratio of 0.35 from samples where buprenorphine could be quantified, indicated higher relative placental transfer of buprenorphine in humans under chronic dosing conditions compared with that observed in human placental tissue *in vitro* models of 0.10 [17]. The only human study [22] to have reported buprenorphine concentrations in umbilical cord plasma reported concentrations between 0.101 and 0.137 ng ml⁻¹, in cords obtained from three infants at delivery. Maternal samples from the same study collected post-delivery observed concentrations between 0.115 and 0.798 ng ml⁻¹. Slightly higher cord and maternal concentrations in the current study (cord: 0.13–0.75 ng ml⁻¹; maternal: 0.14–3.43 ng ml⁻¹) were unlikely to be due to maternal dosing as doses used in the current study were slightly lower than those used in the previous study (7.2 ± 0.82 (mean ± SD) mg day⁻¹ and 9.33 ± 1.33 mg day⁻¹ [22]).

The relative transfer of the active (R)- methadone enantiomer responsible for methadone's opioid effects, was also higher than the 10% of the maternal dose observed in the foetal circulation in human placental tissue *in vitro* models [19].

When only samples with quantifiable drug concentrations were included in the statistical analysis, the cord : maternal concentration ratios for (R)- methadone and buprenorphine were not significantly different. However, it should be noted that half of the cord buprenorphine samples had concentrations below the assay LLOQ, indicating that a proportion of infants born to mothers maintained on buprenorphine could potentially be exposed to very low concentrations of the drug at birth. If a value of 0.0625 ng ml⁻¹ (midway between 0 and 0.125 ng ml⁻¹) (using a previously described methodology [29]) is imputed for the nine buprenorphine samples with concentrations below the LLOQ, then the recalculated mean cord : maternal plasma concentration of 0.3 (median); 0.05–0.47 (range), with supplementary statistical analysis, is significantly lower ($P < 0.05$, 95% CI –0.221, –0.0314) than the (R)-methadone cord : maternal plasma concentration reported above. These data merely suggest a lesser relative exposure of the infant to maternal buprenorphine dose compared with methadone, which may in part explain the lower level of NAS associated with buprenorphine maintenance during pregnancy [2, 4–6].

Norbuprenorphine (a full opioid agonist with a potentially long half-life) [8, 30], showed higher relative transfer compared with buprenorphine. If high concentrations of a long acting opioid agonist (norbuprenorphine) are present in the infant following birth, this may have a pro-

TECTIVE effect on infant withdrawal and reduce the requirement for morphine subsequent to reduced severity of NAS. However, the parent drug has the potential to block the agonist properties of norbuprenorphine as has previously been shown in relation to respiratory depression in animals [30]. The effects of norbuprenorphine in humans, however, is not known to date.

If infants with cord buprenorphine concentrations below LLOQ are taken into account, the results suggest that infants are exposed to no more of the percentage of the maternal dose compared with methadone and potentially even relatively less buprenorphine reaches the foetal circulation compared with methadone. The difference in the relative transfer of methadone and buprenorphine to the foetal circulation is complex and could be due to a number of mechanisms. One such mechanism may involve the differences in placental metabolism of methadone and buprenorphine. CYP19 (aromatase) is responsible for the majority of both buprenorphine (apparent K_m 12 ± 4 μM) [31, 32] and methadone (apparent K_m 424 ± 92 μM) [33, 34] metabolism by the placenta. However, apparent K_m values of methadone and buprenorphine for CYP19 in relation to concentrations reported in the current study are unlikely to explain differences in relative transfer of methadone and buprenorphine to the foetal circulation.

Drug transporter mechanisms could contribute to concentration differences between methadone and buprenorphine appearing in the foetal circulation. Methadone is regulated in the placenta by the drug transporter P-gp [18]. P-gp has been shown to play a role in the transport of buprenorphine across the blood brain barrier in *in vivo* rat studies [35], but not in human placenta tissue *in vitro* studies [36]. Therefore differences in cord : maternal concentration ratios for methadone and buprenorphine are unlikely to be due to differences in affinities for P-gp in the placenta. Other yet unknown drug transporters may be responsible for differences in concentration ratios.

The higher cord : maternal plasma (R)-methadone ratio compared with the cord : maternal (S)-methadone ratio as well as the significantly higher (R) : (S)-methadone concentration ratio in cord, compared with maternal plasma may be explained by several different mechanisms, including protein binding, metabolism and active transport.

(R)- and (S)-methadone are stereoselectively bound to alpha 1-acid glycoprotein (AAG) ((R)-methadone unbound fraction 14%, (S)-methadone unbound fraction 10% [37]). AAG concentrations are lower in cord plasma than in maternal plasma [38, 39]. Therefore as less (R)-methadone, compared with (S)-methadone, is bound to AAG in the maternal circulation, this would allow for greater transfer of (R)-methadone to the cord circulation. This would result in increased (R)-methadone cord : maternal concentration compared with (S)-methadone cord : maternal concentration as observed in the current study.

As yet no work has been performed assessing the stereoselective metabolism of (R)- and (S)-methadone by

CYP19 (aromatase) in the placenta. It is therefore unknown as to whether this may contribute to differences in concentrations of (R)- and (S)-methadone observed in maternal and foetal circulations and subsequently differences observed in concentration ratios.

With regard to the effect of drug transport, methadone has been shown to be a substrate for P-gp which is found in placental tissue [18]. A weak stereoselectivity in methadone transport by P-gp has been observed towards the (S)-enantiomer [40] in pig kidney epithelial cells. This would support the significantly higher cord compared with maternal (R) : (S)-methadone concentration observed in the current study. However, the observed stereoselectivity of P-gp towards the (S)-enantiomer seen by Crettol *et al.* [40], was for concentrations that far exceeded those in the current study, and is therefore unlikely to have played a role in observed differences in this instance.

These results indicate that the transfer of both methadone and buprenorphine across the placenta under chronic dosing conditions in humans is higher than previously reported in human placental tissue *in vitro* models. In addition, infants are exposed to no more of the percentage of the maternal dose of buprenorphine compared with methadone, and potentially even relatively less buprenorphine reaches the foetal circulation compared with methadone and may contribute to reduced withdrawal severity observed in buprenorphine exposed infants. Results also indicate that there is stereoselectivity in the transfer of the individual enantiomers of methadone across the placenta to the foetal circulation which has not previously been studied. The precise mechanisms explaining the differences between the stereoselective transfer of methadone enantiomers, and in the amount of methadone and buprenorphine reaching the foetal circulation, remain unknown and require further investigation.

Registration of clinical trials

This trial was registered with the Therapeutic Goods Administration of Australia (Clinical Trial Notification Number: 2002/418).

Competing interests

Funding for this study was provided by an education grant from Reckitt Benckiser. Reckitt Benckiser had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.

Authors ALG, OVL and JMW have previously received reimbursement from Reckitt Benckiser for attending Reckitt Benckiser symposia. ALG and JMW have received a fee for speaking at these symposia.

We are grateful to staff and subjects from Warinilla, Northern and Southern Clinics of Drug and Alcohol Services South Australia and to the private prescribers who assisted with the study. We are also grateful to staff and subjects from Women's and Children's Hospital South Australia. We would also like to thank Aaron Farquharson and Charlotte Goess for their assistance with sample collection.

This research was supported by an educational grant from Reckitt Benckiser. Andrea Gordon was supported by a Royal Adelaide Hospital Postgraduate Dawes Scholarship and a University of Adelaide scholarship.

Contributors

Authors Andrea Gordon, Olga Lopatko and Jason White designed the clinical study and wrote the protocol. Authors Andrew Somogyi and David Foster designed the sample assays and wrote the protocol for these. Author Andrea Gordon managed all of the sample collection and Author David Foster was responsible for all of the sample analysis. Author Andrea Gordon managed the literature searches and summaries of previous related work, undertook the data analysis, and wrote the first draft of the manuscript. Authors Olga Lopatko, Jason White, Andrew Somogyi and David Foster edited the manuscript. All authors contributed to and have approved the final manuscript.

REFERENCES

- 1 Kaltenbach K, Berghella V, Finnegan L. Opioid dependence during pregnancy. Effects and management. *Obstet Gynecol Clin North Am* 1998; 25: 139–51.
- 2 Colombini N, Elias R, Busuttill M, Dubuc M, Einaudi MA, Bues-Charbit M. Hospital morphine preparation for abstinence syndrome in newborns exposed to buprenorphine or methadone. *Pharm World Sci* 2008; 30: 227–34.
- 3 Ebner N, Rohrmeister K, Winklbaur B, Baewert A, Jagsch R, Peternell A, Thau K, Fischer G. Management of neonatal abstinence syndrome in neonates born to opioid maintained women. *Drug Alcohol Depend* 2007; 87: 131–38.
- 4 Fischer G, Ortner R, Rohrmeister K, Jagsch R, Baewert A, Langer M, Aschauer H. Methadone versus buprenorphine in pregnant addicts: a double-blind, double-dummy comparison study. *Addiction* 2006; 101: 275–81.
- 5 Jones HE, Johnson RE, Jasinski DR, O'Grady KE, Chisholm CA, Choo RE, Crocetti M, Dudas R, Harrow C, Huestis MA, Jansson LM, Lantz M, Lester BM, Miliio L. Buprenorphine versus methadone in the treatment of pregnant opioid-dependent patients: effects on the neonatal abstinence syndrome. *Drug Alcohol Depend* 2005; 79: 1–10.
- 6 Lejeune C, Simmat-Durand L, Gourarier L, Aubisson S. Prospective multicenter observational study of 260 infants

- born to 259 opiate-dependent mothers on methadone or high-dose buprenorphine substitution. *Drug Alcohol Depend* 2006; 82: 250–57.
- 7 Kakko J, Heilig M, Sarman I. Buprenorphine and methadone treatment of opiate dependence during pregnancy: comparison of fetal growth and neonatal outcomes in two consecutive case series. *Drug Alcohol Depend* 2008; 96: 69–78.
 - 8 Kuhlman JJ, Jr, Lalani S, Magluilo J, Jr, Levine B, Darwin WD. Human pharmacokinetics of intravenous, sublingual, and buccal buprenorphine. *J Anal Toxicol* 1996; 20: 369–78.
 - 9 Kuhlman JJ, Jr, Levine B, Johnson RE, Fudala PJ, Cone EJ. Relationship of plasma buprenorphine and norbuprenorphine to withdrawal symptoms during dose induction, maintenance and withdrawal from sublingual buprenorphine. *Addiction* 1998; 93: 549–59.
 - 10 McAleer SD, Mills RJ, Polack T, Hussain T, Rolan PE, Gibbs AD, Mullins FG, Hussein Z. Pharmacokinetics of high-dose buprenorphine following single administration of sublingual tablet formulations in opioid naive healthy male volunteers under a naltrexone block. *Drug Alcohol Depend* 2003; 72: 75–83.
 - 11 Meresaar U, Nilsson MI, Holmstrand J, Änggård E. Single dose pharmacokinetics and bioavailability of methadone in man studied with a stable isotope method. *Eur J Clin Pharmacol* 1981; 20: 473–78.
 - 12 Nilsson MI, Änggård E, Holmstrand J, Gunne L-M. Pharmacokinetics of methadone during maintenance treatment: adaptive changes during the induction phase. *Eur J Clin Pharmacol* 1982; 22: 343–49.
 - 13 Verebely K, Volavka J, Mule S, Resnick R. Methadone in man: pharmacokinetic and excretion studies in acute and chronic treatment. *Clin Pharmacol Ther* 1975; 18: 180–90.
 - 14 Walsh SL, Preston KL, Stitzer ML, Cone EJ, Bigelow GE. Clinical pharmacology of buprenorphine: ceiling effects at high doses. *Clin Pharmacol Ther* 1994; 55: 569–80.
 - 15 Wolff K, Rostami-Hodjegan A, Shires S, Hay AWM, Feely M, Calvert R, Raistrick D, Tucker GT. The pharmacokinetics of methadone in healthy subjects and opiate users. *Br J Clin Pharmacol* 1997; 44: 325–34.
 - 16 Kandall SR, Doberczak TM, Jantunen M, Stein J. The methadone-maintained pregnancy. *Clin Perinatol* 1999; 26: 173–83.
 - 17 Nanovskaya T, Deshmukh S, Brooks M, Ahmed MS. Transplacental transfer and metabolism of buprenorphine. *J Pharmacol Exp Ther* 2002; 300: 26–33.
 - 18 Nanovskaya T, Nekhayeva I, Karunaratne N, Audus K, Hankins GD, Ahmed MS. Role of P-glycoprotein in transplacental transfer of methadone. *Biochem Pharmacol* 2005; 69: 1869–78.
 - 19 Nekhayeva IA, Nanovskaya TN, Deshmukh SV, Zharikova OL, Hankins GD, Ahmed MS. Bidirectional transfer of methadone across human placenta. *Biochem Pharmacol* 2005; 69: 187–97.
 - 20 Blinick G, Inturrisi CE, Jerez E, Wallach RC. Methadone assays in pregnant women and progeny. *Am J Obstet Gynecol* 1975; 121: 617–21.
 - 21 Doberczak TM, Kandall SR, Friedmann P. Relationship between maternal methadone dosage, maternal-neonatal methadone levels, and neonatal withdrawal. *Obstet Gynecol* 1993; 81: 936–40.
 - 22 Johnson RE, Jones HE, Jasinski DR, Svikis DS, Haug NA, Jansson LM, Kissin WB, Alpan G, Lantz ME, Cone EJ, Wilkins DG, Golden AS, Huggins GR, Lester BM. Buprenorphine treatment of pregnant opioid – dependent women: maternal and neonatal outcomes. *Drug Alcohol Depend* 2001; 63: 97–103.
 - 23 Foster DJ, Morton EB, Heinkele G, Mürdter TE, Somogyi AA. Stereoselective quantification of methadone and a d6-labelled isotopomer using high performance liquid chromatography-atmospheric pressure chemical ionization mass-spectrometry: application to a pharmacokinetic study in a methadone maintained subject. *Ther Drug Monit* 2006; 28: 559–67.
 - 24 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.
 - 25 Totah RA, Sheffels P, Roberts T, Whittington D, Thummel K, Kharasch ED. Role of CYP2B6 in stereoselective human methadone metabolism. *Anesthesiology* 2008; 108: 363–74.
 - 26 Jensen ML, Foster DJ, Upton RN, Kristensen K, Hansen SH, Jensen NH, Nielsen BN, Skram U, Villesen HH, Christrup L. Population pharmacokinetics of buprenorphine following a two-stage intravenous infusion in healthy volunteers. *Eur J Clin Pharmacol* 2007; 63: 1153–59.
 - 27 Mercolini L, Mandrioli R, Conti M, Leonardi C, Gerra G, Raggi MA. Simultaneous determination of methadone, buprenorphine and norbuprenorphine in biological fluids for therapeutic drug monitoring purposes. *J Chromatogr B Analyt Technol Biomed Life Sci* 2007; 847: 95–102.
 - 28 Oechsler S, Skopp G. Buprenorphine and major metabolites in blood specimens collected for drug analysis in law enforcement purposes. *Forensic Sci Int* 2010; 195: 73–7.
 - 29 Beal SL. Ways to fit a PK model with some data below the quantification limit. *J Pharmacokinet Pharmacodyn* 2001; 28: 481–504.
 - 30 Megarbane B, Marie N, Pirnay S, Borron SW, Gueye PN, Risede P, Monier C, Noble F, Baud FJ. Buprenorphine is protective against the depressive effects of norbuprenorphine on ventilation. *Toxicol Appl Pharmacol* 2006; 212: 256–67.
 - 31 Deshmukh SV, Nanovskaya TN, Ahmed MS. Aromatase is the major enzyme metabolizing buprenorphine in human placenta. *J Pharmacol Exp Ther* 2003; 306: 1099–105.
 - 32 Kobayashi K, Yamamoto T, Chiba K, Tani M, Shimada N, Ishizaki T, Kuroiwa Y. Human buprenorphine N-dealkylation is catalyzed by cytochrome P450 3A4. *Drug Metab Dispos* 1998; 26: 818–21.
 - 33 Hieronymus TL, Nanovskaya TN, Deshmukh SV, Vargas R, Hankins GD, Ahmed MS. Methadone metabolism by early gestational age placentas. *Am J Perinatol* 2006; 23: 287–94.

- 34** Nanovskaya TN, Deshmukh SV, Nekhayeva IA, Zharikova OL, Hankins GD, Ahmed MS. Methadone metabolism by human placenta. *Biochem Pharmacol* 2004; 68: 583–91.
- 35** Suzuki T, Zaima C, Moriki Y, Fukami T, Tomono K. P-glycoprotein mediates brain-to-blood efflux transport of buprenorphine across the blood-brain barrier. *J Drug Target* 2007; 15: 67–74.
- 36** Nekhayeva IA, Nanovskaya TN, Hankins GD, Ahmed MS. Role of human placental efflux transporter P-glycoprotein in the transfer of buprenorphine, levo-alpha-acetylmethadol, and paclitaxel. *Am J Perinatol* 2006; 23: 423–30.
- 37** 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: 338–46.
- 38** Israili ZH, Dayton PG. Human alpha-1-glycoprotein and its interactions with drugs. *Drug Metab Rev* 2001; 33: 161–235.
- 39** Sudhakaran S, Rayner CR, Li J, Kong DC, Gude NM, Nation RL. Differential protein binding of indinavir and saquinavir in matched maternal and umbilical cord plasma. *Br J Clin Pharmacol* 2007; 63: 315–21.
- 40** Crettol S, Digon P, Golay KP, Brawand M, Eap CB. In vitro P-glycoprotein-mediated transport of (R)-, (S)-, (R,S)-methadone, LAAM and their main metabolites. *Pharmacology* 2007; 80: 304–11.