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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Modelling and simulation based on the population pharmacokinetic approach provides estimates of infant exposure to drugs in breast milk.
- However, complex mechanistic modelling using both blood and breast milk concentrations of drug has been challenging, which makes risk assessment less complete.

WHAT THIS STUDY ADDS

- Applying the population pharmacokinetic approach to milk concentration data of fluoxetine (an active parent drug) and norfluoxetine (an active metabolite), we show the feasibility of constructing a simple prediction model for milk concentration profiles of the parent and active metabolite without using blood concentration data.

AIMS

Population pharmacokinetic (pop PK) modelling can be used for PK assessment of drugs in breast milk. However, complex mechanistic modelling of a parent and an active metabolite using both blood and milk samples is challenging. We aimed to develop a simple predictive pop PK model for milk concentration–time profiles of a parent and a metabolite, using data on fluoxetine (FX) and its active metabolite, norfluoxetine (NFX), in milk.

METHODS

Using a previously published data set of drug concentrations in milk from 25 women treated with FX, a pop PK model predictive of milk concentration–time profiles of FX and NFX was developed. Simulation was performed with the model to generate FX and NFX concentration–time profiles in milk of 1000 mothers. This milk concentration-based pop PK model was compared with the previously validated plasma/milk concentration-based pop PK model of FX.

RESULTS

Milk FX and NFX concentration–time profiles were described reasonably well by a one compartment model with a FX-to-NFX conversion coefficient. Median values of the simulated relative infant dose on a weight basis (sRID: weight-adjusted daily doses of FX and NFX through breastmilk to the infant, expressed as a fraction of therapeutic FX daily dose per body weight) were 0.028 for FX and 0.029 for NFX. The FX sRID estimates were consistent with those of the plasma/milk-based pop PK model.

CONCLUSIONS

A predictive pop PK model based on only milk concentrations can be developed for simultaneous estimation of milk concentration–time profiles of a parent (FX) and an active metabolite (NFX).

Introduction

Breastfeeding has major benefits for infants including reduction in morbidity and mortality, reduction of infection and positive impact on cognitive functions [1–7]. At present, the reported breastfeeding initiation rate is as high as 90% in some countries but 66% to 80% of women receive medications during the post-partum period [8–12]. As a result, there is a high likelihood for the infants to be exposed to the medications through breast milk. Although the average amount of most drugs ingested by infants through breast milk is much less than that received by mothers even on a body weight adjusted basis [13, 14], individual variations are poorly understood and cases of drug toxicity through breast milk are reported (summarized in Drugs and Lactation Database 'LactMed') [15].

Pharmacokinetic (PK) assessment of drug excretion into milk provides important information necessary for clinical management of breastfeeding women on drugs. However, conventional PK studies, which require multiple samples from each participant, are difficult to conduct particularly in this population due to the demanding feeding schedule and inconvenience for mothers and infants. A population pharmacokinetic (pop PK) approach with model-based simulation of a population offers an attractive alternative [16, 17].

Previously, we conducted a proof-of-concept study to establish a pop PK model of fluoxetine (FX) disposition in the context of breastfeeding using data of maternal blood and breast milk samples [17]. This mechanistic model, however, was not able to account for its active metabolite, norfluoxetine (NFX), partly due to the imbalance between the data quantity and the complexity of the physiologically based mechanistic model with multiple blood and milk compartments.

The objective of this proof-of-concept study was to develop a simple pop PK model predictive of FX and NFX milk concentrations without referring to plasma concentrations, thereby negating the need for a complex mechanistic model and intensive PK blood sampling while allowing simultaneous prediction of a parent drug and its active metabolite.

Methods

Patients' data

We used the datasets of FX and NFX concentrations in milk, which were published elsewhere [17]. These data were originally retrieved from two previous studies [18, 19], and were de-identified for our purposes. FX treatment was started at a median of 70 days (range 13–750) [18] and 41 days (range 15–166 days) prior to the study [19], and we assumed a steady-state. Each of the two studies was approved by its local ethics committee, and informed consent was obtained from every participant.

The dataset of breast milk FX and NFX concentrations in 10 women represented the average concentrations of the pre- and post-feeding samples [18]. In the remaining four women in the same study [18], samples were aliquot parts of milk emptied from both breasts at a given post-dose time. The datasets from the other study [19] consisted of milk level data of 10 women: pre-feed samples (three women), post-feed samples (two women), pooled samples of pre-feed and post-feed milk (one woman) and unknown timing (four women).

Pharmacokinetic modelling

Model development and parameter estimation Modelling was performed using NONMEM® version 7.2 (ICON development solutions). Our base model, which was published previously [17], addressed FX concentrations in maternal plasma and milk in a two compartment model, but NFX concentrations were not accounted for. In the present analysis, we aimed to describe milk concentrations of both FX and NFX without referring to maternal plasma concentrations. To this end, we first modelled FX milk concentrations using one and two compartment models. After selecting the best model, the model was expanded to describe both FX and NFX concentrations in milk. Model selection was based upon the likelihood ratio test using minimum objective function values (OFV), pharmacokinetic parameter estimates and their confidence intervals (CIs), goodness-of-fit plots, and consistency with our previous results [17]. The stochastic approximation expectation maximization (SAEM) method with ADVAN 5 subroutine was used in the model development.

Covariate model Maternal body weight (BW) was examined as a covariate, using a stepwise forward addition. Each given parameter was log-transformed (θ), and modelled linearly with this covariate (BW), as shown by the equation $\theta = \theta_a + \theta_b \cdot BW$, where θ_a is the mean estimate of population and θ_b is the deviation due to the covariance. Improvement of the model with a new covariate was accepted if there was a significant decrease in the minimum OFV. A decrease in OFV more than 10.8 ($P < 0.001$ in chi-square test) was considered to be significant.

Error model Interindividual variabilities were assessed by exponential error models as follows:

$$P_i = \theta \cdot e^{\eta i}$$

where P_i is the value of the model parameter for the ith individual, θ is the population mean estimate for parameter P and ηi is the normally distributed interindividual random variability with a mean of zero and variance ω^2.

In order to describe the intra-individual variability (residual error), an exponential error model was used, provided by the equation below:

$$Yj = Fj \cdot e^{\varepsilon j}$$

where Y_j is an observed value of each parameter for the jth individual, F_j is an individually predicted value, and ε_j is a normally distributed random variable with a mean of 0 and a variance of σ^2.

Model validation The final model was evaluated using both internal and external validation methods. We performed bootstrap with 200 time replacement and repeat procedure to assess the stability of the final model and CI of each pharmacokinetic parameter. Visual predictive check (VPC) was used for assessment of the predictive performance. In order to collect published FX and NFX milk data for external validation, the MEDLINE database was searched, with the key words 'fluoxetine' and either 'breast feeding' or 'breast milk', from 1946 to 2011. Four articles were eligible for external validation for VPC [20–23]. All concentration values for VPC were normalized to a 20 mg maternal dose of FX for the purposes of comparison.

Bootstrap and VPC were performed with NONMEM® and PLT tools® (version 4.6.7.; "P Less Than", San Francisco, CA, USA)

Simulation We took a two stage approach for the simulation. First, based on the final population pharmacokinetic estimates and variances, fluoxetine milk concentrations at steady-state were simulated in a population of 1000 individuals. The women's weight was fixed arbitrarily at 70 kg, and the maternal dose was fixed at 20 mg every day, a standard adult dose. Second, using 'R' statistical language (V 2.14.1; R development Core Team 2011. R: A language and environment for statistical computing. R foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org), we randomly generated feeding-related parameters with normal distribution as indicated in the previously published data [24], and assigned them to each of the simulated infants. The assigned parameters obtained from the above mentioned study [24] included feeding numbers per day (mea 11; SD 3), amount of milk ingested at each feeding (mean 76 g per feeding; SD 12.6 g), feeding intervals (mean 138 min; SD 43 min), and infant age (mean 15.3 weeks; SD 5.9 weeks).

In order to derive the body weight-adjusted FX dose an infant is predicted to ingest, we needed to assign body weight to the simulated infants. The above-mentioned study [24] showed infant ages but no data on body weight was provided. We thought that estimation of a body weight solely from the age of the infant without accounting for simulated milk intake amount was likely to introduce unwanted wide variations into the weight-adjusted dose, because daily milk intake appears to be closely related to body weight of the infant [25]. Because daily milk intake per body weight shows relatively tight intra-age group variations [25], we took advantage of that parameter and calculated body weight of each of the simulated infants as follows. We averaged the milk consumption per body weight in infants of the seven age groups described in the study (4 weeks to 6 months of age) [25], which are similar to our simulated infant age range, and obtained a mean milk intake of 127.5 ml (123.7 g) kg^{-1} day^{-1} across the age range. The specific gravity of breast milk was assumed to be 1.031 [26]. Each infant's body weight was calculated by dividing the simulation-estimated daily milk intake (ml day^{-1}) with 127.5 ml kg^{-1} day^{-1}. The mean of the estimated body weight was 5.43 kg (SD 1.29 kg). For validation purposes, we confirmed that 2 SD of these estimated body weights were within 2 SD of the age-specific weight data collected in the World Health Organization (WHO) Multicentre Growth Reference Study (http://www.who.int/childgrowth/en/).

The results of FX milk concentration and infants' exposure were compared with our previously published model which consisted of maternal blood and milk compartments for FX alone [17]. All modelling and simulations were conducted in NONMEM 7.2, PLT tools, and 'R'.

Estimation of FX and NFX amount ingested by infants We converted NFX to FX-equivalent concentrations (ng ml^{-1}) using their respective molecular weight (FX 309.3 and NFX 295.3), so that the amount of NFX ingested by the infant can be compared with the maternal FX dose. Therefore, NFX is expressed as FX-equivalent unless otherwise stated.

The individual infant daily dose of FX or NFX was derived as follows:

$$\text{Infant daily dose} = \sum_{k=1}^{n} Ck \cdot mvk$$

where Ck (ng ml^{-1}) is the simulated FX or NFX concentration at a kth feeding time after administration of FX to the mother, n is the number of feeds per day, and mvk is the volume of the milk at a kth feeding (ml). n and mvk of each simulated infant were derived as described above based on the published data [24]. Because NFX is an active metabolite [27], we also calculated the sum of infant dose of FX and NFX. Because FX undergoes stereoselective metabolism, and because both enantiomers of NFX may be less active than FX enantiomers [27–29], we note that this total FX + NFX is likely to overestimate infant doses, providing conservative predictions.

The ratio between the infant daily dose per body weight and the weight-adjusted maternal therapeutic dose (i.e. 20 mg day^{-1} for a 70 kg woman is about 0.3 mg kg^{-1} day^{-1}) was defined as a simulated relative infant dose (sRID). A sRID was calculated for FX, NFX and

the sum of both. A sRID ≦ 0.1 (i.e., infant daily dose is equal, or less than 10% of the maternal therapeutic dose of FX on a kg body weight basis) served as a reference point of drug exposure for a breastfed infant [13, 14].

Results

Patients' characteristics

The training data set was the same as our previous article [17]. The original data [18, 19] from 24 women taking FX with a mean daily dose of 29.4 mg (range: 7.5–80 mg day^{-1}) and their 25 breastfeeding infants (one pair of twins) provided 112 breast milk FX and NFX concentration values that were used in the pop PK analyses. The mean parametric values (± SD) were maternal age, 31.8 ± 5.6 years (range 22.7–44 years), maternal body weight, 64.5 ± 13.5 kg (range 31–85 kg), infant age, 6.3 ± 6.8 months (range 0.13–25 months) and infant body weight, 5.3 ± 2.1 kg (range 2.8–10 kg).

Population PK analysis

First, one and two compartment models with absorption were tested for the prediction of breast milk concentration of FX. We selected a one compartment model with absorption over a two compartment model, which was not chosen because the inter-individual variations of parameters could not be estimated. The differential equations of the model are shown as follows:

$$\frac{dA(FX)}{dt} = K_a \cdot Dose - K_e \cdot A(FX)$$

where Dose is the amount of FX administered per dose per day, A(FX) is the amount of FX in the compartment, K_a is an absorption rate constant and K_e is an elimination rate constant.

Second, we attempted to describe FX and NFX milk concentrations simultaneously, by expanding the FX one compartment model (above) to a two compartment model (i.e. a FX compartment and an NFX compartment). However, variations of some parameter estimates could not be reduced to a reasonable level (data not shown). Given the fact that the observed milk concentration–time profiles of FX and NFX were similar (Supplementary Figure S1), an FX-to-NFX conversion coefficient (KFN) was used instead as a scaling factor:

$$NFX\ milk = KFN \times FX\ milk$$

where NFX milk is NFX concentration in milk, FX milk is FX concentration in milk, and KFN is the conversion coefficient. Adding absorption lag time did not improve the OFV and covariates were not found to improve the model fit.

Table 1

Final estimated parameters of the optimal model and bootstrap

	Population mean estimate	RSE(%)*	Bootstrap evaluation Median	95%CI
Fixed effects (exp(θ))				
K_a (h^{-1})	0.016	13.3	0.016	0.0027, 0.041
***V* (l)**	20.5	3.5	20.3	7.24, 72.0
CL (l h^{-1})	13.4	6.9	13.1	10.6, 16.7
KFN	1.01	20.2	0.99	0.79, 1.2
Random effects				
Interindividual variability (ω)				
K_a (CV%)	111.4	174.2	137.2	52.2, 268.2
***V* (CV%)**	22.8	133.9	84.9	32.4, 248.3
CL (CV%)	48.1	64.9	46.7	36.4, 58.3
KFN	48.1	46.8	47.5	30.8, 60.7
Residual variability (σ)				
FX(CV%)	28.1	58.2	26.9	20.5, 33.8
NFX(CV%)	29.8	56.1	29.1	19.4, 36.3

*RSE: relative standard error (percentage of standard error). KFN FX-to-NFX conversion coefficient.

The final population estimates and variability are presented in Table 1. Goodness-of-fit plots of FX and NFX milk concentrations (Figure 1) showed that when based on the model with the population mean parameter estimates, the concentration data are diversely scattered around the line of unity in the observation–prediction space (Figure 1A and B, left panel). Variations were reduced substantially when model prediction was performed using the individual data (Figure 1A and B, right panel).

Internal and external model validation

Bootstrap Bootstrap results (Table 1) showed that the parameter values conformed well with those of the population mean, except for the inter-individual variability of volume of distribution (coefficient of variation of 22.8% vs. the bootstrap median of 84.9%).

VPC VPC of FX (Figure 2A) and NFX (Figure 2B) in milk showed good model performance. The model prediction was also assessed against a data set from published studies (shown as closed circles in Figure 2), which were not used for model development. Overall, the internal and external validation indicates reasonable model performance to predict milk concentrations of FX and NFX.

Simulation Based upon the final pop PK estimates and variances, FX and NFX concentrations in milk were simulated at steady-state in 1000 women, and infants' exposure through breast milk was estimated. As described in the methods, the average daily milk ingestion of the infants was estimated to be 127.5 ml kg^{-1} day^{-1} [25], and each infant's body weight was calculated according to this weight-adjusted milk intake and the milk intake per day for each of the simulated infants. The details of infant expo-

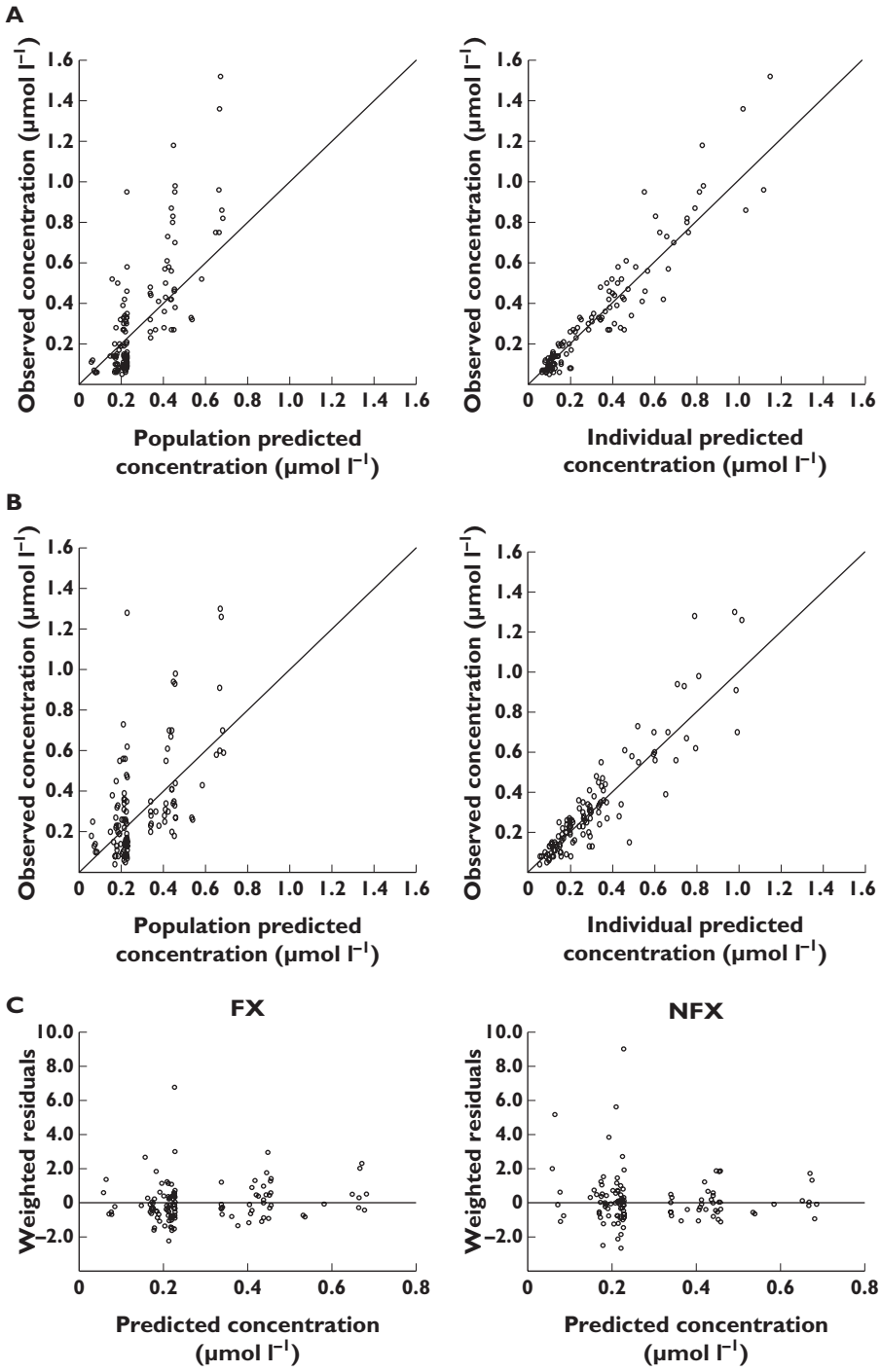

Figure 1

Goodness-of-fit plots. The observed milk FX (Figure 1A) and NFX (Figure 1B) concentrations are plotted against the mean population predicted values (left), and the individually predicted (Post Hoc) predictive values (right). Figure 1C shows weighted residuals of FX (left) and NFX (right)

sure level to FX and NFX are shown in Table 2, and graphically presented in Figure 3.

The simulation yielded a median infant FX exposure of 0.0080 mg kg^{-1} day^{-1} (95% CI 0.0077, 0.0083). The median sRID of FX was 0.028 (95% CI 0.027, 0.029), which was in close proximity with our previous report (median 0.031; 95% CI 0.030, 0.032) (Figure 3A). The 99th percentile of FX sRID was 0.083, which means most of the infants were exposed to less than 8.3 % of maternal dose adjusted by body weight.

The median infant NFX exposure level was 0.0083 mg kg^{-1} day^{-1} (95% CI 0.0079, 0.0088). The 99th

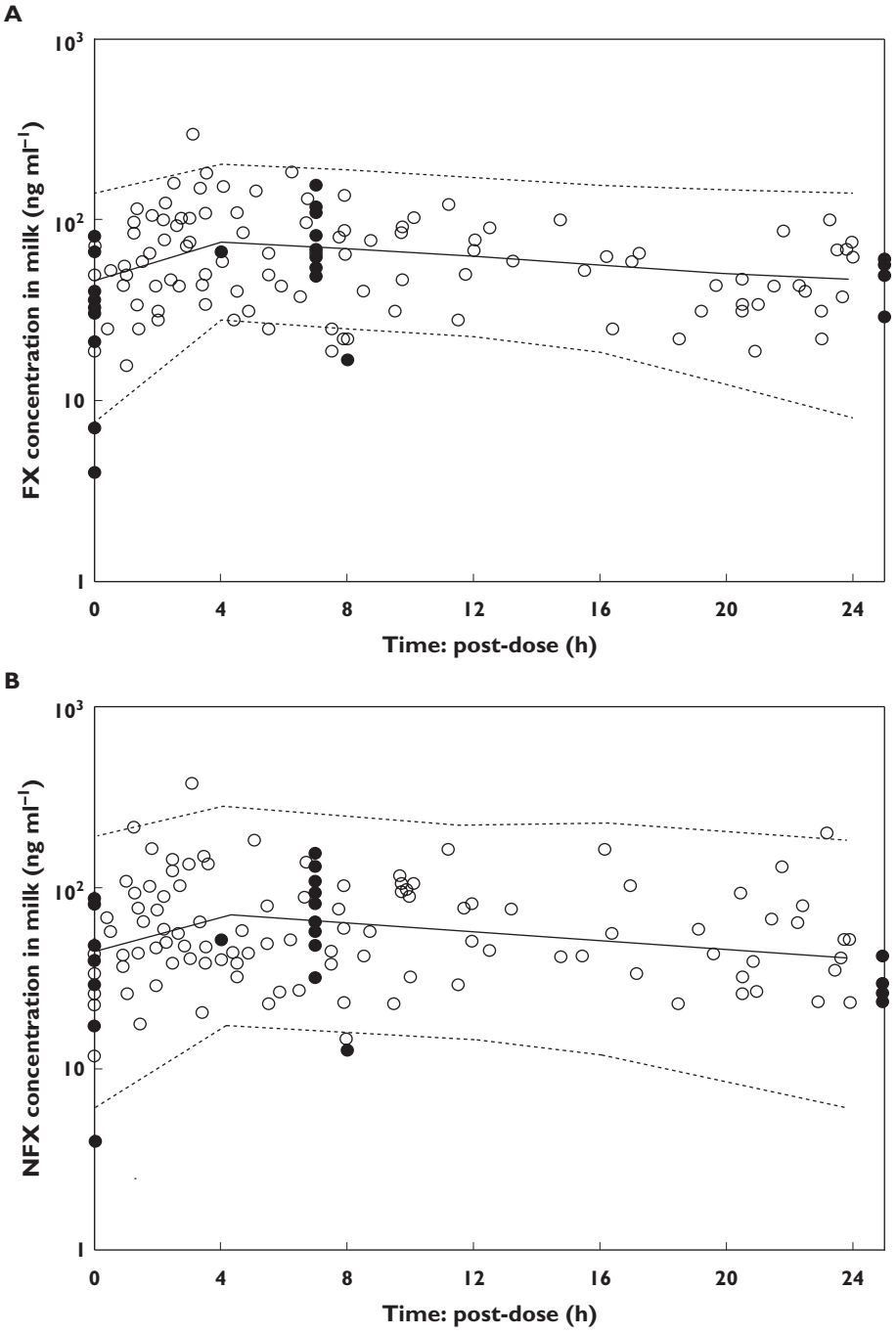

Figure 2

Visual predictive check (VPC) in FX (panel A) and NFX (panel B). Observed FX or NFX concentrations are shown (white circles) with the median (solid line), 2.5th percentile (lower broken line), and 97.5th percentile predictions from the model. NF or NFX concentrations from the published article are also shown (black circles). Data points in those articles without specified post-dose sampling time are shown outside the 24 h time frame. Concentrations described as 'peak' without specific post-dose time [22] are plotted at 7 h post-dose because a reported average post-dose time of a peak concentration of FX was 6 to 8 h [38]. (A) —, median (model prediction); - - -, 2.5th–97.5th percentile (model prediction); ○, observed FX data (present study); ●, observed FX data from other studies (external validation). (B) —, median (model prediction); - - -, 2.5th–97.5th percentile (model prediction); ○, observed NFX data (present study); ●, observed NFX data from other studies (external validation)

Table 2
Simulated infant exposure of FX, NFX and the total of both

	FX	NFX (equivalent to FX)	Sum (FX + NFX)
Simulated infant dose through breastfeeding (mg day^{-1} kg^{-1})			
Range	0.0019 to 0.035	0.0009 to 0.084	0.0034 to 0.11
Median	0.0080	0.0083	0.017
95% CI of median	0.0077, 0.0083	0.0079, 0.0088	0.016, 0.018
Simulated relative infant dose (fraction of the maternal weight-adjusted dose)			
Range	0.0068 to 0.12	0.0032 to 0.29	0.012 to 0.37
99th percentile	0.083	0.15	0.23
Median	0.028	0.029	0.059
95% CI of median	0.027, 0.029	0.028, 0.031	0.057, 0.062

percentile of sRID was 0.15. The median of combined exposure to both FX and NFX was 0.017 mg kg^{-1} day^{-1} as FX equivalents. The 99th percentile of sRID of FX + NFX was 0.23 or 23% of the maternal weight-adjusted FX dose.

Discussion

The first step of a risk assessment of adverse drug effects in an infant breastfed by a woman on a medication is to obtain an estimate of the amount of the drug ingested by the infant. This estimate could be derived from published data on drug concentrations in milk, multiplied by an assumed infant milk intake. Although published data are valuable, they are often based on case reports, and the derived point estimate of the infant dose through milk may not accurately reflect actual infant exposure. For example, the highest reported concentration of the drug in milk is often used to estimate the infant dose per day. This intentional overestimate is to provide a 'worst case' scenario (i.e. the highest exposure level) by assuming that the drug concentration in milk remains at the reported highest level throughout the feeding cycle. However, this approach generates a dose estimate which significantly deviates from a population distribution curve of infant drug exposure levels, potentially jeopardizing risk assessment and clinical decision making.

Lack of large scale studies in this population poses another challenge. Even if combined, small sample sizes of published studies make it difficult to estimate variations of the drug exposure levels in a population of breastfeeding women and their infants. As a consequence, the likelihood of infant exposures to a certain level of drug in milk is difficult to predict. A large scale pharmacokinetic study with intense sampling provides required information, but such a study is difficult to conduct in this population. In this context, a pop PK approach offers an attractive solution because a sparse sampling design can be applied, and a derived pop PK model can be used for simulation analyses of a population of breastfed infants.

In this proof-of-concept study using FX and NFX concentration data in milk without referring to plasma concentrations, we have shown that a relatively simple non-mechanistic pop PK model may address the parent (e.g. FX) and its active metabolite (e.g. NFX) disposition in milk. If the purpose of modelling analyses is to gain insight into mechanisms of mammary drug disposition, a mechanistic model provides a powerful approach. However, biologically (or physiologically) based models tend to be complex (i.e. an increasing number of parameters) and demand datasets which represent multiple compartments (e.g. blood and milk). On the other hand, if prediction of drug concentration–time profiles in milk (i.e. infant exposure levels) is the main goal of the modelling analyses, then a simple, non-mechanistic model based on milk concentrations can be used without referring to plasma concentrations. Our present model showed that a non-mechanistic simple model using only milk data (without maternal blood concentration) could sufficiently estimate FX excretion into breast milk, and the results were consistent with our previous model with both milk and blood data [17]. There have been at least two articles published where excretion into human breast milk of both parent drugs and their metabolites were estimated by pop PK modelling [30, 31], although in these two studies the mechanistic models were developed using both maternal plasma and milk concentration data. Whether our approach of using milk concentration profiles to obtain a reasonable prediction model can be applied to other drugs with active metabolites requires further investigation.

FX is reported to demonstrate non-linear pharmacokinetic profiles in higher dosage [32] which may have implications in our dose-standardized VPC (Figure 2). However, the mean FX dose in the dataset (29.4 mg day^{-1}) is relatively low. Therefore, it is unlikely that FX shows non-linear pharmacokinetics in this population, justifying our dose-standardized VPC.

Both FX and NFX concentrations in milk are approximately 1.5 to 2 times higher in post-feed than in pre-feed samples [18]. In our dataset, milk concentrations used for

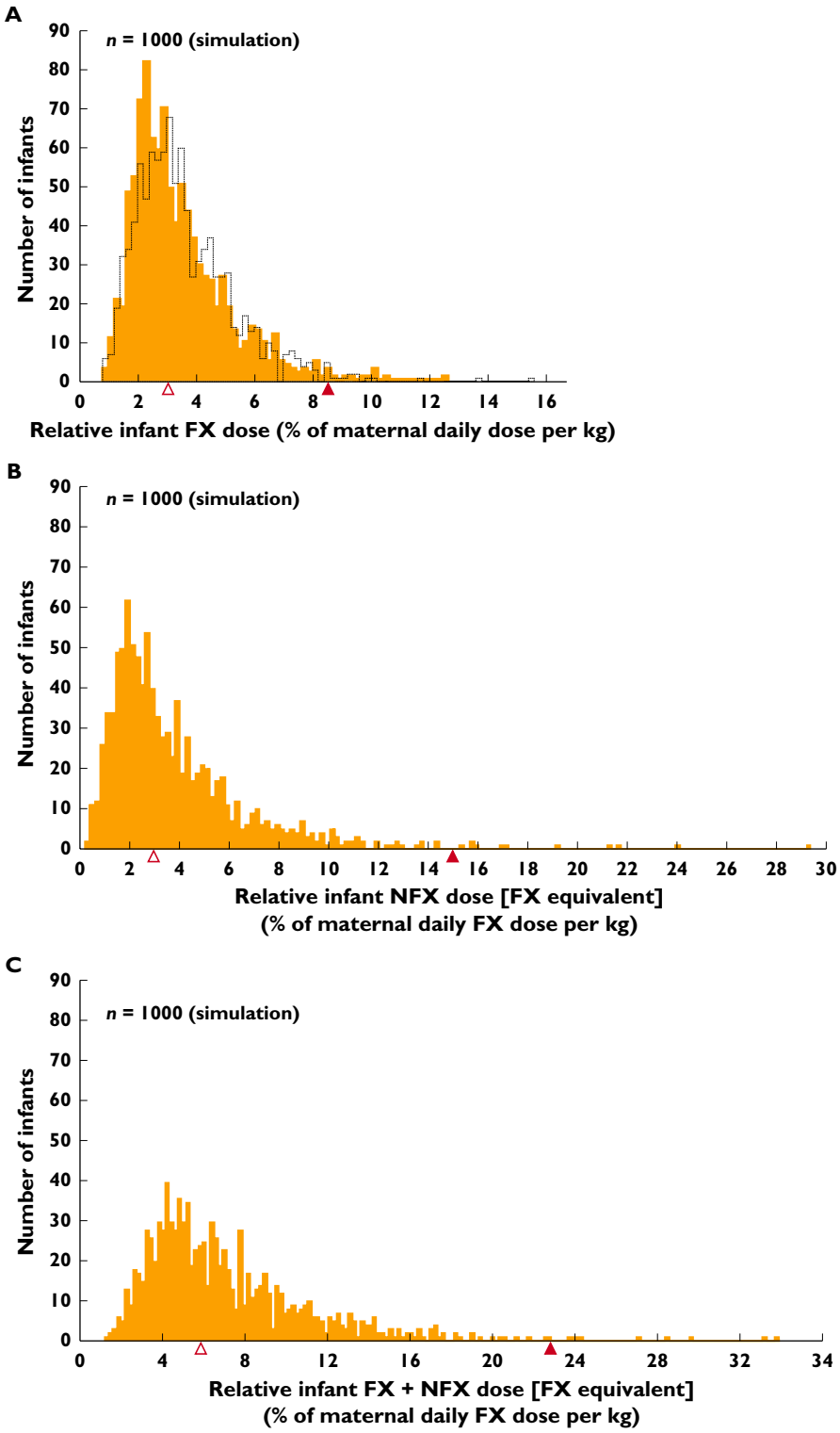

Figure 3

A histogram of simulated relative infant dose (sRID) of FX, NFX, and FX + NFX. One thousand infants were simulated using the final model with randomly assigned feeding parameters according to characteristics of milk intake of infants aged between 4 weeks and 6 months [24, 25]. The simulation results are shown as the probability distribution of infant exposure levels in the form of a histogram of sRID of FX (panel A), NFX (panel B), and FX+NFX (panel C). sRID is a dose the infant would ingest per day, which is expressed as % of the standard dose kg^{-1} of the mother (see *Methods*). Median (open triangles) and 99th percentile values (closed triangles) of sRID were also shown. NFX results were converted to FX equivalent on a molar basis. In Figure 3A, FX simulation results are shown with a histogram of the previous study [17], which was based on a model of blood and milk FX concentrations. (A) ▬, present study; △, median (2.8%); ▲, 99th percentile (8.3%); ▭, Panchaud et al. [17]. (B) △, median (2.9%); ▲, 99th percentile (15.0%). (C) △, median (5.9%); ▲, 99th percentile (23.0%)

the modelling analyses included mainly averages of pre- and post-feed milk levels, aliquot concentrations, and those determined in pre- or post-feed samples [18, 19]. Therefore, the milk concentration data are unlikely to be biased in one particular direction (i.e., pre- or post-feeding samples). Nevertheless, standardization of sampling timing is important to design a study of drug excretion into milk. FX is metabolized to NFX mainly by CYP2D6 [33]. Both FX and NFX are known to have antidepressant activity [27]. FX is a racemic mixture (1:1) of R-fluoxetine and S-fluoxetine enantiomers [34], and is metabolized to R-norfluoxetine and S-norfluoxetine. Both in animal and human studies, S-norfluoxetine, R-fluoxetine and S-fluoxetine act equally [27–29]. On the other hand, R-norfluoxetine is significantly less potent than these three enantiomers [27–29]. In this simulation, we conservatively assumed that the activity of NFX was higher than that reported in animal studies (i.e. we assumed that the activity ratio of NFX to FX is 1:1). Based on this assumption, we calculated a sum of FX and NFX in milk, which showed that the 99th percentile of sRID (FX + NFX) was relatively high (23% of the maternal weight-adjusted dose), although the median was 5.9%. Our model predicts that infant doses of NFX (Figure 3B) are similar to those of FX (Figure 3A), causing combined doses of FX and NFX (Figure 3C) to be approximately two-fold higher than each of the FX and NFX doses. In theory, intake of this dose range of FX and NFX for a prolonged period of time may result in steady-state plasma concentrations at near therapeutic concentrations in individuals with significantly reduced clearance. Whether this happens in infants is not clear, as ontogeny of FX and NFX clearance has not been fully revealed.

There are several limitations and challenges in this study: First, our sample size was relatively small. A pop PK approach requires large numbers of subject to develop a valid model and address interindividual variations. Because we used data from previous studies, only 24 mothers with 25 infants were available. This is a relatively small sample size, potentially increasing uncertainty of parameter estimates. Secondly, the model did not take into account maternal pharmacogenomic aspects of FX metabolism. PK modelling of drugs metabolized by CYP2D6, which is characterized by large inter-individual differences in function due to genetic polymorphism, requires information on genetic variants, which was not available in our dataset. The maternal CYP2D6 genotype information may improve the model performance. On the other hand, CYP2D6 genotypes are less likely to play a major role in neonates and infants, because their CYP2D6 function is poorly developed [35]. Third, relative paucity of data on CYP2D6 development [36, 37] poses a challenge when estimated infant drug intake (Figure 3) is interpreted. In this study, we provide estimated distributions of the infant doses, which have different implications depending on infant drug metabolizing capacities. Because the activity of CYP2D6 may be as low as 20% of the adult level in 8 to 30-day-old infants [35–37], clearance of FX in infants may be low as well. Similarly, NFX clearance in neonates and infants, which is mainly through glucuronidation, may be lower than adults due to its developmental process. However, data on FX and NFX clearance in infants are lacking.

Despite these limitations and challenges, our approach opens a door to pop PK analyses to predict concentrations of other drugs with active metabolites in human milk. To validate this approach, prospective studies of model development and validation will be needed.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare no support from any organization for the submitted work with a conflict of interest. AT has received grants from Pfizer, Natus, and Ferndale outside the submitted work in the previous 3 years. The other authors did not have any relationship to disclose in these 3 years besides the submitted work. During the period of this project RT received personal funding support from Tokyo Children's Cancer Study Group, Japan and from Joseph M. West Family Memorial Fund from the Post Graduate Medical Education at University of Toronto, Toronto, Canada. No other relationships or activities that could appear to have influenced the submitted work are reported.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1
The observed concentrations of FX and NFX in each patient. FX and NFX concentration–time profiles in milk were largely similar