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Microarray Determination of the Expression of Drug Transporters in Humans and Animal Species Used for the Investigation of Nasal Absorption

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Abstract

Purpose—Mice and rats are commonly used to investigate in vivo nasal drug absorption, yet their small nasal cavities limit their use for *in vitro* investigations. Bovine tissue explants have been used to investigate drug transport through the nasal respiratory and olfactory mucosae, yet limited information is available regarding the similarities and differences among these animal models compared to humans. The aim of this study was to compare the presence of a number of important drug transporters in the nasal mucosa of these species.

Methods—DNA microarray results for nasal samples from humans, rats and mice were obtained from GenBank, while DNA microarray and RT-PCR were performed on bovine nasal explants. The drug transporters of interest include multidrug resistance, cation, anion, peptide, and nucleoside transporters.

Results—Each of the species (mouse, rat, cow and human) shows similar patterns of expression for most of the important drug transporters. Several transporters were highly expressed in all the species, including MRP1, OCTN2, PEPT2 and γ -LAT2.

Conclusion—While some differences in transporter mRNA and protein expression were observed, the transporter expression patterns were quite similar among the species. The differences suggest that it is important to be aware of any specific differences in transporter expression for a given compound being investigated, yet the similarities support the continued use of these animal models during preclinical investigation of intranasally administered therapeutics.

Introduction

“Carriers” or “transporters” are proteins associated with cellular membranes and are responsible for the intake and the efflux of crucial endogenous substances such as sugars, amino acids, nucleosides and inorganic ions. The activity of transporters, however, is not

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limited only to their physiological substrates but also includes a variety of exogenous compounds. Drugs with structural similarities to endogenous transporter substrates can also be transported across cell membranes thus modulating their absorption, disposition and elimination.¹

Nasal drug delivery has been recognized for its utility in administering drugs locally to treat nasal conditions such as congestion and allergies. The nasal route is also an attractive alternative to the oral and parenteral routes to deliver drugs systemically, and it offers many advantages including rapid drug absorption and quick onset of action, enhanced bioavailability by avoiding gastrointestinal and hepatic first-pass metabolism, and noninvasive administration.^{2–4} The nasal route does possess some limitations, however, including a short residence time due to mucociliary clearance, a low volume capacity, and variable absorption primarily due to administration and user differences. The nasal route may also offer a potential means to deliver drugs directly to the central nervous system while bypassing the blood-brain barrier, and several recent reviews describe the potential for drug transporters to act as chaperones to enhance the nose to brain transport of substrate drug compounds.^{3–6} A more complete understanding of the mechanisms regulating drug absorption in the nasal cavity will help identify drug candidates with suitable characteristics for nasal delivery. Tremendous progress has been made in the identification and characterization of transporters influencing drug absorption and distribution in the gastrointestinal tract and in clearance of drugs via the hepatic or renal systems. The role of transporter function at other administration sites, including the lungs, eyes, and oral mucosa has also been the subject of active investigation.^{7, 8} Only a limited number of transporters have been investigated for their role in nasal drug absorption, however.^{2, 6, 9–11} Previous investigations clearly demonstrated the expression of p-glycoprotein in the nasal mucosa of mice and cows and demonstrated its important role in limiting the transfer of drugs into the brain following nasal administration or limiting their transfer across excised nasal mucosal tissues.^{12, 13} Investigations of uptake transporter activities have also used Western blotting and immunohistochemistry to confirm the expression of OCT2, OCTN2, LAT1, LAT2, CNT3, and ENT1 in cows.^{10, 12, 14–16} Further in vivo investigations have shown that OCT2 and OCTN2 are able to modulate the transfer of drugs across the nasal mucosa and the activity of these transporters significantly enhances nose to brain transport of substrate drug compounds.

The transporters of greatest current interest in drug disposition belong to one of two major classes: ATP-binding cassette (ABC) and solute carrier (SLC) transport families. ABC transporters are primarily active transporters that mediate the efflux of compounds out of cells,^{1, 17} and the overexpression of ABC transporters has long been known to be related to drug resistance.^{18–20} The most studied drug transporters belonging to the ABC family include multidrug resistance protein 1 (MDR1) or P-glycoprotein (P-gp), the multidrug resistance-associated proteins 1–9 (MRPs 1–9), and breast cancer resistance protein (BCRP). The majority of known drug uptake transporters belong to the SLC transporter family. Among the families that have been studied extensively for their role in drug absorption, the organic cation, organic cation/carnitine, organic anion, peptide, monocarboxylate, and nucleoside transporters are of particular current interest.¹ The amino acid transporters, another SLC family, are divided into two subfamilies: the cationic amino

acid transporters (CAT family, SLC7A1–4) and the glycoprotein-associated amino acid transporters (gpaAT family, SLC7A5–7) which are heterodimeric amino acid transporters (HAT).²¹ LAT 1 and LAT2 are HAT transporters that interact with large neutral amino acids and have been suggested to contribute to the transport of several drugs including L-DOPA, gabapentin, and thyroid hormones.²²

The presence of transporters in the nasal mucosa helps in explaining and/or predicting the permeation of specific substrates across the nasal mucosa. The major focus of nasal drug delivery studies is on the utility of this route for administration of different therapeutic compounds and on identifying methods to enhance nasal permeability. Only a few studies have been pursued to clearly identify the mechanisms regulating nasal permeability of various compounds, especially the involvement of transporters.^{2, 9, 10, 12, 13, 23, 24} For example, Chemutruri and Donovan showed that dopamine permeation across the nasal mucosa was concentration dependent and was influenced by the activity of organic cation transporters.¹² In addition, others found that the transport of substances such as chlorpheniramine, chlorcyclizine and verapamil across the nasal mucosa was enhanced in the presence of compounds known to inhibit efflux transporters.^{13, 23, 24}

In order to further characterize the role of the transporters expressed in the nasal mucosa, the gene expression of drug transporters in the nasal mucosa of a variety of frequently used animal models (mice, rats, and cows) was investigated, since knowledge of the presence of drug transporters in the nasal mucosa can assist in the identification of drug compounds whose absorption may be enhanced due to the activity of an uptake transporter or reduced as the result of efflux. Preclinical investigations of drug candidates frequently involve the use of in vitro cell culture and excised tissues models along with preliminary biodistribution, safety, and efficacy studies in rodent models. The purpose of this investigation was to use the power of DNA microarray technology to identify the important drug transporters which may be expressed in typical preclinical models and in humans in order to better inform model selection for improved experimental protocol development and to improve the ability to use the results obtained from preclinical investigations to predict human response.

Materials and Methods

Materials

TRIzol® reagent and the SuperScript™ II Reverse Transcriptase kit were purchased from Invitrogen (Life Technologies, Carlsbad, CA). The PCR kit was from New England-Biolabs (Ipswich, MA). For microarray analysis, the Ovation RNA Amplification System v2 was purchased from NuGEN Technologies (San Carlos, CA), QIAGEN QIAquick PCR Purification Kits from QIAGEN (Hilden, Germany) were used. Streptavidin-phycoerythrin was obtained from Molecular Probes, Inc. (Eugene, OR) and the anti-streptavidin antibody was from Vector Laboratories, Inc. (Burlingame, CA). Affymetrix GeneChip Bovine Genome Array and other reagents used in the microarray analysis were from Affymetrix, Inc. (Santa Clara, CA). All reagents used were of research grade.

Tissue preparation and RNA extraction

Excised bovine respiratory and olfactory nasal tissues were obtained from Bud's Custom Meats (Riverside, IA). The tissues were collected immediately after decapitation, and the nasal tissues were retrieved by removing the nasal septum to expose the nasal cavity. The dorsal and the ventral conchae are lined by the respiratory mucosa, and the ethmoid and the middle conchae have regions lined by the olfactory mucosa.²⁵ The conchae were excised using a sharp knife, and the tissues were snap-frozen using liquid nitrogen. Tissues from three 24 month old male cows were used as the source for the microarray samples.

RNA was extracted from the tissues with TRIzol® Reagent using the manufacturer's recommended protocol. The samples used for DNA-microarray analysis were treated with DNase to remove any genomic DNA. The RNA integrity was evaluated using an RNA LabChip® with an Agilent 2100 Bioanalyzer (Agilent Technologies, San Clara, CA). An RIN > 6 was used for inclusion of the sample in the microarray analysis.

Primer design

Primers were designed using the Primer-BLAST website (<http://www.ncbi.nlm.nih.gov/tools/primer-blast/>). *Bos taurus* mRNA sequences for the drug transporters of interest were found using the NCBI nucleotide search website (<http://www.ncbi.nlm.nih.gov/nucleotide/>). The sequence (NCBI reference number) was submitted to Primer-BLAST for primer selection. To avoid amplifying genomic DNA that might be present in the samples, the primers were selected to span an exon-exon junction. In addition, primers were selected so that their binding site did not include any known single nucleotide polymorphisms (SNP). The selected primers used in these investigations are listed in Table 1.

cDNA production and PCR

cDNA was synthesized using SuperScript™ II Reverse Transcriptase according to the manufacturer's protocol. Oligo dT was used as the primer. PCR was performed for 35 cycles with 55 or 58°C as the annealing temperatures. While the 55 °C annealing temperature was suitable for the PCR of most of the genes, a higher annealing temperature of 58 °C was used for MRP2, PEPT1, and PEPT2. The expression of the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene, a "housekeeping gene", was used as an internal control. The PCR products were analyzed using 2% agarose gel electrophoresis, and the gel was imaged using an AlphaImager 2200, V5.5 (Alpha Innotech Corporation, San Leandro, CA).

Microarray analysis

Fifty nanograms of total RNA were converted by Single Primer Isothermal Amplification (SPIA®) to cDNA using the Ovation RNA Amplification System v2 according to the manufacturer's recommended protocol. The amplified SPIA® cDNA product was purified through a QIAGEN QIAquick PCR purification column according to modifications recommended by NuGEN. A sample (3.75 ug) of cDNA was fragmented (average fragment size = 85 bases) and biotin labeled using the NuGEN FL-Ovation cDNA Biotin Module v2 per the manufacturer's recommended protocol. The resulting biotin-labeled cDNA was mixed with Affymetrix eukaryotic hybridization buffer, placed onto Affymetrix GeneChip

Bovine Expression arrays, and incubated at 45 °C for 18 h at 60 rpm in an Affymetrix Model 640 Genechip Hybridization Oven (Affymetrix, Inc., Santa Clara, CA). Following hybridization, the arrays were washed, stained with streptavidin-phycoerythrin, and the signal was amplified with anti-streptavidin antibody using the Affymetrix Model 450 Fluidics Station. Arrays were scanned with an Affymetrix Model 3000 scanner with 7G upgrade, and data were collected using the GeneChip operating software (GCOS) v1.4. Expression value estimates were generated using the Microarray Analysis Suite (MAS) v5.0 software.

Microarray data analysis

Microarray expression data for human, mouse, and rat nasal mucosa were identified and collected from NCBI's Gene Expression Omnibus. Only samples from normal (nondiseased, nontreated) tissues were used. A total of 71 samples from humans (GSE11348, GSE2395, GSE8987, GSE9150), 15 samples from mice (GSE1680, GSE2437, GSE3455, GSE4915, GSE4927), and 34 samples from rats (GSE5019, GSE5349, GSE7002) were analyzed. These samples were all run on 3' IVT arrays from Affymetrix, from which the expression present/absent calls were obtained using the MAS5 algorithm provided from Affymetrix. In addition, microarray results from 6 samples (three samples each from olfactory and respiratory epithelia) from three cows were determined experimentally. Expression results from the Affymetrix Bovine Genome Array were normalized, and gene-level expression values were obtained using Partek® Genomics Suite™ software (Partek, Inc., St. Louis, MO). Expression level (high, moderate, absent) was identified by determining the fraction (percentile) of the arrays indicating presence of the gene. Genes expressed above the 50th percentile were defined as highly expressed; expression values between the 25th and 50th percentile were classified as moderately expressed; expression on less than 25% of the arrays was classified as weak/absent. Orthologs of the genes of interest were identified using Ensembl BioMart resource, a web tool provided by EMBL-EBI and the Wellcome Trust Sanger Institute (<http://useast.ensembl.org/info/website/tutorials/compara.html>).

Results

Although bovine nasal tissues are common *in vitro* nasal models, little information is known about transporter expression in the bovine nasal mucosa. The results of RT-PCR and microarray analysis for bovine samples are presented in Table II. Of the 19 transporters genes that were annotated on the GeneChip Bovine Genome Array and also had RT-PCR performed on bovine mucosal samples, six genes showed disagreement between the reported microarray expression and RT-PCR including MRP4, OCT1, MCT1, LAT2, and ENT2. Each of these genes which were indicated to be highly expressed in the microarray analysis but could not be identified using RT-PCR. Additional work in our laboratories has demonstrated the weak expression of LAT2 in the bovine nasal mucosa.¹⁵ MDR1 mRNA, which was not determined to be significantly expressed from the microarray, was readily detected by RT-PCR. The false positives may be the result of several factors, including a lack of probe specificity to distinguish some genes, the limited information on the variabilities inherent within the bovine genome, and the presence of paralogous sequences for many transporters. While microarrays are excellent at simultaneously measuring

expression levels from tens of thousands of genes, their functionality is limited by the information available during their design. As a result, RT-PCR was used as a complementary technique to validate the microarray results as well as to interrogate the expression of genes for which microarray probes were unavailable (e.g., CNT1, CNT2).

Using CIMminer heatmap analysis (Genomics and Bioinformatics Group, Laboratory of Molecular Pharmacology (LMP), Center for Cancer Research (CCR) National Cancer Institute (NCI)) (Figure 1), several open regions (white) in the bovine section and to a lesser extent in the rat section indicate data unavailable due to missing probes. The heat map was constructed to cluster genes that are more highly expressed near the top and the genes with low expression at the bottom. Due to the limited bovine sample size, the heatmap cannot be used for quantitative comparison, but it can help in the initial determination of agreement and disagreement of the level of expression of the transporters between the species. For example, ABCC1 (MRP1) and SLC7A6 (γ -LAT2) are highly expressed (shades of red) in all species while SLC22A6 (OAT1) and SLC22A7 (OAT2) are weakly expressed (blue).

To assist with the comparison of drug transporter mRNA expression between the species, the microarray data are also displayed as bar charts. When evaluating the efflux transporters (Figure 2, Table II), MRP1 mRNA (ABCC1) is highly expressed in the nasal mucosa of all the species and MRP 3, 4, and 5 (ABCC 3, 4, and 5) are also moderately to highly expressed in these species. MDR1 (ABCB1) is expressed in humans, rats, and mice, and while the microarray results indicated it was not expressed in bovine nasal mucosa, RT-PCR, however, confirmed its mRNA expression in cows. In comparison, MRP 2 (ABCC 2) is weakly expressed in humans and mice (no data available for MRP8) and no probe datasets were available for rats or cows.

The level of expression for some efflux transporters showed variation between humans and the animal models, however. For example, MRP6 (ABCC6) was more highly expressed in humans compared to all of the animal models while BCRP was highly expressed in all of the animal models but was weakly expressed in humans.

Organic cation transporters (OCTs) and organic cation/carnitine transporters (OCTNs) are two additional families of drug transporters whose mRNA was found to be expressed in the nasal mucosa of all the species investigated (Figure 3, Table II). OCTN1 and 2 (SLC22A4 and 5) were highly expressed in the human nasal mucosa, but microarray data from the animal models were only available for OCTN2 (SLC22A5); they also showed high expression of this transporter. OCT 1, 2 and 3 (SLC22A 1, 2 and 3) were weakly expressed in the human nasal mucosa, while their expression varied among the animal models. mRNAs for organic anion transporters (OATs), on the other hand, were minimally expressed or not expressed in any of the species (Figure3, Table II). Finally, several peptide, monocarboxylate, and amino acid transporters were also observed to be highly expressed in the human nasal mucosa as well as in the other species investigated, especially γ -LAT2 (SLC7A6) and PEPT2 (SLC15A2) (Figure 4 and 5, Table II).

Several nucleoside transporter mRNA sequences were also found in the nasal mucosa of humans and animals. The concentrative nucleoside transporter, CNT3 (SLC28A3), was

shown to be moderately to highly expressed in all species, and both equilibrative transporters, ENT 1 and 2 (SLC29A 1 and 2), were also expressed in all of the species except for the lack of reported expression of ENT1 in mice (Figure 6, Table II).

For gene profiling of the bovine nasal mucosa, two distinct regions of the nasal mucosa were investigated: the respiratory region, which covers most of the mucosal surface area, and the olfactory region, which is located in the uppermost regions the nasal cavity. Both play an important role in drug absorption, and it is believed that the olfactory mucosa plays a key role in the direct nose to brain transport route.^{3–6} Microarray analysis results showed the same the level of mRNA expression for the transporters in both regions of the bovine nasal cavity. The intensity of the bands of the RT-PCR results also showed limited differences between the two regions.

Discussion

Uptake and efflux transporters are important determinants of the disposition of a variety of drugs. As a result, identifying transporters in absorptive mucosae has been pursued extensively, especially in the intestinal mucosa. The gene expression patterns of membrane transporters and channel proteins in the human intestine have been identified using microarray.^{26, 27} Moreover, the relative abundance of the transporters' mRNA at different regions of the small intestine has been evaluated using q-PCR.^{28–30} In addition, several studies have also compared the transporter expressions in human intestine with the human intestinal cell line, Caco-2, and with animal models such as rats and mice.^{28–31}

The nasal mucosa offers an alternative pathway to the oral route to deliver drugs to the systemic circulation and/or the central nervous system. However, compared to the intestinal mucosa, little is known about transporter expression in the nasal mucosa. A limited number of studies describe efflux and/or uptake transporters in the nasal mucosa, and expression has only been described for Pgp, organic anionic, and several organic cationic, nucleoside, and monocarboxylate transporters.^{9, 11, 12, 32–41} Based on the microarray results, mRNAs for several transporters were shown to be highly expressed in the nasal mucosa of all the species, particularly MRP1 (ABBC1), OCTN2 (SLC22A5), PEPT2 (SLC15A2), and y +LAT2 (SLC7A6), while anion transporters were weakly expressed in all the species.

Gene expression analysis can assist with identification of a greater number of key transporters which could play major roles in drug transport in the nasal mucosa. While the presence of the transcribed product (mRNA) may not necessarily indicate the presence of the encoded protein, examining gene expression is a convenient and acceptable method to determine the likely presence of proteins. Table II shows that the gene expression of transporters identified in this study using microarray or RT-PCR techniques agreed well with reports of transporter protein presence detected by Western blotting and immunohistochemistry in humans, mice, rats, and cows. In addition, the microarray and RT-PCR results of this study agreed with gene expression results found by other methods such as RT-PCR and branched DNA technology (Table II). Only a few conflicting results were noted, including OCT1 (SLC22A1) which was determined to be very weakly expressed in the human nasal mucosa using microarray but was observed to be highly expressed using

other techniques.^{40, 41} Similarly, microarray analysis of OCT3 (SLC22A3) in the rat nasal mucosa revealed that it was moderately to highly expressed, which contradicted what was previously reported using branched DNA technology.³²

RT-PCR was also performed using bovine nasal samples to verify some of the results obtained from the microarray analysis. While PCR depends on the specific hybridization of predetermined primers and the defined targeted sequence, DNA microarray depends on the hybridization of targeted nucleotide sequences to the probes which are placed on the microarray chip. Since primers used for PCR can be purposely designed, one can design more specific primers for PCR, and PCR can be used to evaluate the expression of genes of known sequence that are not present on a commercial microarray. This is of particular importance in the case of bovine gene expression, because the bovine genome is not as well documented as the human, mouse, or rat genomes, and the currently available bovine microarray is not as extensively annotated as the microarrays for the other species. When conflicting results are obtained between microarray and RT-PCR analyses, the RT-PCR results are likely more accurate, given the detection of the encoding gene by microarray is the result of a statistical evaluation of the “probe-hit” pattern for multiple nucleotide sequences while PCR primers are designed to interact specifically with an RNA region identified as unique to the gene of interest. Even RT-PCR results can be erroneous if primer sequences are not carefully designed or if sufficiently unique sequences are not identified during primer development. In order to verify that the mRNA encoding for a protein are translated and the resulting protein is expressed and functional, further evaluations, frequently using Western blotting or immunohistochemistry, to detect the location of the expressed protein within a cell or tissue are conducted. Both of these protein-detection techniques suffer from a lack of specificity, however, but when combined with other techniques, including DNA microarray or RT-PCR, provide strong evidence for the expression of a protein.

Table II provides a detailed comparison of the evidence for transporter expression in the nasal mucosa of mice, rats, cows, and humans using a number of these techniques, including microarray, RT-PCR, and immunohistochemistry. Table III provides a summary of these results where any experimental evidence of mRNA or protein expression was interpreted as evidence the transporter may be present in the nasal mucosa. This limited-exclusion summary of the current results is warranted given the limitations regarding the availability of experimental details for datasets uploaded to GeneBank, the lack of complete annotation of the bovine genome on the GeneChip Bovine Genome Array, and the lack of specific antibodies available to probe for the expression of all of the transporter proteins of interest.

One of the objectives for this investigation was to determine whether many of the transporters expressed in the human nasal mucosa were also expressed in various animal models in order to assist with model selection during preclinical drug development. The summary-level information contained in Table III provides a guide for the selection of potential, nasally administered drug candidates and key factors in their evaluation, based on known transporter interactions for the specific drug. For example, the absence of expression of the OATs indicates that these transporters would be poor targets for enhanced drug

uptake in the nasal mucosa and limited mucosal-level OAT transporter effects on disposition would be anticipated to occur.

Even though information regarding the nasal cavity regions from which the human nasal samples were obtained was not available, the rat and mouse nasal samples were reportedly obtained from the olfactory region. The bovine samples were purposely taken from both the respiratory and olfactory regions to investigate any significant and distinct transporter expression patterns in order to draw conclusions about the roles of these transporters in the nasal absorption of drugs. The respiratory region occupies the largest surface area of the nasal cavity and is highly vascularized, thus it can play a significant role in the systemic absorption of compounds from the nasal cavity. The close anatomical location and neuronal connections of the olfactory region to the brain enable it to potentially play a role in the direct transport of the drugs into the CNS from the nasal cavity. In general, there were no differences in the gene expression profiles between bovine nasal respiratory and olfactory tissues. The nasal respiratory and the olfactory mucosa may have different levels of transporter proteins expressed in these tissues, or their cellular locations may differ within the different regions, however. For instance, OCT2 (SLC22A2) and OCTN2 (SLC22A5) proteins were shown to have different levels of expression in the bovine nasal respiratory mucosa (higher in OCTN2) compared to the olfactory mucosa (higher in OCT2) as revealed by ELISA analysis.¹⁴ Moreover, immunohistochemistry showed that OCT2 was expressed on the apical surface of the rat nasal olfactory mucosa as well as in the Bowman glands while it was primarily detected in the secretory granules in the rat respiratory mucosa.³² Other transporters have also shown varied immunolocalization in rat nasal respiratory and olfactory mucosae, and these include ENT1 (SLC29A1), ENT2 (SLC29A1), and MRP1 (ABCC1).³² Careful characterization of the location of specific transporters is still necessary when determining their role in the transport of substrates within the tissues.

Determining the gene expression of drug transporters in the nasal mucosa can have a tremendous impact on the planning and evaluation of nasal drug delivery studies. Identifying the transporters present in the nasal mucosa helps in predicting the absorption behavior of drug substrates after intranasal administration and can assist in the determination of the suitability of the nasal route as a site for drug administration. For example, the nasal mucosa showed a high expression of peptide transporters, which suggests that the nasal route could offer a promising site for the absorption of peptide-like prodrugs, such as valacyclovir. In addition, the presence of several efflux transporters in the nasal mucosa has assisted in the explanation of the limited nasal absorption for some drugs.^{13, 23, 24} Moreover, the observed differences in the gene expression of drug transporters may help to identify drugs which may show bioavailability differences between humans and the animal species commonly used in the preclinical studies. For example, LAT1 (SLC7A5) is expressed in higher levels in rats and mice than in humans, which may result in the overestimation of the absorption of a substrate drug in the preclinical animal model. Further investigations of the differential levels of protein expression and transporter functionality would need to be carried out to fully evaluate the potential differences in behaviors between species, but even these initial results can be used to identify situations where these further investigations are warranted to best assist in the translation of preclinical results to those expected in humans.

Conclusion

The presence of a number of important drug transporter systems in the nasal mucosa suggests that they can play a significant role in the absorption and distribution of substrate compounds following nasal administration. Information regarding the expression of transporters in the nasal mucosa of humans and commonly used preclinical animal models can assist in the selection of model systems when the drug under investigation is a transporter substrate. In addition, knowledge of species differences in transporter expression can help in translating results from animal models to humans. For the majority of the transporters investigated in this study, there was a significant correspondence in the expression of mRNA or of the protein itself across the four species (Table III). While specific levels of expression and the cellular locations of the transporters should be further investigated when comparing nasal absorption and disposition results between species, microarray results provide a rapid and convenient source for initial preclinical model selection and guidance regarding the translation of transporter-related effects between animals and humans.

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Abbreviations

ABC	ATP-binding cassette
Asc-1	alanine–serine–cysteine transporter
ATP	adenosine triphosphate
b⁰+AT	B(0,+)-type amino acid transporter, glycoprotein-associated amino acid transporter
b-DNA	branched DNA technology
BCRP	breast cancer resistance protein
cDNA	complementary DNA
CCR	Center for Cancer Research
CNS	central nervous system
CNT	concentrative nucleoside transporter
CSF	cerebrospinal fluid
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
ENT	equilibrative nucleoside transporter
GAPDH	glyceraldehyde 3-phosphate dehydrogenase

gpaAT	glycoprotein- associated amino acid transporters
HAT	heterodimeric amino acid transporters
IHC	Immunohistochemistry
IVT	In Vitro Transcription
LAT	L-amino acid transporter
L-DOPA	L-3,4-dihydroxyphenylalanin
LMP	Laboratory of Molecular Pharmacology
MCT	monocarboxylate transporter
MDR	multidrug resistance-associated protein
mRNA	messenger RNA
MRP	multidrug resistance-associated protein
NCBI	National Center for Biotechnology Information
NCI	National Cancer Institute
OAT	organic anion transporter
OCT	organic cation transporter
OCTN	organic cation/carnitine transporters
PCR	polymerase chain reaction
P-gp	P-glycoprotein
PEPT	peptide transporter
q-PCR	quantitative PCR
RIN	RNA integrity number
RNA	ribonucleic acid
RT-PCT	reverse transcriptase PCR
SLC	solute carrier
sMCT	small monocarboxylate transporter
SNP	single nucleotide polymorphism
SPIA	single primer isothermal amplification
Wb	Western blotting
xCT	cystine/glutamic acid transporter
y+LAT	cationic L-amino acid transporter

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Figure 1. CIMminer heatmap representation of drug transporter expression in the nasal mucosa in humans, mice, rats and cows obtained from microarray analysis.

Figure 2.

Expression levels of gene transcripts (as fraction/percentage of samples in which gene is observed) determined from analysis of microarray data obtained from NCBI's Gene Expression Omnibus (human, mouse and rat) and from Affymetrix microarray analysis (GeneChip Bovine Genome Array) for **efflux transporters**. *indicates no probes were present on the microarray. ▲ indicates zero expression.

Figure 3.

Expression levels of gene transcripts (as fraction/percentage of samples in which gene is observed) determined from analysis of microarray data obtained from NCBI's Gene Expression Omnibus (human, mouse and rat) and from Affymetrix microarray analysis (GeneChip Bovine Genome Array) for **cationic and anionic transporters**. *indicates no probes were present on the microarray. ▲ indicates zero expression.

Figure 4.

Expression levels of gene transcripts (as fraction/percentage of samples in which gene is observed) determined from analysis of microarray data obtained from NCBI's Gene Expression Omnibus (human, mouse and rat) and from Affymetrix microarray analysis (GeneChip Bovine Genome Array) for **peptide and monocarboxylate transporters**. *indicates no probes were present on the microarray. ▲ indicates zero expression.

Figure 5.

Expression levels of gene transcripts (as fraction/percentage of samples in which gene is observed) determined from analysis of microarray data obtained from NCBI's Gene Expression Omnibus (human, mouse and rat) and from Affymetrix microarray analysis (GeneChip Bovine Genome Array) for **amino acids transporters**. *indicates no probes were present on the microarray. ▲ indicates zero expression.

Figure 6. Expression levels of gene transcripts (as fraction/percentage of samples in which gene is observed) determined from analysis of microarray data obtained from NCBI's Gene Expression Omnibus (human, mouse and rat) and from Affymetrix microarray analysis (GeneChip Bovine Genome Array) for **nucleoside transporters**. *indicates no probes were present on the microarray. ▲ indicates zero expression.

Table 1

List of primers designed for bovine gene analysis using Primer-BLAST.

Transporter	Gene	NCBI reference number	Primer	Primer sequence
MDR1	ABCB1	XM_002686731.2	Sense	TGACCGCCACAGAAAGAGTGC
			Anti-sense	AATAGCGAAAGCGAGCCGAGGG
MRP1	ABCC1	NM_174223.1	Sense	CCAAAGTCCAGAAAGCAGCCGGT
			Anti-sense	TGATGAACAGCAGCGCCGTGT
MRP2	ABCC2	XM_002698487.1	Sense	TCCTCGTTGTTGGATAGCCCGGA
			Anti-sense	ACACACCATCGCCTGTGTGT
MRP3	ABCC3	NM_001192756.1	Sense	TGGTCTTGCTAATCCCGCTCAACG
			Anti-sense	ACACACTGGTCTGGGCCAGGTT
MRP4	ABCC4	XM_002683737.1	Sense	TGTGATTGGTGTGGTGGGCGTG
			Anti-sense	AGACAGGACCAAGGCCAACGTGT
MRP5	ABCC5	NM_001077907.1	Sense	ACCAGCAATTCAGGGCAGCACCC
			Anti-sense	CCACTGGTGTGGTGGTTTGA
BCRP	ABCG2	NM_001037478.2	Sense	ACAGAGCCGGGTGCTCTTCTT
			Anti-sense	ATGCTGCAAGCCGCATAGCC
OCT3	SLC22A3	XM_002690372.1	Sense	TTCTGCTGCTGTGCCTAACGG
			Anti-sense	ACACGAGGTCAAACTCGCTGACG
PEPT1	SLC15A1	NM_001099378.1	Sense	TGCGTGTCTGCATTGGCGGA
			Anti-sense	GGATGGCGTTACCCGTCTGCAT
PEPT2	SLC15A2	NM_001079582.1	Sense	GCCATCTCCGAAATCTGTGGCT
			Anti-sense	AGCAATGGCTGCTCCAGGAT
MCT1	SLC16A1	NM_001037319.1	Sense	AAGCAAGCCCAAGACCTCCA
			Anti-sense	AAGCAAGCCCAAGACCTCCA
MCT2	SLC16A7	NM_001076336.2	Sense	CAGTGGCCGAGACCAGCAGTT
			Anti-sense	TCACCACTGGCCGGCTACCAT
MCT3	SLC16A8	XM_002687932.1	Sense	ATGCTTTACGGCACAGGCCCC
			Anti-sense	AGGCGATGCAGAAAGGCGACAA
MCT4	SLC16A3	NM_001109980.1	Sense	CAGCCTCTGTTCAGGACGCAGAA

Transporter	Gene	NCBI reference number	Primer	Primer sequence
			Anti-sense	TTGAGCGCCAAACCCAAAGCCA
sMCT1	SLC5A8	NM_001164861.1	Sense	TGCACACAGACCAGCTCATGCC
			Anti-sense	AAGCCAGTGCAGCCATGCCAA
sMCT2	SLC5A12	NM_001101059.1	Sense	TCATGTCCGCCGCTCACTGTCT
			Anti-sense	GGCAGCCACGGTGTCTCAAGTT
γ -LAT1 Light chain	SLC7A7	NM_001075151.1	Sense	TTCAGGGCTGGGGAAGGGCAAT
			Anti-sense	CCCACAAATCAGGCACACAGCCCAT
$b^0 +$ AT Light chain	SLC7A9	NM_001035054.1	Sense	TGCCGGCTGCCATCTTGCTCAT
			Anti-sense	AGCGGCGCGATGGTTGAAAA
Asc-1 Light chain	SLC7A10	NM_001104989.1	Sense	TGGCTTGCTCTGATGCTCTTTGACG
			Anti-sense	AGCGTGACGTGTCTCTCCCACGA
xCT Light chain	SLC7A11	XM_002694373.1	Sense	ATGCGTACGCCCGGCTGGTTTT
			Anti-sense	AAGCTGGGATGAACAGCGGCA
ENT1	SLC29A1	NM_001034398.1	Sense	AGCGCCTGGAAGGCCTACTTCA
			Anti-sense	AGCACAGGCCATGAGCAAGCA
ENT2	SLC29A2	NM_001103269.1	Sense	TTCCTGTGGCCCGACGAAAGACA
			Anti-sense	ACACCAGCTGCACGGTTGACA
CNT1	SLC28A1	BC108101.1	Sense	TGTGTGGCCGGGATCCTCTACA
			Anti-sense	TTCTGTCCCGCTACGCACAGA
CNT2	SLC28A2	XM_002690906.1	Sense	TGACGATGCACACCCCTTTCCT
			Anti-sense	ACCGGGTAGTGAAACCCGCAA
CNT3	SLC28A3	NM_001192167.1	Sense	TGGTGGCGAAACATTGCCCGTGA
			Anti-sense	AGCCATGGAAAGTGAGTCCACCGA

Table II

Drug transporter expression reported in the nasal mucosa of humans, mice and rats and the method used for determination. Expression levels of gene transcripts for known drug transporter expression were determined from analysis of Affymetrix microarray and RT-PCR performed on bovine nasal samples. For microarray, gene expression above the 50th percentile was defined as **High**; expression values between the 25th and 50th percentile were classified as **Moderate**; and expression levels below the 25th percentile were defined as **Not/Weak**.---: data not available. For RT-PCR, **Yes** indicates the expression was observed in at least two of the three samples and **No** expression was observed (Wb: Western blotting, IHC: Immunohistochemistry and B-DNA: branched DNA technology).

Transporter Family	Human Gene Name	Human Family Member	Human			Mouse		Rat	
			Microarray	Other methods		Microarray	Other methods	Microarray	Other methods
Multidrug resistance protein (MDR)	ABCB1	MDR1	Not/Weak	Yes and IHC ¹³	Yes	IHC and RT-PCR ^{*,†42, 43}	High	Moderate	Yes RT-PCR, Wb and IHC ³³
	ABCC1	MRP1	High	Yes	Yes	IHC and RT-PCR ^{†42}	High	High	--
	ABCC2	MRP2	--	No	--		Not/Weak	--	--
	ABCC3	MRP3	High	Yes	--		High	--	--
	ABCC4	MRP4	High	No	--		Moderate	--	--
	ABCC5	MRP5	Moderate	Yes	--		High	High	--
	ABCC6	MRP6	Not/Weak	--	--		Not/Weak	Not/Weak	--
	ABCC10	MRP7	High	--	--		Not/Weak	--	--
	ABCG2	BCRP1	High	Yes	--		High	High	--
	SLC22A1	OCT1	High	No ¹⁴	Yes	RT-PCR, Wb and IHC ^{*,40,41}	Moderate	Not/Weak	weak B-DNA ³²
Organic cation transporter (OCT)	SLC22A2	OCT2	--	Yes ¹⁴ and IHC and Wb ¹²	No	RT-PCR, Wb and IHC ^{*,40,41}	High	Moderate	Yes RT-PCR, B-DNA, and IHC ^{32, 38}
	SLC22A3	OCT3	--	No	Yes	RT-PCR, Wb and IHC ^{*,40,41}	Not/Weak	Moderate/High	weak B-DNA ³²
	SLC22A4	OCTN1	--	Yes ¹⁴	Yes	RT-PCR, Wb and IHC ^{*,40,41}	Not/Weak	--	weak B-DNA ³²
Organic cation/cartinine transporter (OCTN)	SLC22A5	OCTN2	High	Yes ¹⁴	Yes	RT-PCR, Wb and IHC ^{*,40,41}	High	High	--
	SLC22A6	OAT1	Not/Weak	--	--		Not/Weak	Not/Weak	--
	SLC22A7	OAT2	Not/Weak	--	--		Moderate	Not/Weak	--
Organic anion transporter (OAT)	SLC22A8	OAT3	Not/Weak	--	--		Not/Weak	Not/Weak	Yes RT-PCR ³⁸
	SLCO1A2	OATP1A2	High	--	--		--	Not/Weak	Yes (OATP2) Yes (OATP3) RT-PCR ³³ B-DNA and IHC ³²
	SLCO1B1	OATP1B1	--	--	--		Not/Weak	Not/Weak	--

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Transporter Family	Human Gene Name	Human Family Member	Cow		Human		Mouse		Rat	
			Microarray	RT-PCR	Microarray	Other methods	Microarray	Other methods	Microarray	Other methods
	SLC02A1	OATP2A1	High	--	High	--	Moderate	--	Not/Weak	--
	SLC02B1	OATP2B1	High	--	Moderate/High	--	Moderate	--	High	--
	SLC03A1	OATP3A1	High	--	High	--	High	--	High	--
	SLC04A1	OATP4A1	High	--	Moderate	--	Moderate	--	High	--
	SLC04C1	OATP4C1	High	--	High	--	Moderate	--	--	--
	SLC15A1	PEPT1	High	Yes	Moderate	--	Moderate	--	--	--
Peptide transporter (PEPT)	SLC15A2	PEPT2	High	Yes	High	--	High	--	High	Yes
Mono-carboxylate transporters (MCTs)	SLC16A1	MCT1	High	No	High	--	Moderate	--	High	Yes
	SLC16A7	MCT2	--	Yes	High	--	Moderate	--	High	Yes
	SLC16A8	MCT3	--	Yes	Not/Weak	--	Not/Weak	--	Not/Weak	--
	SLC16A3	MCT4	High	Yes	High	--	Weak	--	High	--
	SLC5A8	sMCT1	--	Yes	Not/Weak	--	Moderate	--	High	--
	SLC5A12	sMCT2	--	No	Moderate	--	Not/Weak	--	Moderate	--
Heterodimeric amino acid transporters	SLC7A5	LAT1	High	Yes ¹⁵	Moderate	--	High	--	High	--
	SLC7A8	LAT2	High	No ¹⁵	High	--	Moderate	--	High	--
	SLC7A7	y+LAT1	High	Yes	Moderate	--	Moderate	--	High	--
	SLC7A6	y+LAT2	High	--	High	--	High	--	High	--
	SLC7A9	b ⁰ +AT	Moderate	Yes	Not/Weak	--	Moderate	--	Not/Weak	--
	SLC7A10	Asc-1	--	Yes	Not/Weak	--	Moderate	--	Not/Weak	--
	SLC7A11	xCT	--	Yes	High	--	Moderate	--	--	--
	SLC28A1	CNT1	--	Yes	Moderate	No	--	--	Not/Weak	--
	SLC28A2	CNT2	--	No	Moderate	Yes	Not/Weak	--	--	--
Nucleoside transporters (CNT, ENT)	SLC28A3	CNT3	High	Yes	High	Yes	Moderate	--	Moderate	--
	SLC29A1	ENT1	High	Yes	High	--	Moderate	--	High	Yes
	SLC29A2	ENT2	High	No	Moderate	--	Not/Weak	--	High	Yes

* Source was human nasal turbinate explant⁴²

Source was polarized primary cultures of normal human nasal epithelial cells 43
* Source was human nasal turbinate explant 40,41
* Source was human nasal turbinate explant(6)

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Table III

Comparison of the expression of selected drug transporters in the nasal mucosa among three pre-clinical animal species (mouse, rat, cow) and humans. Evidence for transporter expression was either via DNA microarray analysis, RT-PCR amplification of mRNA, Western blotting, or immunohistochemistry. Positive evidence (✓) from any one technique was viewed as sufficient to suggest the transporter was expressed, (-) indicates no evidence for expression was detected or reported.

Transporter	Gene	Evidence for Expression			
		Mice	Rats	Cows	Humans
MDR1	ABCB1	✓	✓	✓	✓
MRP1	ABCC1	✓	✓	✓	✓
MRP2	ABCC2	-	-	-	-
MRP3	ABCC3	✓	✓	✓	✓
MRP4	ABCC4	✓	-	✓	✓
MRP5	ABCC5	✓	✓	✓	✓
MRP6	ABCC6	-	-	-	✓
MRP7	ABCC10	-	-	✓	✓
BCRP	ABCG2	✓	✓	✓	-
OCT1	SLC22A1	✓	✓	✓	✓
OCT2	SLC22A2	✓	✓	✓	-
OCT3	SLC22A3	-	✓	-	✓
OCTN1	SLC22A4	✓	✓	✓	✓
OCTN2	SLC22A5	✓	✓	✓	✓
OAT1	SLC22A6	✓	-	-	-
OAT2	SLC22A7	✓	-	-	-
OAT3	SLC22A8	-	✓	-	-
OATP1A2	SLC01A2	-	✓	✓	✓
OATP1B1	SLC01B1	-	-	-	✓
OATP2A1	SLC02A1	✓	-	✓	✓
OATP2B1	SLC02B1	✓	✓	✓	✓
OATP3A1	SLC03A1	✓	✓	✓	✓
OATP4A1	SLC04A1	✓	✓	✓	✓

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Transporter	Gene	Evidence for Expression			
		Mice	Rats	Cows	Humans
OATP4C1	SLC04C1	✓	-	✓	✓
PEPT1	SLC15A1	✓	-	✓	✓
PEPT2	SLC15A2	✓	✓	✓	✓
MCT1	SLC16A1	✓	✓	✓	✓
MCT2	SLC16A7	✓	✓	✓	✓
MCT3	SLC16A8	-	-	✓	-
MCT4	SLC16A3	✓	✓	✓	✓
sMCT1	SLC5A8	✓	✓	✓	-
sMCT2	SLC5A12	-	✓	-	✓
LAT1	SLC7A5	✓	✓	✓	✓
LAT2	SLC7A8	✓	✓	✓	✓
γ+LAT1 Light chain	SLC7A7	✓	✓	✓	✓
γ+LAT2	SLC7A6	✓	✓	✓	✓
b ⁰⁺ AT Light chain	SLC7A9	✓	-	✓	-
Asc-1 Light chain	SLC7A10	✓	-	✓	-
xCT Light chain	SLC7A11	✓	-	✓	✓
ENT1	SLC29A1	✓	✓	✓	✓
ENT2	SLC29A2	-	✓	✓	✓
CNT1	SLC28A1	-	-	✓	✓
CNT2	SLC28A2	-	-	-	✓
CNT3	SLC28A3	✓	✓	✓	✓