Pharm Res (2013) 30:2410–2422
DOI 10.1007/s11095-013-1085-5

RESEARCH PAPER

Contribution of Protein Binding, Lipid Partitioning, and Asymmetrical Transport to Drug Transfer into Milk in Mouse *Versus* Human

Naoki Ito • Kousei Ito • Hiroki Koshimichi • Akihiro Hisaka • Masashi Honma • Takashi Igarashi • Hiroshi Suzuki

Received: 14 January 2013 / Accepted: 19 May 2013 / Published online: 31 May 2013

ABSTRACT

Purpose Drug transfer into milk is a general concern during lactation. Because data are limited in human subjects, particularly for new drugs, experimental animal models of lactational drug transfer are critical. This study analyzed drug transfer into milk in a mouse model, as well as the contribution of similar and dissimilar host factors.

Methods Milk/plasma drug concentration ratios (M/P) in humans were obtained from the literature, while those in mice were determined experimentally after intraperitoneal implantation of osmotic pumps containing drugs of interest. Unbound drug fractions in plasma and milk were determined *in vitro* for both species.

Results M/P values were determined for 27 drugs in mice and compared with those in human. These values were increased in mice for 21 drugs; the geometric mean ratio of M/P between mice and humans was 2.03 (95% CI, 1.42–2.89) for all 27 drugs. These results were reasonably explained by the relatively high protein and lipid content in mouse milk. Moreover, species-specific asymmetrical transport systems were suggested for 9 drugs.

Conclusions In addition to species-specific differences in milk protein and lipid content, variances in asymmetrical drug transport across the mammary epithelium may yield discordant M/P values in humans and mice.

KEY WORDS breastfeeding · lactation · mammary epithelium · species-specific differences · transporter

ABBREVIATIONS

ABC	ATP-binding cassette
AUC	area under the drug concentration-time curve
BCRP	breast cancer resistance protein/ABC transporter G2
BEH	bridged ethyl hybrid
C_m	drug concentration in milk
$C_{m,lipid}$	drug concentration in lipid fraction of milk
$C_{m,skim}$	drug concentration in skim milk
$C_{m,unbound}$	unbound drug concentration in skim milk
C_p	drug concentration in plasma
$C_{p,unbound}$	unbound drug concentration in plasma
DMSO	dimethyl sulfoxide
f_m	unbound drug fraction in skim milk
$f_{m,total}$	fraction of drug free from binding to milk protein and lipid
f_p	unbound drug fraction in plasma
K_f	milk lipid-to-water partition coefficient
LC-MS/MS	liquid chromatography-tandem mass spectroscopy

Electronic supplementary material The online version of this article (doi:10.1007/s11095-013-1085-5) contains supplementary material, which is available to authorized users.

N. Ito
Department of Pediatrics, The University of Tokyo Hospital
Faculty of Medicine, The University of Tokyo
7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan

K. Ito (✉) · H. Koshimichi · M. Honma · H. Suzuki
Department of Pharmacy, The University of Tokyo Hospital
Faculty of Medicine, The University of Tokyo
7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
e-mail: kousei-tky@umin.ac.jp

A. Hisaka
Pharmacology & Pharmacokinetics, The University of Tokyo Hospital
Faculty of Medicine, The University of Tokyo
7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan

T. Igarashi
National Center for Child Health & Development
2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan

M/P	ratio of drug concentration in milk to that in plasma
$M/P_{unbound}$	ratio of unbound drug concentration in milk to that in plasma
$M/P_{unbound,pred}$	$M/P_{unbound}$ ratio predicted based on pH partition theory
OCT	organic cation transporter
SDS	sodium dodecyl sulfate
SLC	solute carrier
UPLC	ultra-performance liquid chromatography

INTRODUCTION

Breastfeeding is the optimal method of feeding for the healthy growth and development of infants, given the high nutritional value and absorption and digestion rate of breast milk. The impact of breastfeeding has been well established in recent years (1). Breastfeeding protects against several infectious diseases, such as respiratory tract illnesses, diarrhea, otitis media in infancy, and bacterial translocations in the innate sterile intestinal tissues of newborns. Several morbidities associated with allergic diseases are also decreased in breastfed infants, because the newborns acquire both humoral and cell-mediated immunity *via* breast milk. For example, breast milk contains elevated levels of transforming growth factor-β, an essential anti-allergy cytokine, during early lactation. Furthermore, accelerated neurocognitive development is associated with infants who are provided with breast milk instead of artificial milk (2), which is possibly related to the long-chain polyunsaturated fatty acid content in breast milk (3). Finally, trustful and reciprocal mother-infant bonds are created *via* breastfeeding and/or skin-to-skin contact, particularly among very preterm infants in intensive care units (4–6). Indeed, breastfeeding of infants under 2 years of age has the greatest potential impact on child survival of all preventive interventions, with the potential to prevent 1.3 million deaths in children under 5 years of age in the developing world (7).

A matter of importance for infant health is that most maternally ingested drugs or chemical compounds transfer, at least to some extent, into breast milk. However, information on drug transfer into and retention by breast milk is only available for a limited number of medications in humans, because such information is not usually requested when a new drug is approved for use by lactating mothers. Given the vagaries of drug transfer, human data cannot be anticipated, although information stemming from pertinent animal models is sometimes accessible. On the other hand, it is not well established whether animal data qualitatively and quantitatively reflect drug distribution in human milk. This lack of knowledge has caused mothers who require medication to give up nursing on their own accord, or based on the advice of medical practitioners. Consequently, their infants do not receive the established benefits of breast milk, necessitating methods to better predict drug transfer into human milk.

Several models have been proposed to address this issue (8). For example, the pH-partition theory was applied to predict drug distribution between plasma and milk in early animal studies (9). Atkinson and Begg extended this theory to advance the "phase distribution model", in which drug distribution into milk was evaluated by a combination of the pH-partition theory, protein binding, and distribution into milk lipids (10,11). The phase distribution model is based on the hypothesis that a rapid equilibrium of drug concentrations exist between plasma and milk; however, a major limitation of this model is that the drug concentration-time courses in plasma and milk do not usually increase or decrease in parallel (12,13).

We recently conducted an exhaustive search of the literature regarding human clinical data of drug concentration-time profiles in plasma and milk. By fitting these data to one or two compartment model, reliable secretion and reuptake clearance data across the mammary epithelium were obtained for 49 out of a total of 64 drugs analyzed (14). Based on these data, we proposed equations predicting secretion and reuptake clearance from physicochemical parameters. These equations proved useful not only for predicting the milk to plasma area under the drug concentration-time curve (AUC) ratio, but also the drug concentration-time profile in human milk (14). Nonetheless, our prediction methods were not always reliable, because asymmetrical transport processes were not considered (8,15–17). This is a major concern, given that some drugs are conveyed by transporters or other transfer mechanisms including exocytotic transport and vesicular transcytosis across the mammary epithelium (18). If the contribution of these transfer sysytems was taken into account, a more general and dependable prediction method could be constructed.

Breast cancer resistance protein/ATP-binding cassette (ABC) transporter 2 (BCRP) is the only example to date of a transporter whose contribution to drug transfer into milk is clearly demonstrated in humans as well as experimental animals (19,20). Although transporters other than BCRP are expressed at the mRNA level in mammary epithelial cells during lactation, it is not clear whether these transporters are actually involved in drug transport across the mammary epithelium to affect the drug content in milk (21,22). To address such a fundamental issue, information regarding the unbound drug concentration ratio between milk and plasma is critical. If the ratio is higher or lower than that explained by the pH-partition theory, asymmetrical conveyance is likely involved. However, to the best of our knowledge, a comprehensive analysis has not yet been conducted of milk/plasma drug ratios in humans or in experimental animals.

The objective of the present research was thus to systematically compare drug distribution in breast milk for a large number of compounds in humans and mice, and to assess the contribution of similar *versus* dissimilar host factors to this process. The investigated factors include protein binding in plasma and skim milk, partitioning of drugs into the milk lipid fraction, and asymmetrical transport across the mammary epithelium. Thirty-two drugs were selected as model compounds whose transfer into human milk was analyzed in our previous report (14). The mouse was chosen as an experimental animal model, because it is often employed in the preclinical drug development process.

MATERIALS AND METHODS

Drugs

Acetaminophen, cephalothin sodium salt, clindamycin hydrochloride, disopyramide phosphate salt, labetalol hydrochloride, nitrofurantoin, (±)-propranolol hydrochloride, terbutaline hemisulfate salt and (±)-verapamil hydrochloride were obtained from Sigma-Aldrich (St. Louis, MO). Acyclovir, alprazolam, atenolol, anhydrous caffeine, cefotaxime sodium salt, cephapirin sodium salt, diltiazem hydrochloride, metronidazole, nitrazepam, prednisolone, 6-propyl-2-thiouracil (propylthiouracil) and trazodone hydrochloride were from Wako (Osaka, Japan). Chloramphenicol, cimetidine and theophylline were from Nacalai Tesque (Kyoto, Japan). Fluconazole, metoprolol, mirtazapine, praziquantel and quetiapine fumarate were from LKT Laboratories, Inc. (St. Paul, MO). Triprolidine hydrochloride was from MP Biomedicals, LLC (Solon, OH). Metformin was from Alexis Biochemicals (San Diego, CA). Moclobemide was from Toronto Research Chemicals (Brisbane, Canada). All other reagents were of analytical grade.

Sample Collection and Animal Handling

Primiparous pregnant female mice (ddY strain), mating age = 9 weeks and gestational age = 16 days, were purchased from Japan SLC (Hamamatsu, Japan). Following parturition and during lactation, mothers were individually paired-housed with their pups in a room maintained at 25°C and 50% relative humidity. The animals had *ad libitum* access to a normal chow diet (CMF diet, Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water. The litter size was standardized to eight pups (four males and four females) at 4 days postpartum. The research protocol adhered to the "Principles of Laboratory Animal Care" (National Institutes of Health (NIH) publication #85-23, revised in 1985) and was approved by the Animal Studies Committee of the University of Tokyo.

Implantation of Micro-Osmotic Pumps

Cassette dosing was used for drug administration to mice ($n=6$ for each group). The 32 drugs were divided into five groups (group A, 7 drugs in one pump; group B, 6 drugs in one pump; group C, 10 drugs in two pumps; group D, 5 drugs in three pumps; and group E, 4 drugs in one pump), taking into consideration possible competition between the drugs for the known transport systems in mammary epithelia, compatible drug solvents in each osmotic pump, the detergent to be employed in the pretreated ultrafiltration devices, and the gradient program for liquid chromatography (Supplementary Material Table 1). For example, acyclovir, cimetidine and nitrofurantoin are transported by BCRP in the mammary epithelium (19,20) and were thus divided into different groups. Regarding the solvents, 50% dimethyl sulfoxide (DMSO) or distilled water was used according to the manufacturer's instructions for each drug. The drug dose (mg/kg/day) was customized according to the clinical dose and the detection limit for each drug (Supplementary Material Table 1).

Micro-osmotic pumps (Alzet® model 1003D, Durect Corporation, Cupertino, CA) containing each drug combination were implanted intraperitoneally into lactating mice at the postnatal age of 14 days. The pumps had a reservoir volume of 97 ± 7 μL and a constant flow rate of 0.95 ± 0.02 μL/h over a period of 72 h. Each mouse received ether anesthesia *via* inhalation and was intraperitoneally implanted with one to three pumps containing the appropriate combination of drugs (Supplementary Material Table 1). After the operation, mice were returned to their home cages with their pups and fed *ad libitum*. All mice survived the surgical procedure and appeared to be lactating normally, given that all pups thrived throughout the course of the experiment, with a constant increase in body weight.

Collection of Milk

Milk was collected from nursing mice at 60 h after implantation of the pumps. The mother mice were separated from their pups at 8 h before the indicated milking time and injected with 10 units of oxytocin (Sigma-Aldrich). A milking apparatus for laboratory animals (Automated Milker WAT-2008, Little Leonardo Corporation, Tokyo, Japan) was used in order to gather the milk samples. The settings were as follows: −140 mmHg for the pressure, 60 times/min for the pulsation frequency, and 60% for the suction. Inhaled ether anesthesia was used to sedate the mice. Suction was applied to one nipple of a supine mouse by using the teat cup of the milker, with manual massaging. After a small amount of milk was obtained, suction was subsequently applied to another nipple in a similar fashion. Milking was finished after all 10 nipples of the nursing mother were treated in this manner, resulting in the collection of around 200 μL of milk per mouse.

The collected milk was placed into a siliconized eppendorf tube and frozen at −80°C until analysis. Next, blood was drawn from the jugular vein, collected into a heparinized and siliconized tube (Watson, Tokyo, Japan), and centrifuged for 3 min at 13,000 rpm. The supernatant representing the plasma sample was frozen at −80°C until analysis.

Protein concentrations in total milk and skim milk (prepared as described below) were measured by the BCA method (Pierce BCA Protein Assay Kit, Thermo Fisher Scientific, Rockford, IL). A creamatocrit measurement was performed to evaluate the lipid contents of the milk. Briefly, the milk was centrifuged in a hematocrit capillary tube, and the length of the cream layer was expressed as a percentage of the length of the milk column (23).

Measurement of Drug Concentrations in Milk and Plasma

Concentrations of the drugs in milk (C_m) and plasma (C_p) were measured as follows. An aliquot (50 μL) of thawed milk or plasma sample was mixed with acetonitrile (500 μL) containing 100 nM carbamazepine as an internal standard, vortexed, and deproteinized by centrifugation for 10 min at 3,500 rpm for the milk and at 15,000 rpm for the plasma. An aliquot (450 μL) of each supernatant was condensed and dried by using a centrifugal concentrator (SpeedVac, Thermo Fisher Scientific). Desiccated milk samples were dissolved in 40% acetonitrile (200 μL) and n-hexane (100 μL) and vortexed in order to remove the lipids. An aliquot (120 μL) of the acetonitrile layer was applied to the sampling plates. Plasma samples were dissolved in 40% acetonitrile (200 μL), and an aliquot of the acetonitrile layer (120 μL) was applied to the plates. Carbamazepine was used as the internal standard.

Liquid chromatography-tandem mass spectroscopy (LC-MS/MS) was performed by using an ultra-performance liquid chromatography (UPLC) system and a Quattro Premier XE mass spectrometer (Waters, Milford, MA) with a 1.7 μm particle Acquity UPLC™ BEH (bridged ethyl hybrid) C_{18} analytical column (2.1 mm × 100 mm, Waters). Samples were kept at 4°C in the sample injector, and aliquots of 7.5 μL were injected. The oven temperature was 40°C, and the flow rate was 0.3 mL/min. Acetonitrile or methanol was selected as the organic solvent in the mobile phase. The mobile phase consisted of formic acid-acetonitrile (0.1:99.9, v/v) or formic acid-methanol-20 mM ammonium acetate (0.1:97.9:2.0, v/v/v) (see Supplementary Material Table 1 for a summary of detailed LC-MS/MS analytical conditions).

Determination of the Unbound Fraction and Partition Ratio *In Vitro*

Because the amount of milk and plasma obtained from each mouse was limited, it was not practical to measure unbound drug concentrations *in vivo* in milk and plasma. Alternatively, *in vitro* experiments were conducted to independently determine the unbound fraction in milk and plasma, as described below.

Blank mouse milk samples were collected from lactating ddY mice (11–13 weeks old) at several time points. These mice were treated in the same manner as the experimental mice, without the implantation of the pumps. In addition, blank human milk samples were obtained with written consent from healthy nursing mothers whose children were admitted to the University of Tokyo Hospital. The mothers did not take any medications for at least 72 h prior to milk sampling. The research protocol followed the tenets of the Declaration of Helsinki promulgated in 1964 and was approved by Research Ethics Committee at the Faculty of Medicine and the Graduate School of Medicine of the University of Tokyo.

Pooled mouse and pooled human milk samples were centrifuged (2,000 × g, 15 min, room temperature) and separated into skim and lipid portions. The specimens were then reconstituted by mixing the skim and lipid portions to ensure lipid contents of 20% for the mouse milk and 5% for the human milk, respectively. The protein concentrations in the milk were 92.0 mg/mL and 13.9 mg/mL for mouse and human samples, respectively. The pH of the reconstituted milk was 7.2. An aliquot of reconstituted milk (1.35 mL) was combined with the appropriate drug mixture (150 μL; the same drug combinations that were employed above for the *in vivo* experiments were also employed for the *in vitro* experiments; Supplementary Material Table 1) to yield a milk sample (1.5 mL) containing final drug concentrations of 1 μM and lipid concentrations of 18% and 4.5% for the mouse and human samples, respectively.

Samples were then transferred to siliconized 1.5 mL tubes (Watson, Tokyo, Japan), gently turned upside down and right side up 10 times, and incubated for 5 min at 37°C to achieve equilibration of protein binding and distribution into milk lipid. After collecting an aliquot (50 μL) for the measurement of drug concentration (C_m), the remaining 1.45 mL was centrifuged at 2,000 × g for 15 min. Next, the sample was snap frozen in liquid nitrogen, and skim milk was obtained by removing the upper lipid layer. An aliquot was thawed and used for the measurement of drug concentration in the skim milk ($C_{m,skim}$), and the remaining sample was centrifuged for 5 min at 15,000 rpm to remove aggregates. An aliquot of the supernatant (300 μL) was then transferred to a Nanosep 10 K Omega ultrafiltration device (molecular weight cutoff> 10,000, Pall Corporation, Washington, NY) pretreated with a surfactant (5% Tween-20/5% sodium dodecyl sulfate (SDS) or 5% Triton X-100) to minimize nonspecific drug adsorption (see Supplementary Material Table 1) and centrifuged (5,000 × g, 5 min, room temperature) to separate out proteins.

Nonspecific adsorption to the device was corrected by employing the recovery ratio of each drug dissolved in dually ultrafiltrated blank milk. The ultrafiltrated sample (50 μL) was mixed with 2% formic acid (50 μL) and subjected to LC-

MS/MS analysis for the determination of $C_{m,\ unbound}$ (unbound drug concentration in skim milk). C_m and $C_{m,skim}$ were measured according to the same method as described above.

The unbound fraction in skim milk, f_m, was calculated by dividing $C_{m,unbound}$ by $C_{m,skim}$ (24). The fraction of drug free from binding to milk protein and lipid, $f_{m,total}$, was calculated by dividing $C_{m,unbound}$ by C_m. The drug concentration in the lipid fraction of milk, $C_{m,lipid}$, was calculated by Eq. 1.

$$C_{m,lipid} = \frac{C_{m,total} - C_{m,skim} \times (1 - Creamatocrit \times 10^{-2})}{Creamatocrit \times 10^{-2}} \quad (1)$$

The milk lipid-to-water partition coefficient, K_f, was calculated by dividing $C_{m,lipid}$ by $C_{m,unbound}$ (24).

Next, blank mouse plasma samples were collected from non-nursing ddY female mice (11–13 weeks old of the age). Pooled mouse plasma samples (450 μL) were spiked with the appropriate drug mixture (50 μL; again, the same drug combinations that were employed for the *in vivo* experiments were also employed for the *in vitro* experiments; Supplementary Material Table 1) to yield a final concentration of 500 nM for each drug. Samples were gently turned upside down and right side up 10 times and incubated for 5 min at 37°C to achieve equilibration. An aliquot (50 μL) was reserved to measure the drug concentration in plasma (C_p), and 300 μL of the remaining sample was transferred to pretreated Nanosep 10 K Omega ultrafiltration devices (Pall Corporation) and processed in the same way as described for $C_{m,unbound}$ to measure $C_{p,unbound}$ (unbound drug concentration in the plasma).

The unbound fraction in plasma, f_p, was calculated by dividing $C_{p,unbound}$ by C_p for the mouse. Human f_p values were obtained from the DrugBank database (http://www.drugbank.ac/) or the package insert for each drug. The ratio of the drug concentration in milk to that in plasma, M/P, was calculated by dividing C_m by C_p for the mouse. The human M/P(AUC) values described in our previous report (14) were employed as M/P values in the current investigation.

The ratio of unbound drug concentration in milk to that in plasma, $M/P_{unbound}$, was initially calculated by Eq. 2. On the other hand, $M/P_{unbound}$ is only anticipated to reach equilibrium for uncharged drugs, with equalized drug concentrations in the milk and the plasma. Accordingly, the ratio was predicted ($M/P_{unbound,pred}$) with Eqs. 3 and 4 for acidic and basic drugs, respectively, based on the pH-partition theory. Drug pKa values were obtained from the DrugBank database (http://www.drugbank.ac/).

$$M/P_{unbound} = M/P \times \frac{f_{m,total}}{f_p} \quad (2)$$

$$M/P_{unbound,pred} = \frac{1 + 10^{(milkpH - pKa)}}{1 + 10^{(7.4 - pKa)}} \quad (3)$$

$$M/P_{unbound,pred} = \frac{1 + 10^{(pKa - milkpH)}}{1 + 10^{(pKa - 7.4)}} \quad (4)$$

RESULTS

Measurement of Drug Concentration Ratio in Mouse Plasma and Milk

The production rate and composition of milk changes extensively during the first several days after parturition in mice, but remain relatively stable at later time periods (*i.e.*, 2 weeks after parturition) (25). Considering these early changes, we designed the osmotic pump implantation experiments so as to administer drugs to the animals at 14 days after partition. Next, milk was collected at 3 days after pump implantation to maximize the volume of milk obtained and to minimize inter-individual variability of its constituents. Sixty hours after implantation of the osmotic pumps, the production of milk was well preserved. Neither milk protein content nor creamatocrit value were not significantly affected by capsule implantation (Supplementary Material Fig. 1). These parameters were also not significantly different among mice groups, implanted with different number of capsules containing different kinds of drugs (Supplementary Material Fig. 1).

Drug concentrations in both plasma and milk were successfully determined for 27 out of 32 drugs in mice (Table I and Fig. 1). Notably, M/P values were higher in mouse than in human for 21 of these 27 drugs. The geometric mean ratio of M/P between mouse and human was 2.03 (95% confidence interval (CI), 1.42–2.89). M/P values were more than 3-fold higher in mouse relative to human for diltiazem (3.3-fold), metformin (7.7-fold), praziquantel (5.9-fold), propylthiouracil (45.6-fold), quetiapine (3.1-fold), and terbutaline (8.7-fold). In mice, M/P values fluctuated from the lowest value of 0.20 for cefotaxime and trazodone, to the highest value of 13.3 for terbutaline (Fig. 1). On the other hand, the lowest human M/P value was 0.08 for cefotaxime, and the highest was 4.18 for cimetidine (Table I). Drug concentrations in plasma were under the detection limit for cephapirin (< 8 nM), mirtazapine (< 0.8 nM), and nitrofurantoin (< 20 nM), while concentrations in milk were under the detection limit for cephalothin (< 8 nM), cephapirin (< 8 nM), and prednisolone (< 4 nM). Known BCRP substrates (acyclovir and cimetidine) were highlighted with closed circles in Fig.1. Higher M/P values of acyclovir (4.2) and cimetidine (10.3) were similar to those reported previously in mice (19). It indicated that our evaluation method can detect such asymmetrical transport system.

Table I M/P and $M/P_{unbound}$ Values in Mouse and Human

	Observed concentration in mouse (nM)					M/P					$M/P_{unbound}$				Major ionizing functional group		Charge at pH6.8–7.2	$M/P_{unbound,pred}$
Drug	C_p	±SD	C_m	±SD	n	mouse	±SD	human[a]	±SD	Ratio	mouse	±SD	human	Ratio		pKa	(+ or−)	average at pH6.8–7.2
Acetaminophen	39	20	37	6.9	6	1.16	0.51	1.24	0.15	0.93	0.81	0.36	1.35	0.60	Acidic	9.5		1.00
Acyclovir	112	21	430	373	6	4.21	4.04	1.58		2.66	3.29	3.16	1.64	2.01	Acidic	8.0		0.89
Alprazoram	31	16	40	26	6	1.32	0.35	0.46		2.85	1.57	0.41	1.48	1.06	Basic	5.1		1.01
Atenolol	894	312	1659	1321	5	1.99	1.36	3.12		0.64	2.53	1.74	3.75	0.68	Basic	9.7	+	2.77
Caffein	321	197	282	166	6	0.90	0.11	0.71	0.21	1.25	1.10	0.14	1.03	1.07	Neutral			1.00
Cefotaxime	1010	1098	201	280	6	0.20	0.22	0.08	0.03	2.63	0.13	0.14	0.13	1.03	Acidic	3.2	−	0.44
Cephalothin	50	24	ND		6	ND		0.15					0.26		Acidic	3.6	−	0.44
Cephapirin	ND		ND		6	ND		0.15					0.25		Acidic	3.6	−	0.44
Chloramphenicol	48	29	30	15	6	0.80	0.54	0.50	0.17	1.62	0.71	0.48	0.90	0.78	Acidic	7.6		0.78
Cimetidine	116	66	898	116	6	10.33	5.68	4.18	1.85	2.47	6.42	3.53	4.51	1.42	Basic	6.9	+	1.43
Clindamycin	43	31	20	16	6	0.69	0.81	0.94		0.73	1.20	1.41	11.39	0.11	Basic	7.6	+	2.09
Diltiazem	23	14	61	36	6	3.29	2.20	0.99		3.33	6.20	4.14	2.00	3.09	Basic	7.9	+	2.35
Disopyramide	461	237	790	534	5	1.78	1.11	2.82		0.63	1.40	0.88	5.69	0.25	Basic	10.4	+	2.78
Fluconazole	4105	560	3574	1152	6	0.86	0.17	0.87		0.99	0.85	0.17	0.70	1.22	Basic	2.3		1.00
Labetalol	1583	796	1973	481	6	1.46	0.53	1.01		1.44	1.15	0.42	1.24	0.92	Basic	9.7	+	2.77
Metformin	361	173	1317	672	6	3.68	1.21	0.48	0.12	7.70	4.35	1.43	0.52	8.39	Basic	11.6	+	2.78
Metoprolol	217	157	514	379	5	2.98	3.09	2.79		1.07	3.14	3.26	2.95	1.06	Basic	9.7	+	2.77
Metronidazole	825	162	701	182	6	0.87	0.28	0.91		0.97	1.14	0.37	0.79	1.44	Basic	3.1		1.00
Mirtazapine	ND		31	24	4	ND		0.97					2.51		Basic	6.6	+	1.24
Modobemide	3.6	2.9	2.9	1.7	6	1.41	1.55	0.61		2.32	1.00	1.10	0.89	1.12	Basic	6.0		1.07
Nitrazepam	165	94	119	56	6	0.79	0.28	0.40		1.95	0.36	0.13	1.52	0.24	Basic	2.6		1.00
Nitrofurantoin	ND		82	28	6	ND		6.29					11.71		Acidic	9.2		0.99
Praziquantel	2.3	2.1	2.1	1.1	6	1.42	1.05	0.24	0.09	5.87	0.96	0.71	0.60	1.61	Neutral			1.00
Prednisolone	39	23	ND		6	ND		0.13	0.06				1.35		Acidic	12.6		1.00
Propranplol	755	397	655	154	6	1.06	0.46	0.39	0.28	2.72	2.41	1.05	1.55	1.55	Basic	9.7	+	2.77
Propylthiouracil	162	84	795	273	3	5.84	3.84	0.13		45.6	1.06	0.70	0.50	2.14	Acidic	8.1		0.91
Quetiapine	20	10	21	11	6	1.11	0.33	0.36		3.06	1.98	0.59	0.67	2.93	Basic	7.1	+	1.60
Terbutaline	16	8	146	93	5	13.34	12.20	1.54	0.05	8.68	13.17	12.04	1.63	8.05	Basic	9.6	+	2.77
Theophyline	480	257	464	455	6	0.85	0.29	0.64		1.33	0.64	0.22	1.07	0.60	Acidic	7.8		0.84
Trazodone	716	485	98	59	6	0.20	0.15	0.14		1.42	0.45	0.33	0.36	1.24	Basic	7.1	+	1.60

Table I (continued)

Drug	Observed concentration in mouse (nM)				M/P			M/$P_{unbound}$			Major ionizing functional group		Charge at pH6.8–7.2	M/$P_{unbound,pred}$
	C_p	±SD	C_m	±SD	n	mouse	±SD	human[a]	±SD	Ratio	mouse	±SD	human	Ratio		pKa	(+ or −)	average at pH6.8–7.2
Triprolidine	11.2	9.2	6.9	4.4	6	0.77	0.49	0.77		1.00	0.54	0.35	1.55	0.35	Basic	8.6	+	2.68
Verapamil	108	60	100	61	6	0.95	0.45	0.87		1.10	0.33	0.15	1.91	0.17	Basic	9.7	+	2.77

Observed mean drug concentration in plasma (C_p) and milk (C_m) are shown with SD values ($n = 3–6$)

M/P values of mouse were calculated in individual mouse and then averaged

[a] M/P values of human were derived from our prevous report (Koshimichi *et al.* 2010). Data without SD are single or average of duplicate data

M/$P_{unbound}$ values were calculated using M/P values and free fraction in plasma (f_p) and milk ($f_{m,total}$)

Mouse to human ratios of M/P and M/$P_{unbound}$ are shown aside

Major ionizing functional group (acidic/basic) is shown with the pKa value. Data were obtained from the DrugBank database (http://www.drugbank.ac/)

Charge (+ or −) at pH6.8–7.2 is shown. Blank column means neutral charge. Data were obtained from the DrugBank database (http://www.drugbank.ac/)

M/$P_{unbound,pred}$ average at milk pH range of 6.8–7.2 was calculated based on pH partition theory with respective pKa value at standard milk pH of 6.8 and 7.2 as described in the "Materials and Methods"

ND not detected

Comparison of f_p, $f_{m,total}$, f_m and K_f Values Between Human and Mouse

The higher M/P values observed for mouse *versus* human (Fig. 1) are potentially ascribable to species-specific differences in the unbound fraction of drugs in plasma and/or milk; for example, higher mouse f_p values and/or lower mouse $f_{m,total}$ values. This is because, in principle, only unbound (not associated with protein or lipid) and unionized drug molecules can diffuse across the mammary epithelium, while molecules associated with protein and/or lipid droplets are retained in the blood or milk compartment (10,24). Therefore, we next compared f_p and $f_{m,total}$ values in human and mouse (Table II). The experimentally determined mouse f_p values were similar but slightly higher than the corresponding human values; the geometric mean ratio of f_p values between mouse and human was 1.24 (95% CI, 1.04–1.49), and a linear correlation was observed between the f_p values in the two species ($y = 0.860x - 0.004$, $r^2 = 0.737$, Fig. 2a). On the other hand, the $f_{m,total}$ values were significantly lower in mouse than in human; in this case, the geometric mean ratio between mouse $f_{m,total}$ and human $f_{m,total}$ was 0.64 (95% CI, 0.52–0.77). This relationship was more evident for drugs with $f_{m,total}$ values of less than 0.75 in mouse (Fig. 2b).

Furthermore, we separately determined f_m and K_f for mouse and human to investigate the detailed cause of the species-specific differences between $f_{m,total}$ values (Table II). The f_m values varied largely in mouse compared with human (Fig. 3a), with significantly lower f_m values in mouse (Fig. 3a); the geometric mean ratio between mouse f_m and

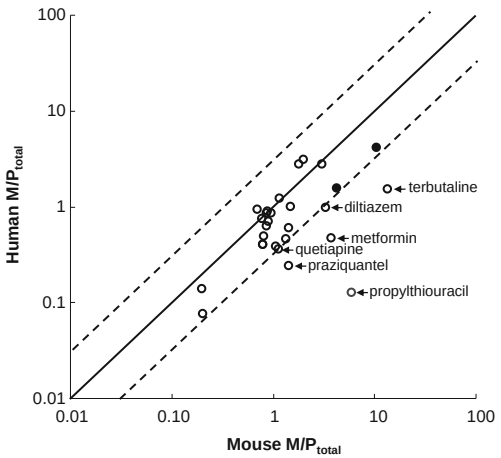

Fig. 1 Comparison of M/P between human and mouse. The M/P value for each drug was calculated for each individual mouse and then expressed as the average of the ratios for all mice employed in the experiment ($n = 3–6$). The human M/P value for each drug was obtained from our previous report (14). *Solid lines* represent best fit lines (1:1), and *dashed lines* represent 3-fold deviations. Names of drugs with M/P value of more than 3-fold higher in mouse *versus* human are shown aside. Known BCRP substrates including acyclovir and cimetidine are shown with *closed circles*.

Table II Parameters Related to Protein Binding and Lipid Partition

	f_p			$f_{m,total}$				f_m				K_f			
Drug	mouse	±SD	human[a]	mouse	±SD	human	±SD	mouse	±SD	human	±SD	mouse	±SD	human	±SD
Acetaminophen	0.66	0.06	0.75	0.46	0.04	0.82	0.05	0.40	0.04	0.82	0.05	0.46	1.84	1.43	2.11
Acyclovir	1.00[b]		0.79	0.78	0.03	0.82	0.07	0.84	0.05	0.93	0.04	1.70	0.55	4.26	1.05
Alprazoram	0.21	0.05	0.20	0.25	0.03	0.64	0.03	0.29	0.02	0.73	0.03	7.18	2.57	5.94	0.82
Atenolol	0.86	0.04	0.89	1.10	0.11	1.05[c]		0.99	0.03	0.97	0.05	0.49	0.45	1.32	3.79
Caffein	0.82	0.11	0.70	1.01	0.25	1.00		0.55	0.02	0.96		-		0.22	
Cefotaxime	0.77	0.10	0.44	0.49	0.03	0.71	0.03	0.54	0.04	0.78	0.04	2.83	0.75	3.93	0.71
Cephalothin	0.47	0.07	0.28	0.59	0.06	0.48	0.05	0.51	0.05	0.54	0.10	0.51	0.99	6.05	2.32
Cephapirin	0.66	0.06	0.46	0.77	0.13	0.77	0.06	0.70	0.11	0.80	0.04	0.75	0.29	2.48	3.71
Chloramphenicol	0.52	0.08	0.45	0.46	0.14	0.82	0.16	0.27	0.09	0.87	0.13	-		2.94	2.19
Cimetidine	0.87	0.03	0.83	0.54	0.19	0.89	0.04	0.42	0.03	0.75	0.01	0.50	3.70	-	
Clindamycin	0.39	0.04	0.07	0.69	0.09	0.85	0.04	0.64	0.07	0.85	0.10	1.00	0.39	1.25	1.65
Diltiazem	0.21	0.11	0.25	0.39	0.05	0.51	0.08	0.45	0.01	0.90	0.13	4.49	2.21	20.53	3.16
Disopyramide	0.86	0.05	0.43	0.68	0.05	0.86	0.02	0.64	0.09	0.85	0.03	1.04	0.46	0.96	0.88
Fluconazole	0.81	0.06	0.89	0.80	0.05	0.71	0.05	0.89	0.06	0.80	0.08	1.77	0.58	4.47	0.53
Labetalol	0.28	0.06	0.50	0.22	0.02	0.61	0.05	0.19	0.02	0.58	0.03	0.88	2.52	-	
Metformin	0.99	0.04	1.00	1.17	0.11	1.05[c]		1.00[b]		1.00[b]		0.93	0.42	2.88	2.28
Metoprolol	0.80	0.07	0.88	0.85	0.02	0.93	0.01	0.80	0.01	0.94	0.05	0.88	0.23	1.15	1.36
Metronidazole	0.94	0.03	0.90	1.22[c]		0.78	0.02	1.00[b]		0.80	0.03	-		1.90	0.54
Mirtazapine	0.21	0.05	0.15	0.16	0.07	0.39	0.07	0.27	0.00	1.00[b]		20.64	13.28	37.87	6.05
Moclobemide	0.66	0.07	0.50	0.47	0.01	0.73	0.06	0.46	0.06	0.63	0.04	1.86	0.89	-	
Nitrazepam	0.17	0.05	0.15	0.08	0.01	0.56	0.04	0.12	0.04	0.89	0.09	36.32	8.85	15.66	2.07
Nitrofurantoin	0.48	0.08	0.60	0.58	0.12	1.05[c]		0.61	0.03	1.00[b]		2.31	1.97	0.26	1.40
Praziquantel	0.20	0.03	0.18	0.14	0.02	0.43	0.03	0.31	0.04	0.68	0.08	26.33	4.56	20.11	4.30
Prednisolone	0.11	0.02	0.08	0.44	0.22	0.83	0.40	0.37	0.16	1.00[b]		0.64	1.12	10.10	13.41
Propranplol	0.14	0.01	0.12	0.31	0.02	0.47	0.02	0.29	0.02	0.62	0.03	2.22	2.49	12.76	1.55
Propylthiouracil	0.71	0.09	0.18	0.13	0.02	0.70	0.16	0.14	0.01	0.74	0.22	9.96	5.65	2.80	7.22
Quetiapine	0.06	0.02	0.17	0.11	0.02	0.31	0.02	0.14	0.01	0.49	0.02	16.77	5.28	27.75	3.62
Terbutaline	0.80	0.05	0.80	0.79	0.07	0.85	0.09	0.75	0.05	0.82	0.09	0.94	0.71	0.25	2.09
Theophyline	0.72	0.23	0.60	0.54	0.02	1.00		0.50	0.07	0.95		1.04	0.98	0.15	
Trazodone	0.08	0.04	0.08	0.17	0.02	0.21	0.02	0.22	0.04	0.64	0.05	11.39	3.48	74.22	7.60
Triprolidine	0.32	0.08	0.10	0.23	0.09	0.20	0.00	0.29	0.06	0.30	0.02	10.91	5.78	38.59	6.73
Verapamil	0.23	0.12	0.10	0.08	0.01	0.22	0.01	0.12	0.00	0.87	0.10	30.69	13.95	76.37	6.26

Unbound fraction in plasma (f_p), milk ($f_{m,total}$), and skim milk (f_m) were determined *in vitro* except for human f_p[a] which is derived from our prevous report (Koshimichi *et al.* 2010)

The milk lipid-to-water partition coefficient (K_f) was calculated as described in the "Materials and Methods"

Data are mean ± SD of triplicate determinations except for caffein and theophyline data in human

"-"; not calculable because the values were lower than 0

[b], f_p and f_m values calculated as higher than theoretical maximum (1.00), were fixed to 1.00

[c], $f_{m,total}$ values calculated as higher than theoretical maximum (1.22 for mouse and 1.05 for human) were fixed to 1.22 and 1.05 for mouse and human, respectively

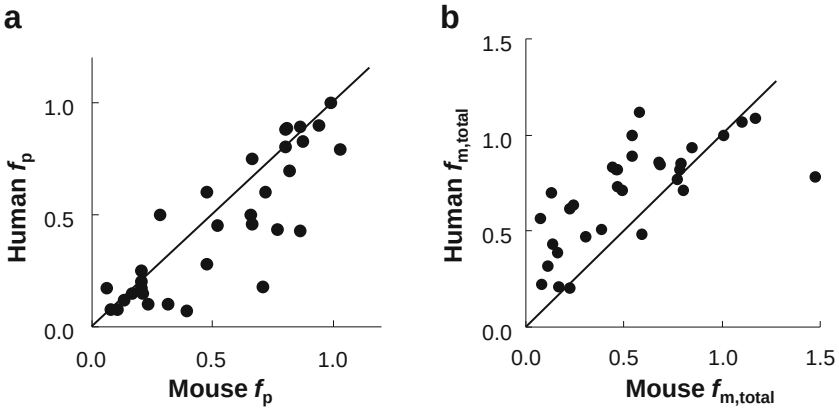

Fig. 2 Comparison of f_p (**a**) and $f_{m,total}$ (**b**) between human and mouse. Human f_p values were obtained from the literature. Human $f_{m,total}$, mouse f_p and mouse $f_{m,total}$ values were determined *in vitro*. Solid lines represent best fit lines (1:1).

human f_m was 0.53 (95% CI, 0.43–0.65). These observations signify that protein binding by drugs in skim milk is significantly higher in the mouse. Next, we successfully calculated the mouse and human K_f values for 26 drugs. The K_f values in mouse were slightly lower than those in human; the geometric mean ratio between mouse K_f and human K_f was 0.61 (95% CI, 0.40–0.95), and a linear correlation was observed between their logarithmic values ($y=0.837x+0.286$, $r^2=0.489$, Fig. 3b). Taken together, these results indicate that the higher M/P values in mouse can be mostly explained by the lower $f_{m,total}$ values. The lower $f_{m,total}$ values in mouse can in turn be explained by the increased binding of drugs to protein in skim milk, in addition to a massive partitioning of drugs into the lipid fraction due to the higher creamatocrit percentage in mouse *versus* human milk (18.0% *versus* 4.5%).

Unbound Drug Concentration Ratio Between Milk and Plasma

The contribution of asymmetrical transport across the mammary epithelium was next examined by comparing unbound drug concentration ratios between plasma and milk ($M/P_{unbound}$) for mice and humans. Asymmetrical transport process should be considered in cases where the $M/P_{unbound}$ value significantly deviates from the value predicted by the pH-partition theory (10,24). $M/P_{unbound}$ values were calculated by using unbound fraction parameters for 27 drugs (Table I). In contrast to the results for M/P (Fig. 1), no trend indicated that the overall $M/P_{unbound}$ values were higher or lower in mouse compared to human. Furthermore, the ratio between mouse $M/P_{unbound}$ and human $M/P_{unbound}$ were close to 1.0 with some exceptions (see below and Discussion) (Fig. 4). The geometric mean ratio between mouse $M/P_{unbound}$ and human $M/P_{unbound}$ was 1.03 (95% CI, 0.70–1.52).

$M/P_{unbound}$ values predicted based on the pH-partition theory are also shown in Table I, assuming a pH of 6.8–7.2 for standard milk. Classification of drugs based on the observed and predicted $M/P_{unbound}$ values are summarized in Fig. 5. The $M/P_{unbound}$ values for 18 out of 27 drugs were within a 3-fold higher or lower range of the predicted values (average $M/P_{unbound}$ at pH6.8–7.2), while the $M/P_{unbound}$ values of the other 9 drugs were not (Table I, Fig. 4). Among these 9 drugs, the $M/P_{unbound}$ values of 5 drugs (cefotaxime, human and mouse; metformin, human; triprolidine, mouse; trazodone, human and mouse; and verapamil, mouse) were more than 3-fold lower than predicted. In contrast, the

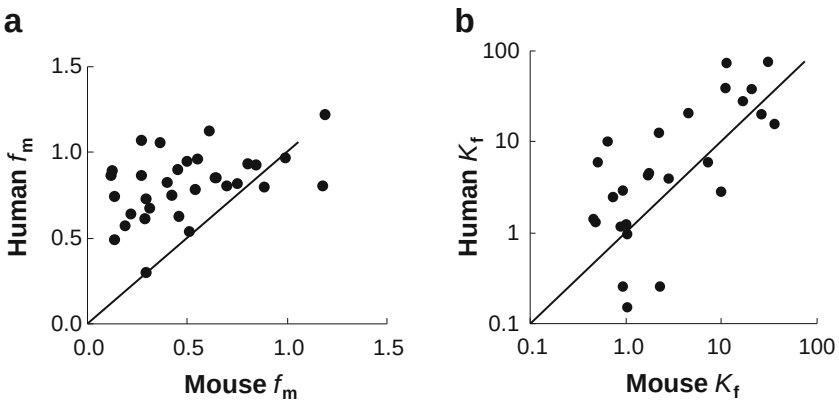

Fig. 3 Comparison of f_m (**a**) and K_f (**b**) between human and mouse. *Solid lines* represent best fit lines (1:1).

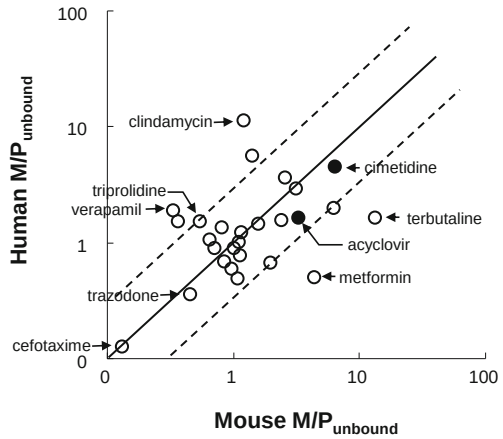

Fig. 4 Comparison of $M/P_{unbound}$ between human and mouse. Human and mouse $M/P_{unbound}$ values were calculated using M/P values shown in Table I and f_p and $f_{m,total}$ values shown in Table II. *Solid line* represents best fit line (1:1), and *dashed lines* represent 3-fold deviations. Drugs with $M/P_{unbound}$ values of more than 3-fold higher or lower than the predicted average values based on the pH-partition theory (at the milk pH range of 6.8–7.2) are highlighted with their names. Known BCRP substrates including acyclovir and cimetidine are shown with *closed circles*.

$M/P_{unbound}$ values of the other 4 drugs (acyclovir, mouse; cimetidine, human and mouse; clindamycin, human; and terbutaline, mouse) were more than 3-fold higher than predicted. These results suggest that there is little species-specific variation regarding the $M/P_{unbound}$ for cimetidine, cefotaxime, or trazodone (the $M/P_{unbound}$ values were more than 3-fold higher or lower than predicted for both species), whereas species-specific differences do exist for acyclovir, clindamycin, metformin, terbutaline, triprolidine, and verapamil ($M/P_{unbound}$ values were more than 3-fold higher or lower than predicted for one of the species, but not the other).

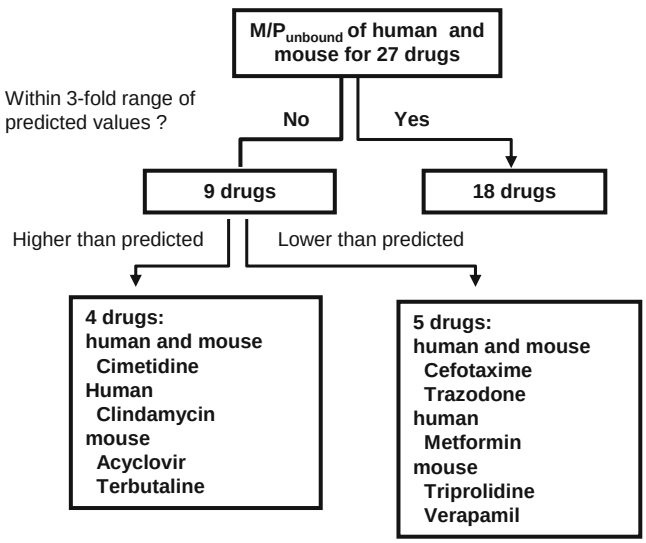

Fig. 5 Classification of drugs based on the observed and predicted $M/P_{unbound}$ values. $M/P_{unbound}$ values observed in mouse and human were compared with theoretically predicted average $M/P_{unbound}$ values ($M/P_{unbound,pred}$) based on the pH-partition theory at the milk pH of 6.8–7.2 (see Table I). As a result, 9 drugs were found to deviate by more than 3-fold from $M/P_{unbound,pred}$ values in either human or mouse (Fig. 4). These 9 drugs were divided into two groups according to whether the values were higher or lower than predicted, and also according to species-specific differences.

DISCUSSION

This is the first report in which the transfer of drugs with physiologically diverse actions into breast milk was comprehensively compared between mouse and human, with consideration of the possible contribution of carrier-mediated transport across the mammary epithelium. Our major findings are as follows: 1) M/P values were higher in mouse relative to human for most compounds; 2) $M/P_{unbound}$ values were similar in the two species with some exceptions (see below); 3) drug binding to proteins and partitioning into the lipid fraction contributed to the more extensive drug distribution in mouse *versus* human milk; and 4) a significant contribution of asymmetrical transport processes was suggested for some compounds in both humans and mice. These findings are discussed in detail in the following paragraphs, along with possible interpretations.

First, the trend toward higher M/P values in mice (Fig. 1) is not surprising given the higher protein and lipid content in mouse *versus* human milk. The protein constituents of mouse milk range from 97.0–213.6 mg/mL, depending on the mouse strain (26). These figures are about 10–20 times higher than the protein concentration in human milk (9.2 mg/mL (27)). Moreover, the creamatocrit percentage in mouse milk is also higher than that in human milk (18.8% *versus* 4.5%, on average (14)). As shown in Fig. 2, $f_{m,total}$ values were generally lower in the mouse, while f_p values were similar between human and mouse. Higher binding to milk proteins and partitioning into the lipid fraction in mouse milk results in the retention of the drug in the milk compartment. The lower protein binding in human milk may ensue from the lower serum albumin content in human milk (0.5 g/L) (28), which is less than one-tenth of that in mouse milk (4.8–10.2 mg/mL) (29). On the other hand, the concentration of serum albumin was similar in human plasma (44 mg/mL) (30) and mouse plasma (38 mg/mL) (31). As a result, the correlation between f_p and f_m was better in mice ($y=0.682x+0.128$, $r^2=0.598$) than in humans ($y=0.210x+0.702$, $r^2=0.147$) (Supplementary Material Fig. 2). To our knowledge, no other reports have provided evidence supporting the general importance of species-specific differences in the concentration of milk albumin, a major drug-binding component in breast milk, to drug distribution during lactation.

In this study, the contribution of additional drug-binding protein(s) in milk was also suggested for a particular drug, propylthiouracil. Indeed, the protein binding of propylthiouracil in mouse plasma was low (f_p=0.71), while that in milk was high (f_m=0.14), indicating the presence of a specific binder other than serum albumin in mouse milk. This observation may account for the relatively high concentration of propylthiouracil in mouse milk (M/P=5.84) compared with human milk (M/P=0.13). Interestingly, propylthiouracil is the considered safest of the anti-thyroid medications for use in lactating human mothers, whereas the pharmacological impact of propylthiouracil on breastfed rat pups is well known (32,33). Although the M/P of propylthiouracil was not determined in the rat study, it is probably also high in this experimental animal.

$M/P_{unbound}$ values of 27 drugs were mostly centered around 1.0, consistent with the commonly accepted pH-partition theory. However, for drugs with $M/P_{unbound}$ values higher or lower than predicted, an asymmetrical transport system should be suspected. As summarized in Fig. 5, 9 out of 27 drugs fell into this category. These 9 drugs were further classified based on different points of views. From the viewpoint of asymmetrical drug transport, 4 drugs (acyclovir, cimetidine, clindamycin, and terbutaline) were categorized into a group associated with secretory passage across the mammary epithelium, and the other 5 (cefotaxime, trazodone, triprolidine, metformin, and verapamil) were categorized into a group associated with absorptive reuptake transport. From the viewpoint of species-specific variations in drug transport, 6 drugs (acyclovir, clindamycin, metformin, terbutaline, triprolidine, and verapamil) were categorized into a group with extensive species-specific differences, while the other 3 (cefotaxime, cimetidine, and trazodone) were categorized into a group with little species-specific disparity.

Maternally ingested cimetidine is concentrated in the milk of mouse, rat, and human, but not rabbit (12,15–17). The contribution of BCRP to the secretion of cimetidine into milk was previously established by using gene knockout mice (19,20). Similarly, acyclovir is also concentrated in the milk of mouse by BCRP. Our current results, showing a more than 3-fold higher than predicted $M/P_{unbound}$ value for cimetidine and acyclovir in mice, are consistent with these previous observations (19). However, it is unknown whether terbutaline and clindamycin are also substrates of BCRP.

Expression profiles have been demonstrated for many potential candidate transporters in the mammary gland (21,22), including various solute carriers (SLC) and ABC transporters. For example, organic cation transporter (OCT) 1 (also known as SLC family member 22A1 (SLC22A1)) and OCT3 (also known as SLC22A3) mRNAs are both expressed in mammary epithelial cells. Moreover, OCT1 is induced during lactation in humans (21) and rats (14). Among the 27 drugs investigated in the current study, cimetidine (34) and metformin (35) are representative substrates of OCT family proteins. Furthermore, disopyramide reportedly interacts with rat OCT1 and OCT2 (also known as SLC22A2) (36) and is transported by human OCT3 (37), but the functional significance of OCT family members in the passage of drugs across the mammary epithelium has not yet been reported.

Our results showed that the $M/P_{unbound}$ value of metformin was 4.35 in mouse, which was quite similar to the predicted value (3.98−1.58 at milk pH of 6.8–7.2) based on the pH-partition theory, whereas the $M/P_{unbound}$ value in human (0.53) was far lower than the predicted value (Table I). This suggests that active transport by OCT family members is not necessarily involved in the secretory transport of metformin into the milk. Moreover, the M/P value of cimetidine in wild-type mice (approximately 13–14) was significantly reduced to as low as 2–3 in BCRP knockout mice (19). From the $M/P_{unbound,pred}$, f_p and $f_{m,total}$ values for cimetidine determined in this study (Table II), M/P can be predicted to be 1.8–2.8, which is consistent with the value observed in BCRP knockout mice. These considerations also imply that BCRP on the apical membrane is sufficient for the secretory transport of cimetidine in wild-type mice. Strictly speaking, however, the possibility cannot be excluded that uptake transporters, such as OCT family members, are necessary for the entry of drugs from the blood into mammary epithelial cells, which are subsequently secreted into the milk by BCRP. To conclusively address this issue, experiments employing OCT knockout mice would be advantageous.

Predictability of drug transfer into milk could be improved by the quantification and correct integration of asymmetrical transport, though it is not established in human as well as in experimental animals. Actually, $M/P_{unbound}$ values of 4 compounds including clindamycin, nitrazepam, triprolidine, and verapamil, were higher in human than mouse by more than 3-fold. From the safety point of view, under-prediction of $M/P_{unbound}$ in human should be avoided. Better understanding of this machinery in mammary epithelia in human and experimental animals would improve the predictability of drug transfer into milk in human and finally secure the infants from unnecessary exposure to drug.

CONCLUSIONS

This study is the first of its kind to comprehensively show species-specific differences in drug transfer into milk during lactation. In addition to the large quantitative difference in milk protein and lipid content in mice

and humans, important similarities and dissimilarities in host transporter systems for certain drugs emerged. Consideration of asymmetrical transport processes is anticipated to improve the predictability of drug transfer into milk.

ACKNOWLEDGMENTS AND DISCLOSURES

This work was supported by a research scholarship from the Japan Research Foundation for Clinical Pharmacology, and a Grant-in-Aid for Scientific Research on Innovative Areas HD-Physiology [Grant 22136015] from the Ministry of Education, Science and Culture of Japan.

REFERENCES

1. Breastfeeding and the use of human milk. Pediatrics. 2012;129:e827–41.
2. Kramer MS. "Breast is best": the evidence. Early Hum Dev. 2010;86:729–32.
3. Guxens M, Mendez MA, Molto-Puigmarti C, Julvez J, Garcia-Esteban R, Forns J, et al. Breastfeeding, long-chain polyunsaturated fatty acids in colostrum, and infant mental development. Pediatrics. 2011;128:e880–9.
4. Flacking R, Ewald U, Starrin B. "I wanted to do a good job": experiences of 'becoming a mother' and breastfeeding in mothers of very preterm infants after discharge from a neonatal unit. Soc Sci Med. 2007;64:2405–16.
5. Renfrew MJ, Craig D, Dyson L, McCormick F, Rice S, King SE, et al. Breastfeeding promotion for infants in neonatal units: a systematic review and economic analysis. Health Technol Assess. 2009;13:1–146. iii–iv.
6. Flacking R, Ewald U, Nyqvist KH, Starrin B. Trustful bonds: a key to "becoming a mother" and to reciprocal breastfeeding. Stories of mothers of very preterm infants at a neonatal unit. Soc Sci Med. 2006;62:70–80.
7. Jones G, Steketee RW, Black RE, Bhutta ZA, Morris SS. How many child deaths can we prevent this year? Lancet. 2003;362:65–71.
8. Fleishaker JC. Models and methods for predicting drug transfer into human milk. Adv Drug Deliv Rev. 2003;55:643–52.
9. Miller GE, Banerjee NC, Stowe Jr CM. Diffusion of certain weak organic acids and bases across the bovine mammary gland membrane after systemic administration. J Pharmacol Exp Ther. 1967;157:245–53.
10. Atkinson HC, Begg EJ. Prediction of drug distribution into human milk from physicochemical characteristics. Clin Pharmacokinet. 1990;18:151–67.
11. Begg EJ, Atkinson HC, Duffull SB. Prospective evaluation of a model for the prediction of milk:plasma drug concentrations from physicochemical characteristics. Br J Clin Pharmacol. 1992;33:501–5.
12. Somogyi A, Gugler R. Cimetidine excretion into breast milk. Br J Clin Pharmacol. 1979;7:627–9.
13. Shyu WC, Shah VR, Campbell DA, Venitz J, Jaganathan V, Pittman KA, et al. Excretion of cefprozil into human breast milk. Antimicrob Agents Chemother. 1992;36:938–41.
14. Koshimichi H, Ito K, Hisaka A, Honma M, Suzuki H. Analysis and prediction of drug transfer into human milk taking into consideration secretion and reuptake clearances across the mammary epithelia. Drug Metab Dispos. 2011;39:2370–80.
15. Dostal LA, Weaver RP, Schwetz BA. Excretion of high concentrations of cimetidine and ranitidine into rat milk and their effects on milk composition and mammary gland nucleic acid content. Toxicol Appl Pharmacol. 1990;102:430–42.
16. McNamara PJ, Burgio D, Yoo SD. Pharmacokinetics of cimetidine during lactation: species differences in cimetidine transport into rat and rabbit milk. J Pharmacol Exp Ther. 1992;261:918–23.
17. Gerk PM, Oo CY, Paxton EW, Moscow JA, McNamara PJ. Interactions between cimetidine, nitrofurantoin, and probenecid active transport into rat milk. J Pharmacol Exp Ther. 2001;296:175–80.
18. McManaman JL, Neville MC. Mammary physiology and milk secretion. Adv Drug Deliv Rev. 2003;55:629–41.
19. Jonker JW, Merino G, Musters S, van Herwaarden AE, Bolscher E, Wagenaar E, et al. The breast cancer resistance protein BCRP (ABCG2) concentrates drugs and carcinogenic xenotoxins into milk. Nat Med. 2005;11:127–9.
20. Merino G, Jonker JW, Wagenaar E, van Herwaarden AE, Schinkel AH. The breast cancer resistance protein (BCRP/ABCG2) affects pharmacokinetics, hepatobiliary excretion, and milk secretion of the antibiotic nitrofurantoin. Mol Pharmacol. 2005;67:1758–64.
21. Alcorn J, Lu X, Moscow JA, McNamara PJ. Transporter gene expression in lactating and nonlactating human mammary epithelial cells using real-time reverse transcription-polymerase chain reaction. J Pharmacol Exp Ther. 2002;303:487–96.
22. Gilchrist SE, Alcorn J. Lactation stage-dependent expression of transporters in rat whole mammary gland and primary mammary epithelial organoids. Fundam Clin Pharmacol. 2010;24:205–14.
23. Lucas A, Gibbs JA, Lyster RL, Baum JD. Creamatocrit: simple clinical technique for estimating fat concentration and energy value of human milk. Br Med J. 1978;1:1018–20.
24. Fleishaker JC, Desai N, McNamara PJ. Factors affecting the milk-to-plasma drug concentration ratio in lactating women: physical interactions with protein and fat. J Pharm Sci. 1987;76:189–93.
25. Jara-Almonte M, White JM. Milk production in laboratory mice. J Dairy Sci. 1972;55:1502–5.
26. Piletz JE, Ganschow RE. Genetic variation of milk proteins in mice. Biochem Genet. 1981;19:1023–30.
27. Mitoulas LR, Kent JC, Cox DB, Owens RA, Sherriff JL, Hartmann PE. Variation in fat, lactose and protein in human milk over 24 h and throughout the first year of lactation. Br J Nutr. 2002;88:29–37.
28. Montagne P, Cuilliere ML, Mole C, Bene MC, Faure G. Immunological and nutritional composition of human milk in relation to prematurity and mother's parity during the first 2 weeks of lactation. J Pediatr Gastroenterol Nutr. 1999;29:75–80.
29. Boumahrou N, Andrei S, Miranda G, Henry C, Panthier JJ, Martin P, et al. The major protein fraction of mouse milk revisited using proven proteomic tools. J Physiol Pharmacol. 2009;60 Suppl 3:113–8.
30. Bezerra FF, Laboissiere FP, King JC, Donangelo CM. Pregnancy and lactation affect markers of calcium and bone metabolism differently in adolescent and adult women with low calcium intakes. J Nutr. 2002;132:2183–7.
31. Stock B, Dean M, Levy G. Serum protein binding of drugs during and after pregnancy in rats. J Pharmacol Exp Ther. 1980;212:264–8.
32. Cooke PS, Kirby JD, Porcelli J. Increased testis growth and sperm production in adult rats following transient neonatal goitrogen treatment: optimization of the propylthiouracil dose and effects of methimazole. J Reprod Fertil. 1993;97:493–9.
33. Blake HH, Henning SJ. Effect of propylthiouracil dose on serum thyroxine, growth, and weaning in young rats. Am J Physiol. 1985;248:R524–30.

34. Urakami Y, Okuda M, Masuda S, Saito H, Inui KI. Functional characteristics and membrane localization of rat multispecific organic cation transporters, OCT1 and OCT2, mediating tubular secretion of cationic drugs. J Pharmacol Exp Ther. 1998;287:800–5.
35. Wang DS, Jonker JW, Kato Y, Kusuhara H, Schinkel AH, Sugiyama Y. Involvement of organic cation transporter 1 in hepatic and intestinal distribution of metformin. J Pharmacol Exp Ther. 2002;302:510–5.
36. Urakami Y, Okuda M, Masuda S, Akazawa M, Saito H, Inui K. Distinct characteristics of organic cation transporters, OCT1 and OCT2, in the basolateral membrane of renal tubules. Pharm Res. 2001;18:1528–34.
37. Hasannejad H, Takeda M, Narikawa S, Huang XL, Enomoto A, Taki K, et al. Human organic cation transporter 3 mediates the transport of antiarrhythmic drugs. Eur J Pharmacol. 2004;499:45–51.