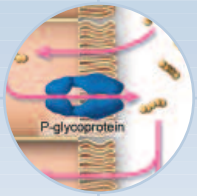

Adverse Drug Reactions in Herding-Breed Dogs: The Role of P-Glycoprotein*

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ABSTRACT: P-glycoprotein, encoded by the *MDR1* gene, plays an important role in the absorption, distribution, metabolism, and excretion of many drugs. Thus it is not surprising that lack of P-glycoprotein, which occurs in many herding-breed dogs, leads to increased susceptibility to drug toxicosis (e.g., use of ivermectin and other drug sensitivities in collies). By using a genetic test, it is now possible to predict which dogs will experience drug sensitivity and which will tolerate routine doses of drugs.

For decades, veterinarians have witnessed wide interindividual variation in response to drug therapy; however, the cause of this variation is multifactorial. Some patients respond well to a particular therapy, whereas others given routine doses of a particular drug fail to respond or experience unexplained (i.e., idiosyncratic) toxicosis. Many small animal clinicians have been informed by a dog owner or breeder that their dog's breed "can't handle" a particular type of medication or that the breed is "sensitive to" a particular type of medication. These anecdotal reports may be explained by pharmacogenetic differences among breeds.

Pharmacogenetics is the branch of pharmacology that studies variation in drug response and/or drug behavior based on an individual's genetic makeup. Pharmacogenetics can explain adverse drug reactions and the phenomenon of drug responders and nonresponders and offers a major step toward individualized effective and safe pharmacotherapy. Pharmacogenetics has also been used to explain idiosyncratic drug reactions in veterinary patients (many of which appear to be breed related).

Ideally, information about breed-related adverse drug reactions would be readily available to veterinarians so that they could make appropriate decisions about drug therapy. Although this information is not available for all breeds and drugs, recent research in veterinary pharmacogenetics has yielded some clinically useful information on a variety of drugs that affect many herding breeds. Some herding-breed dogs have a single mutation in a gene coding for a protein (i.e., P-glycoprotein) that drastically affects the absorption, distribution, metabolism, and excretion of a variety of drugs used in veterinary medicine. This article reviews the *MDR1-1Δ* mutation, which affects P-glycoprotein function and is responsible for ivermectin and other drug hypersensitivity in collies and other herding-breed dogs.[1,2]

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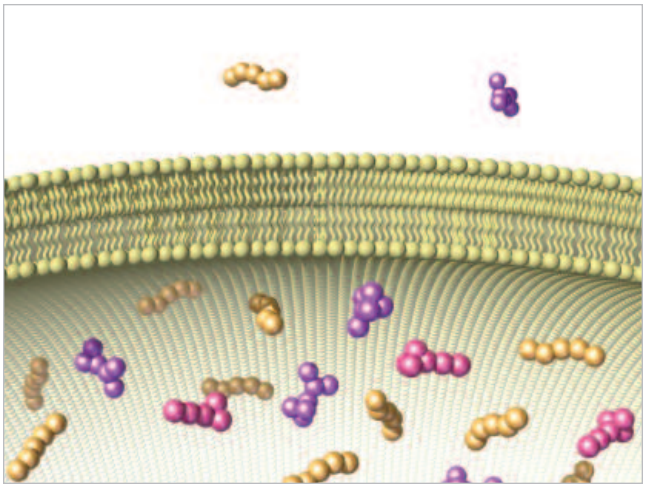
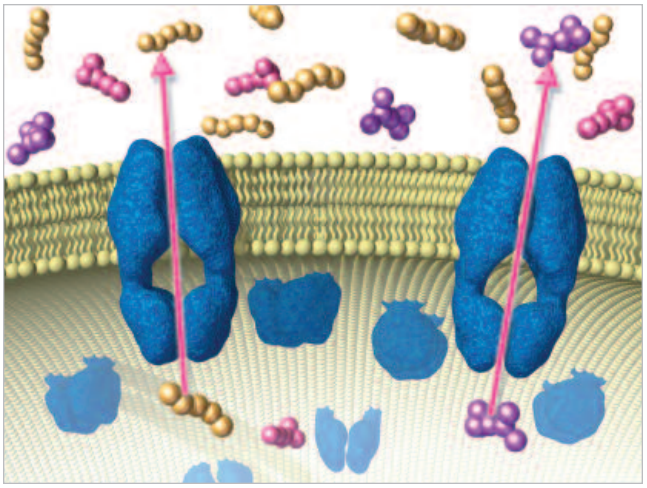
Figure 1. Illustration of P-glycoprotein's role in chemotherapeutic drug resistance. Because P-glycoprotein actively pumps many chemotherapeutics out of the cell, it becomes resistant to multiple chemotherapeutics.



This cancer cell does not express P-glycoprotein.



This cancer cell does express P-glycoprotein on the plasma membrane.

Illustrations by Felecia Paras

P-GLYCOPROTEIN

P-glycoprotein is a pump that transports substrate drugs across cell membranes. P-glycoprotein first attracted attention in the 1980s when it was discovered that P-glycoprotein expression by tumor cells contributed to chemotherapeutic multidrug resistance (Figure 1) in many types of human cancers.[3,4] Hence, the gene encoding P-glycoprotein was named *MDR1* (multidrug resistance gene; the most recent nomenclature is *ABCB1*). Subsequent work in canine lymphoma and osteosarcoma cell lines demonstrated that P-glycoprotein also contributes to chemotherapeutic resistance in canine cancers.[5,6] Researchers are still investigating P-glycoprotein–mediated chemotherapy resistance in tumor cells, but a more recent discovery[61] (in the 1990s) revealed an equally, if not more, important physiologic function mediated by P-glycoprotein in nonneoplastic mammalian tissue.

P-glycoprotein is a large (i.e., approximately 170 kD) protein that contains 12 membrane-spanning motifs. P-glycoprotein has two drug-binding sites and two ATP-binding sites located along the intracytoplasmic portion of the transporter. If a drug is a substrate for P-glycoprotein, the drug is actively transported from the intracellular to the extracellular space. Drug transport by P-glycoprotein is an ATP-dependent, active process that can function against steep concentration gradients.[4,7] P-glycoprotein is normally expressed in various mammalian tissues, including the apical border of intestinal epithelial cells,[8] brain capillary endothelial cells,[9] biliary canalicular cells,[10] renal proximal tubular epithelial cells,[11] placenta,[12] and testes.[13] P-glycoprotein actively pumps substrate drugs from within the cell back into the lumen of the intestine, brain capillary, bile canaliculus, or renal tubule, respectively. Consequently, it is tempting to speculate that P-glycoprotein functions in a protective capacity by decreasing exposure of the organism to potentially toxic xenobiotics in the environment.

P-glycoprotein homologues also exist in lower organisms, including *Plasmodium falciparum*, *Candida albicans*, *Saccharomyces cerevisiae*, and *Lactococcus lactis*.[14] In these lower organisms, P-glycoprotein–like multidrug transport pumps play an important role in conferring drug resistance on pathogens. For example, the *MDR1* homologue gene in *P. falciparum* (i.e., malaria) is associated with chloroquine resistance.[15] P-glycoprotein homologues in *C. albicans* and *L. lactis* appear to contribute to azole[16] and antibiotic[17] resistance, respectively. P-glycoprotein homologues also contribute to anthelmintic resistance such as ivermectin resistance in *Haemonchus* spp and other nematodes.[18] Thus it seems likely that the physiologic role of P-glycoprotein in both simple and complex organisms is to minimize an organism's exposure to potentially toxic xenobiotics by pumping them out of protected sites and promoting their excretion in the intestinal, biliary, and urinary tracts.

Mammalian P-glycoprotein has wide substrate specificity, transporting a number of drugs with diverse chemical structures, including anticancer agents (i.e., vinca alkaloids, doxorubicin), immunosuppressants (i.e., cyclosporine, tacrolimus), antiparasitic agents (i.e., iver-

Selected P-Glycoprotein Substrates[20,22–24,59]

Anticancer agents
- Doxorubicin
- Docetaxel[a]
- Vincristine[a]
- Vinblastine[a]
- Etoposide[a]
- Actinomycin D

Steroid hormones
- Aldosterone
- Cortisol[a]
- Dexamethasone[a]
- Methylprednisolone

Antimicrobial agents
- Erythromycin[a]
- Ketoconazole
- Itraconazole[a]
- Tetracycline
- Doxycycline
- Levofloxacin
- Sparfloxacin

Opioids
- Loperamide
- Morphine
- Butorphanol

Cardiac drugs
- Digoxin
- Diltiazem[a]
- Verapamil[a]
- Talinolol

Immunosuppressants
- Cyclosporine[a]
- Tacrolimus[a]

Miscellaneous
- Ivermectin and other avermectins
- Amitriptyline
- Terfenadine[a]
- Ondansetron
- Domperidon
- Acepromazine
- Vecuronium

[a]Substrate of CYP3A.

mectin, selamectin), steroid hormones (e.g., cortisol, dexamethasone), and others[19,20] (see box on this page). The mechanism by which P-glycoprotein can recognize and transport such a structurally diverse range of compounds is unknown, and attempts to predict whether a drug will be a P-glycoprotein substrate based purely on chemical structure have not been successful.[21] Interestingly, most P-glycoprotein substrates are natural compounds or synthetic derivatives of natural compounds. Vinca alkaloids, opioids, and digoxin are plant-derived drugs, whereas avermectins, doxorubicin, and cyclosporine are derived from fungal organisms.

A list of drugs relevant to veterinary medicine that are reportedly P-glycoprotein substrates is provided (see box on this page). More comprehensive lists of P-glycoprotein substrates are available elsewhere.[20,22–24] Many drugs have not been specifically evaluated regarding their status as P-glycoprotein substrates, so other drugs used in veterinary medicine will likely be identified as such and added to these lists. To date, it appears that P-glycoprotein substrate specificity is relevant across species. For instance, drugs determined to be substrates for murine P-glycoprotein are also substrates for both human and canine P-glycoprotein.[1,6,25] However, studies specifically addressing this question have not been conducted. One study[26] using tumor cell lines provides evidence that doxorubicin and vincristine are substrates for feline P-glycoprotein.

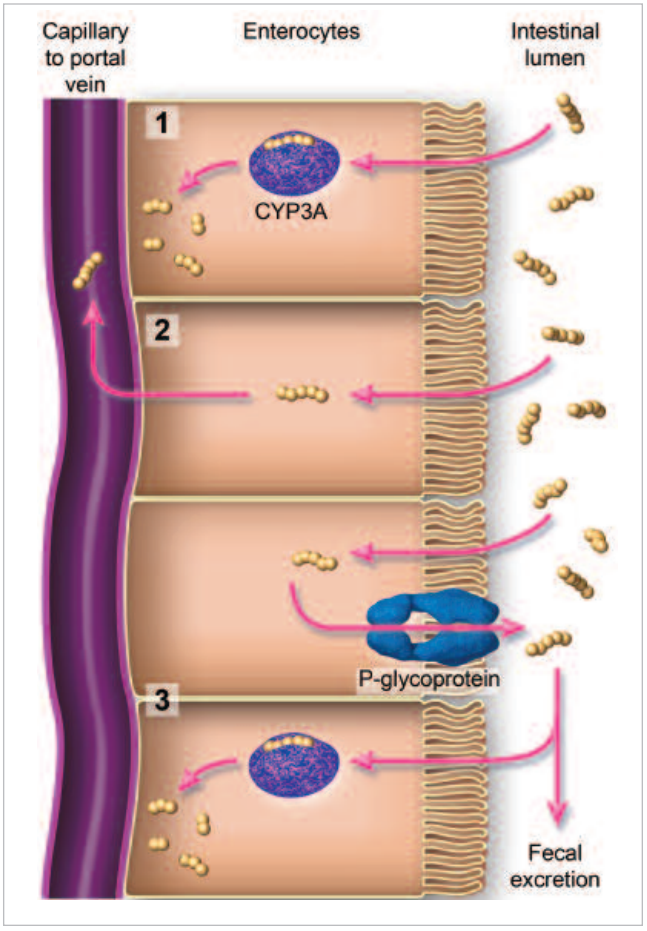


Illustration by Felecia Paras

Figure 2. Illustration of the interactive role of P-glycoprotein and CYP3A in modulating oral absorption of substrate drugs. Three possible outcomes exist for substrate drugs: (**1**) the drug may be metabolized by CYP3A, (**2**) the drug may enter the systemic circulation, or (**3**) the drug may be extruded by P-glycoprotein back into the intestinal lumen, where it may enter another enterocyte at a more distal site along the digestive tract, thus allowing further access to CYP3A. The net result is that non–P-glycoprotein/CYP3A substrate drugs pass through the enterocyte only once, whereas P-glycoprotein substrates may be continually cycled between the enterocyte and the gut lumen, resulting in repeated access of CYP3A to the drug molecule or fecal excretion of the drug due to repeated P-glycoprotein efflux.

ROLE OF P-GLYCOPROTEIN IN DRUG DISPOSITION

The initial discovery that P-glycoprotein was an important determinant in drug disposition was

serendipitous. A group of investigators studying P-glycoprotein genetically engineered a strain of mice that do not express P-glycoprotein (*mdr1a[-/-]* knockout mice).[27] These researchers were initially surprised and perhaps somewhat disappointed that these mice were healthy and fertile and appeared to live a normal life span without P-glycoprotein expression in their tissue. No anatomic malformations were detected in these mice, and there were no abnormalities in the results of complete blood cell counts, serum biochemistry profiles, or other physiologic parameters.[27,28] It was concluded that under normal laboratory conditions, P-glycoprotein was not essential for the basic physiologic functioning of an organism. Interest in the *mdr1a(-/-)* knockout mouse would have waned had it not been for a mite infestation in this colony. The infected mice were treated according to standard operating procedures for the facility by spraying a solution of ivermectin. Within 24 hours, nearly all of the *mdr1a(-/-)* mice had died, whereas none of the wild-type (i.e., normal mice that express P-glycoprotein) mice showed abnormal signs. The researchers ultimately determined that the blood–brain barrier in *mdr1a(-/-)* mice was defective, resulting in 100-fold greater concentrations of ivermectin in brain tissue of the knockout mice compared with wild-type mice.[27–29] This discovery paved the way for literally hundreds of subsequent studies investigating the role of P-glycoprotein in drug disposition, including studies of ivermectin sensitivity in collies.

For years, ivermectin sensitivity has been described in a subpopulation of collies and other herding breeds.[30] These dogs appear to display the same phenotype as do *mdr1a(-/-)* knockout mice after exposure to ivermectin. Not surprisingly, a mutation in the *MDR1* gene has been identified in these dogs. A four base-pair deletion mutation produces a frame shift in the gene of affected dogs.[1] This frame shift generates a premature stop codon in *MDR1*, resulting in a severely truncated, nonfunctional protein. Dogs homozygous for this mutation (i.e., *MDR1-1Δ*) experience adverse neurologic effects after a single dose (i.e., 100 µg/kg) of ivermectin.

Role of P-Glycoprotein in Intestinal Drug Absorption

P-glycoprotein is expressed on the lumenal border of intestinal epithelial cells (Figure 2), where it transports substrate drugs from the cytoplasm back into the intestinal lumen. In *mdr1a(-/-)* knockout mice, the oral bioavailability of paclitaxel, a chemotherapeutic agent known to be a P-glycoprotein substrate, is threefold greater than in wild-type mice.[31] Similar results have been documented with other orally administered P-glycoprotein substrates, including cyclosporin A,[32] β-adrenergic antagonists,[33] opioids,[34,35] ivermectin,[36] digoxin,[28,37] dexamethasone,[28] fluoroquinolones,[38] and other drugs used in mice. Peak plasma concentrations of the P-glycoprotein substrate morphine were also twofold higher in humans pretreated with a P-glycoprotein inhibitor (i.e., a drug that inhibits P-glycoprotein function) than in patients receiving morphine alone.

A mutation in the gene encoding P-glycoprotein occurs in some herding-breed dogs, rendering them extremely susceptible to toxicosis resulting from administration of ivermectin, loperamide, vincristine, doxorubicin, and other drugs.

These results indicate that P-glycoprotein plays a major role in limiting intestinal absorption of many drugs in human and rodent studies.

In dogs, there is also evidence that P-glycoprotein limits oral absorption of some drugs. Oral absorption of paclitaxel is enhanced in dogs treated with drugs that block P-glycoprotein function.[39] When paclitaxel was administered to dogs in conjunction with a P-glycoprotein inhibitor, peak plasma concentrations were 15-fold greater than when paclitaxel was administered alone. Similarly, coadministration of cyclosporin with grapefruit juice, an inhibitor of both P-glycoprotein and cytochrome P450 3A (CYP3A), increased oral absorption of cyclosporin in dogs.[40] Digoxin toxicity was recently documented in a collie homozygous for the *MDR1-1Δ* mutation.[41] This patient developed an unusually high serum digoxin concentration that led to digoxin toxicosis despite administration of a reduced (i.e., 60% of the calculated daily dose) digoxin dosage (i.e., 0.002 mg/kg in the morning and 0.004 mg/kg in the evening; recommended dosage: 0.005 mg/kg bid).[42] Because other factors that precipitate digoxin toxicosis, such as obesity, hypokalemia, or azotemia, were not

present, it seems likely that lack of P-glycoprotein led to increased oral bioavailability and decreased intestinal and/or renal excretion of digoxin in this collie.

Role of P-Glycoprotein in Drug Distribution

P-glycoprotein is an important component of the blood–brain, blood–placenta, and blood–testes barriers, minimizing the distribution of substrate drugs to these tissues. Dogs that lack P-glycoprotein (i.e., those homozygous for the *MDR1-1Δ* mutation) experience profound neurologic effects when given "normal" doses of ivermectin and loperamide (Imodium, McNeil Consumer Healthcare). For example, 300 µg/kg of ivermectin (i.e., low end of dosing range for demodectic mange) is well tolerated by most dogs but induces neurologic toxicosis (i.e., hypersalivation, mydriasis, ataxia, impaired postural and cranial nerve reflexes, stupor, coma) in dogs with the *MDR1-1Δ* mutation. The recommended dose of loperamide (0.2 mg/kg) also causes neurologic toxicosis, with clinical signs similar to those of ivermectin toxicosis, in dogs with the *MDR1-1Δ* mutation but produces no adverse effects in dogs that express P-glycoprotein normally (*MDR1* wild-type dogs).[43] Loperamide is an opioid that is generally devoid of central nervous system (CNS) activity because it is excluded from the brain by P-glycoprotein.[35,44] Dogs with the *MDR1-1Δ* mutation also appear to have increased susceptibility to neurologic adverse effects of other macrocyclic lactones, including milbemycin, selamectin,[45] and moxidectin.[46] Similarly, I have observed apparent sensitivity to acepromazine and butorphanol in dogs homozygous for the *MDR1-1Δ* mutation compared with *MDR1* wild-type dogs receiving the same dose. *MDR1-1Δ*–mutant dogs experienced more pronounced and protracted CNS depression than did wild-type dogs.

Selected P-Glycoprotein Inhibitors[19,22,25,35,60,61]

Antidepressants
- Fluoxetine
- Saint John's Wort
- Paroxetine

Antimicrobials
- Erythromycin[a,b]
- Itraconazole[a]
- Ketoconazole[a,b]

Opioids
- Methadone
- Pentazocine

Cardiac drugs
- Verapamil[a]
- Amiodarone[a]
- Carvedilol
- Quinidine[a]
- Nicardipine[a]

Immunosuppressants
- Cyclosporine[a]
- Tacrolimus[a]

Miscellaneous
- Bromocriptine
- Chlorpromazine
- Tamoxifen[a]
- Grapefruit juice[b]

[a]CYP3A substrate.
[b]CYP3A inhibitor.

Role of P-Glycoprotein in Drug Metabolism

Although P-glycoprotein itself does not have metabolic functions, it was recently shown to be an important component of intestinal drug metabolism in humans by working in conjunction with CYP3A. Because CYP3A is one of the most abundant cytochrome P450 enzymes and is known to be responsible for the metabolism of about 60% of currently known human drugs, it is considered the major phase-one drug-metabolizing enzyme family in mammals.[47,48] CYP3A and P-glycoprotein are expressed at high levels in the villus tip of enterocytes in the gastrointestinal (GI) tract, the primary absorptive site for orally administered drugs (Figure 2). Because so many drugs are substrates for both CYP3A and P-glycoprotein, it appears that the two proteins work in concert to prevent oral absorption of many drugs (see box on page 25). Once a drug is absorbed by the intestine, three possible outcomes exist in the enterocyte: The drug may be metabolized by CYP3A, may enter the systemic blood circulation, or may be extruded by P-glycoprotein back into the intestinal lumen, where it may be reabsorbed distally into another enterocyte. The net result is that non–P-glycoprotein/CYP3A substrate drugs pass through the enterocyte only once, whereas P-glycoprotein substrates may continually undergo cycling between the enterocyte and the gut lumen, resulting in repeated access of CYP3A for drug metabolism, or fecal excretion of the drug due to repeated P-glycoprotein efflux in the intestinal lumen.

P-glycoprotein plays an important role in the absorption, distribution, metabolism, and excretion of a wide variety of drugs used in veterinary medicine.

Breeds in Which the *MDR1-1Δ* Mutation Has Been Documented

Herding breeds
- Collie
- Australian shepherd (standard and miniature)
- English shepherd
- McNab
- Old English sheepdog
- Shetland sheepdog
- German shepherd[a]
- Border collie[b]

Sighthounds
- Longhaired whippet
- Silken windhound

[a]Frequency in white dogs is greater than in dogs of other colors.
[b]Based only on dog's appearance (these dogs were not registered in the American Kennel Club).

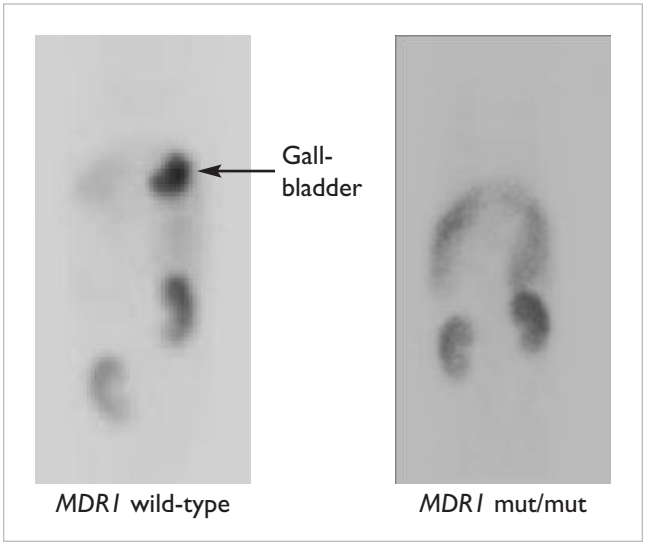


Figure 3. Nuclear scintigraphic image of an *MDR1* wild-type *(left)* and *MDR1* (mut/mut; *right*) dog 60 minutes after intravenous injection of a radiolabeled P-glycoprotein substrate (99mTc-sestamibi). The gallbladder can be easily observed in one dog (*left*) because sestamibi is pumped into the bile by P-glycoprotein. The gallbladder is not visible in another dog (*right*) because it lacks P-glycoprotein.

P-Glycoprotein Inhibition

Many veterinarians have, perhaps unknowingly, affected the P-glycoprotein/CYP3A system when using ketoconazole to increase the oral bioavailability of cyclosporine.[49,50] Although the mechanisms are not entirely clear, ketoconazole inhibits both CYP3A metabolic activity and P-glycoprotein efflux activity. While this particular drug interaction is used for therapeutic benefit, a number of other potentially adverse drug interactions exist between CYP3A and P-glycoprotein substrates and inhibitors. A number of drugs are known to inhibit P-glycoprotein and CYP3A in humans (see box on page 27). Concurrent use of drugs listed in the box on page 25 with drugs listed in the box on page 27 should be avoided, or plasma drug concentrations should be measured to avoid drug toxicosis.

Role of P-Glycoprotein in Biliary and Renal Drug Excretion

Renal and biliary excretion are the most important pathways of drug elimination, but excretion may also occur by other routes, such as the intestine (as already described). As noted previously, P-glycoprotein is expressed on renal tubular and biliary canalicular cell membranes. Concurrent administration of a P-glycoprotein inhibitor decreases the biliary and renal clearance of doxorubicin in rats.[51] Figure 3 demonstrates accumulation of the radiolabeled P-glycoprotein substrate (i.e., 99mTc-sestamibi) in the gallbladder of an *MDR1* wild-type dog but a distinct lack of radioactivity in the gallbladder area in a dog with the *MDR1-1Δ* mutation, providing visual evidence that P-glycoprotein plays an important role in biliary excretion of P-glycoprotein substrates in dogs (unpublished research by the author). Altered biliary and/or renal excretion may play a role in the apparent increased sensitivity of herding breeds to chemotherapeutic drugs that are P-glycoprotein substrates. For example, dogs with the *MDR1-1Δ* mutation are extremely susceptible to myelosuppression and GI toxicosis induced by the chemotherapeutic agents vincristine and doxorubicin (which are P-glycoprotein substrates), even at low doses, but appear to tolerate cyclophosphamide (which is not a P-glycoprotein substrate) at the full dose.[52] It is likely that deficient P-glycoprotein in these animals results in delayed renal and/or biliary excretion and subsequent toxicosis.

ASSESSMENT OF *MDR1* GENOTYPE IN CLINICAL PATIENTS

To date, the *MDR1-1Δ* mutation has been identified in 10 different dog breeds (see box on this page). In a study[53] involving collies from the northwestern United States, over 75% had at least one mutant allele (i.e., 35% were homozygous for the mutant allele, and 42% were heterozygous). A similar distribution was described in separate studies involving over 250 collies, primarily from the United States,[54] and in collies from France[55]

and Australia,[56] indicating that most collies have at least one mutant allele. Surprisingly, the longhaired whippet also has a high frequency of the *MDR1-1Δ* mutation, with roughly 65% having at least one mutant allele (i.e., 16% were homozygous for the mutant allele, and 52% were heterozygous).[54] Based on discussions with some owners, I believe that the mutation may have been introduced into the breed by a Shetland sheepdog or other herding-breed dog in an effort to enhance the desired longhaired trait. The frequency of the *MDR1-1Δ* mutation is lower in other affected breeds.

It has been proposed that the mutation originated in a working sheepdog, likely one that lived in the United Kingdom in the 1800s, predating the emergence of formal breeds.[54] The first formal breeds to emerge from working sheepdog populations were the collie, Old English sheepdog, and Shetland sheepdog. Other breeds affected by the *MDR1-1Δ* mutation are thought to have been derived from these breeds.

Clinical Implications

The presence of the *MDR1-1Δ* mutation in a particular 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 *MDR1-1Δ* mutation causes severe GI toxicosis and myelosuppression, even after 25% dose reductions.[57] Use of loperamide (Imodium) for treating diarrhea or ivermectin for treating mange (200 to 400 µg/kg) in a dog homozygous for the *MDR1-1Δ* mutation causes severe neurotoxicity. Apparently, dogs heterozygous for the *MDR1-1Δ* mutation are susceptible to vincristine and doxorubicin sensitivity (i.e., phenotypically sensitive) but have an intermediate phenotype with respect to ivermectin sensitivity. Some heterozygous dogs have been successfully treated with ivermectin (300 µg/kg) for demodectic and sarcoptic mange. However, it is important to note that dogs were very closely monitored for neurologic toxicity, and discontinuation of ivermectin for 2 to 3 weeks was necessary in some instances. In each of these instances, the *MDR1-1Δ* genotype of the patient was known before ivermectin was administered, so the owner and veterinarian were highly aware of the risk of toxicity.

A commercially available test is now available so that all veterinarians can screen dogs for the *MDR1* genotype before administering drugs that are substrates for P-glycoprotein (see box on this page). Rather than heeding the adage "white feet, don't treat" regarding ivermectin use, clinicians can use a DNA-based test in most herding-breed dogs to determine whether they will benefit from treatment with ivermectin for demodectic or sarcoptic mange. Similarly, dogs can be genotyped before treatment with other P-glycoprotein substrate drugs. For example, *MDR1* genotyping is being used by veterinary oncologists to determine whether lower chemotherapeutic drug doses (or alternative drugs) should be adminis-

Information on Testing for the *MDR1-1Δ* Genotype in Clinical Patients

Veterinary Clinical Pharmacology Laboratory (VCPL)
College of Veterinary Medicine
Washington State University
Web: www.vetmed.wsu.edu/vcpl
Phone: 509-335-3745

Price
$60 for one dog
$51 per sample for five or more dogs submitted as one batch

Sample required
Cheek swab sample to collect DNA

Processing requirements
Cheek swab testing kits are obtained by contacting VCPL. Instructions for obtaining an appropriate sample are on the Web site (above) and are included with the test kit. Samples do not require refrigeration and can be returned via regular mail.

Turnaround time
For samples received by Tuesday afternoon, results are available by Friday afternoon (Pacific time).

A genetic test is now available to screen herding-breed dogs for the mutation, allowing veterinarians to determine whether P-glycoprotein drugs can be safely administered to a particular patient or should be avoided.

Table 1. Summary of Reported Adverse Drug Reactions in Patient(s) with the *MDR1-1Δ* Mutation

Drug(s)	Toxic Dose	Nature of Adverse Drug Reaction	Supportive Evidence/ Data Source
Antiparasitics			
Ivermectin	>100 µg/kg (20× label dose for heartworm prevention)	CNS toxicosis[1,2,62]	Prospective studies Case reports Author's unpublished data Package insert Personal communications
Selamectin	10× label dose	Mild CNS toxicosis at 10× label dose	Manufacturer
Moxidectin	30× label dose	CNS toxicosis	Manufacturer
Milbemycin	10–20× label dose	CNS toxicosis	Manufacturer
Abamectin (pesticide)	Unknown	CNS toxicosis	Author's unpublished data
Antineoplastics			
Doxorubicin	22.5 mg/M^2 causes toxicosis in dogs heterozygous for the *MDR1-1Δ* mutation	Myelosuppression Severe diarrhea Anorexia	Case report Author's unpublished data Personal communications
Vincristine (similar effects would be expected for vinblastine)	0.56 mg/M^2 causes toxicosis in dogs heterozygous for the *MDR1-1Δ* mutation	Myelosuppression Vomiting Anorexia	Case report Author's unpublished data Personal communications
Paclitaxel	Unknown	Myelosuppression and GI effects likely because paclitaxel is a P-glycoprotein substrate	None available
Opioids			
Loperamide	0.2 mg/kg causes toxicosis in dogs homozygous for the *MDR1-1Δ* mutation Effects in heterozygous animals are unknown	Severe CNS depression	Case report Author's unpublished data Personal communications
Butorphanol	0.05 mg/kg IV caused greater and more prolonged CNS depression in *MDR1*-mutant dogs than in *MDR1* wild-type dogs	CNS depression Recommend 50% of normal dose in dogs homozygous for the *MDR1-1Δ* mutation	Author's unpublished data
Morphine	Unknown	Likely CNS depression because morphine is reportedly a P-glycoprotein substrate	None available

Table 1. Summary of Reported Adverse Drug Reactions in Patient(s) with the *MDR1-1Δ* Mutation *(continued)*

Drug(s)	Toxic Dose	Nature of Adverse Drug Reaction	Supportive Evidence/ Data Source
Miscellaneous			
Acepromazine	0.04 mg/kg caused greater and more prolonged CNS depression in *MDR1*-mutant dogs than in *MDR1* wild-type dogs	CNS depression Recommend 50% of normal dose in dogs homozygous for the *MDR1-1Δ* mutation	Author's unpublished data
Dexamethasone	Unknown	*MDR1* mutant dogs experience greater HPA axis suppression than do *MDR1* wild-type dogs	Manuscript submitted (prospective study)
Digoxin	60% of the calculated daily digoxin dose resulted in a serum concentration of digoxin four times greater than the recommended therapeutic range	Anorexia Vomiting Cardiotoxicity	Case report (submitted)
Ondansetron	0.4 mg/kg caused toxicosis in a collie homozygous for the *MDR1-1Δ* mutation	CNS toxicosis, including depression and altered mentation	—

HPA = hypothalamic–pituitary–adrenal axis.

tered to canine cancer patients carrying a mutant *MDR1* allele.[52] Alternatively, if all herding breeds were treated with lower doses of doxorubicin, vincristine, or other P-glycoprotein substrate chemotherapeutic drugs, these animals would be at risk of shorter remission durations, which tend to be proportional to drug dose.[58] *MDR1* genotyping represents the first step toward incorporating pharmacogenetics into clinical veterinary medicine.

CONCLUSION

Lack of functional P-glycoprotein, as occurs in dogs with the *MDR1-1Δ* mutation, alters the absorption, distribution, metabolism, and excretion of a variety of commonly used drugs in veterinary medicine, increasing the risk of adverse drug reactions in these patients. Patients with the *MDR1-1Δ* mutation may have increased oral bioavailability of substrate drugs because P-glycoprotein is not present in the GI tract to pump drug back into the intestinal lumen. Lack of P-glycoprotein at the blood–brain barrier results in increased brain concentrations of P-glycoprotein substrate drugs, greatly enhancing the risk of neurotoxicity. P-glycoprotein expressed on enterocytes pumps substrate drugs into the intestinal lumen; on biliary canalicular cells, it pumps substrates into bile; and on renal tubular epithelial cells, it pumps substrates into urine. Dogs homozygous for the *MDR1-1Δ* mutation lack the capacity for P-glycoprotein–mediated drug excretion, enhancing the risk of adverse drug reactions. Table 1 contains a summary of reported adverse drug reactions in patient(s) with the *MDR1-1Δ* mutation.

REFERENCES

1. Mealey KL, Bentjen SA, Gay JM, Cantor GH: Ivermectin sensitivity in collies is associated with a deletion mutation of the *mdr1* gene. *Pharmacogenetics* 11:727–733, 2001.
2. Nelson OL, Carsten E, Bentjen SA, Mealey KL: Ivermectin toxicity in an Australian shepherd dog with the *MDR1* mutation associated with ivermectin sensitivity in collies. *J Vet Intern Med* 17:354–356, 2003.
3. Ueda K, Cardarelli C, Gottesman MM, Pastan I: Expression of a full-length cDNA for the human *MDR1* gene confers resistance to colchicine, doxorubicin, and vinblastine. *Proc Natl Acad Sci USA*:3004–3008, 1987.
4. Juranka PF, Zastawny RL, Ling V: P-glycoprotein: Multidrug-resistance and a superfamily of membrane-associated transport proteins. *FASEB J* 3:2583–2592, 1989.
5. Bergman PJ, Ogilvie GK, Powers BE: Monoclonal antibody C219 immunohistochemistry against P-glycoprotein: Sequential analysis and predictive ability in dogs with lymphoma. *J Vet Intern Med* 10:354–359, 1996.
6. Mealey KL, Barhoumi R, Rogers K, Kochevar DT: Doxorubicin induced

expression of P-glycoprotein in a canine osteosarcoma cell line. *Cancer Lett* 126, 187–192, 1998.
7. Cornwell MM: Molecular biology of P-glycoprotein. *Cancer Treat Res* 57:37–56, 1991.
8. Li M, Hurren R, Zastawny RL, et al: Regulation and expression of multidrug resistance (MDR) transcripts in the intestinal epithelium. *Br J Cancer* 80:1123–1131, 1999.
9. Cordon-Cardo C, O'Brien JP, Casals D, et al: Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood–brain barrier sites. *Proc Natl Acad Sci USA*:695–698, 1989.
10. Thiebaut F, Tsuruo T, Hamada H, et al: Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc Natl Acad Sci USA*:7735–7738, 1987.
11. Hori R, Okamura N, Aiba T, Tanigawara Y: Role of P-glycoprotein in renal tubular secretion of digoxin in the isolated perfused rat kidney. *J Pharmacol Exp Ther* 266:1620–1625, 1993.
12. Lankas GR, Wise LD, Cartwright ME, et al: Placental P-glycoprotein deficiency enhances susceptibility to chemically induced birth defects in mice. *Reprod Toxicol* 12:457–463, 1998.
13. Melaine N, Lienard MO, Dorval I, et al: Multidrug resistance genes and P-glycoprotein in the testis of the rat, mouse, guinea pig, and human. *Biol Reprod* 67:1699–1707, 2002.
14. van Veen HW, Konings WN: The ABC family of multidrug transporters in microorganisms. *Biochim Biophys Acta* 1365:31–36, 1998.
15. Volkman SK, Cowman AF, Wirth DF: Functional complementation of the *ste6* gene of *Saccharomyces cerevisiae* with the *pfmdr1* gene of *Plasmodium falciparum*. *Proc Natl Acad Sci USA*:8921–8925, 1995.
16. Ramage G, Bachmann S, Patterson TF, et al: Investigation of multidrug efflux pumps in relation to fluconazole resistance in *Candida albicans* biofilms. *J Antimicrob Chemother* 49:973–980, 2002.
17. Konings WN, Poelarends GJ: Bacterial multidrug resistance mediated by a homologue of the human multidrug transporter P-glycoprotein. *IUBMB Life* 53:213–218, 2002.
18. Xu M, Molento M, Blackhall W, et al: Ivermectin resistance in nematodes may be caused by alteration of P-glycoprotein homolog. *Mol Biochem Parasitol* 91:327–335, 1998.
19. Ford JM, Hait WN: Pharmacologic circumvention of multidrug resistance. *Cytotechnology* 12:171–212, 1993.
20. Sakaeda T, Nakamura T, Okumura K: *MDR1* genotype-related pharmacokinetics and pharmacodynamics. *Biol Pharm Bull* 25:1391–1400, 2002.
21. Seelig A: A general pattern for substrate recognition by P-glycoprotein. *Eur J Biochem* 251:252–261, 1998.
22. Marzolini C, Paus E, Buclin T, Kim RB: Polymorphisms in human *MDR1* (P-glycoprotein): Recent advances and clinical relevance. *Clin Pharmacol Ther* 75:13–33, 2004.
23. Schwab M, Eichelbaum M, Fromm MF: Genetic polymorphisms of the human *MDR1* drug transporter. *Annu Rev Pharmacol Toxicol* 43:285–307, 2003.
24. Sakaeda T, Nakamura T, Okumura K: Pharmacogenetics of *MDR1* and its impact on the pharmacokinetics and pharmacodynamics of drugs. *Pharmacogenomics* 4:397–410, 2003.
25. Page RL, Hughes CS, Huyan S, et al: Modulation of P-glycoprotein–mediated doxorubicin resistance in canine cell lines. *Anticancer Res* 20:3533–3538, 2000.
26. Okai Y, Nakamura N, Matsushiro H, et al: Molecular analysis of multidrug resistance in feline lymphoma cells. *Am J Vet Res* 61:1122–1127, 2000.
27. Schinkel AH, Smit JJ, van Tellingen O, et al: Disruption of the mouse *mdr1a* P-glycoprotein gene leads to a deficiency in the blood–brain barrier and to increased sensitivity to drugs. *Cell* 20(77):491–502, 1994.
28. Schinkel AH, Wagenaar E, van Deemter L, et al: Absence of the *mdr1a* P-glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. *J Clin Invest* 96:1698–1705, 1995.
29. Schinkel AH, Mol CA, Wagenaar E, et al: Multidrug resistance and the role of P-glycoprotein knockout mice. *Eur J Cancer* 31A:1295–1298, 1995.
30. Paul AJ, Tranquilli WJ, Seward RL, et al: Clinical observations in collies given ivermectin orally. *Am J Vet Res* 48:684–685, 1987.
31. Sparreboom A, van Asperen J, Mayer U, et al: Limited oral bioavailability and active epithelial excretion of paclitaxel (Taxol) caused by P-glycoprotein in the intestine. *Proc Natl Acad Sci USA*:2031–2035, 1997.
32. Lown KS, Mayo RR, Leichtman AB, et al: Role of intestinal P-glycoprotein (*mdr1*) in interpatient variation in the oral bioavailability of cyclosporine. *Clin Pharmacol Ther* 62:248–260, 1997.
33. Schwarz UI, Gramatte T, Krappweis J, et al: Unexpected effect of verapamil on oral bioavailability of the beta-blocker talinolol in humans. *Clin Pharmacol Ther* 65:283–290, 1999.
34. Bouer R, Barthe L, Philibert C, et al: The roles of P-glycoprotein and intracellular metabolism in the intestinal absorption of methadone: In vitro studies using the rat everted intestinal sac. *Fundam Clin Pharmacol* 13:494–500, 1999.
35. Wandel C, Kim R, Wood M, Wood A: Interaction of morphine, fentanyl, sufentanil, alfentanil, and loperamide with the efflux drug transporter P-glycoprotein. *Anesthesiology* 96:913–920, 2002.
36. Kwei GY, Alvaro RF, Chen Q, et al: Disposition of ivermectin and cyclosporin A in CF-1 mice deficient in *mdr1a* P-glycoprotein. *Drug Metab Dispos* 27:581–587, 1999.
37. Westphal K, Weinbrenner A, Giessmann T, et al: Oral bioavailability of digoxin is enhanced by talinolol: Evidence for involvement of intestinal P-glycoprotein. *Clin Pharmacol Ther* 68:6–12, 2000.
38. Yamaguchi H, Yano I, Saito H, Inui K: Pharmacokinetic role of P-glycoprotein in oral bioavailability and intestinal secretion of grepafloxacin in vivo. *J Pharmacol Exp Ther* 300:1063–1069, 2002.
39. McEntee M, Silverman JA, Rassnick K, et al: Enhanced bioavailability of oral docetaxel by coadministration of cyclosporin A in dogs and rats. *Vet Comp Oncol* 2:105–112, 2003.
40. Amatori FM, Meucci V, Giusiani M, et al: Effect of grapefruit juice on the pharmacokinetics of cyclosporine in dogs. *Vet Rec* 154:180–181, 2004.
41. Henick RA, Kellum HB, Bentjen SA, Mealey KL: Digoxin and mexiletine sensitivity in a collie with the *mdr1* mutation. *J Vet Intern Med*, accepted for publication, 2005.
42. Boothe D: *Small Animal Clinical Pharmacology and Therapeutics.* Philadelphia, WB Saunders, 2001.
43. Sartor LL, Bentjen SA, Trepanier L, Mealey KL: Loperamide toxicity in a collie with the *MDR1* mutation associated with ivermectin sensitivity. *J Vet Intern Med* 18:117–118, 2004.
44. Ericsson CD, Johnson PC: Safety and efficacy of loperamide. *Am J Med* 88:10S–14S, 1990.
45. Package insert: Revolution, Pfizer, 2005.
46. Tranquilli WJ, Paul AJ, Todd KS: Assessment of toxicosis induced by high-dose administration of milbemycin oxime in collies. *Am J Vet Res* 52:1170–1172, 1991.
47. Patel J, Mitra AK: Strategies to overcome simultaneous P-glycoprotein mediated efflux and CYP3A4 mediated metabolism of drugs. *Pharmacogenomics* 2:401–415, 2001.
48. Zhang Y, Benet LZ: The gut as a barrier to drug absorption: Combined role of cytochrome P450 3A and P-glycoprotein. *Clin Pharmacokinet* 40:159–168, 2001.
49. Dahlinger J, Gregory C, Bea J: Effect of ketoconazole on cyclosporine dose in healthy dogs. *Vet Surg* 27:64–68, 1998.
50. Patricelli AJ, Hardie RJ, McAnulty JE: Cyclosporine and ketoconazole for the treatment of perianal fistulas in dogs. *JAVMA* 220:1009–1016, 2002.
51. Kiso S, Cai SH, Kitaichi K, et al: Inhibitory effect of erythromycin on P-glycoprotein–mediated biliary excretion of doxorubicin in rats. *Anticancer Res* 20:2827–2834, 2000.
52. Mealey KL, Bentjen SA: Unpublished data, Veterinary Clinical Pharmacology Laboratory, Pullman, WA, 2005.
53. Mealey KL, Bentjen SA, Waiting DK: Frequency of the mutant *MDR1* allele associated with ivermectin sensitivity in a sample population of collies from the northwestern United States. *Am J Vet Res* 63:479–481, 2002.
54. Neff MW, Robertson KR, Wong AK, et al: Breed distribution and history of canine *MDR1*-1(Delta), a pharmacogenetic mutation that marks the emergence of breeds from the collie lineage. *Proc Natl Acad Sci USA*, 2004.
55. Hugnet C, Bentjen SA, Mealey KL: Frequency of the mutant *MDR1* allele associated with multidrug sensitivity in a sample of collies from France. *J Vet Pharmacol Ther* 27:227–229, 2004.
56. Mealey KL, Munyard KA, Bentjen SA: Frequency of the mutant *MDR1* allele associated with multidrug sensitivity in a sample of herding breed dogs living in Australia. *Vet Parasitol* 131:193–196, 2005.

57. Mealey KL, Northrup NC, Bentjen SA: Increased toxicity of P-glycoprotein-substrate chemotherapeutic agents in a dog with the *MDR1* deletion mutation associated with ivermectin sensitivity. *JAVMA* 223:1434, 1453–1455, 2003.
58. Frei E: Curative cancer chemotherapy. *Cancer Res* 45:6523-6537, 1985.
59. Fromm MF: Genetically determined differences in P-glycoprotein function: Implications for disease risk. *Toxicology* 181–182, 299–303, 2002.
60. Choo EF, Leake B, Wandel C, et al: Pharmacological inhibition of P-glycoprotein transport enhances the distribution of HIV-1 protease inhibitors into brain and testes. *Drug Metab Dispos* 28:655–660, 2000.
61. Sadeque AJ, Wandel C, He H, et al: Increased drug delivery to the brain by P-glycoprotein inhibition. *Clin Pharmacol Ther* 68:231–237, 2000.
62. Roulet A, Puel O, Gesta S, et al: *MDR1*-deficient genotype in collie dogs hypersensitive to the P-glycoprotein substrate ivermectin. *Eur J Pharmacol* 460:85–91, 2003.

ARTICLE #1 CE TEST

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1. P-glycoprotein is not expressed in
a. the stratum corneum.
b. the epithelial lining of the GI tract.
c. brain capillary endothelial cells.
d. biliary canalicular cells.

2. Which is not a substrate for P-glycoprotein?
a. vinca alkaloids
b. loperamide
c. digoxin
d. alkylating agents (i.e., cyclophosphamide)

3. Animals deficient in P-glycoprotein function are susceptible to ivermectin-induced neurotoxicity because P-glycoprotein
a. metabolizes ivermectin to inactive compounds.
b. is an efflux pump that pumps ivermectin out of the brain.
c. binds ivermectin covalently and prevents it from being absorbed from the GI tract.
d. is a neurotransmitter that antagonizes the action of ivermectin in the brain.

4. The *MDR1-1Δ* mutation has been identified in
a. collies.
b. Shetland sheepdogs.
c. Old English sheepdogs.
d. all of the above

5. In which pharmacokinetic parameter may P-glycoprotein play a role?
a. absorption
b. distribution
c. metabolism
d. all of the above

6. If contemplating treatment of a collie with demodectic mange, a veterinarian should
a. follow the adage "white feet, don't treat" and offer amitraz dips only.
b. offer *MDR1* genotyping to determine whether the dog has the *MDR1-1Δ* genotype and then decide whether ivermectin can be safely used in the dog.
c. treat the dog with at least 300 µg/kg q24h of ivermectin until three negative skin scrapings are obtained.
d. apply ivermectin topically to affected areas.

7. Which statement regarding pharmacogenetics is true?
a. Breed-related adverse drug reactions in dogs are a figment of breeders' imaginations.
b. Pharmacogenetics can explain all adverse drug reactions in veterinary and human patients.
c. Pharmacogenetics explains some adverse drug reactions in veterinary and human patients.
d. Genetic testing for susceptibility to adverse drug reactions in dogs is not currently feasible for any drugs used in veterinary medicine.

8. The frequency of the *MDR1-1Δ* mutation in collies is high, with over 75% of collies tested having at least _____ mutant allele(s).
a. one
b. two
c. three
d. four

9. P-glycoprotein
a. is present in hepatic cells and has intrinsic metabolic capacity.
b. is present on biliary canalicular cells and transports substrates into the bile.
c. in the liver works in conjunction with CYP2A to metabolize and excrete a variety of drugs.
d. is not present in the hepatobiliary system.

10. P-glycoprotein
a. function can be inhibited by several drugs, including ketoconazole.
b. is present on the glomerulus and transports substrates into the urine.
c. in the kidneys works in conjunction with CYP3A to metabolize and excrete a variety of drugs.
d. is not present in the kidneys.