Short Communication

BASOLATERAL ACTIVE UPTAKE OF NITROFURANTOIN IN THE CIT3 CELL CULTURE MODEL OF LACTATION

(Received September 18, 2002; accepted February 24, 2003)

This article is available online at http://dmd.aspetjournals.org

ABSTRACT:

Nitrofurantoin and other agents are actively transported into human and rodent milk. The purpose of this study was to determine whether nitrofurantoin active transport across mammary epithelia occurs basolaterally or apically, using the CIT3 cell culture model of lactation. The CIT3 model actively transports nitrofurantoin in the basolateral to apical direction. Basolateral to apical permeability [92.9 ± 6.6 (μl/h)/cm²] was differentially decreased by unlabeled nitrofurantoin (250 μM) on the basolateral, apical, or both sides [49.5 ± 1.8, 57.9 ± 1.4, or 48.5 ± 1.6 (μl/h)/cm², respectively]. Apical to basolateral permeability [27.6 ± 1.8 (μl/h)/cm²] was increased in the presence of unlabeled nitrofurantoin (250 μM) on the basolateral, apical, or both sides [36.4 ± 1.5, 39.9 ± 0.7, 42.4 ± 1.1 (μl/h)/cm², respectively]. These data indicate a basolateral active uptake mechanism for nitrofurantoin, which remains to be identified. This mechanism may influence the exposure of suckling infants to xenobiotics, as well as having potentially toxic effects on the lactating mammary epithelium and possibly altering the nutritional quality of the milk.

Breastfeeding has numerous benefits but also some disadvantages, including potential xenobiotic exposure to the suckling infant (Gerk et al., 2001a). Although most drugs enter milk by passive diffusion (Fleishaker and McNamara, 1988), some drugs are actively transported into milk. Previously, we have demonstrated active transport of nitrofurantoin into human and rat milk, reaching milk-to-serum concentration ratios of 20 to 100 times those predicted by diffusion, respectively (Gerk et al., 2001a,b). Furthermore, active, saturable transport of nitrofurantoin across a murine cell culture model of lactation has been demonstrated (Toddywalla et al., 1997). Previously published data in lactating rats and/or the CIT3 model shows that nitrofurantoin transport is also sodium-dependent; inhibited by cimetidine, diprydamole, and purine nucleosides; but insensitive to probenecid, pyrimidine nucleosides, and nucleobases (Gerk et al., 2001b, 2002). However, the mechanism has not been identified and further elucidation is needed to gain a better understanding of how certain drugs are concentrated into milk.

For nitrofurantoin to be concentratively transported from the blood (or serum) into milk, it must cross the basolateral (blood-facing) and apical (milk-facing) cell membranes. As a result, the active transport step may occur as either basolateral uptake or apical efflux. A basolateral active uptake mechanism for nitrofurantoin, which remains to be identified, the location of the active transport step has functional consequences and may yield insights into the nitrofurantoin transport mechanism. Therefore, the purpose of this study was to determine whether nitrofurantoin active transport across mammary epithelia occurs basolaterally or apically.

Materials and Methods

The 14C-nitrofurantoin (58 mCi/mmol) was obtained from Chemsyn Labs (Lexena, KS) and was purified as described previously (Gerk et al., 2002). Dulbecco’s modified Eagle’s medium with Ham’s F12 medium was obtained from Invitrogen (Carlsbad, CA); hydrocortisone and insulin were from Sigma-Aldrich (St. Louis, MO); ovine prolactin was from the National Hormone and Pituitary Program (Dr. A. F. Parlow, Torrance, CA); and fetal bovine serum was from Gemini BioProducts (Calabasas, CA). All other chemicals were obtained from Sigma-Aldrich.

CIT3 cells were cultured according to the previously published protocol (Toddywalla et al., 1997). Briefly, the cells (passages 15 to 21) were grown in a growth medium containing Dulbecco’s modified Eagle’s medium with Ham’s F12 medium supplemented with 2% fetal bovine serum, epidermal growth factor (5 ng/ml), insulin (10 μg/ml), penicillin (100U/ml), and streptomycin (100 μg/ml). The cells were seeded on 3407 Snapwell polycarbonate 0.4 μm, 1 cm² filter inserts (Corning Inc., Acton, MA), then differentiated in a secretion medium, in which epidermal growth factor was replaced with prolactin (3 μg/ml) and hydrocortisone (3 μg/ml).

The experiments were performed at pH 7.4 in an isotonic saline buffer free of antibiotics, serum, hormones, or proteins, containing 111 mM NaCl, 22 mM NaHCO3, 4.2 mM KHCO3, 1.05 mM CaCl2, 0.41 mM MgSO4, 0.14 mM MgCl2, 20 mM glucose, and 10 mM HEPES. The calculated osmolarity was adjusted to 280 to 305 mOsm/liter with mannitol. The directionality and inhibition of 14C-nitrofurantoin concentrative transport permeability were determined by placing 14C-nitrofurantoin on either the basolateral (B1) or the apical (A) side and using cold nitrofurantoin on the donor side; ANOVA, analysis of variance.
Comparisons are shown by dashed lines where $p$ values (Pe) were determined, by rearranging the equation lateral to apical (B-A) or apical to basolateral (A-B) effective permeability.

80, 100, and 120 min and analyzed by liquid scintillation counting. Baso-

mean

Data were analyzed by one-way ANOVA with Bonferroni

lysed overnight with 2 ml of 10% trichloroacetic acid. A 1-ml aliquot of the

the snapwells were rinsed quickly three times in ice-cold buffer, excess fluid

was complete, and less than 5% of the radiolabel was transferred to the

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opposite side in 120 min. By contrast, an apical efflux mechanism would function

association with the snapwells in the presence of cold nitrofurantoin.

As described previously (Gerk et al., 2002), the diffusion chambers were assembled and

filled with 5 ml of the buffer with or without cold nitrofurantoin. Samples

(100 µl) were taken from the basolateral and apical sides at 1, 20, 40, 60, 80, 100, and 120 min and analyzed by liquid scintillation counting. Baso-

lateral to apical (B-A) or apical to basolateral (A-B) effective permeabilities

(Pe) were determined, by rearranging the equation $J = A^*Pe^*Cd$ to

solve for $Pe = J/(A^*Cd)$, where $J$ is flux, $A$ is snapwell area (1 cm²), and

Cd is the 14C-nitrofurantoin concentration on the donor side. Mass balance was complete, and less than 5% of the radiolabel was transferred to the opposite side in 120 min.

At the end of the experiments, the diffusion chambers were disassembled, the snapwells were rinsed quickly three times in ice-cold buffer, excess fluid was drained, and the snapwells were placed in 50-ml centrifuge tubes, then lysed overnight with 2 ml of 10% trichloroacetic acid. A 1-ml aliquot of the lysate was taken for liquid scintillation counting. Permeability and association data were analyzed by one-way ANOVA with Bonferroni’s multiple comparisons post hoc with $\alpha = 0.05$, where the same prospectively determined comparisons are indicated in Fig. 1, B and C.

Results and Discussion

To characterize nitrofurantoin active transport, the directionality and inhibition were examined. Figure 1A demonstrates the linearity of nitrofurantoin flux during the course of the experiment. As expected in B-A active transport, flux was highest in the B-A direction and lowest in the opposite direction (A-B). Flux in either direction collapsed to a common value in the presence of 250 µM cold nitrofurantoin, indicating effective inhibition of the active transporter. We consider this flux diffusion since it is independent of concentration up to 2 mM, near the solubility limit of nitrofurantoin at pH 7.4 (Gerk et al., 2002).

The effects of a saturating concentration of nitrofurantoin on the directional permeability of nitrofurantoin shown in Fig. 1B indicate a basolateral uptake mechanism as an important step in nitrofurantoin active transport across this model. First, the direction of active transport is basolateral to apical, as expected for active transport into milk and as demonstrated previously (Toddywalla et al., 1997; Gerk et al., 2002). The difference in directional transport was eliminated when cold nitrofurantoin was present on both sides, consistent with a saturable mechanism. Basolateral to apical permeability was inhibited as effectively by placing cold nitrofurantoin only on the basolateral (cis) side as it was by placing it on both sides. B-A permeability was less effectively inhibited when cold nitrofurantoin was placed only on the apical (trans) side. This suggests a basolateral active uptake mechanism. A-B permeability increased when cold nitrofurantoin was present on either or both sides, consistent with inhibiting a basolateral active uptake mechanism trying to maintain a concentration gradient. Since nitrofurantoin has a high diffusional permeability (half of total B-A permeability), diffusion may result in concentrations near the transporter high enough to inhibit it.

The snapwell association data are consistent with a basolateral active uptake mechanism. Since only 1.3 to 15% of the radioactivity was bound to the blank filters, the majority of 14C-nitrofurantoin bound to the snapwells was associated with the cell layer (85 to 98.7%), so these data mainly represent accumulation and binding in the cell layer rather than the filter. 14C-Nitrofurantoin association with CIT3 snapwell filters was greater when 14C-nitrofurantoin was presented on the basolateral side than when it was presented on the apical side, or in the presence of cold nitrofurantoin on either or both sides.

The other comparisons performed were not significant. The data are consistent with a basolateral uptake mechanism, which would be expected to result in less accumulation in the cell layer when the transporter is saturated or when nitrofurantoin was presented on the opposite side. By contrast, an apical efflux mechanism would function to minimize cellular entry from the apical compartment, and inhibiting it would increase the intracellular concentration. However, when nitrofurantoin was presented to the apical side, there was no increased association with the snapwells in the presence of cold nitrofurantoin on either or both sides, again supporting a basolateral active uptake mechanism.

An implication of a basolateral active uptake mechanism concentrating its substrates into cells would be the potential for cellular toxicity, as seen with organic anion transporter 1 concentrating ochratoxin A into renal proximal tubule cells (Tsuda et al., 1999). For nitrofurantoin, oxidative stress occurs through reduction to its radical anion by NADPH-cytochrome P450 reductase, which generates superoxide anion radicals, and causes formation of hydrogen peroxide, leading to depletion of reduced glutathione and protein thiols, and lipid peroxidation in rat lungs (Suntres and Shek, 1992). Also, nitrofurantoin causes dose-dependent depletion of reduced glutathione and protein thiols as well as increased bile flow in the isolated perfused rat
liver (Hoener et al., 1989), and time- and concentration-dependent enhancement of membrane damage due to hydroperoxides or diamide in rat liver mitochondria (Carbonera et al., 1988). As a result of basolateral active uptake, high intracellular concentrations of nitrofurantoin could cause oxidative stress, in the lactating mammary epithelial cells, depending on the activity of NADPH-cytochrome P450 reductase or other enzymes in the lactating mammary epithelium. Furthermore, if nitrofurantoin displaces uptake of an endobiotic needed to support cell metabolism and/or milk production, the health of the lactating mammary epithelial cells and/or the nutritive qualities of the milk could also be adversely affected. The uptake activity of this transporter could expose the suckling infant to xenobiotics, and toxicity to the lactating mammary epithelium could affect the mother as well as the nutritional quality of the milk.

The present results, along with previously published data on nitrofurantoin active transport in lactating rats and/or the CIT3 model, can help eliminate several nitrofurantoin transporter candidates. Previous data in lactating rats and/or the CIT3 model show that nitrofurantoin transport is saturable, sodium-dependent, inhibited by cimetidine, dipyridamole, and purine nucleosides, but relatively insensitive to probenecid, pyrimidine nucleosides, and nucleobases (Gerk et al., 2001b, 2002). These data indicate that nitrofurantoin active transport is inconsistent with the ABC drug-resistance transporters (Litman et al., 2001), bile salt transporters (Meier and Stieger, 2002), organic anion transporters (Dresser et al., 2001), and known nucleoside/nucleobase transporters. Future studies could compare the expression patterns of other transporter candidates with the basolateral location of the nitrofurantoin active transporter.

In conclusion, nitrofurantoin transport across the CIT3 model involves a basolateral active uptake mechanism as well as nonsaturatable components. This mechanism may influence the exposure of suckling infants to xenobiotics as well as potentially having toxic effects on the lactating mammary epithelium. Further studies are needed to determine the identity of the nitrofurantoin active transporter.

Acknowledgments. We thank Linda Hanson and Dr. Peggy Neville for help with CIT3 cultures and acknowledge the support of Dr. Mary Vore for the preparation of this manuscript.

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