Adverse Drug Reactions in Veterinary Patients Associated with Drug Transporters

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KEYWORDS
• ATP-binding cassette transporters • ABCB1 • ABCG2 • Solute carrier
• Pharmacogenetics

KEY POINTS
• Because drug transporters play an important role in drug absorption, distribution, and excretion, alterations in drug transporter function can result in adverse drug reactions.
• The ABCB1 polymorphism in dogs and drug interactions involving P-glycoprotein can enhance the toxicity of many drugs.
• The species-wide ABCG2 defect in cats is responsible for fluoroquinolone-induced retinal toxicity.
• Because drug transporters play an important role in drug disposition, a thorough understanding of drug transporters in companion animals is critical in drug discovery and development.

INTRODUCTION
Most veterinarians consider an adverse drug reaction to be something that happens when excessive drug accumulation results in drug toxicity. Another type of adverse drug event, lack of efficacy, occurs when a drug fails to reach effective concentrations at the site of action. Both types of adverse drug events (lack of drug efficacy or drug toxicity) can be deleterious to the patient, so both should be avoided. Optimal drug therapy is achieved when drugs reach effective concentrations at the site of action but do not reach toxic concentrations in susceptible tissues.

Any factor that influences plasma and/or tissue drug concentrations will influence the optimization of drug therapy. Some obstacles to achieving optimal drug therapy have been discussed in previous articles. Alterations in the function of drug-metabolizing enzymes, whether due to genetic polymorphisms or drug-drug

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interactions, can increase or decrease plasma and tissue drug concentrations. Other factors that may influence plasma and tissue drug concentrations include liver disease, renal disease, patient age, dietary interactions, and interactions with nondrug supplements such as herbas and so-called nutraceuticals (nutritional supplements).

For many drugs used in veterinary practice, plasma and tissue concentrations are also highly dependent on the activity of drug transporters. These transporters are large transmembrane proteins that function as either drug efflux or uptake pumps. The transporters are expressed to some degree on a variety of tissues while being highly expressed on the surface of tissues that are responsible for drug absorption, metabolism, and excretion, such as liver, intestinal lumen, biliary canaliculi, and renal tubular epithelium. In addition, these transporters are expressed on the endothelium of “sanctuary” or protected sites, including brain, retina, testes, and placenta (Fig. 1).

Two superfamilies of drug transport proteins are considered to be of major importance in determining drug disposition in human patients: the adenosine triphosphate (ATP)-binding cassette (ABC) superfamily and the solute carrier (SLC) superfamily. Research in human patients and/or knockout mice models have demonstrated clinically significant changes in drug disposition resulting from altered function of these drug transporters. Although specific examples of drug transporter defects in veterinary species are scarce, those that have been documented dramatically illustrate the importance of the ABC transporter family in drug disposition. Altered function of drug transporters either intrinsically (ie, genetic polymorphisms) or extrinsically (ie, drug-drug interactions) can result in decreased drug efficacy or increased risk of toxicity in affected patients. Whether one can extrapolate data from human or mouse studies and apply it to other species depends on the specific transporter and drug involved, because there may be species differences in tissue expression and/or substrate specificity of the transporter.

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**Fig. 1.** Major drug transporters of the ABC and SLC superfamilies, their tissue localization, and direction of drug transport. ABC, ATP-binding cassette; BCRP, breast cancer resistance protein; MATEs, multidrug and toxin extrusion transporters; MRP, multidrug resistance–related protein; OATs, organic anion transporters; OATPs, organic anion–transporting polypeptides; OCTs, organic cation transporters; P-gp, P-glycoprotein; SLC, solute carrier.
This article describes how functional changes in drug transporters, whether mediated by genetic variability or drug-drug interactions, affect drug disposition and, ultimately, drug safety and efficacy in veterinary patients. A greater understanding of species, breed, and individual (genetic) differences in drug transporter function, as well as drug-drug interactions involving drug transporters, will result in improved strategies for drug design and will enable veterinarians to incorporate individualized medicine in their practices.

ATP-BINDING CASSETTE SUPERFAMILY

There are more than 40 members of the ABC protein superfamily. These proteins use ATP to transport substrates across biological membranes (often against steep concentration gradients). Substrates for ABC transporters include ions, peptides, hormones, conjugated metabolites, and xenobiotics, including drugs. Only 3 members of the ABC transporter superfamily are known to transport drug molecules as their substrates: P-glycoprotein (P-gp) (encoded by the ABCB1, formerly MDR1, gene); breast cancer resistance protein (BCRP) (encoded by the ABCG2 gene); and multidrug resistance–related protein (MRP) (encoded by the ABCC1 gene).

The ABC drug transporters P-gp, BCRP, and MRP are membrane-spanning proteins that function as transmembrane efflux pumps. One or more of these proteins are normally expressed by a variety of mammalian tissues that serve as barriers to drug absorption (apical border of intestinal epithelial cells), and enhance drug elimination from the body (biliary canalicular or renal tubular epithelial cells) or on capillary endothelial cells at so-called sanctuary sites (blood-brain barrier, blood-retina barrier, testes, placenta). Because of their strategic locations and their ability to prevent drug absorption, enhance drug excretion, and prevent drug entry into specialized tissues, it is presumed that ABC drug transporters provide a protective role for the organism by decreasing exposure to potentially toxic xenobiotics. Thus it is not surprising that deficient ABC transporter function can result in significantly enhanced exposure to substrate drugs. Animals with defects in an ABC transporter experience extreme drug sensitivity when exposed to drugs that are substrates for the defective transporter.

**ABCB1 (P-Glycoprotein)**

P-gp was the first ABC transporter characterized, and is well known for its ability to modulate multidrug resistance in cancer cells in many species. P-gp substrates include many anticancer drugs (anthracyclines, vinca alkaloids, epipodophyllotoxins), macrocyclic lactones (ivermectin, selamectin, milbemycin, and so forth), loperamide, acepromazine, butorphanol, ondansetron, and dozens of other drugs. Several studies have addressed P-gp’s role in drug absorption, distribution, and excretion, and how these pharmacokinetic parameters affect safety and efficacy of P-gp substrate drugs in dogs. Much of this information has been generated from studies and clinical observations of dogs affected by a mutation in the ABCB1 (MDR1) gene. The ABCB1 polymorphism in dogs consists of a 4-base-pair deletion mutation. This deletion results in a shift of the reading frame that generates several premature stop codons. Because protein synthesis is terminated before even 10% of the protein product is synthesized, dogs with 2 mutant alleles exhibit a P-gp null phenotype, similarly to abcb1 (mdr1) (−/−) knockout mice. Heterozygous dogs with one mutant allele and one wild-type allele (ABCB1 normal/mutant) have an intermediate phenotype. Affected dogs include many herding breeds (Table 1). The frequency of the ABCB1-1Δ mutation is very high in some breeds, with affected dogs displaying the
“multidrug sensitivity” phenotype. Studies have determined that roughly 75% of collies and 50% of Australian Shepherds in the United States,7 Europe,8 Japan,9 and Australia10 have at least 1 mutant allele. Thus, an understanding of P-gp’s role in drug distribution and elimination is critically important when dogs of these breeds are treated with any drug that might be a substrate for P-gp.

Drug distribution, and therefore the risk of drug toxicity, can be dramatically affected by alterations in P-gp function. Because P-gp is a component of the blood-brain barrier, the blood-testes barrier, and the placenta, distribution of P-gp substrate drugs to these tissues is greatly enhanced in ABCB1(mutant/mutant) dogs and moderately enhanced in ABCB1(mutant/normal) dogs. Macrocyclic lactones such as ivermectin can cause neurologic toxicity in any animal at high doses (>2 mg/kg). ABCB1(mutant/mutant) dogs experience adverse neurologic effects after a single dose of ivermectin (>120 µg/kg) and will experience life-threatening neurologic toxicity at the antiparasitic dose of 300 µg/kg because the defective blood-brain barrier allows ivermectin to accumulate in brain tissue of these animals.6,11 The concentration of ivermectin in brain tissue of abcb1(−/−) knockout mice is 100 times greater than the concentration of ivermectin in brain tissue of wild-type mice.12 A similar difference in brain accumulation of ivermectin in ABCB1(mutant/mutant) versus ABCB1(normal/normal) dogs would be expected. It is important that the dose of ivermectin used in commercial heartworm preventive products (6 µg/kg per month) will not cause neurologic toxicity even in ABCB1(mutant/mutant) dogs. Dogs with the ABCB1 mutation also have increased susceptibility to neurologic adverse effects of other macrocyclic lactones (milbemycin, selamectin, and moxidectin) as well as the antidiarrheal drug loperamide. Loperamide is an opioid that is generally devoid of central nervous system (CNS) activity because it is excluded from the brain by P-gp. At routinely used therapeutic doses, loperamide causes profound CNS depression in ABCB1(mutant/mutant) dogs.13,14 This effect can be reversed by the opioid antagonist naloxone. Less information is available regarding P-gp and the blood-brain barrier in cats. The

| Table 1
<p>| Approximate frequencies (%) of MDR1 genotypes in affected dog breeds&lt;sup&gt;a&lt;/sup&gt; |</p>
<table>
<thead>
<tr>
<th>Breed</th>
<th>MDR1(Mutant/Mutant)</th>
<th>MDR1(Mutant/Normal)</th>
<th>MDR1(Normal/Normal)</th>
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<td>Australian Shepherd</td>
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<tr>
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<td>85</td>
</tr>
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<td>8</td>
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<td>10</td>
<td>89</td>
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<td>35</td>
</tr>
<tr>
<td>McNab</td>
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<td>28</td>
<td>70</td>
</tr>
<tr>
<td>Mixed breed</td>
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<td>89</td>
</tr>
<tr>
<td>Old English Sheepdog</td>
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<td>93</td>
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<tr>
<td>Shetland Sheepdog</td>
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<td>15</td>
<td>83</td>
</tr>
<tr>
<td>Silken Windhound</td>
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<td>33</td>
<td>66</td>
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</tbody>
</table>

<sup>a</sup> Represent combined results from several different investigations. Data from Refs.7–10,39–41
author has received anecdotal reports of ivermectin toxicity in cats after therapeutic doses (100–300 μg/kg), but whether the underlying cause is a result of altered P-gp expression or function is not currently known.

P-gp also affects drug excretion. Although P-gp is expressed on both renal tubular cells and biliary canalicular cells, evidence is lacking to support an important role for P-gp with respect to renal drug excretion. On the contrary, studies in different species demonstrate the important role P-gp plays in biliary drug excretion. Altered P-gp function has been shown to dramatically alter the disposition of several P-gp substrate drugs. For example, concurrent administration of a P-gp inhibitor decreases the biliary clearance of doxorubicin (P-gp substrate) in rats, resulting in increased plasma concentrations of doxorubicin.15 Similarly, administration of verapamil (a P-gp inhibitor) to rats decreased biliary excretion of the P-gp substrate irinotecan by half.16 Studies comparing biliary excretion of the P-gp substrate 99mTc-sestamibi in ABCB1(normal/normal) and ABCB1(mutant/mutant) dogs demonstrate that ABCB1(mutant/mutant) dogs are not able to excrete this compound into bile.17 99mTc-sestamibi is essentially undetectable in gallbladders of ABCB1(mutant/mutant) dogs but is highly concentrated in gallbladders of ABCB1(normal/normal) dogs. Diminished biliary drug excretion may play a role in the apparent increased sensitivity of herding breeds to toxicity caused by chemotherapeutic drugs that are P-gp substrates. For example, ABCB1(mutant/mutant) and ABCB1(normal/mutant) dogs are significantly more likely than ABCB1(normal/normal) dogs to develop hematologic toxicity (neutropenia and thrombocytopenia) after treatment with the P-gp substrate vincristine.18 However, these dogs tolerate cyclophosphamide (a drug that is not a P-gp substrate) at full doses. In dogs, vincristine is eliminated primarily via biliary excretion of parent drug with some urinary excretion of parent drug and metabolites. Therefore, reduced doses of P-gp substrate chemotherapeutic agents including vincristine, doxorubicin, and vinblastine should be given to ABCB1(mutant/mutant) and ABCB1(normal/mutant) dogs to avoid severe toxicity.

**ABCG2 (Breast Cancer Resistance Protein)**

BCRP is expressed on enterocytes, the canalicular membrane of hepatocytes, proximal renal tubule cells, erythrocytes, and hematopoietic stem cells.19 It is also expressed on endothelial cells of the brain and retina, where it functions as an important component of the blood-brain barrier and the blood-retina barrier. At these locations BCRP functions in a protective capacity, excreting substrate drugs into the intestinal lumen, bile, and urine, or restricting access of substrate drugs to sensitive brain and retinal tissues. In addition, BCRP is expressed in mammary tissue where it transports substrates into milk.20 Several polymorphisms in ABCG2 have been described in humans. The most well-characterized polymorphism, ABCG2 421C>A, results in a glutamine (polar) to lysine (nonpolar) amino acid change within an important functional domain, the ATP-binding domain.21 The ATP-binding domain is critical to protein function because ATP binding supplies the energy to pump substrates against a concentration gradient. ABCG2 expression in affected individuals is decreased and ABCG2 transport capacity is reduced when compared with individuals with the wild-type allele. Compared with patients with wild-type ABCG2, oral bioavailability of topotecan is 1.34-fold greater in patients with the ABCG2 421C>A variant.22 Human patients with the ABCG2 421C>A variant also experience altered pharmacokinetics and/or increased drug-induced toxicity when administered substrate drugs such as gefitinib, irinotecan, sulfasalazine, and other drugs in comparison with individuals with the wild-type allele.22 Thus, functional changes in ABCG2 are also likely to have therapeutic implications for veterinary patients.
Whereas canine ABCG2 has not been well characterized, characterization of feline ABCG2 has been described. Feline ABCG2 was sequenced and the consensus amino acid sequence compared with that of 10 other mammalian species, including humans. Four feline-specific amino acid changes in conserved regions of ABCG2 were identified. One of the amino acid changes was similar to the human variant ABCG2 421C>A, in that it occurs within the ATP-binding domain and consists of a glutamate (polar) to methionine (nonpolar) shift. Therefore one would predict a similar defect in ABCG2 function. The effect of these feline-specific amino acid changes on ABCG2 function was assessed using transfection experiments. cDNA constructs for feline and wild-type human ABCG2 were inserted in a pcDNA3 expression vector and expressed in HEK 293 cells. ABCG2 transport function was assessed using 2 standard ABCG2 substrates (mitoxantrone and BODIPY-prazosin). As predicted, feline ABCG2 transport of either substrate was defective when compared with wild-type human ABCG2. These results have several important clinical consequences, particularly when one considers that these amino acid changes appear to affect all cats and do not represent an ABCG2 variant (as is the case in humans). Based on this information, in comparison with other species cats would be expected to exhibit enhanced susceptibility to toxicity of ABCG2 substrate drugs.

For more than 20 years, fluoroquinolone antimicrobials have been used in veterinary patients. Domestic cats experience an unusual, and apparently species-specific, adverse drug reaction associated with fluoroquinolones: acute retinal degeneration and blindness. Fluoroquinolone-induced retinal toxicity was first documented with enrofloxacin, which is structurally similar to the human-approved fluoroquinolone ciprofloxacin. Enrofloxacin received approval from the Food and Drug Administration (FDA) in 1990 for use in cats at an oral dose of 2.5 mg/kg/d. In 1997, a flexible dosing label for enrofloxacin was approved, which consisted of a dose range of 5 to 20 mg/kg, per os, either once daily or as a divided daily dose. Shortly after this flexible dosing label was introduced, veterinarians began diagnosing blindness in cats receiving enrofloxacin, particularly after once-daily administration of doses at the high end of the flexible dose range. In a prospective study, enrofloxacin was administered orally at a dose of 50 mg/kg to 12 cats while 12 cats received saline control. All enrofloxacin-treated cats developed retinal degeneration as early as 3 days after enrofloxacin administration, whereas none of the control cats developed retinal lesions. Histopathologic changes included severe vacuolization progressing to necrosis in the photoreceptor layer, outer nuclear layer, and outer plexiform layer of the retina. Thus, high doses of enrofloxacin are acutely toxic to the retina of cats. Orbifloxacin, another fluoroquinolone approved for use in cats and dogs, also causes retinal damage in cats (www.fda.gov freemedom of information summary NADA 141-081).

In human patients, cutaneous photosensitivity is known as one of the more common adverse reactions to fluoroquinolones. When exposed to light, fluoroquinolones generate reactive oxygen species (ROS) that attack cellular lipid membranes, causing tissue damage. With the exception of skin, the organ most susceptible to phototoxicity is the eye, including the retina. Retinal degeneration and blindness can result from extensive phototoxic damage to the retina. Under normal conditions, the retina is protected from photoreactive compounds by the blood-retina barrier. The blood-retina barrier consists of both capillary endothelial cell tight junctions and retinal pigment epithelial cell tight junctions, as well as important transporters including ABCG2 that function to prevent distribution of drugs to the retina. ABCG2 is strategically positioned on the luminal membrane of retinal capillary endothelial cells, where it actively transports substrates from the endothelial cell back into the capillary lumen.
Because fluoroquinolones are substrates for ABCG2, the distribution of these drugs to retinal tissues is normally restricted by ABCG2.

Dysfunction of ABCG2 in cats results in a dysfunctional blood-retina barrier. Cats experience accumulation of photoreactive fluoroquinolones in their retina in comparison with other species. Exposure of the retina to light then generates ROS, which create the characteristic retinal degeneration and blindness documented in cats receiving high doses of fluoroquinolones. Defective ABCG2 function in cats may also contribute to their sensitivity to acetaminophen. In dogs and humans, acetaminophen is metabolized primarily by glucuronidation and sulfation to nontoxic metabolites. Cytochrome P450 enzymes, which generate a toxic metabolite (NAPQI), are not involved. Relative to other species, cats have low hepatic levels of uridine diphosphate glucuronyltransferase and are unable to use glucuronidation pathways. Thus, acetaminophen metabolism is shifted to the sulfation pathway. In most species ABCG2 mediates the biliary excretion of acetaminophen sulfate. However, in abcg2 knockout mice, biliary excretion of acetaminophen sulfate was negligible. Because feline ABCG2 is also dysfunctional, a similar phenomenon would be expected to occur in cats. This process would result in shunting of acetaminophen metabolism away from sulfation in favor of cytochrome P450-mediated pathways where the toxic metabolite NAPQI is generated. Thus, defective ABCG2 function in cats may help explain enhanced susceptibility of cats to acetaminophen toxicity.

A long list of substrates and inhibitors of ABCG2 already exists, and the list continues to expand. With regard to cancer therapy, important substrates of ABCG2 include mitoxantrone, methotrexate, etoposide, and, of particular interest, many tyrosine kinase inhibitors. Two tyrosine kinase inhibitors (toceranib and masitinib) have recently received FDA approval for the treatment of canine mast cell tumors. Of note, tyrosine kinase inhibitors are reported to be both substrates for and inhibitors of ABCG2. Whether defective ABCG2 function in cats will affect efficacy and/or toxicity of tyrosine kinase inhibitors has yet to be determined.

**Inhibitors of ABC Transporters Can Mimic Pharmacogenetically Mediated Adverse Reactions**

Because P-gp and ABCG2 contribute to multidrug resistance in patients with cancer, numerous drugs have been developed to inhibit these transporters in an effort to improve outcomes in patients treated with chemotherapy. Clinical trials involving inhibitors of ABC transporters have resulted in unexpected and undesired pharmacokinetic interactions between the inhibiting agents and the chemotherapeutic drugs used for treating patients with cancer. For example, inhibiting agents often block renal and/or biliary excretion of anticancer drugs by the ABC transporter, enhancing their accumulation in plasma and thereby increasing the risk of toxicity (ie, myelosuppression, gastrointestinal adverse effects). Inhibiting ABC transporters not only blocks excretion of drugs but also alters their distribution. Inhibiting P-gp has been shown to enhance brain penetration of substrate drugs (mimicking what is seen in MDR1 mutant/mutant dogs). For example, ketoconazole increases brain penetration of ivermectin in MDR1(normal/normal) dogs, causing neurologic toxicity (author’s personal observation). Ketoconazole inhibits biliary excretion of 99mTc-sestamibi in MDR1(normal/normal) dogs. For P-gp substrate drugs with a narrow therapeutic index (ie, vincristine, doxorubicin), concurrent administration of a drug that inhibits P-gp function should be avoided. Drugs known to inhibit P-gp function in dogs include ketoconazole, cyclosporine, and spinosad.

While less is known about the clinical consequences of ABCG2 inhibition, caution should be exercised. For example, pharmacologic inhibition of ABCG2 would be
expected to enhance retinal accumulation of substrate drugs such as fluoroquinolones. Thus, concurrent administration of a fluoroquinolone and an ABCG2 inhibitor (such as a tyrosine kinase inhibitor) may cause retinal degeneration and blindness in any species.

**ABCC1 (Multidrug Resistance-Related Protein)**

Like many ABC transporters, ABCC1 expression was first identified in cancer cells that were resistant to multiple chemotherapeutic drugs. Subsequently ABCC1 expression was identified in many normal tissues including lung, testis, kidney, placenta, and muscle. ABCC1 substrates include a variety of structurally diverse compounds, including some of the same drugs transported by P-gp (anthracyclines, vinca alkaloids, and some human immunodeficiency virus–1 protease inhibitors). Endogenous substrates for ABCC1 include oxidized glutathione, cysteinyl leukotrienes, and glucuronide and sulfate conjugates. Expression of MRP1 has been reported in both canine and feline tumor cells, but ABCC1 variants in veterinary species have not been reported.

Despite its similarities to other important ABC drug transporters (eg, tissue expression and substrate specificity), altered ABCC1 function in human patients has not been shown to alter drug disposition in a clinically significant manner. This finding may be due to the fact that ABCC1 polymorphisms identified to date do not result in major disruption to ABCC1 transport function. It is also possible that alternative pathways and overlapping substrate specificity with other ABC transporters mitigates the impact of ABCC1 on drug disposition. However, one human ABCC1 variant (ABCC1 Gly67Val) is significantly associated with doxorubicin-induced cardiomyopathy without evidence of altered doxorubicin pharmacokinetics.

**SOLUTE CARRIER SUPERFAMILY**

The SLC superfamily of transporters uses several different processes (facilitated diffusion, ion coupling, ion exchange) to transport substrates across biological membranes. Some members of the SLC superfamily are uptake transporters (transport substrates into cells) whereas others are efflux transporters (transport substrates out of cells). In humans and rodents, members of the SLC superfamily mediate transport of a variety of structurally diverse compounds, including endogenous and exogenous substances. There is some overlap of substrates among members of the SLC superfamily in humans and rodents. For example, fluvastatin (a cholesterol-lowering drug) is a substrate for at least 3 different members of the human SLC superfamily. Less is known about substrate specificity of canine and feline SLC transporters. In humans, rodents, and, presumably, dogs and cats, SLC transporters are expressed along the body’s functional “barrier” tissues including intestine, brain capillary endothelial cells, placenta, liver, and kidney, as well as other tissues. These strategic locations allow SLC transporters to influence drug absorption, distribution, metabolism, and excretion. Members of the SLC superfamily include organic cation transporters (OCTs), multidrug and toxin extrusion transporters (MATEs), organic anion transporters (OATs), and organic anion–transporting polypeptides (OATPs).

**Organic Cation Transporters**

Among the organic cation transporters in humans, those that appear to play a major role in drug transport include OCT1 (SLC22A1), OCT2 (SLC22A2), and OCT3 (SL22A3). These proteins function as uptake transporters in the tissues where they are expressed. The highest levels of expression of OCT1 and OCT2 are along
the basolateral (capillary side) membranes of hepatocytes and renal proximal tubular cells (see Fig. 1). OCT3 is expressed in a greater variety of tissues. Substrates for OCTs include endogenous cations such as creatinine and monoamine neurotransmitters, as well as exogenous cations such as metformin, ondansetron, and cimetidine.

Dysfunction of OCTs as a result of genetic variation can dramatically affect the pharmacokinetics and clinical response of substrate drugs in human patients. OCT1 loss-of-function mutations are associated with higher plasma concentrations and increased clinical effects of ondansetron. OCT1 normally transports ondansetron from the capillary lumen into hepatocytes, where it is metabolically inactivated. Lack of OCT1 transport function results in decreased hepatocellular uptake of ondansetron, decreased metabolic inactivation, and higher plasma concentrations of active drug. Similar alterations in drug disposition can be demonstrated in OCT1(+/−) knockout mice. Pharmacokinetics and pharmacodynamics of metformin, an OCT1 substrate, differ significantly in wild-type mice in comparison with OCT1(−/−) knockout mice.

In terms of common veterinary species, there are few data on the impact of OCTs on drug disposition. Because of the potential importance of drug/xenobiotic transport into cow’s milk, initial studies have investigated whether OCTs are expressed in an immortalized bovine mammary epithelial cell line. Based on basolateral to apical transport of the OCT substrate tetraethyl ammonium, and the fact that specific OCT inhibitors blocked the transport of tetraethyl ammonium, the investigators concluded that OCTs were expressed by bovine mammary epithelial cells.

**Multidrug and Toxin Extrusion Transporters**

Although the existence of a renal efflux transport system has been recognized since the late 1990s, MATE transporters were not specifically described until 2005. Tissues with the highest levels of MATE expression are the kidney (luminal membrane of renal proximal tubule cells) and liver (canalicular membrane of hepatocytes) (see Fig. 1). Because many of the substrates and inhibitors for MATE transporters overlap with those for OCTs, isolating and identifying the specific actions of MATE transporters is complex and continues to be the subject of ongoing investigations. Although polymorphism in genes encoding MATE transporters have been identified, the consequences of these polymorphisms in clinical patients have not been described. Plasma concentrations of metformin are greater in Mate1(+/−) mice than in wild-type mice, suggesting that alterations in MATE1 transport function, whether genetically mediated or the result of pharmacologic inhibition, would alter the disposition of substrate drugs.

**Organic Anion Transporters**

Members of the OAT family of transporters pump substrates against a concentration gradient by harnessing the power of a sodium gradient generated by Na+/K+-ATPase. The most important OATs with regard to drug transport are OAT1 and OAT3. These 2 transporters have overlapping substrate specificity, but OAT1 is expressed on the basolateral membrane of proximal renal tubular cells, whereas OAT3 is expressed not only on the basolateral membrane of proximal renal tubular cells but also in the choroid plexus. In the kidney OAT1 and OAT3 are uptake transporters, that is, they transport substrates from blood into renal tubular epithelial cells, thus enhancing renal drug clearance (see Fig. 1). OAT substrates include steroid hormones, biogenic amines, and angiotensin-converting enzyme inhibitors. OAT inhibitors include many commonly used drugs such as nonsteroidal anti-inflammatories (NSAIDs) and antimicrobials (many penicillins and fluoroquinolones). Of importance,
loop diuretics (eg, furosemide) and thiazide diuretics rely on OATs to reach their site of action on the luminal membrane of renal proximal tubular cells.

There are several drug interactions that occur via inhibition of OAT-mediated renal tubular secretion of substrates. The classic example, involving probenecid and β-lactam antibiotics, occurs when probenecid inhibits OAT-mediated secretion of β-lactam drugs, resulting in decreased clearance (and enhanced area under the curve) of β-lactams. Another example is the potentially life-threatening interaction involving NSAIDs and methotrexate. NSAIDs inhibit OAT-mediated renal excretion of methotrexate, which has resulted in severe myelosuppression in human patients following prolonged exposure to methotrexate. The contribution of protein-binding interactions with these 2 highly protein-bound drugs might also contribute to the potentially life-threatening drug interaction.

The author is not aware of genetic variants in OATs in veterinary species that have been associated with changes in drug disposition. However, functional OATs have been demonstrated in a cultured bovine mammary epithelial cell line, indicating that this transport family may be responsible for secreting xenobiotics into cow’s milk.

Organic Anion–Transporting Polypeptides

OATPs transport a variety of endogenous compounds including bile acids, thyroid hormones, and glucuronidated and sulfated hormones. Exogenous substrates of OATPs include rifampicin, methotrexate, metformin, and statins (drugs that are not routinely used in veterinary patients). Clinically relevant polymorphisms of OATP1B1 have been described in human patients. OAT1B1 expression in humans occurs exclusively on the basolateral membrane of hepatocytes, where it transports substrates into hepatocytes (see Fig. 1). A loss-of-function polymorphism in OAT1B1 results in decreased uptake of substrates by hepatocytes. For drugs such as the statins, whose site of action is within hepatocytes, individuals with loss-of-function mutations demonstrate a poor response to the lipid-lowering effects of this class of drugs in comparison with patients with wild-type OAT1B1. The author is not aware of any genetic variants of OATPs in veterinary species.

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