**SA251**
# WHAT YOU DON'T KNOW ABOUT P-GLYCOPROTEIN CAN HURT YOU (& YOUR PATIENTS!)

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## Overview of the Issue

P-glycoprotein, encoded by the MDR1 (also known as the ABCB1 gene) plays an important role in the absorption, distribution, metabolism and excretion of many drugs. It is not surprising, then, that dysfunction of P-glycoprotein increases susceptibility to drug toxicity (antiparasitic agents, anti-diarrheal drugs, chemotherapeutic drugs, acepromazine, butorphanol and others). P-glycoprotein dysfunction can occur 2 ways: (i) intrinsic P-glycoprotein dysfunction (inherited as a genetic mutation) or (ii) extrinsic or acquired P-glycoprotein dysfunction (by way of drug interaction--many drugs can inhibit P-glycoprotein function). In susceptible breeds, it is wise to perform MDR1 genotyping prior to administering drugs transported by P-glycoprotein in order to predict which dogs will experience drug sensitivity and which will tolerate routine doses of drugs. For all dog breeds, drugs that inhibit P-glycoprotein should not be used in combination with drugs that are transported by P-glycoprotein unless sufficient dose reductions are made.

## Objectives of the Presentation

At the end of this lecture, the audience should be able to (i) list commonly used drugs that are substrates for P-glycoprotein; (ii) predict the adverse effects of P-glycoprotein drugs if administered to dogs with the MDR1 mutation; (iii) know how to determine the MDR1 genotype of a dog and (iv) know how to avoid serious drug interactions involving P-glycoprotein substrate drugs and P-glycoprotein inhibiting drugs.

## P-glycoprotein (P-gp)

P-gp is a large protein that is expressed by many mammalian tissues that come into contact with drugs: intestinal cells, renal tubular cells, biliary canalicular cells, placenta, brain capillary endothelial cells and others[1]. P-gp has two drug binding sites and two ATP binding sites. If a drug is a substrate for P-gp, the drug is actively transported from the intracellular to the extracellular space. P-gp uses energy from ATP to transport drugs against steep concentration gradients[2]. P-gp is strategically positioned on the cell membranes and functions to actively pump drugs from the cytoplasm back into the lumen of the intestine, renal tubule, bile canaliculus, or brain capillary. Thus P-gp functions to protect the animal by decreasing exposure to potentially toxic xenobiotics that the animal may encounter. P-gp transports a variety of drugs commonly used in companion animals including anticancer agents (Vinca alkaloids, doxorubicin), immunosuppressants (cyclosporine, tacrolimus), antiparasitic agents (ivermectin, milbemycin, moxidectin, selamectin), loperamide , acepromazine, butorphanol, and others[3]. Many drugs have not been specifically evaluated with regard to their status as P-gp substrates, so it is likely that other drugs used in veterinary medicine will be identified as such and added to the list.

**Intrinsic (ABCB1-1□ mutation) and Acquired (P-gp inhibition) P-glycoprotein deficiency in Dogs**

Since the early 1980's, "ivermectin sensitivity" has been described in a subpopulation of Collies and other herding breeds. In susceptible dogs, extralabel doses of ivermectin (and other macrocyclic lactones) cause neurological toxicity (primarily CNS depression varying from mild ataxia to recumbency to coma), while the same dose would cause absolutely no clinical signs in most dogs. In 2001, the cause of this formerly 'idiosyncratic' drug toxicity was identified: a mutation in the ABCB1 (MDR1) gene. A four base-pair deletion mutation was identified. This deletion mutation produces a frame shift in the ABCB1 gene of affected dogs and this frame shift generates several premature stop codons[4]. Thus, dogs with this mutation have a severely truncated (more than 90% of P-gp is missing), nonfunctional protein. Dogs homozygous for this mutation, called ABCB1-1□, experience adverse neurological effects after a single dose of ivermectin > 100 micrograms per kilogram body weight. It is important to keep in mind that the heartworm prevention dose of ivermectin is 6 micrograms per kilogram body weight (thus Heartguard™ is safe even for dogs with the ABCB1-1□ mutation). These dogs are also exquisitely sensitive to adverse effects of other P-gp substrate drugs including other doxorubicin, vincristine, loperamide, acepromazine, butorphanol, and others and should receive reduced doses. Dogs that are heterozygous for ABCB1-1□ have intermediate sensitivity to these drugs and should also receive reduced doses. For example, dogs with the ABCB1-1□ mutation (heterozygotes and homozygotes) are significantly more likely to develop neutropenia and thrombocytopenia after a standard dose of vincristine than are ABCB1 wildtype (normal) dogs[5].

Breeds that may carry the ABCB1-1□ mutation are primarily herding breed dogs (Collies, Australian Shepherds, Shetland Sheepdogs, Old English Sheepdogs, German Shepherds, English Shepherds and herding breed mixes) but two sight hound breeds are also known to carry the ABCB1-1□ mutation (Silken Windhounds and Long-haired Whippets). The frequency of the mutation in each breed varies: Collies ~ 75%; Australian Shepherds and Long-haired Whippets ~ 50%; Shetland Sheepdogs ~ 10%; and other breeds - 10% or less[6].

Just because the dog on your examination table is not a herding breed doesn't mean that you can forget about P-gp-mediated adverse drug reactions. Many drugs are very effective at blocking/inhibiting P-gp function and thus creating an acquired P-gp deficiency. Co-administration of a P-gp inhibitor with a P-gp substrate drug will have the same consequences to a patient as administering a P-gp substrate drug to a dog with the ABCB1-1□ mutation. Commonly used drugs that inhibit P-gp function include ketoconazole, cyclosporine and spinosad.

**Role of P-gp in intestinal drug absorption**

P-gp is expressed on the lumenal border of intestinal epithelial cells, where it transports substrate drugs from the cytoplasm back into the intestinal lumen. In mdr1 (-/-) knockout mice the oral bioavailability of paclitaxel, a chemotherapeutic agent known to be a P-gp substrate, is 3-fold greater than in wildtype mice. Similar results have been documented with other orally administered P-gp substrates, including cyclosporin A, ivermectin, digoxin, dexamethasone, and other drugs in mice[7]. In dogs there is also evidence that P-gp limits oral absorption of some drugs. Oral absorption of paclitaxel is enhanced in dogs treated with

drugs that inhibit P-gp function. When paclitaxel was administered to dogs in conjunction with a P-gp inhibitor, peak plasma concentrations were 15-fold greater than when paclitaxel was administered alone. Similarly, co-administration of cyclosporin with grapefruit juice, an inhibitor of both P-gp and cytochrome P450 3A (CYP 3A) increased oral absorption of cyclosporin in dogs[40]. Digoxin toxicity has been described in a collie homozygous for the *ABCB1-1*□ mutation presumably due to enhanced oral bioavailability of digoxin. This patient developed an unusually high serum digoxin concentration which led to digoxin toxicity despite the administration of a reduced digoxin dosage (60% of the usual daily dose). Because other factors that precipitate digoxin toxicity, such as obesity, hypokalemia, or azotemia, were not present, it seems likely that lack of P-gp led to increased oral bioavailability and decreased intestinal and/or renal excretion of digoxin in this collie.

**Role of P-gp in drug distribution**

P-gp is an important component of the blood-brain barrier, blood-placental barrier, and the blood-testes barrier, minimizing the distribution of substrate drugs to these tissues. Dogs that lack P-gp experience profound neurological effects when given "normal" doses of ivermectin and loperamide (Imodium™). For example, 300 microgram per kg of ivermectin (low end of dosing range for demodectic mange) is well tolerated by most dogs, but will induce neurological toxicity (i.e., hypersalivation, mydriasis, ataxia, impaired postural and cranial nerve reflexes, stupor, coma) in dogs with the *ABCB1-1*□ mutation. The recommended dose of loperamide (0.2 mg/kg) will also cause neurological toxicity with clinical signs similar to those seen with ivermectin toxicity, in dogs with the *ABCB1-1*□ mutation yet will produce no adverse effects in dogs with normal P-gp function. Loperamide is an opioid that is generally devoid of CNS activity because it is excluded from the brain by P-gp. Dogs with the *ABCB1-1*□ mutation mutation also appear to have increased susceptibility to neurologic adverse effects of other macrocyclic lactones including milbemycin, selamectin, and moxidectin. Similarly, the author has observed sensitivity to acepromazine and butorphanol in dogs homozygous for the *ABCB1-1*□ mutation as compared to wildtype dogs receiving the same dose. These dogs experienced more pronounced and protracted CNS depression than did wildtype dogs receiving the same dose.

**Role of P-gp in drug metabolism**

While P-gp itself does not have its own metabolic functions, it works synergistically with cytochrome P450 enzymes, particularly the CYP3A subfamily. CYP enzymes are present not only in hepatocytes, but in enterocytes as well. Both CYP 3A and P-gp are expressed at high levels in the villus tip of enterocytes in the gastrointestinal tract, the primary absorptive site for orally administered drugs. Because so many drugs are substrates for both CYP 3A and P-gp, it appears that the two proteins work in concert to prevent oral absorption of many drugs. Once a drug is absorbed by the intestine (into enterocytes), three possible outcomes exist: (i) the drug may be metabolized by CYP 3A; (ii) the drug may enter the systemic blood circulation; or (iii) the drug may be extruded by P-gp back into the intestinal lumen, where it may be reabsorbed distally into another enterocyte. The net result is that non-P-gp/CYP 3A

substrate drugs will pass through the enterocyte only once, while P-gp substrate drugs may undergo several 'cycles' between the enterocyte and the gut lumen, resulting in repeated access of CYP 3A for drug metabolism, or fecal excretion of the drug due to repeated P-gp efflux in the intestinal lumen. Thus, oral bioavailability of P-gp substrate drugs (cyclosporin, doxorubicin, vincristine, others) is often low.

Many veterinarians have, perhaps unknowingly, manipulated the P-gp/CYP 3A system for the benefits of their patients by using ketoconazole to increase the oral bioavailability of cyclosporine (and thus decrease the dose and cost of cyclosporine). Ketoconazole inhibits both CYP 3A metabolic activity and P-gp efflux activity thus allowing a greater fraction of the cyclosporin dose to be absorbed. While this particular drug interaction is used for therapeutic benefit, a number of other potential adverse drug interactions exist between CYP 3A and P-gp substrates and inhibitors. Concurrent use of P-gp substrate and P-gp inhibiting drugs should either be avoided in some situations. Alternatively, dose reductions and/or therapeutic drug monitoring should be performed in order to avoid drug toxicity.

**Role of P-gp in biliary and renal drug excretion**

Renal and biliary excretion are very important pathways of drug elimination. As noted previously, P-gp is expressed on renal tubular and biliary canalicular cell membranes. Biliary and renal excretion of the parent drug are major means of clearance for doxorubicin, a chemotherapeutic drug that is also a P-gp substrate. When P-gp function is inhibited the biliary and renal clearance of doxorubicin in rats is substantially decreased—enhancing the risk of toxicity. P-gp substrates that are normally cleared by biliary excretion in dogs fail to appear in the gallbladder of dogs that are homozygous for ABCB1-1□□. Heterozygotes have intermediate biliary clearance of P-gp substrate drugs. Altered biliary and/or renal excretion is likely responsible for the increased sensitivity of herding breeds to vincristine and doxorubicin. Dose reductions are necessary in dogs with the ABCB1-1□ mutation (both heterozygotes and homozygotes).

**Assessment of MDR1 genotype in Clinical Patients**

Thus far, the ABCB1-1□ mutation has been identified in roughly a dozen different dog breeds including mixed breed dogs with some breeds having a frequency greater than 50%. The mutation has been identified with the same relative breed frequencies in Europe, North America, Japan, and Australia. It has been proposed that the mutation originated in a working sheepdog, likely one that lived in Great Britain in the 1800's, predating the emergence of formal breeds[9]. The first formal breeds to emerge from working sheepdog populations were the Collie, Old English Sheepdog, and Shetland Sheepdog. Other breeds affected by the ABCB1-1□ mutation (Border Collies, Australian Shepherds, English Shepherds, etc.) are also likely to have derived from these breeds.

**Clinical implications**

The presence of the ABCB1-1□ mutation in a particular canine patient has broad clinical implications with respect to veterinary medicine. For example, use of vincristine or doxorubicin in a collie or related breed with the ABCB1-1□ will cause severe gastrointestinal toxicity and myelosuppression, even after 25% dose reductions. Use of loperamide (Imodium®) for diarrhea or ivermectin at doses used to treat mange (200 to 600 micrograms per kg) in a dog

homozygous for the ABCB1-1□ mutation will cause severe neurotoxicity. Even dogs that are heterozygous for the ABCB1-1□ mutation are susceptible to vincristine and doxorubicin sensitivity, but have an intermediate phenotype with respect to ivermectin sensitivity. Some heterozygous dogs have been successfully treated with ivermectin (300 micrograms per kg) for demodectic and sarcoptic mange. However, it is important to note that these dogs were very closely monitored for neurological toxicity, and discontinuation of ivermectin for a 2 to 3 week period was necessary in some instances. In each of these instances, the ABCB1-1□ genotype of the patient was known prior to administering ivermectin, so the owner and veterinarian were highly aware of the risk of toxicity and the dogs were vigilantly monitored.

A commercial test is available (www.vetmed.wsu.edu/vcpl) so that all veterinarians can screen dogs for the *ABCB1* genotype prior to administering drugs that are substrates for P-gp. Rather than heeding the adage "white feet, don't treat" with regard to ivermectin, a DNA-based test will allow most herding dogs to benefit from ivermectin treatment for demodectic or sarcoptic mange. Similarly, dogs can be genotyped prior to treatment with other P-gp substrate drugs as well. For example *ABCB1* genotyping is considered standard care by veterinary oncologists to determine if lower chemotherapeutic drug doses (or alternative drugs) should be administered to canine cancer patients carrying a mutant MDR1 allele. Alternatively, if all herding breeds were treated with lower doses of doxorubicin, vincristine, or other Pgp substrate chemotherapeutic drugs, these animals would be at risk for shorter remission durations since remission duration tends to be proportional to drug dose.

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