Pharmacokinetics of Caffeine and Its Demethylated Metabolites in Lactation: Predictions of Milk to Serum Concentration Ratios

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INTRODUCTION

Considerable attention over the last twenty years has been directed toward neonatal exposure to methyl xanthines (caffeine, in particular) via breast milk[1-5]. Although caffeine is excreted to a limited extent in breast milk and the dose of caffeine presented to the infant is generally regarded as small (i.e., <5% of maternal dose), clinical concern continues to focus on the inability of the neonate to metabolize caffeine[6,7]. The newborn and, in particular the premature newborn, is at greater risk for accumulating caffeine compared to the adults since their ability to metabolize methyl xanthines may not be fully developed[6,7].

The primary metabolites of caffeine are the demethylated products 1,7-dimethylxanthine (paraxanthine), 3,7-dimethylxanthine (theobromine) and 1,3-dimethylxanthine (theophylline) formed by the cytochrome P-450 enzyme system in the liver[8,9]. Theobromine and theophylline are present in breast milk following their respective administration[2,5] and may also accumulate in the newborn[10,11]. All four of these methyl xanthines (caffeine, paraxanthine, theobromine and theophylline) possess cardiovascular and psychomotor activity[12,13] and, therefore, these metabolites could contribute to a pharmacological effect in newborns.

We have proposed a diffusional model for the distribution of xenobiotics between milk and serum[14-16]. Diffusional model predictions were comparable to in vivo measurements for a number of drugs in lactating New Zealand White rabbits[15,16]. Model predictions for several model compounds agreed with literature values for the milk to serum concentration ratios (M/S) in humans[14]. To date, this model has not been directly tested in humans. The purpose of the present study is to examine the ability of the diffusion model to predict M/S values from in vitro determinations for caffeine and its three monodemethylated metabolites in human volunteers.

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MATERIALS AND METHODS

Chemicals

Caffeine (1,3,7-trimethylxanthine) was obtained from Aldrich Chemical Co. (Milwaukee, WI). Theophylline (1,3-dimethylxanthine), theobromine (3,7-dimethylxanthine), paraxanthine (1,7-dimethylxanthine), and beta-hydroxyethyl-theophylline were obtained from Sigma Chemical Co. (St. Louis, MO).

Drug Administration and Sampling Protocol

The study population consisted of the 5 healthy human volunteers 6-28 weeks post partum. All subjects provided documentation of informed consent according to the University Institutional Review Board prior to their participation in the study. Subjects were asked to refrain from ingesting caffeine containing beverages for 48 hours prior to the study. All subjects were required to stop nursing their infants for the duration of the study; an electronic breast pump (Egnell LACT/E model, Cary, IL) was used to maintain normal milk flow.

Each volunteer received a single oral dose of 200mg caffeine (No Doz^R). Prior to dosing, blank serum and milk samples were obtained for in vitro determinations of pH, skim to whole milk concentration ratio and protein binding. Serial blood samples (5.0 ml) were obtained from a peripheral indwelling venous catheter at 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12 and 24 hr after oral dosing. Following collection, serum samples were harvested and stored at −20°C until drug analysis. Serial milk samples (10.0 ml) were also taken via an electronic breast pump at the sampling times as described above.

In Vitro Measurements

The in vitro measurements were determined by previously reported methods[14,15]. pH measurements in serum and milk were performed anaerobically at 37°C within 1 h of collection. Protein binding in serum and skim milk was determined for caffeine, paraxanthine theophylline and theobromine (to a final concentration of 2 ug/ml of each of the xanthines) by equilibrium dialysis for 5 h. Skim to whole milk concentration ratios (S/W) were also determined.

Assay Methodology

Serum and milk concentrations of caffeine, paraxanthine, theophylline and theobromine were measured by modification of the HPLC method described by Dorrbecker et al[17]. The mobile phase (adjusted to pH 3.5) consisted of tetrahydrofuran:methanol:0.01 M KH_2PO_4 (1:9:90). The flow rate was 1.2 ml/min and the effluent was monitored at 214 nm. The retention times were 5.2, 8.5, 9.4, 10.9, and 16.5 min for theobromine, paraxanthine, theophylline, beta-hydroxyethyl theophylline and caffeine, respectively.

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Table 1. Mean (±S.D.) Values for Pharmacokinetic Parameters for Caffeine and its Demethylated Metabolites (Paraxanthine, Theobromine and Theophylline) After an Oral Dose of 200 mg of Caffeine in Five Lactating Human Volunteers

Parameter	Caffeine	Paraxanthine	Theobromine	Theophylline
Cl_o, ml/min/kg	1.0 ± 0.2	—	—	—
MRT_s^{oral}, h	8.7 ± 2.6	14 ± 4	27 ± 15	22 ± 14
MRT_m^{oral}, h	9.1 ± 2.9	16 ± 3	30 ± 17	21 ± 8
$t_{1/2}$, h	5.6 ± 1.8	6.7 ± 2.8	12 ± 2.5	8.2 ± 3.7

Data Analysis

Serum and milk drug concentration versus time data were analyzed by fitting a mono or biexponential equation to these time profiles using nonlinear regression analysis (RSTRIP, MicroMath, Salt Lake City, UT). Area under the drug concentration-time curve (AUC) and area under the first moment curve (AUMC) were determined. The subscripts s and m refer to serum and milk, respectively.

Observed M/S values were determined from

$$M/S_{obs} = \frac{AUC_m}{AUC_s} \quad (1)$$

Predicted M/S values were calculated using a previously derived relationship[14]:

$$M/S_{pred} = \frac{f_s^{un} f_s}{f_m^{un} f_m (S/W)} \quad (2)$$

where f_{un} and f values referred to fractions unionized and unbound, respectively.

Oral clearance (Cl_{po}) was calculated from

$$Cl_{po} = \frac{Dose}{AUC_s} \quad (3)$$

Mean residence time for an oral dose administration (MRT^{oral}) was determined as

$$MRT_{s\ or\ m}^{oral} = \frac{AUMC_{s\ or\ m}}{AUC_{s\ or\ m}} \quad (4)$$

RESULTS AND DISCUSSION

The distribution of caffeine[1,3,4], theophylline[5], and theobromine[2] into human milk have been reported. The time course of caffeine in milk has been described as monoexponential and roughly parallel to plasma concentrations in humans. Stavchansky et al.[4] demonstrated that human milk concentrations of caffeine coincided with plasma concentrations, and showed no difference in caffeine concentration in milk between breasts when sampled as a function of time. The time course of theobromine[2] and theophylline[6] in human milk following their respective administrations also paralleled their plasma and saliva levels. Consistent with these reports, our data indicated that the time courses of all four xanthines in milk paralleled their respective serum time profiles (Table 1 & Figure 1). The relative concentration order for the xanthines was consistent in all of the subjects (caffeine > paraxanthine > theobromine > theophylline).

M/S values following the direct administration of these methylxanthines, including this study, have been reported to be between 0.7 and 0.9 for caffeine[1,3,4], theophylline[5] and theobromine[2]. In vitro predictions of caffeine, paraxanthine, theophylline and theobromine M/S values were consistent with in vivo observations (Table 2). The correlation across all

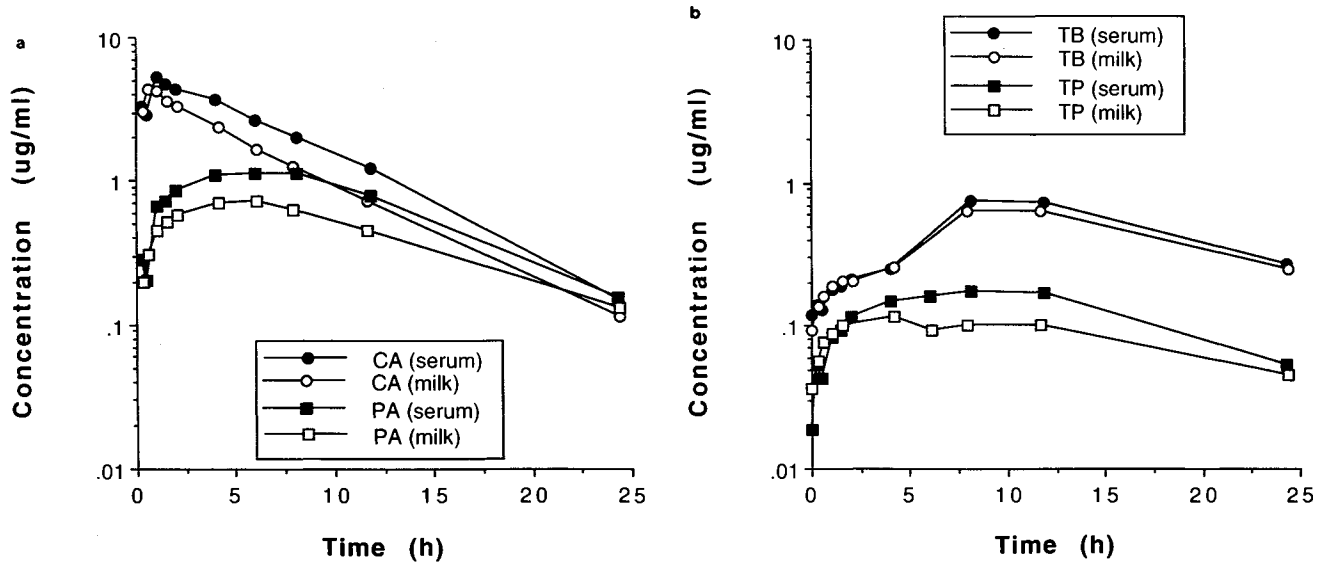

Figure 1. Concentration Time Profile for Caffeine (CA) and Its demethylated metabolites (paraxanthine (PA), theobromine (TB) and theophylline (TP)) following an oral dose of 200 mg of caffeine in a representative lactating human volunteer.

Table 2. Mean (±S.D.) Values for f_s, f_m, S/W and M/S_{pred} Obtained from In Vitro Studies and M/S_{obs} from In Vivo Studies for Five Lactating Human Volunteers

Parameter	Caffeine	Paraxanthine	Theobromine	Theophylline
f_s	0.71 ± 0.02	0.54 ± 0.04	0.93 ± 0.04	0.50 ± 0.04
f_m	0.98 ± 0.02	1.01 ± 0.02	0.94 ± 0.03	1.01 ± 0.01
S/W	1.00 ± 0.02	1.02 ± 0.03	1.01 ± 0.01	1.03 ± 0.02
M/S_{pred}	0.73 ± 0.04	0.52 ± 0.04	0.97 ± 0.04	0.48 ± 0.04
M/S_{obs}	0.70 ± 0.06	0.52 ± 0.14	0.82 ± 0.03	0.57 ± 0.14

pKa values of weak acids of CA = 14.0; PA = 8.65; TB = 10.0; TP = 8.68 (see Pfleiderer V, Nubel G. Zur struktur des xanthines und seiner N-methyl-derivate. Justus Liebigs Ann. Chem. 1961;647:155–160).

four xanthines was good (Figure 2), although for any given xanthine the correlation was weak or non-existant due to the narrow range of M/S values.

The M/S values differed among the structurally similar xanthines. Caffeine and theobromine had a mean M/S_{obs} values which were larger than theophylline and paraxanthine. The greater extent of serum protein binding for paraxanthine and theophylline were largely responsible for their similar M/S values. A similar phenomenon was observed for the M/S values for these methyl xanthines in rabbits[16]. N-demethylation of caffeine at position one (theobromine) decreased the binding of the xanthine moiety to human serum proteins, whereas N-demethylation at position 3 (paraxanthine) or 7 (theophylline) resulted in a considerable increase in the extent of serum binding. The underlying cause for the differences in binding is not readily apparent from their physiochemical properties (i.e., pKa and lipid solubility). Again, these binding results were comparable to those obtained with rabbits[16].

The neonatal dose for caffeine at steady-state may be estimated from the present pharmacokinetic data. Assuming neonatal milk consumption at 150 ml/kg/day, using the observed M/S and a steady state caffeine concentration of 14 ug/ml (Css = AUC / tau), the newborn would ingest an equivalent of 7% of the maternal caffeine dose (on a body weight basis). This estimate is slightly higher than previous values[6,7]. The demethylated metabolites of caffeine possess considerable pharmacological activity[12,13], hence the impact of their ingestion by the newborn following maternal caffeine ingestion should also be addressed. Performing a similar analysis for paraxanthine, theophylline and theobromine following maternal caffeine administration yields a total xanthine dose ingested by the neonate which would be equivalent of 18% of the maternal caffeine dose (total xanthine exposure to the infant on a body weight basis). Hence, active metabolites should be considered when estimating the risk of newborn drug exposure via lactation.

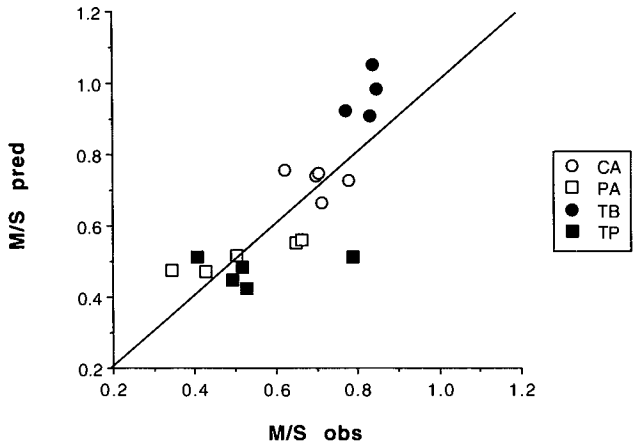

Figure 2. Predicted and Observed M/S Ratios for Caffeine (CA) and its demethylated metabolites (paraxanthine (PA), theobromine (TB), and theophylline (TP)) following an oral dose of 200 mg of caffeine in five lactating human volunteers. Overall regression line (Y = 0.0185 + 0.998 X; R^2 = 0.636).

Consideration must also be given to the ability of the newborn to metabolize methyl xanthines. Caffeine systemic clearance in the premature infant is reported to be ten-fold lower than adult clearance[6]. This lower clearance would result in steady-state concentrations of caffeine that approached maternal concentrations, despite the fact that the infant received less than 10% of the maternal dose via suckling. Neonatal risk assessment should consider not only the dose of the parent drug, but the exposure / dose of active metabolites and the ability of the neonate to clear xenobiotic.

In summary, human M/S ratios for caffeine, and its metabolites, paraxanthine, theobromine and theophylline were predicted from in vitro experiments. These results provide the first prospective evidence in humans which supports diffusion as the principal mechanism for drug transfer into breast milk.

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