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Abstract: The monolayer-forming, human choriocarcinoma cell line, BeWo, was used to study the mechanisms of monocarboxylic acid transport across the human trophoblast. Benzoic acid, acetic acid, and lactic acid were used as markers for monocarboxylic acid carrier-mediated transport. The uptake of benzoic acid by BeWo cells was saturable ($K_t = 0.6 \pm 0.3$ mM) at higher concentrations and significantly inhibited by typical metabolic inhibitors, sodium azide and 2,4-dinitrophenol. A selection of different monocarboxylic acids, including a natural substrate lactic acid, also substantially inhibited the uptake of benzoic acid and acetic acid by BeWo cells, whereas dicarboxylic acids did not affect the uptake of either marker. Monocarboxylic acid uptake was pH-dependent and inhibited by carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP), a protonophore. Kinetic analysis using Lineweaver-Burk plots revealed that monocarboxylic acids competitively inhibited the uptake of benzoic, lactic, and acetic acid by BeWo cells. In transport experiments, the permeation of benzoic acid from apical-to-basolateral side was greater than the permeation from the basolateral-to-apical side, and the transport of benzoic acid from apical-to-basolateral side was inhibited by monocarboxylic acids. The findings obtained in the present study confirm the existence of an asymmetric, carrier-mediated transport system for monocarboxylic acids across the BeWo cell, a representative of the human trophoblast.

Text of paper:

CARRIER-MEDIATED TRANSPORT OF MONOCARBOXYLIC ACIDS IN BEWO CELL MONOLAYERS AS A MODEL OF THE HUMAN TROPHOBLAST

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INTRODUCTION

The placenta, in part, serves as a semipermeable barrier separating the maternal and fetal circulations. The placental barrier is comprised of a single layer of trophoblasts that have an important role in controlling the passage of molecules from mother to fetus. In general, the absorption of drugs across the single layer of trophoblasts can be explained in terms of the pH-partition hypothesis which is well illustrated by passive diffusion mechanisms.[1,2] However, with the use of brush-border-membrane vesicle techniques or cultured cells[3], it has become increasingly clear that there are a number of carrier-mediated transport mechanisms present at the placental barrier including systems for amino acids[3,4], transferrin[5], and dopamine[6]. These carriers take on pharmaceutical relevance due to the fact that nutrients, hormones, as well as, drugs and drugs of abuse can be transported across the placental barrier by these mechanisms.[7]

Carrier-mediated transport mechanisms for monocarboxylic acids exist in many cell types.[8-10] In fact, there are known to be at least seven putative monocarboxylic acid transporter (MCT) isoforms. The mRNA representing five of the seven MCTs has been shown in human placenta[11]. Using brush border membrane preparations, a functional, proton-dependent MCT has been observed in tissues from rat[12,13] and human[14] placenta. A functional, bidirectional, and carrier-mediated mechanism for lactic acid, presumably a MCT, has also been demonstrated in the perfused human placental lobe model[15]. In addition to the transport of lactic acid,[11,14,15] a MCT at the placental barrier presents a potential mechanism by which monocarboxylic acid drugs and drugs of abuse may readily distribute between maternal and fetal compartments. Therefore, the

characterization of a placental MCT system can contribute to an understanding of the trophoblast transport mechanisms controlling fetal exposure to substances possessing a monocarboxylic acid constituent.

The objective of this study was to investigate the presence of a functional MCT in BeWo cell monolayers, a representative human trophoblast culture system.[16] BeWo cell monolayers have been applied to studies of the polarized trans-trophoblast transport of serotonin and transferrin, monoamine uptake processes, and relevant nutrient uptake and transport systems.[16-18] The existence of MCT systems in the BeWo cells would provide an *in vitro* tool representative of the human trophoblast to characterize trans-placental transport mechanisms that influence drug distribution in pregnancy. The BeWo cell line is particularly attractive for the studies of drug distribution at the placenta barrier because it is stable, relatively easy to maintain by passage, and grows to a confluent monolayer in relatively short period of time. Indeed, in contrast to primary cultures, the cell line is one of the few existing trophoblast cell culture systems to form a confluent monolayer that allows trans-trophoblast transport studies. More importantly, the BeWo cell displays morphological properties and biochemical marker enzymes and hormone secretion common to normal trophoblasts.[16]

MATERIALS AND METHODS

Materials. [^{14}C]Benzoic acid (55 Ci/mol), [^{14}C]-L-lactic acid (150 Ci/mmol), and [^{14}C]acetic acid (55 Ci/mol) were obtained from American Radiolabeled Chemicals (St. Louis, MO). Fetal bovine serum (FBS) was from JRH Bioscience (Lenexa, KS). Penicillin-streptomycin as a mixture was from Gibco (Gaithersburg, MD). Translucent

polycarbonate filters (13 mm diameter, 0.4 μm pore size) were purchased from Fisher Scientific. All other chemicals were of the purest available analytical grade and purchased from Fisher or Sigma (St. Louis, MO).

Cell Culture. The BeWo cell line was originally derived from a human choriocarcinoma. The BeWo clone (b30) was obtained from Dr. Alan Schwartz (Washington University, St. Louis, MO). The cells were cultured by the methods previously described and the cells used in these experiments were from passages 28 to 40.[16]. Briefly, the cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% heat-inactivated FBS containing 0.37 % sodium bicarbonate and 1% antibiotics (10,000 U/ml penicillin and 10 mg/ml streptomycin). The cells were maintained in 175-cm^2 flasks at pH 7.4 under 5% CO_2 and 95% humidity at 37°C. The cells were harvested by exposure to a trypsin-EDTA solution (0.25% trypsin and 0.02% EDTA in HBSS) and passed onto 12-well tissue culture plates or polycarbonate membranes coated with rat tail collagen in 100-mm culture dishes. With the seeding density of 10,000 cells/cm^2, the cells formed confluent monolayers between 2 and 3 days and were used for experiments at that time.

Uptake Experiments. The BeWo cells at confluence were washed twice with Hank's balanced salt solution (HBSS; 136.7 mM NaCl, 0.385 mM Na_2HPO_4, 0.441 mM KH_2PO_4, 0.952 mM $CaCl_2$, 5.36 mM KCl, 0.812 mM $MgSO_4$, 25 mM D-glucose and 10 mM HEPES (2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid) for adjustment to pH 7.0 or 7.5 or 10 mM MES (2-morpholinoethanesulfonic acid, monohydrate) for adjustment to pHs <6.5 and then the test solution containing [^{14}C]benzoic acid or [^{14}C]acetic acid or [^{14}C]-L-lactic acid was added at 0.25 μCi/ml as a marker for

monocarboxylic acid carrier-mediated transport. The pH of the test solution was 6.0 except in the pH-dependent uptake experiments. After 30 s, the test solution was aspirated away and the cells were washed with ice-cold HBSS three times. For quantitation of drug uptake, the cells were suspended in 1.0 N NaOH and the suspension was incubated at 37°C overnight at which point, a half volume of 2.0 N HCl was added. Radioactivity was quantitated using a liquid scintillation counter. Cellular protein was quantified using a protein assay kit (Pierce) with bovine serum albumin as a standard. Details of the conditions for each experiment are given in the Figure Legends or Table Footnotes.

In one series of experiments, an equivalent amount of choline chloride replaced sodium chloride in the HBSS and was used as a sodium-depleted buffer.

Transport Experiment. A horizontal Side-Bi-Side diffusion apparatus (Crown Glass) was used to measure the transmonolayer permeability of cells grown on the surface of 0.4 µm pored polycarbonate filters, as previous described.[16] The cells faced the donor chamber as the apical side, and the polycarbonate membrane faced the receiver chamber as the basolateral side. The water jacket surrounding the donor and the receiver chambers was thermostated at 37°C. The contents of each chamber were continuously stirred at 600 rpm with magnetic stir bars. When the apical-to-basolateral transport studies were performed, the pH of the donor (apical) side was 6.0 and that of receptor (basolateral) side was 7.4. When the basolateral-to-apical transport studies were performed, the pH of the donor (basolateral) side was 6.0 and that of receptor (apical) side was 7.4. The concentration of added [^{14}C]benzoic acid was 0.25 µCi/ml. A 0.2-ml aliquot from the receiver chamber was taken at several time points up to 60 min.

Calculation of Apparent Permeability Coefficients. Apparent permeability coefficients for the monolayers of cells, P_e, were calculated from the following relationship

$$1/P_t = 1/P_e + 1/P_m \qquad \text{Eq. (1)}$$

where P_t is apparent permeability coefficient for the collage-coated membrane in the presence of monolayers, and P_m is the apparent permeability coefficient for collagen-coated polycarbonate membrane alone.[19]

Michaelis-Menten Parameters. To estimate the values of the kinetic parameters of saturable uptake by BeWo cells, the uptake rate (J) was fitted to the following equation, which consists of both saturable and nonsaturable linear terms, using a nonlinear least-squares regression analysis program MULTI:[20]

$$J = J_{max} \times C / (K_t + C) + k \times C \qquad \text{Eq. (2)}$$

where J_{max} is the maximum uptake rate for a carrier-mediated process, C is the benzoic acid concentration, K_t is the half-saturation concentration (Michaelis constant), and k is a first-order rate constant.

Statistical Analysis. All results were expressed as means ± standard deviation (SD). Statistical analysis between two groups was performed using Student's t-test, and one-way analysis of variance (ANOVA) was used for single and multiple comparisons. P values of 0.05 or less were considered to indicate a statistically significant difference.

RESULTS

[^{14}C]Benzoic acid was rapidly accumulated in BeWo cells with time (Figure 1). The uptake was linear initially and reached equilibrium at about 2 min. Therefore, all

subsequent uptake studies and kinetic analysis were performed from data collected through 30 s.

Figure 2 shows the relationship between the initial rate of uptake of [^{14}C]benzoic acid and its concentration in the incubation buffer. The results indicated that the uptake of benzoic acid consisted of two processes, a saturable process evident at low concentrations and an apparently nonsaturable process evident at high concentrations. The uptake processes were analyzed according to Eq. (2). The kinetic parameters calculated for benzoic acid uptake were a J_{max} of 0.52 $\pm$ 0.22 nmol/30 s/mg of protein, a K_t of 0.64 $\pm$ 0.26 mM, and a k of 0.11 $\pm$ 0.06 µl/30 s/mg of protein. The upper dotted line represents the uptake for the saturable component calculated from the kinetic parameters. The lower dotted line represents the uptake for the nonsaturable component calculated from the kinetic parameters. At any given concentration, the uptake by saturable component was higher than that by nonsaturable one.

The effects of metabolic inhibitors on the uptake of [^{14}C]benzoic acid were studied to determine whether this uptake required cell-dependent energy expenditure (Table 1). Sodium azide (10 mM), a respiratory chain inhibitor, or 2,4-dinitrophenol (1 mM), an uncoupler of oxidative phosphorylation, significantly inhibited the uptake of [^{14}C]benzoic acid by the BeWo cells. Moreover, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP; 50 µM), a protonophore, significantly inhibited the uptake (Table 1), whereas 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS; 0.1 mM), an anion-exchange inhibitor, had no inhibitory effect on benzoic acid uptake. Finally, the uptake of benzoic acid in a sodium-depleted buffer was not different from the control suggesting the mechanism was not sodium-dependent (Table1). Figure 3

illustrates the effect of incubation buffer pH in the range from 5.0 to 7.5 on [^{14}C]benzoic acid uptake by BeWo cells. The rate of [^{14}C]benzoic acid uptake decreased with increasing pH from an acidic to a neutral pH. These findings, together with the concentration dependence and sensitivity to metabolic inhibitors, strongly suggested that [^{14}C]benzoic acid uptake by BeWo cells was dependent on a proton gradient and was carrier-mediated.

To investigate the properties of the carrier involved in [^{14}C]benzoic acid uptake by BeWo cells, we studied the effects of various mono- and di- carboxylic acids on this uptake (Table 2). Each monocarboxylic acid, including lactic acid, significantly inhibited the uptake of [^{14}C]benzoic acid, whereas none of the dicarboxylic acids had a significant inhibitory effect on the uptake of the marker. We also found that the BeWo cells take up [^{14}C]acetic acid and that this uptake was significantly inhibited by monocarboxylic acids, including lactic acid (Table 2). In contrast, dicarboxylic acids had no marked effect on the uptake of [^{14}C]acetic acid by BeWo cells. These results implied that the carrier that mediates monocarboxylic acid uptake by BeWo cells was a nonspecific monocarboxylic acid carrier. Additionally, the carrier is relatively stereoselective as distinguished by the variable inhibition by the two lactic acid isomers

To study the mechanism of the benzoic acid or acetic acid or lactic acid uptake inhibition by the monocarboxylic acids, we analyzed the inhibitory effect kinetically. Figure 4A shows a Lineweaver-Burk plot for the uptake of [^{14}C]benzoic acid by BeWo cells in the absence or presence of 2 mM acetic acid. Acetic acid competitively inhibited the uptake of benzoic acid by the BeWo cells. Figure 4B shows a Lineweaver-Burk plot for the uptake of [^{14}C]acetic acid by BeWo cells in the absence or presence of 2 mM

benzoic acid. Similarly, Figures 4C and 4D confirm that lactic acid and benzoic acid interact with the BeWo uptake mechanism by a competitive process. Table 3 summarizes the kinetic data and indicates that the K_m's and K_i's for benzoic, acetic, and lactic acid for the apparent moncarboxylic acid uptake mechanism were similar.

The trans-trophoblast passage of [^{14}C]benzoic acid across the BeWo monolayer was found to be asymmetric, with the permeability coefficient greater in the apical-to-basolateral direction than that in the basolateral-to-apical direction, as shown in Table 4. The passage of [^{14}C]benzoic acid across the BeWo monolayers was inhibited by an excess amount of unlabeled benzoic acid or other monocarboxylic acid (valproic acid) as shown in Table 4, affirming that the transcellular passage of the monocarboxylic acid appeared similar to the uptake process.

DISCUSSION

Passive diffusion is the primary mechanism by which xenobiotics cross the placental barrier.[1] However, some carrier-mediated transport systems in the trophoblast have been described using brush-border membrane vesicles or cultured cells,[3,5,6] including functional MCTs.[12-14] These carrier systems are notable for mediating the distribution of both endogenous and exogenous substances across the placental barrier.[7] Recent studies in knockout mice have effectively illustrated the relevance of placental transport mechanisms, e.g., P-glycoprotein, in protecting the fetus from exposure to chemicals.[21] Consequently, the recognition of transport mechanisms is important for developing an understanding of the molecular mechanisms and role of the trophoblast in regulating drug passage across the placental barrier.

There is also a critical need for *in vitro* systems of human origin that allow characterization of mechanisms regulating drug distribution between mother and fetus to aid in the future design and development of drugs that can be safely administered in pregnancy.[22,23]

In the present study, we used benzoic acid uptake and transport, as a marker for the monocarboxlic acid transport[8] in BeWo cell monolayers. The establishment of functional MCT mechanisms in the BeWo cells offers the opportunity to characterize mechanisms of monocarboxylic acid transport at the molecular and biochemical level, extending limited studies performed with brush border preparations[12-14] and the perfused human placental lobe.[15]

The processing of benzoic acid by the BeWo monolayers was characterized by saturable uptake and transport. The processes were significantly inhibited by metabolic inhibitors and indicated that benzoic acid uptake is energy dependent. Benzoic acid transport and uptake were also significantly inhibited by in a competitive manner by unlabeled benzoic acid, acetic acid, and lactic acid. Further, benzoic acid uptake and transport were significantly inhibited by monocarboxylic acids but not selected dicarboxylic acids. The competitive nature of the transport, evidenced by the kinetic parameters, was consistent with observations reported for the transport of monocarboxylic acids in placental brush border vesicles.[12-14]

The advantage of the BeWo monolayer system is that trans-trophoblast transport properties can be characterized in a polarized cell.[16] We were able to show the passage of benzoic acid across the BeWo monolayers was bidirectional and of a faster rate going in the apical-to-basolateral side direction across the monolayers in the

presence of a pH gradient. These observations are in good agreement with the lactate transport studies performed in the perfused human placental lobe model.[15] Further, our studies suggested a functional asymmetry in the carrier mechanism.

Caco-2 cells, which are a well-characterized model of the intestinal epithelium, possess a monocarboxylic acid transport system which is pH dependent.[8] The activation of benzoic acid uptake by acidic pH can be explained by H+ cotransport, OH- exchange system, or a possible change in affinity to the carrier protein depending on the extracellular pH. The uptake of benzoic acid by BeWo monolayers increased with a decreased pH. The lack of an inhibitory effect of DIDS, an inhibitor of anion exchange, suggests that an OH- exchange system with benzoic acid was not involved in uptake or transport by BeWo cells. Many types of transporters are Na+ dependent. However, benzoic acid uptake by BeWo cells was also not inhibited in the Na+-depleted buffer in this study. Benzoic acid is an acidic compound; therefore, a decrease in pH increases the fraction of nonionized benzoic acid. According to the pH-partition hypothesis, this increase in the fraction of nonionized form increases the passive accumulation of benzoic acid across the BeWo cell membrane. Data presented here (e.g., Figure 3), and other studies conducted in absence of pH gradients, where the rate of transfer and asymmetry of transport for representative monocarboxylic acid drugs (e.g, acetylsalicylic acid and ibuprofen) is substantially diminished (data not shown), are alone insufficient to conclude that the uptake of benzoic acid is proton-gradient-dependent. However, the need for a proton gradient was affirmed with our results that showed FCCP, a protonophore, strongly inhibited the uptake of benzoic acid. Collectively then, our results indicated that monocarboxylic acid uptake was proton-

gradient-dependent and were entirely consistent with similar studies conducted with human trophoblast brush border membrane preparations.[14] From a pharmacological and physiological perspective, this is significant since a pH gradient does develop across the placenta with advancing pregnancy. The developing pH gradient does influence drug distribution across the placenta[24] and may possibly influence the MCT carrier's role in regulating the distribution of monocarboxylic acids between the mother and fetus. The physiological role of a MCT in the trophoblast is likely related to the regulation of the distribution of L-lactic acid and related substrates across the placental barrier.[14,15]

Monocarboxylic acids are transported out of muscle and into liver (i.e., the so-called Cori cycle) by proton-coupled transporters, MCTs, that exhibited distinct substrate specificities and could be differentially inhibited by α-cyanocinnamates. MCT1 isolated from a Chinese hamster ovary cell cDNA library, is found in cardiac muscle, erythrocytes, basolateral intestinal epithelium, and skeletal muscle. MCT2, cloned from hamster liver, is functionally similar to MCT1 in terms of transport capabilities, but its tissue distribution is significantly different.[25] A third transporter isoform, MCT3, was identified in chick retinal pigment epithelium. More recently, four new MCT homologs have been cloned and sequenced in human tissue (MCT3-MCT7).[11] There is currently no direct information of the type of functional MCTs associated with BeWo cells or human trophoblasts. Thus, further studies with molecular probes and selective substrates, when available, will be required to precisely identify which of the seven monocarboxylic acid transporter isoforms may be present in the

human placental barrier. Elucidating the precise forms of functional MCTs in the trophoblast remains an ongoing objective of our laboratory.

In summary, our present results support limited evidence in the literature[12-15] that monocarboxylic acids and nutrients, drugs, drugs of abuse and other xenobiotics that have a monocarboxylic acid constituent have the potential to be substrates for proton-gradient-dependent transport across the human trophoblast. This work further demonstrates the possible utility of a human, monolayer-forming cell line, BeWo, to characterize putative trans-trophoblast transport mechanisms and their potential roles in controlling nutrients, drugs, and drugs of abuse distribution across the placental barrier.

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Figure Legends

Figure 1. Time course of [^{14}C]benzoic acid uptake by BeWo cells. The vertical bar through each point represents the SD for four replicate experiments.

Figure 2. Concentration dependency of [^{14}C]benzoic acid uptake by BeWo cells. The uptake of [^{14}C]benzoic acid by BeWo cells was measured at 37ºC for 30 s. The upper dotted line represents the uptake for the saturable component calculated from the kinetic parameters obtained as described in the text. The lower dotted line represents the uptake for the nonsaturable component calculated from the kinetic parameters. The vertical bar through each point represents the SD of four replicate experiments.

Figure 3. pH dependence of [^{14}C]benzoic acid uptake by BeWo cells. The uptake of [^{14}C]benzoic acid by BeWo cells was determined at the indicated pH at 37ºC. The vertical bar through each point represents the SD of four replicate experiments.

Figure 4. Lineweaver-Burk plots for: **A)** The concentration-dependent uptake of [^{14}C]benzoic acid by BeWo cells. Uptake was measured in the absence (open circles) or presence of 2 mM acetic acid (closed circles). **B)** The concentration-dependent uptake of [^{14}C]acetic acid by BeWo cells. Uptake was measured in the absence (open triangles) or presence of 2 mM benzoic acid (closed triangles). **C)** The concentration-dependent uptake of [^{14}C]-L-lactic acid by BeWo cells. Uptake was measured in the absence (open circles) or presence of 2 mM benzoic acid (closed circles). **D)** The concentration-dependent uptake of [^{14}C]benzoic acid by BeWo cells. Uptake was measured in the absence (open circles) or presence of 2 mM L-lactic acid (closed circles). The vertical bar through each data point in the figures represents the SD of four replicate experiments.

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Table 1. Effects of metabolic inhibitors, ionophore (carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; FCCP), anion-exchange (4,4'-diisothiocyanostilbene-2,2'-disulfonic acid; DIDS) inhibitors, and sodium-depleted buffer on [^{14}C] benzoic acid uptake by BeWo cell monolayers at 37°C.[a]

Inhibitor	Concentration	Relative Uptake (Percent of Control)[b]
Sodium azide	10 mM	20.1 ± 5.2 *
2,4-Dinitrophenol	1 mM	13.3 ± 7.4 *
FCCP	50 µM	9.3 ± 1.4 *
DIDS	100 µM	117.5 ± 8.1
Sodium-depleted buffer	No treatment	101.7 ± 11.7

a BeWo cells were pretreated with these agents for 15 min prior to performing an uptake experiment.

b Each value represents the mean ± standard deviation of four experiments.

* $P < 0.001$ versus untreated control (100%).

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Table 2. Effects of selected carboxylic acids on [^{14}C]benzoic acid or [^{14}C]acetic acid uptake by BeWo cell monolayers at 37°C.[a]

		Relative uptake (Percent of control)[b]	
		[^{14}C]benzoic acid	[^{14}C]acetic acid
Monocarboxylic acids	Benzoic acid	10.8 ± 0.2*	19.5 ± 2.7*
	D-Lactic acid	20.4 ± 1.8*	26.5 ± 4.2*
	L-Lactic acid	6.1 ± 1.3*	13.4 ± 1.5*
	Propionic acid	12.7 ± 0.9*	18.9 ± 2.6*
	Acetic acid	10.1 ± 2.0*	36.9 ± 2.9*
	p-Aminobenzoic acid	5.5 ± 0.1*	20.7 ± 3.4*
	Acetylsalicylic acid	12.3 ± 1.3*	11.1 ± 1.0*
	Salicylic acid	3.5 ± 0.1*	9.3 ± 0.7*
Dicarboxylic acids	Glutaric acid	91.0 ± 8.5	107.2 ±12.8
	Fumaric acid	113.8 ± 5.5	98.4 ±10.4
	Maleic acid	92.1 ± 7.3	102.4 ±28.4

a [^{14}C]benzoic acid or [^{14}C]acetic acid uptake by BeWo cells were performed in the presence of 10 mM (2000-fold excess) of the indicated carboxylic acid.

b Each value represents the mean ± standard deviation of four experiments.

* $P < 0.001$ versus untreated control (100%).

Utoguchi, N., Magnusson, M., and Audus, K.L. (1999) Carrier-mediated transport of monocarboxylic acids in BeWo cell monolayers as a model of the human trophoblast. J. Pharm. Sci. 88, 1288-1292. PMID: 10585224. Publisher's official version: <http://dx.doi.org/10.1021/js990173q> . Open Access version: http://kuscholarworks.ku.edu/dspace/.

Table 3. Lineweaver-Burke uptake and inhibition constants for monocarboxylic acid uptake by BeWo cell monolayers at 37°C.

	K_m (mM)[b]	Benzoic acid K_i (mM) [b]	Acetic Acid K_i (mM) [b]	Lactic Acid K_i (mM) [b]
[^{14}C]Benzoic acid[a]	1.0 ± 0.1	-	1.1 ± 0.2	0.7 ± 0.3
[^{14}C]Acetic acid[a]	0.8 ± 0.2	0.8 ± 0.3	-	-
[^{14}C]Lactic acid[a]	1.4 ± 0.2	0.7 ± 0.2	-	-

a [^{14}C]benzoic acid or [^{14}C]acetic acid or [^{14}C]lactic acid uptake by BeWo cells were performed alone and in the presence of 2 mM (1 to 8-fold excess) of the indicated monocarboxylic acid.

b Each value represents the mean ± standard deviation of four experiments.

Utoguchi, N., Magnusson, M., and Audus, K.L. (1999) Carrier-mediated transport of monocarboxylic acids in BeWo cell monolayers as a model of the human trophoblast. J. Pharm. Sci. 88, 1288-1292. PMID: 10585224. Publisher's official version: <http://dx.doi.org/10.1021/js990173q> . Open Access version: http://kuscholarworks.ku.edu/dspace/.

Table 4. Apparent permeability coefficients for the passage of [^{14}C]benzoic acid across BeWo cell monolayers at 37°C.

Direction of benzoic acid permeation across monolayer	Treatment	Permeability Coefficient (x 10^3 cm/sec)
Apical to basolateral	none	4.90 ± 1.01
	+ benzoic acid[a]	0.45 ± 0.04*
	+ valproic acid[a]	0.64 ± 0.21*
Basolateral to apical	none	1.06 ± 0.70*

a [^{14}C]benzoic acid permeation across BeWo cell monolayers was performed in the presence of 10 mM (2000-fold excess) of the indicated carboxylic acid in the apical chamber.

* $P < 0.001$ versus untreated benzoic acid permeation apical to basolateral.