Use of a sparse sampling study design to assess transfer of tramadol and its *O*-desmethyl metabolite into transitional breast milk

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

• There are presently no published data on tramadol transfer into breast milk or on its effects in the breastfed infant.

WHAT THIS STUDY ADDS

• We have provided quantitative data on the absolute and relative infant doses of *rac*-tramadol and it *rac-O*-desmethyl metabolite for the breastfed infant.
• We have also demonstrated a novel sparse sampling data collection method for investigating infant exposure via milk.

AIMS

To investigate the transfer of *rac*-tramadol and its *rac-O*-desmethyl metabolite into transitional milk, and assess unwanted effects in the breastfed infant.

METHODS

Tramadol HCl (100 mg six hourly) was administered to 75 breastfeeding mothers for postoperative analgesia on days 2–4 after Caesarian section. Milk and plasma samples were collected after administration of four or more doses. *Rac*-tramadol and *rac-O*-desmethyltramadol were measured by high performance liquid chromatography. Milk : plasma ratio (M : P) and infant doses were calculated by standard methods. The behavioural characteristics of the exposed breastfed infants and a matched control group of infants not exposed to tramadol were also studied.

RESULTS

At steady-state, mean (95% CI) M : P was 2.2 (2.0, 2.4) for *rac*-tramadol and 2.8 (2.5, 3.1) for *rac-O*-desmethyltramadol. The estimated absolute and relative infant doses were 112 (102, 122) $\mu g\ kg^{-1}\ day^{-1}$ and 30 (28, 32) $\mu g\ kg^{-1}\ day^{-1}$, and 2.24% (2.04, 2.44)% and 0.64% (0.59, 0.69)% for *rac*-tramadol and *rac-O*-desmethyltramadol, respectively. The exposed infants and control breastfed infants had similar characteristics, including Apgar scores at birth and Neurologic and Adaptive Capacity Scores.

CONCLUSIONS

The combined relative infant dose of 2.88% at steady-state was low. The similarity of NACS in exposed infants and controls suggests that there were no significant behavioural adverse effects. We conclude that short-term maternal use of tramadol during establishment of lactation is compatible with breastfeeding.

Introduction

Opioids and nonsteroidal anti-inflammatory drugs (NSAIDs) are effective analgesics after Caesarian section and, except for repeated dosing of pethidine, their breast milk transfer and potential neonatal effects are considered acceptable as breast feeding is established. However, opioids can cause adverse reactions such as sedation, nausea and vomiting, while NSAIDs are contraindicated in women with peptic ulcer or gastro-oesophageal reflux disease, bleeding disorders, renal impairment and hypertension.

In nonobstetric populations, tramadol is now widely used in the treatment of pain of diverse origins [1] and is particularly useful for perioperative pain control [2]. Tramadol is effective against mild to moderate pain [3], and together with its active metabolite acts synergistically through μ-opioid and monoaminergic-mediated mechanisms [3, 4]. It is one of the most widely prescribed analgesics with more than 5 billion patient treatment days arising from 30 years of clinical experience [5]. Although tramadol can cause dizziness, sweating, dry mouth and postural hypotension in some patients, in our hospital it is frequently used after Caesarian section, particularly as a relatively strong oral analgesic for step-down analgesia from neuraxial or intravenous opioid techniques after 24 h postpartum. Maternal concern about the effect of a drug when breastfeeding is a reality and drug administration should ideally be subject to risk *vs.* benefit analysis [6]. The Manufacturer's Product Information on tramadol gives very limited data on the transfer of tramadol and its active *O*-desmethyl metabolite (M1) into breast milk, with unpublished information suggesting that 0.12% of a single 100 mg intravenous dose was found in milk collected over 16 h after dose [7].

This investigation was designed to provide detailed information about the breast milk transfer of tramadol and its pharmacologically active *O*-desmethyl metabolite during repeated tramadol administration for the management of postoperative pain following Caesarian section. The hypothesis tested was that the infant doses of tramadol and *O*-desmethyltramadol received in breast milk of early stage lactating women would be insufficient to cause concern about significant clinical effects in the neonate. Because we anticipated a limited milk supply in our early lactation patient group, we chose to use a sparse sampling study design.

Methods

Study protocol and data collection

The study was approved by the Ethics Committee of the Women's and Children's Health Service at King Edward Memorial Hospital for Women, Subiaco, Western Australia and all participants gave written informed consent. We studied mothers taking prescribed oral tramadol, on the second to fourth postpartum day after Caesarian section. Pilot data indicated that women were likely to be taking 50 or 100 mg four to six times daily. Women who had significant renal dysfunction, who had opted not to breast feed, or who consented but were then unable to provide an adequate sample of breast milk for analysis, were excluded.

Samples of transitional milk [8] produced on days 2–4 postpartum, and plasma, were taken over one dose interval, usually following the 4th consecutive 6-hourly oral tramadol dose when both analytes of interest can be expected to be at >93% of steady-state concentrations. Milk (three samples per patient) was collected in approximately equal aliquots from both breasts (mixed samples of 2–3 ml), using either manual expression or an electric breast pump. At the same time as one of these samples was taken, a single blood sample (5 ml, heparinized) was also collected by venepuncture. The sample collection times for each patient were determined according to a randomized schedule of 1 h blocks over the dose interval, to ensure an even distribution of both milk and plasma samples across the dose interval. In 57% of patients, where the study period corresponded with the last tramadol dose, one of the milk and/or blood samples was collected in the period between 6 and 14 h after dose.

Details of maternal age, weight, concomitant analgesic therapy on the study day, and tramadol doses and the dates/times of doses were recorded. The infants ($n = 75$) of the tramadol-treated mothers, and a comparator group of infants ($n = 75$) of mothers who received other analgesics, were studied. Infant sex, gestational age, and weight were recorded. Apgar scores at 1 and 5 min after birth were recorded from the medical record. On the same day as that of sampling, the wellbeing of all infants was assessed by one of three of the authors (RG, TC or KS), using a standardized clinical physical assessment, including an assessment of alertness (Neurologic and Adaptive Capacity Score; NACS) [9].

Materials
Authentic *rac*-tramadol, and ketamine were obtained from Sigma Chemical Co, St Louis, MO, USA, and *rac-O*-desmethytramadol from Grunenthal GmbH, Aachen, Germany. All other solvents and chemicals were of analytical grade.

Measurement of rac-tramadol and rac-O-desmethyltramadol by high performance liquid chromatography (HPLC)
Milk samples (1 ml) were spiked with internal standard (ketamine 400 ng) alkalinized with 0.4 ml of 5 M NaOH and extracted into 8 ml of hexane : ethyl acetate (80 : 20) by shaking for 10 min. After centrifugation at 1500 *g* for 10 min, supernatant (7 ml) was back extracted into 3 ml of

0.1 M HCl by shaking for 5 min, followed by centrifugation as above. The HCl phase was then alkalinized with 0.4 ml of 5 M NaOH, and re-extracted into 8 ml hexane : ethyl acetate (80 : 20). After centrifugation the upper organic layer (7.5 ml) was aspirated, and evaporated to dryness at 45°C under nitrogen. Residues were reconstituted in 0.1 ml of the HPLC mobile phase and 0.08 ml aliquots were injected onto the column. Separations were performed on a Lichrospher™ RP Select B C_8 column (5 µm, 250 mm × 4 mm id; E Merck GmbH, Damstadt, Germany), with a mobile phase of 14% v/v acetonitrile in 20 mM K_2HPO_4, 0.1% v/v triethylamine (pH 3) that was pumped at 0.9 ml min^{-1}. Analytes were detected at 218 nm. Quantification of chromatograms was undertaken using Chemstation Software (Version 9, Agilent Technology, Waldbronn, Germany). For *rac*-tramadol, the assay intraday relative standard deviations (RSDs) were 5.9, 2.8, and 1.5% at 50 µg l^{-1}, 500 µg l^{-1} and 2000 µg l^{-1}, respectively ($n = 5$), while interday RSDs were 7.4, 7.2 and 5.7% at 50 µg l^{-1}, 500 µg l^{-1} and 2000 µg l^{-1}, respectively ($n = 25$). For *rac*-*O*-desmethyl tramadol, intraday RSDs were 6.3, 5.4 and 3.1% at 50 µg l^{-1}, 250 µg l^{-1} and 500 µg l^{-1}, respectively ($n = 5$), while interday RSDs were 7.4, 5.9, and 4.6% at 50 µg l^{-1}, 250 µg l^{-1} and 500 µg l^{-1}, respectively ($n = 25$). The limits of quantification and detection for *rac*-tramadol were 15 µg l^{-1} and 8 µg l^{-1}, respectively, while for *rac*-*O*-desmethyltramadol they were 10 µg l^{-1} and 5 µg l^{-1}, respectively. Plasma *rac*-tramadol and *rac*-*O*-desmethyltramadol were also measured as above. For *rac*-tramadol, intraday RSDs were 7.1 and 8.3%, while interday RSDs were 3.0 and 5.7% at 50 mg l^{-1} and 2000 mg l^{-1} respectively ($n = 5$). For *rac*-*O*-desmethyl tramadol, intraday RSDs were 6.2 and 6.9%, while interday RSDs were 5.7 and 4.5% at 25 mg l^{-1} and 500 mg l^{-1}, respectively ($n = 5$).

Data analysis

Milk to plasma ratios (M : P) were calculated from the steady-state *rac*-tramadol or *rac*-*O*-desmethyltramadol concentration measurements in the paired milk and plasma samples ($n = 73$). Average steady-state *rac*-tramadol and *rac*-*O*-desmethyltramadol concentrations in milk over a 6 h dose interval (C_{avg}) were calculated as the mean (95% CI) of 165 measurements made in 55 patients. Absolute infant dose (µg kg^{-1} day^{-1}) was calculated as the product of the milk C_{avg} and an average infant milk intake of 0.15 l kg $^{-1}$day^{-1} [10]. Relative infant dose was calculated as absolute infant dose/maternal dose (µg kg^{-1} day^{-1}) and expressed as a percentage [10]. The molar ratio of *rac*-*O*-desmethyltramadol : *rac*-tramadol in plasma was calculated to give an indication of the cytochrome P450 2D6 (CYP2D6) status of the participants. Data have been summarized as mean (95% CI) or median (range, or 25th and 75th percentiles) as appropriate. Statistical analyses were performed using SigmaStat Ver 3.5 (SPSS Inc, Chicago, IL, USA).

Results

Ninety-five women were enrolled over an 18-month period. One woman did not meet the inclusion criteria, two women withdrew consent, seven ceased medication on the study day because it was not required or had caused unacceptable side-effects, and 10 were unable to provide samples of breast milk for analysis. The median (IQR) age of the remaining 75 participants was 29 (25, 32) years and their median weight was 75 (67, 91) kg. The usual prescribed dose of tramadol was 100 mg, although eight women received 50 mg for their first and/or second doses. On the study day, the median (range) number of consecutive doses that each woman had received was 4 (3, 9), while the mean (95% CI) dose interval across all doses was 6.2 (5.9, 6.4) h.

The characteristics of the infants of the women who received tramadol and of a matched control group of infants from mothers who received alternate analgesics are summarized in Table 1. Both groups of infants were similar with respect to gestational age, birth weight, Apgar scores at 1 and 5 min after birth, and their NACS scores on days 2–4 after birth. On the study day, 49% of the tramadol patients and 100% of the controls also took other opiate analgesics (mostly oxycodone) while 61% of tramadol patients and 58% of controls also took NSAIDS (mostly diclofenac).
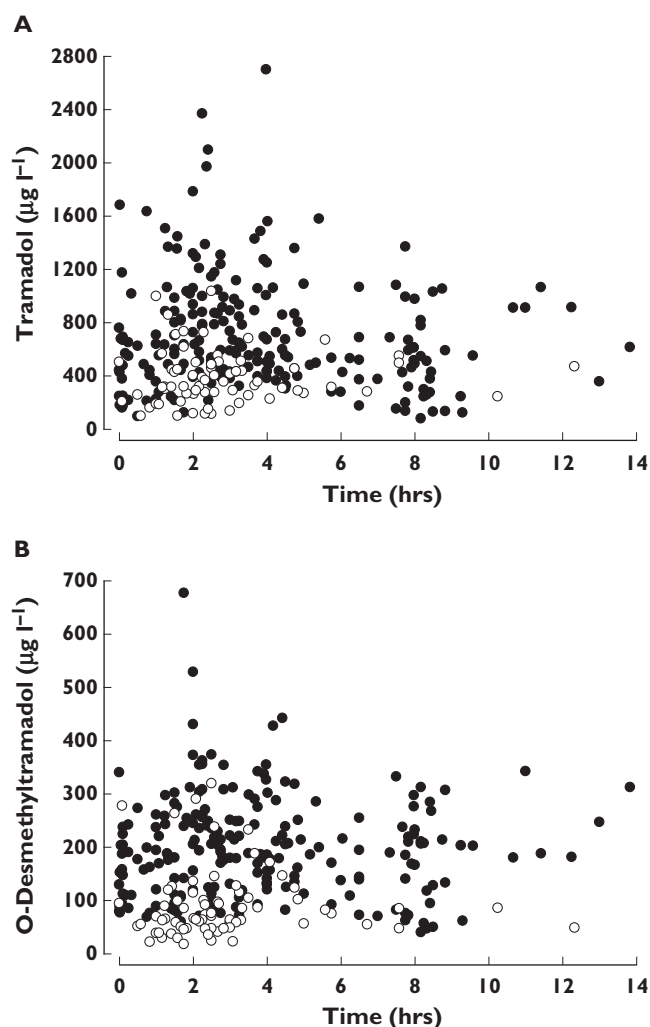
The milk and plasma concentration measurements of *rac*-tramadol and *rac*-*O*-desmethyltramadol are shown in Figure 1. The M : P values for analytes are summarized in Table 2. Steady-state milk C_{avg} (for a 6 h dose interval) and calculated mean absolute and relative infant doses for *rac*-tramadol and *rac*-*O*-desmethyltramadol are also summarized in Table 2. The mean relative infant dose was 2.24% for *rac*-tramadol and 0.64% for *rac*-*O*-desmethyltramadol.

Table 1

Characteristics of the infants of mothers receiving tramadol and comparator group mothers ($n = 75$ in each group unless otherwise specified)

Parameter	Tramadol	Control
Sex ratio (M : F)	1.08	0.89
Gestational age (weeks)	38.7 (38.2, 39.2)*	39.3 (39, 39.6)*
Birth weight (kg)	3.4 (3.3–3.5)*	3.4 (3.3–3.5)*
Apgar score 1 min	9 (9, 10)†	9 (9, 10)†
Apgar score 5 min	9 (9, 10)†	9 (9, 10)†
Assessment day post birth	4 (3, 4)†	3 (2, 4)†
Neurologic and Adaptive Capacity Score (total)	36 (33, 38)†‡	37 (33, 40)†‡
Neurologic score component	28 (26, 29)†‡	27 (26, 30)†‡
Adaptive capacity score component	9 (8, 10)†‡	10 (8, 10)†‡

*mean (95% CI), †median (IQR), ‡$n = 67$ (data for four infants lost, while four infants were not tested because they were in the Special Care Nursery for prematurity (3) or ventilation (1)). Neurologic and Adaptive Capacity Score range 0–40, composed of neurologic score range 0–30 and adaptive capacity score range 0–10.

A

B

Figure 1

Milk (●) and plasma (○) concentration–time observations for *rac*-tramadol (A) and *rac-O*-desmethyltramadol (B) following the fourth or later 100 mg oral dose of tramadol HCl in 75 mothers

A probit plot of the molar ratio of plasma *rac*-tramadol : *rac-O*-desmethyltramadol (range 0.7–33.6) for the 73 patients with data showed a break point at a ratio of around 10, with nine mothers (12.3%) identified as having a putative CYP2D6 poor metabolizer phenotype (graph not shown). None of the women was taking other drugs that are known inhibitors of CYP2D6.

Discussion

Despite the use of tramadol for labour pain and its extensive postoperative clinical use over the last 10 years or so, there are limited data on its pharmacokinetics in both mothers and their neonates. A recent study has shown that following intramuscular administration of tramadol (100–250 mg) for pain relief during labour, both the parent drug

and *O*-desmethyltramadol readily crossed the placenta (cord : maternal ratios of around 0.94 : 1), while in the first 12 h postpartum the elimination $t_{1/2}$ values in mothers and their neonates, respectively, were 7.2 and 7 h for tramadol and 5.5 and 8.5 h for *O*-desmethyltramadol [11]. The authors interpreted their findings to indicate that the neonate has the complete capacity for metabolic formation of *O*-desmethyltramadol, but that immaturity of neonatal renal function delayed its excretion. In addition, based on Apgar scores or NACS performance in the neonates, they reported no drug-related adverse effects. Others have also reported that CYP2D6-mediated *O*-demethylation of tramadol is present in early neonatal life and matures by about 12–16 weeks of life [12]. There is one report of opioid withdrawal in a neonate who was exposed to maternal use of tramadol during pregnancy [13].

To our knowledge, this is the first time that a sparse sampling strategy similar to that used in population pharmacokinetic studies has been used for this type of study. This involved collecting three milk samples and one plasma sample from each of 73 breastfeeding mothers with samples distributed across a dose interval at steady-state. The data show that *rac*-tramadol and its active metabolite can readily transfer from maternal plasma to milk with mean steady-state M : Ps of 2.2 and 2.8, respectively. However, M : P is merely an indicator of transfer capacity and does not of itself have any value in predicting infant exposure [14]. Absolute and relative infant doses estimated from drug concentration in milk and average milk intake are generally the preferred predictors of infant exposure to drugs in milk [10].

Given that in almost all cases, we measured drug concentrations after the fourth or later oral tramadol doses, both *rac*-tramadol and *rac-O*-desmethyltramadol can be expected to be at >93% of steady-state concentration. At steady-state, the absolute infant dose of 112 µg kg⁻¹ day⁻¹ for *rac*-tramadol via milk can be compared with analgesic doses used for postoperative pain in children. One study in children aged 2–8 years used a single intravenous dose of 1000 µg kg⁻¹ [15], while another in children aged 1–6 years used a single rectal dose of 1000–2500 µg kg⁻¹ [16]. Although these are not perfect comparator groups, even when the additional 30 µg kg⁻¹ day⁻¹ from *rac-O*-desmethyltramadol is included, maximum infant exposure in our study is around 14% of a child therapeutic dose. A level of not greater than 10% of the infant therapeutic dose has been suggested as being 'safe' for breastfeeding [17]. The relative infant dose at steady-state was well below the notional 10% level of concern [10], and similar or less than relative infant doses for other commonly used opioid analgesics [14]. Hence the potential for adverse effects in the exposed breastfed infants in our study should be very low.

It should be noted that our method of calculating infant dose at steady-state using milk C_{avg} (mean and 95%

Table 2

M : P values, average concentrations in milk, absolute infant doses and relative infant doses for *rac*-tramadol and *rac*-*O*-desmethyltramadol calculated from the steady-state data*

Parameter	Number of: patients/data points	*rac*-Tramadol	*rac-O-*Desmethyltramadol
M : P	73/73	2.2 (2.0, 2.4)	2.8 (2.5, 3.1)
Milk C_{avg} (μg l^{-1})	55/165	748 (681, 815)	203 (188, 217)
Absolute infant dose (μg kg^{-1} day^{-1})	55/165	112 (102, 122)	30 (28, 32)
Relative infant dose	55/165 (%)†	2.24 (2.04, 2.44)	0.64 (0.59, 0.69)‡

*mean (95% CI), †assuming 100 mg tramadol HCl administered 6 hourly to a 70 kg mother (5020 μg kg^{-1}day^{-1} as tramadol base), ‡as tramadol molar equivalents. M : P, milk to plasma ratio.

CI) measured in a sparse data set collected from a large population over a dose interval is novel. Such calculations are usually derived from measurements of milk C_{max} or C_{avg} in individuals or small groups of patients. Moreover, the sparse dataset population design can be applied without the need for population pharmacokinetic modelling.

We addressed the possibility of adverse behavioural drug-related effects in the infants by including a comparator group in our study. The comparator and tramadol-exposed infants had similar demographic characteristics, including Apgar scores at birth. Although there is no ideal instrument for the assessment of drug effects in the neonate, the NACS has been used to assess drug effects in many previous studies. Scores for the two tramadol-exposed and control groups were similar (both the median and the IQR). It should also be noted that infants from the comparator group were also exposed to maternal analgesics (opioids and NSAIDs). Within these limitations, our findings suggest that exposure to tramadol via milk did not result in additional drug-related clinically adverse effects.

Recently, there has been considerable interest in the pharmacogenetics of tramadol enantiomers, because of polymorphic metabolism to *O*-desmethyltramadol by CYP2D6. These studies show pharmacogenetic-related differences in both the disposition and/or pharmacodynamics of tramadol and its active metabolite [18–21]. Tramadol has also been suggested as a probe drug for CYP2D6 phenotyping [22]. Approximately 12.3% of our mothers (predominantly Caucasians) had a putative CYP2D6 poor metabolizer phenotype, which is comparable with the mean (95% CI) of 10.1 (6.8, 14.2)% reported by Pedersen *et al.* using the urinary ratio of (–)-*O*-desmethyltramadol : (+)-*O*-desmethyltramadol in a Danish population [22].

One limitation of our study could be that we chose to measure *rac*-tramadol and *rac*-*O*-desmethyltramadol, rather than their individual enantiomers. Nevertheless, given our finding of a low level of infant exposure to both *rac*-tramadol and *rac*-*O*-desmethyltramadol via breastmilk, differences in the disposition and pharmacodynamics of their enantiomers are unlikely to be an important influence on effects in exposed infants.

In summary, the combined absolute infant dose for *rac*-tramadol and its active metabolite at steady-state was about 14% of the child therapeutic dose for tramadol. In addition, the combined relative infant doses of 2.88% at steady-state, was also low, and there was no evidence for behavioural adverse effects in the exposed infants. We therefore suggest that short-term maternal use of tramadol in the post Caesarian section context is compatible with breastfeeding. However this does not obviate the need for a full individual risk-benefit analysis when tramadol is used during breastfeeding. We have also shown that a population sampling design with a sparse dataset can be used to estimate infant exposure to drugs without the need for population pharmacokinetic modelling.

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