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Cultured Mammary epithelial Monolayers (BME-UV) Express Functional Organic Anion and Cation Transporters

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Abstract

There is ongoing concern about the potential adverse effects of xenobiotic residues in cows' milk to the human consumer. Although drugs that are intentionally administered to lactating dairy cattle are rigorously regulated to prevent harmful residues, there are numerous other potential sources of exposure that are not as easily controlled. For example, cattle may be exposed to mycotoxins, pesticides and/or persistent organic pollutants through feed, water and inhalation of polluted air. Accurate estimates of the rate and extent of excretion of these compounds into milk is important to assess the risk of exposure through cows' milk. In the present study, the expression of carrier mediated transport processes in cultured mono layers of an immortalized bovine mammary epithelial cell line (BME-UV*) was determined using a flow-through diffusion cell system, selective substrates and inhibitors of organic cation transporters (OCT†) and organic anion transporters (OAT‡). The basal to apical (BL-to-Ap§) flux of tetraethylammonium (TEA**) and estrone sulfate (ES††) significantly exceeded their flux in the opposite direction. The addition of selective inhibitors to the donor compartment significantly decreased the BL-to-Ap flux of either selective substrate. These results suggest that both OCT and OAT are functionally expressed by BME-UV cells.

Keywords

Carrier mediated transport; *in vitro* model; organic cation transporter; organic anion transporter; bovine mammary epithelium

*Bovine Mammary Epithelial Cell line

†Organic Cation Transporters

‡Organic Anion Transporters

§Basal to Apical

**Tetraethylammonium

†† Estrone Sulfate

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INTRODUCTION

Milk and milk products from different species of domesticated animals have been used as important foods by man for thousands of years (Rusoff, 1970). The United States produced more than 185 billion pounds of milk in 2007. Approximately 60% or 111 billion pounds of this total was consumed as fluid milk (USDA, 2007). This high level of consumption, often by vulnerable sub-populations such as infants and children, makes it very important that milk is safe and free of potentially harmful residues. Potential adverse effects of xenobiotic residues to the human consumer include acute toxicity, carcinogenicity, teratogenicity, allergic reactions and the selection of resistant bacteria (Przyrembel *et al.,* 2000; Gehring *et al.,* 2006). Residues can also interfere with the manufacture of dairy products, such as cheese, which depend on bacterial fermentation for their production (Molina *et al.,* 2003).

An integral part of ensuring that consumed milk is the conduct of mandatory residue depletion studies prior to the approval of drugs for use in lactating dairy cattle (FDA). These studies ensure that there are no harmful residues as a result of intentional administration of veterinary drugs, according to label instructions. But there are numerous other potential sources of harmful residues in milk, including exposure to environmental contaminants through feed, water and inhaled air, as well as extra-label use of veterinary drugs. Drug-drug and drug-feed interactions may also lead to changes in the rate and extent of xenobiotic excretion into milk. Environmental contaminants that may result in harmful milk residues include mycotoxins, heavy metals, pesticides and persistent organic pollutants such as polychlorinated biphenyls and dioxins (Westin, 1993; Markaki and Melissari, 1997; Przyrembel *et al.,* 2000; Patra *et al.,* 2008)

Milk concentrations of xenobiotic residues, and therefore the risk of exposure to the human consumer, depend on the rate and extent to which these compounds are excreted into the milk. This, in turn, is dependent on the physicochemical properties of the xenobiotic and how these interact with the biological environment (Gehring and Smith, 2006). Several authors have developed mathematical models to predict milk concentrations based compounds' physicochemical properties, including lipophilicity, pK_a and molecular weight (Atkinson and Begg, 1990; Begg *et al.,* 1992). The extent of ionization and protein binding are also considered in these models. Although these models are successful in predicting exposure to some compounds, *in vivo* studies in a variety of species have identified drugs that are present in milk at higher than predicted concentrations. Among these are acyclovir (Lau *et al.,* 1987), cimetidine (McNamara *et al.,* 1996) and nitrofurantoin (Kari *et al.,* 1997). Contribution of carrier mediated transport systems to the movement of xenobiotics from plasma to milk is the most likely explanation for these higher concentrations (Kimura *et al.,* 2006). Messenger-RNA coding for different members of organic anion and organic cation transporter families have been isolated from human mammary epithelial cells purified from pooled fresh breast milk samples (Alcorn *et al.,* 2002) or from cell cultures (Kimura *et al.,* 2006).

In vitro culture models of polarized epithelial cells grown on permeable supports are used by the pharmaceutical industry to study the movement of drugs across various epithelial barriers. Models have been developed for the gut, respiratory and ocular epithelia, amongst others. These models are considered to be good predictors of the rate and extent of movement of compounds across specific epithelial barriers, since they incorporate not only simple passive diffusion, but also the various other transport mechanisms by which compounds may cross the epithelial barrier (Lehr, 2002). It is, however, important that the chosen cell culture model displays some of the morphological and functional properties that are representative the corresponding *in vivo* cell layers were established (Wilson, 1990). Since the most likely rate-limiting barrier to xenobiotic movement from plasma to milk is

the mammary secretory epithelium (Nguyen and Neville, 1998; Shennan and Peaker, 2000), Kimura *et al.*(2006) investigated the feasibility of developing an *in vitro* model of this barrier using a human mammary epithelial cell line (HMEC). Kimura *et al.* (2006) found OCTs to be expressed in this cell line. Among these is the human mammary epithelial cell (HMEC[‡‡]) culture model that was developed by Kimura *et al.*, (2006). To the authors' knowledge, a model using bovine mammary epithelial cells has not yet been developed, despite the importance of bovine milk as a source of human nutrition, especially for children and infants. The study described in this manuscript was designed to investigate functional expression of OATs and OCTs in the mammary epithelial cell line (BME-UV) which may play a role in the movement of xenobiotic agents from plasma to milk as well as from milk to plasma. Some of these agents are benzylpenicillin, tetracycline, salicylate, acyclovir, cidofovir, and p-aminohippurate which were classified as substrates for OAT, while verapamil, pyrilamine, quinidine, ganciclovir and cimetidine were classified as substrates for OCT (Sai and Tsuji, 2004).

MATERIALS AND METHODS

Chemicals

Tetraethylammonium bromide, estrone sulfate, probenecid, 1,1-Diethyl-2,2-cyanine iodide, disodium ethylenediaminetetraacetic acid and Perdrogen® (30% H_2O_2 w/w) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). [^{3}H]-mannitol (20 Ci/mmol), [^{14}C]-mannitol (55 mCi/mmol), [^{14}C]-tetraethylammonium bromide (55 mCi/mmol) and [^{3}H]-estrone sulfate (50 Ci/mmol) were purchased from American Radiolabeled Chemicals, Inc. (Saint Louis, MO, USA). Biosol® and Bioscint® were purchased from National Diagnostics, Inc. (Atlanta, GA, USA).

Cell culture

The BME-UV cells were cultured under conditions similar to those described previously (Schmidt *et al.*, 2001; Quesnell *et al.*, 2007a; Quesnell *et al.*, 2007b). Briefly, stock cultures were grown to 65-75% confluency in 25 cm^2 plastic culture flasks (Corning, Inc., NY). Cells were dissociated for passage using a solution containing 0.25% trypsin and 2.65 mM disodium ethylenediaminetetraacetic acid (EDTA[§§], Gibco) in phosphate-buffered saline. Then, dissociated and dispersed cells were seeded on permeable polyester inserts, Transwells® (Corning, Inc., NY). These inserts measure 24 mm in diameter and have a pore size of 0.4 μm. Typical bovine medium (TBM[***]), which contains little lactose and has concentrations of electrolytes that closely mirror serum, bathed the basolateral aspect of the cells throughout all experiments. The apical aspect was exposed to apical bovine medium (APM[†††]) of low electrolyte-high lactose composition that resembles the ionic composition of milk. Composition of TBM and APM were reported previously (Schmidt *et al.*, 2001; Quesnell *et al.*, 2007a; Quesnell *et al.*, 2007b). 1.5 ml of APM and 2.5 ml of TBM were added to the top and bottom compartments that were formed by the monolayers of cells, respectively. The cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2. Media on both the apical and basolateral aspects of the cells were refreshed daily. Cells were maintained in culture on permeable supports for 2 weeks to form a confluent, polarized and electrically tight monolayer.

[‡‡]Human Mammary Epithelial Cell
[§§]Disodium Ethylenediaminetetraacetic Acid
[***]Typical bovine medium
[†††]Apical Bovine Medium

Flow-through diffusion cell system

The confluent monolayer of BME-UV cells on the permeable Transwell® support was mounted as a barrier membrane in a flow-through diffusion cell system (Permegear®, Bethlehem, PA). The system had 14 diffusion chambers, automated receptor fluid collection and a high precision multichannel dispenser (Ismatec SA, Switzerland). The flow rate of receptor fluid through the receiver compartment of each diffusion chamber was controlled at 4 ml/h. Test compounds were added to the donor side of the chamber, and sequential samples were collected from the receiver side at predetermined times over a period of 4 hours. Donor compartments of diffusion chambers were capped directly after adding test solutions. A constant temperature was maintained by circulating 37°C water through the diffusion chambers holder.

Transport studies

The flow-through system was used to determine the rate of permeation of selective substrates for OCTs and OATs across the BME-UV monolayer. To characterize the role of transporters in the substrates' permeation across the monolayer, rates were compared from the apical to the basolateral side and vice versa, in the presence and absence of selective transporter inhibitors and over a wide range of concentrations. In the case of carrier mediated transport, the permeation rate constant is expected to be dependent on the direction of the substrate movement across the mammary epithelial cells (Ap-to-BL or BL-to-Ap) and concentration (saturable at higher level) and to decrease significantly in the presence of selective inhibitors.

Test compounds were prepared in both TBM and APM together with mannitol as a cell mono layer integrity marker (Kimura *et al.,* 2006). The transport experiment was initiated by adding 1.5 mL of test solutions containing 100 μM of either [^{14}C]-tetraethylammonium bromide, a known selective substrate for organic cation transporters (Schomig *et al.,* 1993; Goralski and Sitar, 1999; Goralski *et al.,* 2002; Kimura *et al.,* 2006) or [^{3}H]-estrone sulfate, a known selective substrate for organic anion transporters (Kusuhara *et al.,* 1999; Takeda *et al.,* 2001; Dantzler and Wright, 2003; Sai and Tsuji, 2004) to the upper compartment (donor) together with 2 μM of [3H]- or [14C]-mannitol (MNT[‡‡‡]) depending on the radiolabeled test compound that was used in the experiment. Mannitol was used as a marker for paracellular movement to monitor the integrity of the mammary epithelial monolayer since it is hydrophilic and relatively small molecule (mol. wt. 182) (Artursson *etal*.,(1996).

To test if the permeability of the selective substrates was dependent on direction, the cell monolayer was mounted with either the apical or the basolateral side facing the donor compartment. Each of the 14 diffusion chambers was randomly assigned to a direction.

If directionality was observed in the permeation rate constant, the involvement of transporters was confirmed by examining the effect of an inhibitor of OCT (cyanine) (Schomig *et al.,* 1993; Goralski *et al.,* 2002) and an inhibitor of OAT (probenecid) (Takeda *et al.,* 2001; Lash *et et*., 2007) on the permeation rate constants of TEA and ES in both directions. Finally, if the effect of inhibitors was found to be significant, then permeation rate constants for both TEA and ES were determined for a range of concentrations.

Three replicates were performed for each treatment in the above experiments. Two control diffusion chambers with only the permeable support as a separator between the donor and receiver compartments were also included in each experiment. Sequential samples of the receptor fluid were collected at 15-min intervals for the first hour, 30-min intervals for the

‡‡‡Mannitol

next two hours and finally at the fourth-hour of the experiment. At the end of the fourth hour, the residual media in the diffusion chambers were aspirated.

Sample analysis

Substrate concentrations were measured in both the donor and acceptor media for each sampling time. One ml of sample was transferred into a glass scintillation vial and 0.3 mL of 30% H2O2 added for decolorization purposes. After incubating the sample at 55°C for one hour, the sample was cooled to room temperature and 15 ml of liquid scintillation cocktail (Bioscint®) were added. To determine the intracellular content of radioactive isotopes, BME-UV cells were lysed by incubating them with one ml of Biosol® at 55°C for two hours. Fifteen ml of liquid scintillation cocktail (Bioscint®) were then added to the lysed cells after the samples cooled to room temperature. Samples of either [^{14}C]- or [^{3}H]-radiolabeled test compounds and mannitol were assayed by dual labeled β-scintillation counting with quench correction (Beckman LS6500, Beckman Coulter, Inc., CA, USA). Automatic calculation of disintegrations per minute (DPM[§§§]) was used for each sample.

Data analysis

The permeation rate constant (P_{app}[****], cm/s) of each test compound and mannitol was calculated using the following equation (Yamashita *et al.*, 2000):

$$P_{app} - \frac{(dx)}{(dt)} \times \frac{1}{AC_0}$$

Where *(dX)/(dt)* (μmol/s) is the slope of the graph obtained by plotting the cumulative amount of the test compound in the receiver compartment versus time. The slope was measured between time points at which the system was judged to be at pseudo-equilibrium as evidenced by the slope forming a straight line on a linear scale. It can be accepted that sink conditions were maintained throughout the experiment since less than 10 % of the test compound deposited in the donor compartment appeared in receiver compartment and the test compound was continuously removed from the receiver compartment by receptor fluid flow. *A* (cm^2) is the effective diffusion area of the cell culture insert and C^0 (μM) is the compound's initial concentration in the donor compartment.

Statistical analysis

All statistical analyses were performed using the commercially available software SigmaStat® (Systat Software Inc., San Jose, CA). Group comparisons were made by analysis of variance, ANOVA, followed by Tukey's test when ANOVA showed significant differences among treatments to determine the specific pairs of treatments between which statistically significant differences occurred. Comparison between control and other treatments was made by paired sample t-test. The level of significance for all tests was set at $P < 0.05$.

RESULTS

Barrier function of the BME-UV monolayer

The permeation rate constant for both TEA (161×10^{-6} cm/s) and ES (122×10^{-6} cm/s) in the control diffusion chambers that had only a permeable support was at least 30 times and

[§§§]Disintegrations Per Minute
[****]Apparent permeability coefficient

80 times greater than BL-to-Ap and Ap-to-BL permeation rate constants respectively of either test compounds in the diffusion chambers with the monolayer of BME-UV cells as a separator between donor and receiver compartments. In addition, the permeation rate constant of mannitol across monolayer of BME-UV cells was $0.91\pm0.4 \times 10^{-6}$ cm/s. These results indicate that the monolayer of BME-UV cells forms an effective barrier to the movement of these compounds.

Transport study

The cumulative concentrations of TEA and ES in the receiver compartment as a function of time in the absence or presence of inhibitors are shown in figures 1 and 2, respectively. The permeation rate constants of TEA and ES across BME-UV monolayer under different conditions are summarized in Table 1. Transport of TEA in the BL-to-Ap direction was significantly greater by a factor of 2.6 than that in the Ap-to-BL direction (Table 1). Similar directionality was also observed in the ES transport experiment (the BL-to-Ap permeation rate constant was 3.3 times greater than in the other direction). The permeation rate constants for TEA were 3 times and 2 times greater than for ES in the Ap-to-BL and BL-to-Ap directions, respectively.

The permeation rate constant of TEA was decreased significantly by increasing the initial concentration of TEA in the donor compartment from 100 μM to 500 μM and then to 1000 μM (Figure 3). While for ES, it was decreased significantly only when the initial concentration of ES was increased in the donor compartment from 100 μM to 500 μM. When the concentration was increased from 500 μM to 1000 μM, there was no significant difference in the permeation rate constant (Figure 4).

Inhibition study

The permeation rate constant of TEA was decreased significantly (approximately 49%) only in the BL-to-Ap direction by adding cyanine (OCT-inhibitor) to the donor compartment (Table 1). The permeation rate constant of TEA was not changed significantly by adding probenecid (OAT-inhibitor) to the donor compartment in either direction (data not shown). Similar results, but not identical were found for ES in which the permeation rate constant was decreased significantly (approximately 36%) only in the BL-to-Ap direction by adding probenecid (OAT-inhibitor) to the donor compartment (Table 1). The permeation rate constant of ES was not changed significantly by adding cyanine (OCT-inhibitor) to the donor compartment in either direction (data not shown).

DISCUSSION

The immortalized bovine mammary epithelial cell line (BME-UV) that was used for the present study was selected for its ability to establish a cell polarity and form tight junctions between adjacent cells when grown as a monolayer on a filter support that acts as a barrier between dissimilar fluid compartments (Schmidt *et al.*, 2001). Accepted criteria for monitoring the quality of monolayers in transport studies include transepithelial electrical resistance (R_{te}††††), visualization of epithelial integrity and permeability to hydrophilic markers like mannitol (Artursson *et al.*,(1996). The diffusion of mannitol across the epithelial monolayers is limited to the paracellular route (Artursson *et al.*, (1996)). In the present study, the P_{app} of mannitol ($0.91\pm0.4 \times 10^{-6}$ cm/s) across monolayer of BME-UV cells was comparable to those obtained in mouse mammary epithelial monolayer (1.94×10^{-6} cm/s) (Toddywalla *et al.*, 1997) and in human mammary epithelial monolayer ($2.6 \times$

††††Transepithelial Electrical Resistance

10^{-6} cm/s) (Kimura *et al.*, 2006), indicating that the monolayer of BME-UV cells is tight enough to evaluate transcellular drug transport.

The Bronaugh-type flow-through diffusion cell system that was used in this study has numerous advantages. The system allows for automatic sampling of receiver fluid to accurately monitor the absorption/time profile. Multiple replicates of treatment conditions could be obtained cost-effectively. In addition, the permeability to a test compound in two directions, from the Ap-to-BL and BL-to-Ap directions, and both passive and active transport processes could be studied. Most importantly, receiver fluid flow maintained effective infinite sink conditions in the receiver compartment throughout the experiment, which reduces the effect of test compound molecules diffusing back from the basolateral to the apical side or vice versa, thereby isolating directional processes of drug movement across the epithelial barrier (Bronaugh and Stewart, 1985).

The directionality of both TEA and ES transport suggests the functional expression of OCT and OAT, respectively, in the BME-UV cells. A similar polarized transport process for TEA has been identified previously in human mammary epithelial monolayers and attributed to the human organic cation transporters (Kimura *et al.*, 2006). Several *in vivo* studies have also suggested that organic anions (N4-acetylated para-aminohippurate, N4-acetylated sulfanilamide and nitrofurantoin) are actively transported across the bovine, caprine, and rat mammary epithelium into milk, respectively since their observed Milk/Plasma ratio was 3 to 30 times higher than the predicted value which calculated based on the pKa of drug and the pH of both milk and plasma (Rasmussen, 1969a; Rasmussen, 1969b; Kari *et al.*, 1997). Other studies confirmed the presence of a cimetidine transport system in the rat mammary epithelium that is saturable and inhibited by ranitidine (McNamara *et al.*, 1996).

Decreasing values of the permeation rate constant with increasing concentrations being added to the donor compartment serves as further evidence for the involvement of carrier mediated transport in the movement of TEA and ES across the BME-UV monolayer. This is because saturation of the carriers at higher concentrations leads to the rate of movement no longer being proportional to the concentration gradient. The permeation rate therefore decreases as it is normalized to the concentration gradient. The permeation rate constant of TEA decreased significantly when initial concentrations were increased from 100 M to 500 M and then 1000M, suggesting that the carriers were saturated at these high concentrations. Increasing the initial concentration of ES from 100 μM to 500 μM also decreased the permeation rate. However, an increase from 500 |aM to 1000 |aM in the donor compartment had no effect on the permeation rate constant of ES, This may explained by a significant contribution of passive diffusion to the movement of ES across the mono layer of BME-UV cells. The same conclusion was also found by Koljonen *et al* (2008) and Neuhoff *et al* (2005) who studied passive and active transport mechanisms of acidic drugs across Caco-2 cells.

The significant decrease in the permeation rate constant of both TEA and ES in the BL-to-Ap direction after adding OCT and OAT inhibitors, respectively, also supports the hypothesis that the directional movement of organic cations and anions across the BME-UV mono layer is mediated by transporters. Since the test compounds crossed the mono layer in the presence of inhibitors, albeit at a significantly lower rate, passive diffusion may play a role in the movement of these compounds across the mammary epithelium. Further studies are needed to determine the concentrations of inhibitors that are required to saturate the transporters. To the authors' knowledge, this is the first report of the presence of carrier-mediated directional transport in functionally-differentiated bovine mammary epithelial monolayers. The BME-UV monolayer appeared to be more permeable to TEA compared to ES, as evidenced by a lower value for *Papp* for the latter. This could be explained by

differences in the number of organic cation and anion transporters per BME-UV cell. It may also be explained by the differences in the physicochemical properties of these compounds and their affinity to the transporters that are present in the BME-UV cells.

Transporters play a key role in governing bodily drug absorption, distribution, and elimination. They also play a role in drug-drug interactions and interindividual differences in pharmacokinetic profiles (Ito *et al.*, 2005). Knowledge of transporter expression in the mammary epithelium and their apical or basolateral directionality is therefore a necessary step to study the mechanisms that result in xenobiotic residues in milk. This is an important concern to the scientists in both pharmaceutical industry and public health field.

A better understanding of the mechanisms that contribute to the movement of xenobiotics across the mammary epithelium would enhance our ability to predict milk xenobiotic concentrations at specific times following drug administration, or after accidental exposure to environmental contaminants such as mycotoxins, pesticides, and toxic metals. The effect of drug-drug interactions and changes in milk composition could also be explored. Milk loss could thereby be minimized by determining the exact amount of milk that should be discarded based on early information about the transport characteristics of potential xenobiotics into milk. Specific transporters responsible for the excretion of these xenobiotics into milk could also be blocked.

The expression of members of the OCT and OAT families on the membrane of the BMEUV cells may relate to a physiological role of these transporters in milk production. Conversely, they may impart a protective role to the lactating mammary gland extruding potentially toxic xenobiotics into milk. But this increases the risk of milk residues that are potentially harmful to suckling off-spring or the human consumer. The potential involvement of drug efflux transporters, such as P-Glycoprotein (P-gp‡‡‡‡) and MRP, on the monolayer of BME-UV cells remains unknown and further studies should be performed. Further studies are also required to quantify the activity of these transporters and determine the substrate concentrations that lead to saturation.

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References

1. 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. Journal of Pharmacology and Experimental Therapeutics. 2002; 303:487–496. [PubMed: 12388627]
2. Artursson, P.; Karlsson, J.; Ocklind, G.; Schipper, N. Studying transport processes in absorptive epithelia. In: Shaw, AJ., editor. The Epithelial Cell Culture: A Practical Approach. 6th edn.. Oxford; New York: 1996. p. 111-133.
3. Atkinson HC, Begg EJ. Prediction of drug distribution into human milk from physicochemical characteristics. Clinical Pharmacokinetics. 1990; 18:151–167. [PubMed: 2318008]
4. Begg EJ, Atkinson HC, Duffull SB. Prospective evaluation of a model for the prediction of milk:plasma drug concentrations from physicochemical characteristics. BritishJournal of Clinical Pharmacology. 1992; 33:501–505. [PubMed: 1524962]

‡‡‡‡P-Glycoprotein

5. Bronaugh RL, Stewart RF. Methods for in vitro percutaneous absorption studies IV: The flow-through diffusion cell. Journal of Pharmaceutical Sciences. 1985; 74:64–67. [PubMed: 3981421]
6. Dantzler WH, Wright SH. The molecular and cellular physiology of basolateral organic anion transport in mammalian renal tubules. Biochimica Et Biophysica Acta. 2003; 1618:185–193. [PubMed: 14729155]
7. FDA. Guideline For Metabolism Studies And For Selection Of Residues For Toxicological Testing. http://www.fda.gov/cvm/Guidance/guideline3pt1.html
8. Gehring R, Baynes RE, Riviere JE. Application of risk assessment and management principles to the extralabel use of drugs in food-producing animals. Journal of Veterinary Pharmacology and Therapeutics. 2006; 29:5–14. [PubMed: 16420296]
9. Gehring R, Smith GW. An overview of factors affecting the disposition of intramammary preparations used to treat bovine mastitis. Journal of Veterinary Pharmacologyand Therapeutics. 2006; 29:237–241.
10. Goralski KB, Lou G, Prowse MT, Gorboulev V, Volk C, Koepsell H, Sitar DS. The cation transporters rOCT1 and rOCT2 interact with bicarbonate but play only a minor role for amantadine uptake into rat renal proximal tubules. Journal of Pharmacology andExperimental Therapeutics. 2002; 303:959–968.
11. Goralski KB, Sitar DS. Tetraethylammonium and amantadine identify distinct organic cation transporters in rat renal cortical proximal and distal tubules. Journal ofPharmacology and Experimental Therapeutics. 1999; 290:295–302.
12. Ito K, Suzuki H, Horie T, Sugiyama Y. Apical/basolateral surface expression of drug transporters and its role in vectorial drug transport. Pharmaceutical Research. 2005; 22:1559–1577. [PubMed: 16180115]
13. Kari FW, Weaver R, Neville MC. Active transport of nitrofurantoin across the mammary epithelium in vivo. Journal of Pharmacology and Experimental Therapeutics. 1997; 280:664–668. [PubMed: 9023277]
14. Kimura S, Morimoto K, Okamoto H, Ueda H, Kobayashi D, Kobayashi J, Morimoto Y. Development of a human mammary epithelial cell culture model for evaluation of drug transfer into milk. Archives ofPharmacal Research. 2006; 29:424–429.
15. Koljonen M, Rousu K, Cierny J, Kaukonen AM, Hirvonen J. Transport evaluation of salicylic acid and structurally related compounds across Caco-2 cell monolayers and artificial PAMPA membranes. European Journal of Pharmaceutics and Biopharmaceutics. 2008; 70:531–538. [PubMed: 18582575]
16. Kusuhara H, Sekine T, Utsunomiya-Tate N, Tsuda M, Kojima R, Cha SH, Sugiyama Y, Kanai Y, Endou H. Molecular cloning and characterization of a new multispecific organic anion transporter from rat brain. Journal of Biological Chemistry. 1999; 274:13675–13680. [PubMed: 10224140]
17. Lash LH, Putt DA, Xu F, Matherly LH. Role of rat organic anion transporter 3 (Oat3) in the renal basolateral transport of glutathione. Chemico-Biological Interactions. 2007; 170:124–134. [PubMed: 17719021]
18. Lau RJ, Emery MG, Galinsky RE. Unexpected accumulation of acyclovir in breast milk with estimation of infant exposure. Obstetrics & Gynecology. 1987; 69:468–471. [PubMed: 3808527]
19. Lehr, C-M. Cell Culture Models of Biological Barriers: In vitro Test Systems for Drug Absorption and Delivery. Taylor & Francis, Inc.; New York: 2002. p. 143-289.
20. Markaki P, Melissari E. Occurrence of aflatoxin M1 in commercial pasteurized milk determined with ELISA and HPLC. Food Additives & Contaminants. 1997; 14:451–456. [PubMed: 9328529]
21. McNamara PJ, Meece JA, Paxton E. Active transport of cimetidine and ranitidine into the milk of Sprague Dawley rats. Journal of Pharmacology and Experimental Therapeutics. 1996; 277:1615–1621. [PubMed: 8667230]
22. Molina A, Molina MP, Althaus RL, Gallego L. Residue persistence in sheep milk following antibiotic therapy. The Veterinary Journal. 2003; 165:84–89. [PubMed: 12618076]
23. Neuhoff S, Ungell AL, Zamora I, Artursson P. pH-Dependent passive and active transport of acidic drugs across Caco-2 cell monolayers. European Journal of Pharmaceutical Sciences. 2005; 25:211–220. [PubMed: 15911216]

24. Nguyen DA, Neville MC. Tight junction regulation in the mammary gland. Journal of Mammary Gland Biology and Neoplasia. 1998; 3:233–246. [PubMed: 10819511]
25. Patra RC, Swarup D, Kumar P, Nandi D, Naresh R, Ali SL. Milk trace elements in lactating cows environmentally exposed to higher level of lead and cadmium around different industrial units. Science of the Total Environment. 2008; 404:36–43. [PubMed: 18657303]
26. Przyrembel H, Heinrich-Hirsch B, Vieth B. Exposition to and health effects of residues in human milk. Advances in Experimental Medicine and Biology. 2000; 478:307–325. [PubMed: 11065082]
27. Quesnell RR, Erickson J, Schultz BD. Apical electrolyte concentration modulates barrier function and tight junction protein localization in bovine mammary epithelium. The American Journal of Physiology - Cell Physiology. 2007a; 292:C305–318.
28. Quesnell RR, Han X, Schultz BD. Glucocorticoids stimulate ENaC upregulation in bovine mammary epithelium. The American Journal of Physiology - Cell Physiology. 2007b; 292:C1739–1745.
29. Rasmussen F. Active mammary excretion of N4-acetylated p-aminohippuric acid. Acta Veterinaria Scandinavica. 1969a; 10:193–194. [PubMed: 5354237]
30. Rasmussen F. Active mammary excretion of N4-acetylated sulphanilamide. Acta Veterinaria Scandinavica. 1969b; 10:402–403. [PubMed: 5374285]
31. Rusoff LL. Milk: its nutritional value at a low cost for people of all ages. Journal of Dairy Science. 1970; 53:1296–1302. [PubMed: 5468819]
32. Sai Y, Tsuji A. Transporter-mediated drug delivery: recent progress and experimental approaches. Drug Discovery Today. 2004; 9:712–720. [PubMed: 15341785]
33. Schmidt CR, Carlin RW, Sargeant JM, Schultz BD. Neurotransmitter-stimulated ion transport across cultured bovine mammary epithelial cell monolayers. Journal of Dairy Science. 2001; 84:2622–2631. [PubMed: 11814018]
34. Schomig E, Babin-Ebell J, Russ H. 1,1'-diethyl-2,2'-cyanine (decynium22) potently inhibits the renal transport of organic cations. Naunyn-Schmiedeberg's archives of pharmacology. 1993; 347:379–383.
35. Shennan DB, Peaker M. Transport of milk constituents by the mammary gland. Physiological Reviews. 2000; 80:925–951. [PubMed: 10893427]
36. Takeda M, Narikawa S, Hosoyamada M, Cha SH, Sekine T, Endou H. Characterization of organic anion transport inhibitors using cells stably expressing human organic anion transporters. European Journal of Pharmacology. 2001; 419:113–120. [PubMed: 11426832]
37. Toddywalla VS, Kari FW, Neville MC. Active transport of nitrofurantoin across a mouse mammary epithelial monolayer. Journal of Pharmacology and Experimental Therapeutics. 1997; 280:669–676. [PubMed: 9023278]
38. USDA. Trends in Consumer Food Choices Within the MyPyramid Milk Group, Nutrition Insight 35. In: U. S. D. o. A. C. f. N. P. a. Promotion. , editor. U.S. Department of Agriculture; 2007. http://www.cnpp.usda.gov/Publications/NutritionInsights/Insight35.pdf
39. Westin JB. Carcinogens in Israeli milk: a study in regulatory failure. International Journal of Health Services. 1993; 23:497–517. [PubMed: 8375952]
40. Wilson G. Cell culture techniques for the study of drug transport. European Journal of Drug Metabolism and Pharmacokinetics. 1990; 15:159–163. [PubMed: 2200685]
41. Yamashita S, Furubayashi T, Kataoka M, Sakane T, Sezaki H, Tokuda H. Optimized conditions for prediction of intestinal drug permeability using Caco-2 cells. European Journal of Pharmaceutical Sciences. 2000; 10:195–204. [PubMed: 10767597]

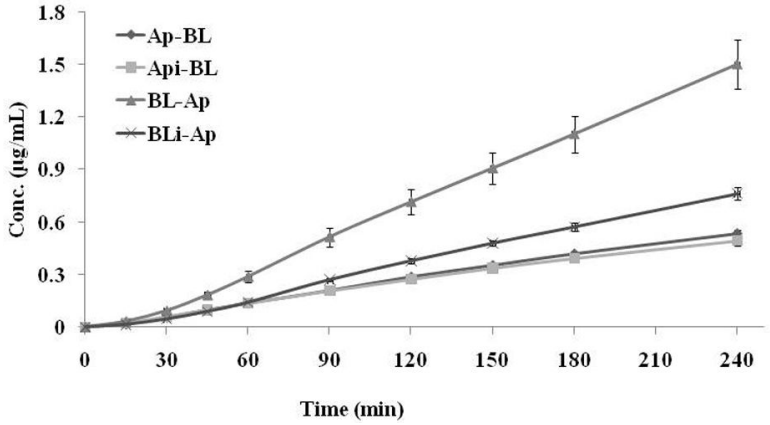

Figure 1.

In vitro cumulative concentration-time profiles of tetraethylammonium permeation across monolayer of BME-UV cells from apical to basolateral (Ap-to-BL) and BL-to-Ap directions in the absence or presence of inhibitors (Api-to-BL) and (BLi-to-Ap). Data represent mean ± SD of three replicates.

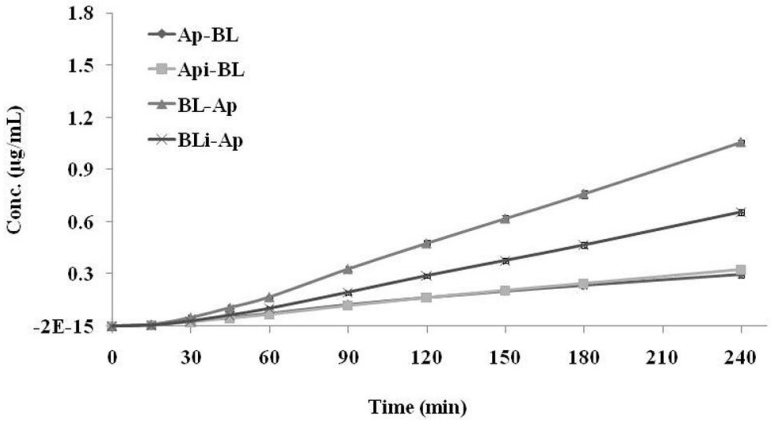

Figure 2.

In vitro cumulative concentration-time profiles of estrone sulphate permeation across monolayer of BME-UV cells from apical to basolateral (Ap-to-BL) and BL-to-Ap directions in the absence or presence of inhibitors (Api-to-BL) and (BLi-to-Ap). Data represent mean ± SD of three replicates.

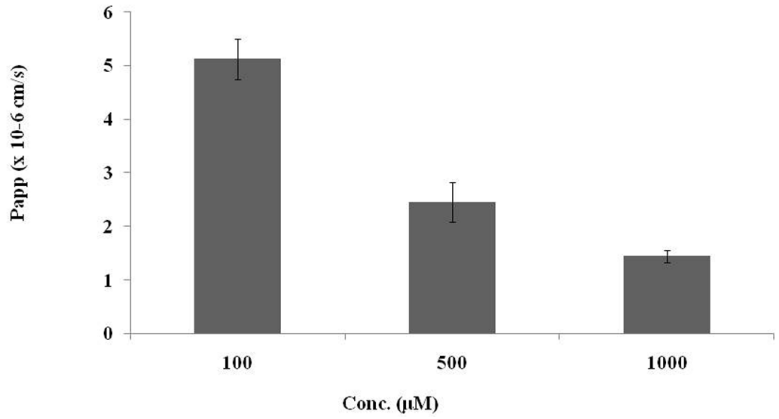

Figure 3.

Permeation rate constant of tetraethylammonium across monolayer of BME-UV cells from BL-to-Ap direction. Data represent mean ± SD of three replicates. Different number of stars indicats the permeation rate constants are significantly different ($P < 0.05$).

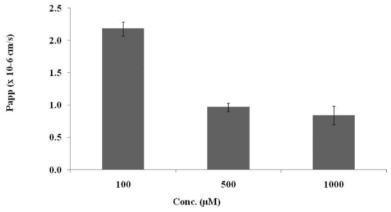

Figure 4.

Permeation rate constant of estrone sulphate across monolayer of BME-UV cells from BL-to-Ap direction. Data represent mean ± SD of three replicates. Different number of stars indicats the permeation rate constants are significantly different ($P < 0.05$).

Table 1

The permeation rate constants (P_{app}) for model substrates across monolayer of BME-UV cells or support alone from apical to basolateral (Ap-to-BL) or BL-to-Ap directions in the absence or presence of inhibitors (Api-to-BL) and (BLi-to-Ap).

	P_{app}±SD(x10^{-6}cm/s)				
	Ap-to-BL¥	**Api-to-BL¥**	**BL-to-Ap¥**	**BLi-to-Ap¥**	**Support**
TEA	2.0±.02	1.9±.06	5.1±.4*	2.6±.2**	161±18***
ES	0.66±.06	0.62±.07	2.2±.1ж	1.4±0.8жж	122±34жжж