Transfer of chloroquine and desethylchloroquine across the placenta and into milk in Melanesian mothers

Irwin Law,¹ Kenneth F. Ilett,^{1,2} L. Peter Hackett,² Madhu Page-Sharp,¹ Francesca Baiwog,³ Servina Gomorrai,³ Ivo Mueller,³ Harin A. Karunajeewa¹ & Timothy M. E. Davis¹

¹School of Medicine and Pharmacology, University of Western Australia, Crawley and ²Clinical Pharmacology & Toxicology Laboratory, PathWest Laboratory Medicine, Nedlands, Australia, and

³Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- The literature on placental and milk transfer of chloroquine and its major bioactive metabolite desethylchloroquine is sparse and incomplete.

WHAT THIS STUDY ADDS

- We have provided data on the transplacental transfer of chloroquine and desethylchloroquine in Melanesian women ($n = 19$), measured transfer of these drugs into breast milk ($n = 16$) and estimated absolute and relative infant doses for the breastfed infant.
- The data for desethylchloroquine are novel.
- In all three areas we have significantly increased both quantity and quality of the available database.

Correspondence

Emeritus Professor Kenneth F. Ilett,
Pharmacology and Anaesthesiology Unit,
M510, School of Medicine and
Pharmacology, University of Western
Australia, Crawley, WA 6009, Australia.
Tel: + 61 8 9346 2985
Fax: + 61 8 9346 3469
E-mail: ken.ilett@uwa.edu.au

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AIMS

To investigate the transfer of chloroquine and its major bioactive metabolite desethylchloroquine across the placenta and into breast milk.

METHODS

In Papua New Guinea, chloroquine (CQ; 25 mg base kg⁻¹) is recommended for prophylaxis of malaria during pregnancy, and at the Alexishafen Health Centre women are routinely prescribed CQ at the time of delivery. Fetal-cord and maternal serum samples were collected at delivery ($n = 19$) and milk samples were collected from day 3 to day 17–21 after delivery ($n = 16$). CQ and its primary active metabolite desethylchloroquine (DECQ) were quantified by high-performance liquid chromatography. For both CQ and DECQ cord/maternal ratios (C/M) were calculated to characterize placental transfer, and infant exposure via milk was estimated by standard methods.

RESULTS

The median (interquartile range) C/M was 1.1 (0.9, 1.6) for CQ and 1.2 (0.5, 1.8) for DECQ. The average concentration in milk over the time of sampling was 167 µg l⁻¹ (27, 340) for CQ and 54 µg l⁻¹ (22, 106) for DECQ. Estimated absolute and relative infant doses were 34 µg kg⁻¹ day⁻¹ (7, 50) and 15 µg kg⁻¹ day⁻¹ (4, 26), and 2.3% (0.5, 3.6) and 1.0% (0.4, 2.0) for CQ and DECQ (as CQ equivalents), respectively.

CONCLUSION

Infant exposure to CQ and DECQ during pregnancy will be similar to that in the maternal circulation, and dependent on maternal dose and frequency. The median CQ + DECQ relative infant dose of 3.2% (as CQ equivalents) was low, confirming that use of CQ during lactation is compatible with breastfeeding.

Introduction

Despite increasing resistance of *Plasmodium falciparum* to conventional antimalarial drugs, chloroquine (CQ) remains widely used for both prophylaxis and treatment of malaria in the tropics because of its relative safety, widespread availability and low cost. Since it is one of the few antimalarial drugs considered safe in pregnancy, CQ still has a role in presumptive treatment and prophylaxis of pregnant women with malaria in a number of tropical countries, including Papua New Guinea (PNG).

The major metabolite of CQ is desethylchloroquine (DECQ), a compound whose therapeutic importance may have been underestimated. Recent data from our group indicate that the area under the DECQ plasma concentration–time curve from the start of a course of CQ to 42 days ($AUC_{0,42 \text{ days}}$) in pregnant women is around 80% of that for CQ (Karunajeewa *et al.*, unpublished observations). In addition, a study of Cameroonian *P. falciparum* field isolates demonstrated that DECQ had significant *in vitro* antimalarial activity, which was relatively preserved in CQ-resistant strains [1]. Given these *in vitro* and pharmacokinetic data, it is likely that DECQ is an important contributor to overall CQ efficacy, especially where parasite resistance to CQ is prevalent. Nevertheless, IC_{50} values may not predict *in vivo* response reliably, while DECQ-associated cardiotoxicity is of potential concern given that previous studies have demonstrated cardiotoxicity for CQ and the closely related di-desethylCQ [2, 3].

Given the extent of CQ use worldwide, it is important to establish its safety in pregnancy and lactation. This would normally be assessed from studies establishing the extent of transfer to the fetus and/or nursing infant, as well as accumulated clinical experience. However, previously published studies [4–9] have included generally small numbers of patients, a wide range of dose regimens and timing in relation to delivery, and variable duration of sampling and methods of calculation of infant doses, whereas DECQ has been assayed in only a minority. In addition, population-specific factors could be important determinants of transfer across the placenta and into breast milk. The aim of the present study was therefore to provide definitive transfer data for CQ and DECQ in a Melanesian population.

Materials and methods

Study protocol and data collection

Ethical approval for the study was obtained from the PNG Medical Research Advisory Committee (approval number 05.22) and all participants gave written informed consent. Patients were recruited from March to July 2006 at the time of their first antenatal clinic visit to Alexishafen Health Centre, Madang Province, situated on the north coast of PNG. The resident population is Melanesian. The area is

hyperendemic for *P. falciparum* and *P. vivax*, and endemic for *P. ovale* and *P. malariae* [10].

We performed a study of cord/maternal (C/M) drug distribution in mothers who had been prescribed CQ (Chloroquin®; Aspen Healthcare Australia Pty Ltd, St Leonards, Australia) at a dose of 750 mg of CQ phosphate (equivalent to 465 mg base) daily for three consecutive days for malaria prophylaxis during pregnancy (intermittent preventive treatment coinciding with clinic visits). In a subset of women from the C/M study, a study was also performed of breast milk transfer following another course of CQ that was administered in the early postnatal period (days 1–3 after delivery).

Cord and maternal serum (1–2 ml) was collected at the time of delivery and milk samples (3–4 ml each of both fore- and hind-milk) were collected using manual expression by the mothers on days 3, 4, 5, 10 and 18–22 after delivery.

Details of maternal age, weight and CQ dosing were recorded at the time of their first antenatal visit after informed consent had been obtained. Details of all subsequent CQ doses during pregnancy and confinement were also recorded.

Materials

Amodiaquine dihydrochloride dihydrate (internal standard) and CQ diphosphate were from Sigma-Aldrich (St Louis, MO, USA), DECQ dioxalate from Starks Associates (Buffalo, NY, USA). All other reagents were of high-performance liquid chromatography (HPLC) or analytical grade.

Measurement of CQ and DECQ in serum and milk by HPLC

CQ and DECQ in plasma were analysed as previously described [11]. For CQ, the intraday relative standard deviations (RSDs) were 8.4, 8.6 and 2.1% ($n = 5$) and interday RSDs 8.6, 6.8 and 2.7% ($n = 15$) at 5, 20 and $2000 \mu\text{g l}^{-1}$, respectively. For DECQ, intraday RSDs ($n = 5$) were 8.9, 9.6 and 1.5%, and interday RSDs were 8.2, 8.3 and 3.5% ($n = 15$) at 3, 123 and $1234 \mu\text{g l}^{-1}$, respectively. The limits of quantification (LOQ) for the assay were $2 \mu\text{g l}^{-1}$ and $1 \mu\text{g l}^{-1}$ for CQ and DECQ, respectively.

For analysis of CQ and DECQ in milk, 1-ml samples were mixed with 375 ng internal standard (amodiaquine) and 0.1 ml 1 M NaOH and extracted by shaking with 7 ml diethylether for 5 min. These mixtures were cooled at -80°C for 15 min to break any emulsions and then allowed to thaw to room temperature. They were then centrifuged at $2000 g$ for 5 min, the ether layer transferred to a clean tube and then back-extracted into 0.2 ml of 0.1 M HCl by shaking for 1 min. After further centrifugation as above, the ether layer was aspirated to waste and the acid layer centrifuged at $2000 g$ for 20 min to remove traces of ether. Aliquots of the acid layer (0.08 ml) were injected onto the HPLC. Separations were achieved on a Merck RP Select B

C₈ column (250×4.1 mm; E Merck & Co, Darmstadt, Germany) using a solvent of 12% v/v CH₃CN in 45 mM KH₂PO₄ buffer (pH 3) pumped at 1.4 ml min⁻¹. Eluting compounds were quantified from their ultraviolet absorption at 330 nm. Retention times were 7.9, 11 and 14 min for DECQ, CQ and amodiaquine, respectively. RSDs for the assay were: CQ, 3.1% and 2.1% intraday and 4.8% and 2.2% interday at 20 µg l⁻¹ and 2000 µg l⁻¹, respectively; and DECQ, 5.0% and 1.9% intraday and 5.5% and 3.6% interday at 12 µg l⁻¹ and 310 µg l⁻¹, respectively. The LOQ for the assays was 2 µg l⁻¹ for both CQ and DECQ.

Measurement of creatinocrit

Creatinocrit for each milk sample was measured as previously described [12].

Data analysis

The AUC_{0,last time} values for CQ and DECQ in milk were by the log-linear trapezoidal rule as implemented in Topfit Ver. 2.0 [13]. In calculating the AUCs, we assumed that the drug concentrations in milk were zero on the day of delivery when the first dose of CQ was administered and that they increased linearly to the concentrations measured on the first day of sampling (the third day after delivery and of administration of the third dose of CQ). No correction was made for CQ or DECQ in milk arising from previous exposure during pregnancy. The average concentration in milk (C_{avg}) over the total duration of the study was calculated as (AUC_{0, last time})/duration of study in days (mean 18 days). Absolute infant dose via milk (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 [14]. Relative infant dose was calculated as absolute infant dose×days of exposure (µg kg⁻¹)/maternal dose (µg kg⁻¹) and expressed as a percentage. Data have been summarized as mean [95% confidence interval (CI)] or median [interquartile range (IQR)]. Differences between group means (for normally distributed data) or medians (for non-normally distributed data) were assessed using a *t*-test or Mann–Whitney Rank Sum

test, respectively (SigmaStat Ver. 3.5; SPSS Inc., Chicago, IL, USA).

Results

Nineteen women were enrolled in the pregnancy arm of the study (C/M drug distribution), of whom 16 participated in the breast milk study (infant dose). The median (IQR) age and mean (95% CI) weight for the whole group were 24 years (22, 27.5) and 54 kg (51, 57), respectively. The breast milk subgroup had statistically similar age and weight (data not shown). The total dose of CQ base received by the women at each 3-day treatment course was 26.3 mg kg⁻¹ (24.9, 27.7).

The median (IQR) concentrations of CQ and DECQ in cord [20 µg l⁻¹ (18, 38) and 17 µg l⁻¹ (16, 25), respectively] and maternal [8 µg l⁻¹ (4, 23) and 8 µg l⁻¹ (4, 19), respectively] serum at delivery were low and consistent with the last dose of treatment being administered 89 days (74, 104) [mean (95%CI)] prior to sampling. The mean C/M distribution ratio was similar for both CQ 1.1 (0.9, 1.6) and DECQ 1.2 (0.5, 1.8).

For the milk study, a preliminary analysis (Mann–Whitney Rank Sum test for paired data) using all available data indicated that median creatinocrit was significantly higher (*P* = 0.008) in hind- [14% (11.1, 18.9)] compared with fore-milk [12.8% (8.6, 16.6)]. Median CQ was similar in both hind-milk [200 µg l⁻¹ (49, 579)] and fore-milk [210 µg l⁻¹ (37, 574)], whereas median DECQ was significantly higher (*P* = 0.042) in hind- [78 µg l⁻¹ (26, 149)] than in fore-milk [69 µg l⁻¹ (22, 141)]. Nevertheless, since the latter concentration differences were small in practical terms, we therefore used the mean of measured CQ or DECQ concentrations in fore- and hind-milk samples when calculating the AUC for the milk concentration–time curves and related indicators of exposure (Table 1).

The CQ and DECQ concentration–time profiles in milk over the duration of the study are shown as spaghetti plots in Figure 1. Despite a uniformity of dosing/weight between

Table 1
Published data for chloroquine (CQ) and desethylchloroquine (DECQ) lactation studies

Maternal CQ dose (mg base kg ⁻¹ ; dose regimen)	Milk Number of subjects	T _{last sample} (days)	Absolute infant dose (µg kg ⁻¹ day ⁻¹)		Relative infant dose* (%)		Reference
			CQ	DECQ	CQ	DECQ†	
9.52; oral, single	3	9.2	9.5	1.8	0.9	0.19	[5]
25; oral, split over 3 days	9	28	79‡	ND	8.8‡	ND	[6]‡
35; oral, split over 5 days	1	9	168	102	4.2	2.9	[7]
3.1; i.m. single	6	1	34	ND	1.1	ND	[4]
10; oral, single	6	1	660§	ND	6.6	ND	[8]
1.67; oral, daily for 10 days	10	3	53	ND	9.5	ND	[9]

*Absolute infant dose × T_{last sample}. 100/(maternal dose kg⁻¹). †As CQ equivalents. ‡Total CQ and metabolites measured by nonspecific ELISA method. §Determined from C_{max} data measured 14.4 h after dose.

subjects, there was wide intersubject variability in drug concentrations in milk. The extremes in $AUC_{0,last}$ varied 61-fold for CQ and 43-fold for DECQ. There was similar variation in C_{max} for CQ and DECQ. The median C_{max} in milk was $408 \mu\text{g l}^{-1}$ for CQ and $109 \mu\text{g l}^{-1}$ for DECQ and both occurred at 3.93 days, shortly after the last of the three daily doses of CQ. The median C_{avg} across the 17.2 days of sampling was $226 \mu\text{g l}^{-1}$ for CQ and $97 \mu\text{g l}^{-1}$ for DECQ. Median absolute and relative infant doses were $34 \mu\text{g l}^{-1}$ and 2.3% for CQ and $15 \mu\text{g l}^{-1}$ and 1.0% (as CQ equivalents) for CQ and DECQ, respectively. Although the IQR for the combined CQ + DECQ relative infant dose was modest, two patients (nos 1 and 19) had very high concentrations of drug (see Figure 1) that resulted in combined absolute and relative infant doses (as CQ equivalents) of

$177 \mu\text{g kg}^{-1} \text{ day}^{-1}$ and 13.9%, and $267 \mu\text{g kg}^{-1} \text{ day}^{-1}$ and 19.6%, respectively.

Discussion

The transfer of CQ across the placenta has been the subject of one previous study. Akintowna *et al.* measured CQ in whole blood in seven pregnant women who received 3.1 mg kg^{-1} of CQ base i.m. during the second stage of labour [4]. The mean (95% CI) concentrations in mixed cord blood, maternal blood and the C/M ratio were $736 \mu\text{g l}^{-1}$ (505, 967), $683 \mu\text{g l}^{-1}$ (609, 757) and 0.98 (0.86, 1.1), respectively. In our 19 pregnant women who received a mean dose of 26.3 mg kg^{-1} significantly earlier (3 months before delivery), there were much lower mean plasma concentrations of both CQ and DECQ in maternal and cord serum, but mean C/M ratios were similar to those of Akintowna *et al.* [4] at 1.1 for CQ and 1.2 for DECQ. These results show that both CQ and its major metabolite DECQ distribute equally across the placenta, suggesting that fetal exposure will be proportional to the maternal dose and its frequency.

The fetal risk from 300 mg CQ weekly for malaria prophylaxis has been investigated in 169 exposed infants and 454 non-exposed controls [15]. In the CQ-exposed group, two infants (1.2%) showed teratogenic effects (teratology of Fallot and congenital hypothyroidism), whereas in the control group there were four with anomalies (0.9%). Thus, despite significant transfer, neither CQ nor DECQ is likely to be a major teratogen. The extensive historical use of CQ for both treatment and prophylaxis of malaria in pregnancy, and the lack of reports of adverse effects in exposed infants, including no cardiotoxicity [16], also support its safety in pregnancy.

The calculation of infant exposure to CQ from previous literature is complicated by differences in doses ($1.7\text{--}35 \text{ mg kg}^{-1}$), study durations (1–28 days), methods of dose calculation (variable infant daily milk intake assumptions and comparisons with maternal dose), specificity of assay methodology (HPLC vs. enzyme-linked immunosorbent assay), and whether or not the major active metabolite DECQ was also quantified (two out of six studies). The data quality is also variable because of small subject numbers in each study (1–10), and wide variability in drug measurements per subject (13–17). Only three studies used AUC data to estimate milk C_{avg} .

To facilitate consideration of the available breast milk literature we have summarized published studies in Table 2. Absolute infant dose was calculated as milk C_{avg} ($\mu\text{g l}^{-1}$) $\times 0.15 \text{ l kg}^{-1} \text{ day}^{-1}$, and relative infant dose as absolute infant dose $\times$ days of therapy ($\mu\text{g kg}^{-1}$) $\times 100/\text{maternal dose}$ ($\mu\text{g kg}^{-1}$). This calculation is different from the usual recommended method for single-dose administrations, which relies on C_{max} as the concentration term [14]. We suggest that our method is a more appropriate measure of

Figure 1

Milk concentration–time observations for chloroquine (CQ) (A) and desethylchloroquine (DECQ) (B) in 16 women who received a mean (95% CI) of 25.5 mg kg^{-1} (24.3, 26.9) CQ base in equally divided doses on the first 3 days postpartum (see arrows)

Table 2

Milk $C_{\max}$, $T_{\max}$, $T_{\text{last sample}}$, AUC, C_{avg} , absolute infant dose and relative infant dose for chloroquine (CQ) and desethylchloroquine (DECQ)

Parameter	CQ*	DECQ*
$C_{\max}$ ($\mu\text{g l}^{-1}$)	408 (89, 1011)	109 (44, 211)
$T_{\max}$ (days)	3.93 (3.67, 5.15)	3.93 (3.67, 5.15)
$T_{\text{last sample}}$ (days)	17.2 (16.1, 18.1)	17.2 (16.1, 18.1)
$\text{AUC}_{0,\text{last}}$ ($\mu\text{g.h l}^{-1}$)	3787 (946, 5767)	1543 (587, 3023)
C_{avg} ($\mu\text{g l}^{-1}$)	226 (44, 336)	97 (26, 175)
Absolute infant dose ($\mu\text{g kg}^{-1} \text{ day}^{-1}$)	34 (7, 50)	15 (4, 26)
Absolute infant dose, $T_{\text{last sample}}$ ($\mu\text{g.days kg}^{-1}$)	568 (142, 865)	232 (88, 454)
Relative infant dose (%)†	2.3 (0.5, 3.6)	1.0 (0.4, 2.0)‡

*Median (IQR). †Absolute infant dose. $T_{\text{last sample}}$, 100/(maternal dose kg^{-1}).

‡Expressed as CQ equivalents.

infant exposure for drugs such as CQ with intermittent dosing regimens and long half-lives. With our dataset, the method yields infant dose estimates that are about 50% lower for CQ, but are similar for DECQ, than those that result from traditional use of $C_{\max}$ values in the dose calculation (see Table 2 for $C_{\max}$ vs. C_{avg} values). Using the modified infant dose calculation as described above, relative infant dose ranged from 0.9 to 9.5% for CQ (six studies) with two single estimates of 0.15% and 2.6% for DECQ. This variability and relative paucity of data is not surprising considering that many milk studies are opportunistic.

We were fortunate to be able to study both CQ and DECQ transfer into milk in 16 patients using C_{avg} derived from AUC measurements made over 17 days. The median absolute and relative infant doses were $34 \mu\text{g kg}^{-1} \text{ day}^{-1}$ and 2.3% for CQ and $15 \mu\text{g kg}^{-1} \text{ day}^{-1}$ and 1.5% for DECQ, respectively. The absolute dose is well below recommended paediatric ($10 \text{ mg base kg}^{-1}$ orally immediately, followed by $5 \text{ mg base kg}^{-1}$ orally at 6, 24 and 48 h, with a total dose of $25 \text{ mg base kg}^{-1}$) [17] and neonatal (loading dose of 20 mg kg^{-1} i.v. followed by a i.v. maintenance of $25\text{--}50 \text{ mg kg}^{-1} \text{ day}^{-1}$ as CQ phosphate or CQ hydrochloride) [18] treatment doses.

The low level of milk transfer we observed also indicates that the breastfed infant will not be protected against malaria. The combined relative infant dose (CQ plus DECQ = 3.2%) is also significantly lower than the recommended 10% safety cut-off [14], and when considered together with the lack of reported adverse effects in exposed infants, CQ can therefore be considered compatible with breastfeeding. Even in the two patients with unusually high concentrations of CQ and DECQ in milk, and correspondingly high relative infant doses, their absolute infant doses on a $\mu\text{g kg}^{-1} \text{ day}^{-1}$ basis were still well below the usual therapeutic infant dose and therefore unlikely to cause problems.

The present study has provided valuable data on transfer of CQ and DECQ across the placenta and into breast

milk when CQ is given during pregnancy and at delivery in PNG women. Although C/M ratios suggest that neonatal plasma concentrations are similar to those in the mother and thus might be sufficient for protection against malaria, infection in the neonatal period is rare. With institution of breast feeding, the plasma CQ concentrations in the infant will fall to subtherapeutic as a result of the low concentrations of CQ and DECQ in breast milk. There do not appear to be any safety concerns for the fetus or breast-fed infant if the mother has been taking CQ in recommended doses.

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