Relative bioequivalence of amoxicillin dissolved in breast milk

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ABSTRACT

Background Oral antibiotics use in infants in developing countries is challenging because liquid formulations are often unavailable. However, dissolving solid formulation of drugs in water poses a risk of gastrointestinal infection. Although mother's milk may be a potential vehicle, no evidence exists to indicate that antibiotics dissolved in human milk are bioequivalent to those dissolved in water. Therefore, we compared pharmacokinetic parameters of an orally administered antibiotic, amoxicillin, dissolved in human milk, to those of water-dissolved amoxicillin.

Methods A pharmacokinetic study was conducted in 16 healthy adult volunteers in a randomised crossover design. Marketed amoxicillin powder for suspension was dissolved in either human milk or water at a final concentration of 50 mg/mL, and 10 mL was given orally in a fasting state. Timed blood samples were obtained and plasma amoxicillin was quantified using liquid chromatography-mass spectrometry.

Findings Results showed that pharmacokinetic parameters, including area-under-the-curve, Cmax and half-life of the water-based and milk-based amoxicillin administration were not significantly different. 90% CIs of the ratios of these parameters in concomitant breast milk administration to those of water were within 89% and 116%, suggesting they are bioequivalent (defined as a range between 80% and 125%).

Interpretation We conclude that oral administration of amoxicillin dissolved in human milk at 50 mg/mL results in pharmacokinetics profiles comparable to amoxicillin dissolved in water. Pharmaceutical interactions between amoxicillin and breast milk are unlikely, suggesting no need to modify dosing schedules.

What is already known on this topic?

In a resource-scarce setting, powder is preferred to liquid formulations for costs and simplicity of storage and transport. Dissolving powder antibiotics in breast milk may be an option for infants due to palatability and sanitary reasons. However, it is not known if dissolving powder of antibiotics in breast milk is bioequivalent to those dissolved in water.

What this study adds?

Amoxicillin powder suspended in human milk at 50 mg/mL is bioequivalent to water-based formulation.

INTRODUCTION

Although parenteral administration of antibiotics remains the treatment of choice for severe bacterial infection in neonates, oral use of antibiotics emerges as a practical alternative in resource-scarce settings.[1] Although suspensions and liquid formulations are suited for oral administration in infants and children, solid formulations may be preferred in developing countries because of their relatively inexpensive and less complicated manufacturing, transporting and storage processes. However, administering solid formulations to infants and children is challenging. Dissolving medicines in water may be acceptable, but safety of drinking water for infants in developing countries and water solubility of the drug itself are major concerns. To circumvent this hurdle, use of mother's milk as a vehicle of drugs, such as antibiotics, is an option. However, little evidence is currently available to prove bioequivalence of antimicrobial drugs dissolved in breast milk compared with those dissolved in water.

Even when suspensions of antibiotics are available, influence of milk intake on their absorption has been a recurring theme in paediatrics. McCracken *et al*[2] reported marked reduction of oral absorption of penicillin V, penicillin G and cephalexin, given as suspensions, in infants and children (aged 2–46 months) when milk or formula feeding followed immediately after the dosing. The reduction of peak serum concentrations and the area-under-the-curve (AUC) was 40– 60%, indicating significant impact on absorption. Amoxicillin is a widely used β-lactam antibiotic with high bioavailability (>90%), and short half-life of about 1.5 h (in children)[3 4] and 3–6 h (in neonates)[5] through predominant renal excretion.[6] Importantly, bioavailability characteristics of amoxicillin between infants and adults are nearly identical.[7] Ginsburg *et al*[8] reported on the influences of milk/formula coadministration on absorption of amoxicillin given as oral suspension in infants and children. They showed that average serum concentrations at 30 min and 1 h of administration tended to be lower when amoxicillin suspension was given with milk or formula, compared with fasting, although overall AUC appeared to be similar. Taken together, these data hint at altered absorption kinetics of amoxicillin if coadministered with breast milk, which may necessitate dosing

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modifications. The first step to address this issue is a bioequivalence evaluation of compatibility between human milk and amoxicillin. To date, however, no such study is available.

In this study, we examined pharmacokinetics (PK) of oral amoxicillin dissolved in breast milk to characterise its bioequivalence, or lack thereof, compared with water-based administration.

METHODS

Ethics approval and registration

The study was approved by the research ethics board of the Hospital for Sick Children, WHO, and the Mother's Milk Bank of Ohio, and registered to Health Canada (Clinical Trial Application: #144700) and the National Institute of Health (clinicaltrial.gov: NCT01435824).

Design and participants

We conducted a randomised 2×2 crossover single-dose PK study in healthy adult volunteers to characterise basic PK parameters of amoxicillin dissolved in human milk or water. All participants signed the informed consent after detailed explanation of the study. The washout period between the water and human milk phases was 1–2 weeks. A 2×2 crossover design was chosen, based on the FDA-recommended design for bioavailability studies.[9] Sixteen young adult volunteers with ages between 20 and 40 years old (eight male, and eight female) were enrolled. Participants underwent an interview including basic vital sign checking, and filled a health status questionnaire to declare lack of any significant medical conditions such as acute and chronic gastrointestinal problems. Urine pregnancy tests, with consents, were performed before dosing for female participants.

Human milk

Frozen human milk was obtained from the Mother's Milk Bank of Ohio (USA). The milk bank collects human breast milk from screened volunteers. Most of the milk is sent to neonatal intensive care units, but the milk bank allocates a small amount for research projects.

Drug administration

Amoxicillin powder marketed as 'powder for suspension' (Novamoxin 250 from Novopharm, Toronto, Canada: Lot#35422063A) was used to make amoxicillin suspension (50 mg/mL) in human milk or water. Ten millilitres of the suspension (500 mg amoxicillin) was measured using a syringe, and orally administered to each volunteer. The concentration we used (ie, 50 mg/mL) is similar to that of a teaspoonful of 250 mg amoxicillin dissolved in human milk, which may be given to a 5 kg infant at a standard dose of 50 mg/kg. The subjects refrained from food intake overnight (10 h; from 22:00 the previous night to 4 h after the dosing in the morning). Water and fluids were restricted from 2 h before dosing up to 2 h after the dosing, and the subjects did not engage in strenuous physical activity for 8 h after the dosing.

Blood sampling

An intravenous line was inserted and maintained using a heparin lock. The sampling schedule was as follows: time 0, 0.25, 0.5, 1, 1.5, 3, 4 and 8 h postdose. Five millilitres blood was taken in a heparinised tube, plasma was separated immediately after the sampling, and stored at −80°C until analyses. Subjects were not allowed to recline at least for 2 h after drug ingestion.

Table 1 Subject characteristics and pharmacokinetic parameters

Subject characteristics		
Age (year: mean±SD)		36.3±9.0
Sex (M/F)		8/8
Weight (kg: mean±SD)		67.1±14.3
Height (cm: mean±SD)		167.6±12.3
	Water	**Breast milk**
Pharmacokinetics parameters (mean±SD)		
AUC $_{0-8}$ (mg min mL^{-1})	1355.8±254.2	1435.0±241.7
AUC $_{\infty}$ (mg min mL^{-1})	1394.1±256.1	1477.2±260.7
Cmax (ng mL^{-1})	8653.8±2217.6	8690.6±1972.4
Tmax (min)	101.3±52.4	106.9±49.0
Ke (min^{-1}×10^{-3})	9.29±1.88	9.29±1.22
Elimination half-life (min)	77.7±17.0	76.1±11.7
CL/F (mL kg^{-1} min^{-1})	5.63±1.05	5.34±1.22
Vdβ/F (L kg^{-1})	0.63±0.18	0.59±0.16

AUC, area-under-the-curve.

Amoxicillin measurement

Amoxicillin plasma concentrations were determined by HPLC-MS/mass spectrometry (MS), according to the method published by Andrew *et al*[10] at the Analytical Facility For Bioactive Molecules in the Centre for the Study of Complex Childhood Diseases at the Research Institute of Hospital for Sick Children. The quantitation limit was 5 ng/mL, and intraday and interday coefficient of variations was 4.0% and 10.0%, respectively.

PK parameter estimation

Amoxicillin PK parameters were estimated using a model-independent approach. Specifically, the log-trapezoidal method was used to calculate AUC_{0-8} (AUC from time 0 to 8 h), and AUC_{∞} was estimated using an elimination rate constant (K_{el}) of the terminal log-linear phase (β phase) of the concentration-time profile extrapolating to time infinity as follows:

$$AUC_{\infty} = AUC_{0-8} + [C]_{8h}/K_{el};$$ where $[C]_{8h}$ is the plasma concentration at time 8 h.

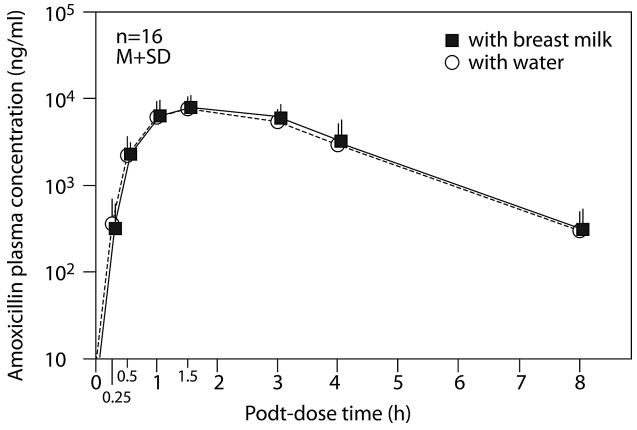

Figure 1 Mean plasma amoxicillin concentration-time profiles. In a cross-over study, 500 mg amoxicillin dissolved in 10 mL breast milk (■, solid line) or water (○, dotted line) was given to 16 adult volunteers (see text). To visualise similarities between the two curves, the curve for amoxicillin dissolved in breast milk is shifted slightly rightward in this figure.

Table 2 Bioequivalence parameters

Parameters	Ratio of milk-based to water-based administration (%)		
	Mean	Lower 90% CI	Upper 90% CI
AUC_{0-8}	106.2	97.5	115.7
AUC_{∞}	106.1	97.5	115.4
Cmax	101.3	89.4	114.9
Tmax	107.9	84.5	137.8
Ke	100.0	91.1	112.1
Elimination half-life	99.0	89.2	109.8

AUC, area-under-the-curve

Other parameters were derived as follows:

$$CL/F = Dose/AUC_{\infty}$$
$$Vd\beta/F = (Dose/AUC_{\infty})/K_{el}$$
$$t_{1/2} = \ln 2/K_{el}$$

where CL is total body clearance, F is bioavailability, Dose is amoxicillin dose per body weight, Vdβ is volume of distribution (β phase), $t_{1/2}$ is elimination half-life, and Kel is an elimination rate constant of the terminal log-linear phase (β phase) of the concentration-time curve.

Statistical analyses

Data were presented as means and SD, and geometric means were also shown when appropriate. According to a common regulatory definition of bioequivalence,[9] a drug preparation is considered bioequivalent to a reference preparation of the drug if 90% CIs of ratios between the two preparations for Cmax, Tmax, AUC_{last} and AUC_{∞} are within a range between 0.80 and 1.25. Therefore, we calculated these ratios between human milk and water-dissolved amoxicillin for comparison. Means of the two arms were compared using paired Student t test, when appropriate.

RESULTS

Demographic parameters of the study volunteers are summarised in table 1. As shown in figure 1, plasma concentration-time profiles indicated apparent mono-exponential elimination patterns. The average concentration-time profiles between the water and milk-based administration were virtually identical. Summary values of pharmacokinetic parameters are shown in table 1. There was no statistically significant difference in these parameters between the breast milk and the water arm.

Table 2 shows ratios and CIs of the bioequivalence parameters between the two arms; 90% CIs of all parameters fell between 80% and 125%, satisfying the bioequivalence criteria[9] except for upper 90% CI of Tmax. Concentration-time profiles of individual subjects further demonstrate that, despite the interindividual (between-subjects) differences of the concentration profiles, the intraindividual (within-subject) variations between water-based and milk-based administration are relatively small (figure 2).

DISCUSSION

Concomitant intake of milk may interfere with absorption of antibiotics. A classical example is tetracycline forming insoluble and hardly absorbable complexes with calcium in milk.[11] Penicillin, including amoxicillin is not known to form complexes

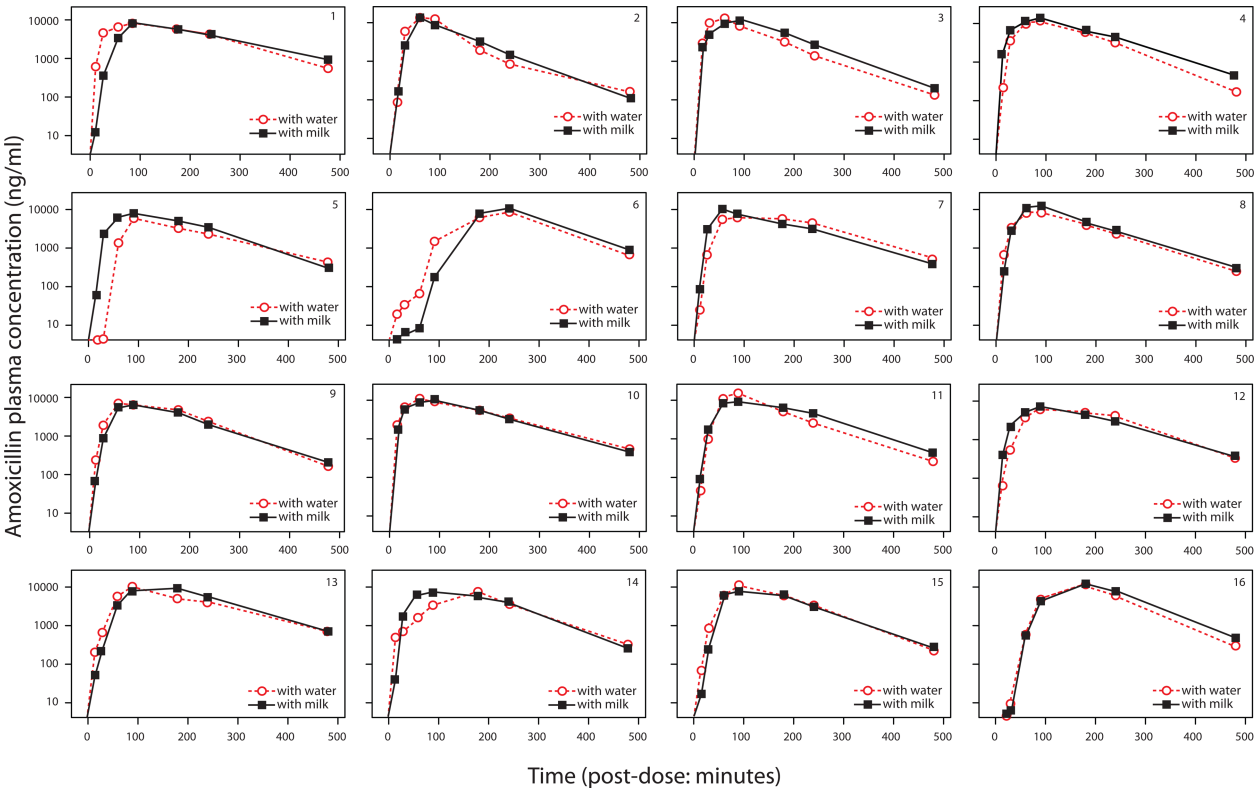

Figure 2 Individual plot of amoxicillin plasma concentration—time profiles. Amoxicillin plasma concentration profiles of 16 subjects are shown to reveal the within-subject similarities.

with any milk components, but a previous study showed a trend toward lower peak serum concentrations of amoxicillin when taken with milk/formula, suggesting slower absorption, although AUC was similar.[8] Our findings clearly show that oral administration of amoxicillin dissolved in human milk has plasma concentration profiles comparable to those from water-dissolved amoxicillin, including Cmax and AUC. In fact, both formulations can be formally considered bioequivalent according to the criteria of regulatory agencies. In resource-scarce settings, parenteral administration of antibiotics may become challenging, and oral administration of antibiotics, such as amoxicillin, represent a practical and effective alternative.[1] The results from our study justify dissolving amoxicillin powder for suspension into human milk at a concentration of 50 mg/mL, instead of water, for oral administration to infants.

Several factors warrant discussion to explain the apparent discrepancy between the present and the previous study,[8] which showed a trend toward altered absorption of amoxicillin in infants who were fed with milk or formula after the drug ingestion. First, our study was a fasting-state study, and each subject ingested amoxicillin dissolved in 10 mL of human milk or water, a negligible fluid volume intake for an adult. In the previous study,[8] infants were fed about 120 mL of milk or formula immediately after ingesting amoxicillin in suspension. Therefore, we cannot rule out a possibility that a larger milk volume (per body weight) changes absorption profiles of the drug through influences on gastrointestinal motility and function, although relatively recent evidence suggests otherwise.[12] Second, although highly speculative, the formulation which Ginsburg *et al*[8] used, may have a pharmaceutical property different from those currently in use. Third, in our study, subjects were adults, because we chose the study design for precise bioequivalence assessment. In general, PK parameters in adults are different from those reported in neonates and infants. However, our study focus was on bioequivalence between the two administration methods. In fact, absorption profiles of oral amoxicillin are similar between infants and adults.[7] Lastly, measuring devices for antibiotic oral suspensions may cause substantial dosing inaccuracies, particularly measuring spoons,[13] making it challenging to interpret existing studies on oral suspensions of antibiotics. However, it is not clear if this factor played any role in the apparent discrepancy between the present and the previous study.

In conclusion, when administered orally, amoxicillin dissolved in breast milk at a concentration of 50 mg/mL (equivalent to 250 mg amoxicillin in a teaspoonful of breast milk) is likely to follow pharmacokinetic profiles similar to amoxicillin dissolved in water. Therefore, it is not necessary to modify doses and dosing schedules of amoxicillin when the drug is dissolved in human milk at this concentration range (50 mg/mL).

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Contributors SI and FG-B designed the study; SI oversaw the study, and obtained funding; PY-B, HF, and RT performed the pharmacokinetic study sessions; PY-B, and FG-B analysed the data; SI led the writing of the paper.

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Competing interests None.

Ethics approval The study was approved by the research ethics board of the Hospital for Sick Children, WHO, and the Mother's Milk Bank of Ohio, and registered to Health Canada (Clinical Trial Application: #144700) and the NIH (clinicaltrial.gov: NCT01435824).

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