Br J clin Pharmac 1993; **36**: 413–416

Distribution of indomethacin in human milk and estimation of its milk to plasma ratio *in vitro*

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1 The distribution of indomethacin in fat and protein fractions of colostrum and mature milk as well as its milk to plasma drug concentration ratio (M/P ratio) were determined *in vitro*.

2 The extent of plasma protein binding of indomethacin (5–20 $\mu g\ ml^{-1}$) was $\geq 99.6\%$. The protein binding of indomethacin in colostrum was 46.0% at pH 7.4. The lower protein content of mature milk compared with colostrum was associated with a significant decrease in the extent of drug protein binding (46 ± 1.93 to 35 ± 1.0 s.e. mean). Protein binding was also decreased significantly in 8% fat mature milk (20.3 ± 2.4 s.e mean) but was constant over the pH range 7.4 to 6.8.

3 About 40% of indomethacin added to milk was associated with the fatty layer. The indomethacin M/P ratio determined by equilibrium dialysis was less than 0.01. Hence the maximum infant daily dose was estimated to be 0.006 mg kg^{-1}.

4 Our results indicate that indomethacin transfers to milk by simple diffusion according to its physicochemical properties, and that treatment with indomethacin is not a contraindication to breast feeding.

Keywords indomethacin breast feeding NSAIDs lactation

Introduction

Guidance on whether breast-feeding mothers may take indomethacin is contradictory. Thus, whereas the *American Academy of Pediatrics* [1] recommends that indomethacin can be taken while breast feeding, Eeg-Olofsson *et al.* [2] reported that the ingestion of indomethacin in breast milk caused seizures in a 7 day old infant. However, no milk or plasma samples were taken to confirm the latter hypothesis and, based on this single report, some reviewers [3] recommend that indomethacin should be avoided during breast feeding.

Lebedevs *et al.* [4] found traces of indomethacin in seven out of 14 milk samples. In the other cases, the median M/P ratio was 0.36. However, this figure must be questioned since some plasma and milk samples were not taken at the same time. Other plasma samples were taken 6 h after drug administration, when plasma drug concentrations are relatively low, this would give rise to a spuriously high M/P ratio if drug elimination from milk is slower than that from plasma.

The M/P ratio is the most commonly used index of drug transfer into milk. Its value is influenced by factors such as maternal free drug concentration, milk protein binding and the solubility of the drug in the milk fat layer. The M/P ratio may be used to estimate drug concentrations in milk from those in maternal plasma [5]. The dose to the infant may then be compared with maternal doses or with doses used therapeutically in infants.

To avoid ethical constraints on studying drug transfer to milk *in vivo*, we have developed an *in vitro* method involving equilibrium dialysis [6, 7]. This allows the reconstitution of milk to simulate physiological variations in pH and lipid and protein concentrations found in colostrum as opposed to mature milk, and in foremilk as compared with hind milk.

The aim of this work was to measure the distribution of indomethacin between the fat and protein milk layers and to estimate its M/P ratio with a view to calculating the dose to infant.

Methods

Sample collection and preparation

Eight Caucasian mothers, between 25 and 32 years old (28 ± 0.85), with a normal body mass index took part in

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this study. All were non-smokers and abstained from alcohol. The mean duration of pregnancy was 39.51 ± 0.37 weeks and all deliveries were uncomplicated. Each patient signed a consent form and the study was approved by the Hospital Ethics Committee.

Milk samples (5 ml) were withdrawn 4 hourly for 24 h, at the beginning and at the end of the feed, on days 4 (colostrum), 12 (intermediate milk) and 26 (mature milk) after delivery. Samples taken at the beginning and the end of each nursing session were pooled. On the day of sampling the mothers were requested to take a diet normal in lipids.

Blood samples were collected in Vacutainer® tubes. The tubes were centrifuged at 1500 *g* for 10 min to obtain plasma. Plasma and milk samples were stored at −20° C until assayed.

Analytical methods

The total protein content of the milk was determined using a micro-adaptation of the Lowry method [8]. Total fat content was determined by the creamatocrit method as described by Lucas *et al.* [9]. Milk and plasma drug concentrations were measured by h.p.l.c. after extraction with anhydrous diethyl ether (ACP Chemicals). A LKB Ultrapac Spherisorb ODS-2, 5 μm, (4 × 250 mm) column and a LKB Lichrosorb RP8, 5 μm, (4 × 10 mm) pre-column were used. The mobile phase was acetonitrile/acetic acid 0.1 M (55/45) and u.v. detection was at 254 nM. The limits of determination were 0.5 μg ml^{-1} in plasma and 0.25 μg ml^{-1} in milk. Recovery of indomethacin (10 μg ml^{-1}) was 77% in plasma and 86% in milk. Calibration curves for plasma and milk were linear for all concentrations studied (0.5–20 μg ml^{-1}). Intra-assay coefficients of variation for plasma and milk assays were 0.28% and 0.85%, respectively (10 μg ml^{-1}, n = 6). Inter-assay coefficients of variation were 5.4% for plasma and 1.0% for milk (0% fat) (10 μg ml^{-1}, n = 3). The coefficient of variation was higher (13.2%) for the assay of 8% fat milk.

Protein binding

Indomethacin was dissolved in h.p.l.c. grade methanol and added to 1 ml plasma to give concentrations of 5, 10 and 20 μg ml^{-1}. In colostrum and mature milk, adjusted to 0 or 8% fat, 10 μg ml^{-1} was used (n = 3 to 6). The milk and plasma pH values were adjusted to 6.8 and 7.4 respectively, using NaOH or HCl 0.1 M. Samples (n = 6) were maintained at 37° C for 1 h before the addition of the drug. After incubation for a further 10 min to allow maximal protein binding, the sample was assayed for total drug concentration (C). The unbound concentration (Cu) was determined by ultrafiltration (2000 *g*, 15 min, 23° C) using a YMT membrane (Centrifree®, Amicon, USA, molecular cut-off of 20000). The bound concentration was calculated from ($C-C$u).

Distribution in the fatty layer

Milk samples with high (8%) and low fat content were prepared by centrifugation (n = 11). The distribution of indomethacin between the fat and water phases of 8% fat milk was determined after shaking at 37° C in a rotatory agitator (Environmental shaker, Lab-Line Instruments, USA) for 20 min. Total drug concentrations were measured in 300 μl aliquots of whole milk. The remainder was centrifuged at 10000 *g* for 15 min at 4° C. The upper layer (fat) was removed and the drug concentration in fat was calculated from $C-C_{skim}$. The C_{skim}/C ratio (S/W) was also calculated.

Equilibrium dialysis

M/P ratios were determined *in vitro* by equilibrium dialysis using Plexiglas® cells (Scienceware, Pequannock NJ, USA). The compartments were separated by a semipermeable membrane (molecular weight cut-off of 6000) (Scienceware, Pequannock NJ, USA). Indomethacin was added to plasma to give a concentration of 20 μg ml^{-1} (n = 6). One ml of plasma and milk were then placed on either side of the membrane. The cells were shaken gently at 37 ± 1° C and samples were taken at 0, 3, 6, 9 and 21 h. Equilibrium dialysis was consistent with colostrum (18 g l^{-1} protein) and mature milk (10 g l^{-1} protein) containing 0 and 8% fat. The pH was 7.4 except for mature milk (0% fat) when it was 6.8.

Calculation of dosage

The dose per day given to the infant was estimated using the equation proposed by Wilson [5] i.e. *Maternal plasma drug concentration* × *M/P ratio* × *volume of milk ingested* = *dose per day*. A fixed milk volume of 1000 ml day^{-1} [10] was assumed. Estimated doses were adjusted to body weight which was assumed to be 5 kg for the infant and 60 kg for the mother [10]. Calculations were also made assuming 150 ml kg^{-1} day^{-1} as the quantity of milk ingested by the infant. The maternal plasma drug concentration was set at 3 μg ml^{-1} [11].

Statistical analysis

Results are expressed as mean ± s.e. mean. The significance of differences was determined using the paired Student's t-test. The null hypothesis was rejected when $P \leq 0.05$.

Results

The extent of plasma binding of indomethacin was ≥ 99.6% at concentrations from 0 to 20 μg ml^{-1}. The protein binding of indomethacin (10 μg ml^{-1}) in colostrum (pH 7.4) containing 18 g l^{-1} of protein and no fat was 46.0 ± 1.93% (Table 1). Similar binding was observed in the 8% fat milk.

At pH 7.4 drug binding in mature milk was significantly lower than in colostrum, reflecting the decrease in protein content. A 24% decrease in protein binding was observed in mature skimmed milk compared with skimmed colostrum. Binding to 10g l^{-1} milk protein was constant at 35.01 ± 1.0% when the pH was lowered from 7.4 to 6.8.

In 8% fat milk, a 55% decrease in protein binding was found in mature milk compared with colostrum (45.3 ± 2.25% to 20.33 ± 2.4%) and a 42% decreased was seen compared with skimmed mature milk (Table 1).

Table 1 Binding of indomethacin to milk proteins and distribution in the lipid layer*

Protein concentration ($g\ l^{-1}$) and pH	*% Protein binding* *Milk lipid 0%*	*Milk lipid 8%*	*Distribution into lipid (Milk lipid 8%)*
Colostrum			
$18g\ l^{-1}$	46.0 ± 1.9^a	45.3 ± 2.6^d	43.3 ± 2.9^f
pH 7.4	(41.0–51.0)	(38.6–52.4)	(35.3–50.1)
Colostrum			
$18g\ l^{-1}$			39.7 ± 1.2^g
pH 6.8	n.d.	n.d.	(36.3–43.2)
Mature milk			
$10g\ l^{-1}$	35.0 ± 1.0^b	20.3 ± 2.4^e	38.7 ± 0.8^h
pH 7.4	(30.7–39.3)	(10.0–30.7)	(36.0–40.0)
Mature milk			
$10g\ l^{-1}$	35.4 ± 1.0^c		
pH 6.8	(32.0–38.2)	n.d.	n.d.
	a,b = 0.034	b,e = 0.02	f,g,h = NS
P	b,c = NS	c,e = 0.056	
	a,d = NS	d,e = 0.001	

*Data are expressed as mean ± standard error with 95% cofidence intervals in brackets.
n.d. = not determined.
NS = not significant.

The fatty layer of the 8% fat milk contained 40.8 ± 1.4% of the indomethacin added to whole milk (S/W ratio = 0.60 ± 0.01). The skim to whole milk ratios of indomethacin in 8% fat colostrum and 8% fat mature milk were similar.

The indomethacin M/P ratio was less than 0.01 irrespective of the fat or protein content and of the pH of the milk.

Assuming that the maternal plasma concentration of indomethacin should not exceed 3 $\mu g\ ml^{-1}$, the dose of indomethacin received by the infant from breast milk was estimated to be $\leq 30\ \mu g\ day^{-1}$ or $4.5\ \mu g\ kg^{-1}\ day^{-1}$ assuming a milk intake of $150\ ml\ kg^{-1}\ day^{-1}$.

Discussion

The extent of plasma binding of indomethacin observed in this study was similar to that (>99%) reported by Hultmark *et al.* [12] and Mason & McQueen [13].

A decrease in milk drug binding in relation to protein content, as seen in our study, has also been reported for diazepam and propranolol by Fleishaker *et al.* [14]. For some drugs this decrease may lead to a lower concentration in milk and, therefore, a lower M/P ratio. However, this is immaterial in the case of indomethacin since its transfer to milk is negligible.

The 40% distribution of indomethacin into milk fat is consistent with its high octanol-water partition coefficient (log P 3.08) [17]. This finding is in agreement with that of Atkinson & Begg [15].

During breastfeeding, lipid concentration is highest in the mature hindmilk. However, because indomethacin concentrates in lipids does not imply a high concentration in hindmilk, even though distribution into milk lipids is associated with a higher drug concentration in milk [16]. Given a concentration of $0.25\ \mu g\ ml^{-1}$ in skimmed milk, to which should be added the 40% indomethacin present in the lipid layer at the end of a breast feeding session, the indomethacin content of milk should not be very much higher at the end of the feed than at the beginning.

An infant dose per kilogram which is less than 10% of the maternal dose per kilogram is generally considered to be safe [17]. The dosage of indomethacin in premature infants treated for patent ductus arteriosus at birth is $200\ \mu g\ kg^{-1}\ day^{-1}$ [18]. This dose is associated with a plasma drug concentration of 0.1 to 1.6 $\mu g\ ml^{-1}$ in the infant. During breast feeding, an infant could receive up to $6\ \mu g\ kg^{-1}\ day^{-1}$ of indomethacin from maternal milk, which will be 3% of the pediatric dose and 0.5% of the maternal dose ($75\ mg\ day^{-1}$). Therefore, we suggest that it is safe for nursing mothers to take indomethacin.

This research was supported by the National Health Research and Development Program (PNRDS) Canada. We thank Pierre De Grandpré for technical advice and André Auclair for the collection of milk samples.

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(Received 6 May 1993, accepted 23 July 1993)