Importance of assessing P-glycoprotein substrate status for canine and feline medications

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1. P-glycoprotein's top 3 contributions to drug disposition in dogs and cats

Hundreds of reviews on P-glycoprotein (P-gp) have been published. Many describe over a dozen different mammalian tissues that express P-gp including the intestines, biliary canalicular cells, kidneys, testes, placenta, adrenal gland, brain capillary endothelial cells, and others. At each of these tissue locations P-gp functions to efflux substrate drugs thereby limiting their concentrations within those tissues and protecting the organism from potentially toxic xenobiotics. In canine and feline patients, only three of these tissue locations seem to alter drug disposition sufficiently to impact clinical decision-making. These tissue locations and P-gp's function at those sites are detailed below.

Brain capillary endothelial cells (Blood-brain barrier). The importance of P-gp at the blood brain barrier has perhaps been demonstrated best by experiments comparing brain concentrations of various P-gp substrates in wildtype mice and mdr1a knockout (P-gp null) mice. Brain concentrations of the P-gp substrates ivermectin, loperamide and ondansetron were 88-fold, 13-fold and 4-fold greater than those in wildtype mice. Enhanced brain penetration of these substrates in dogs and cats with P-gp deficiency (intrinsic or acquired as described section 2 below) result in neurological cabnormalities at doses well-tolerated by animals with normal P-gp function. Consequently, any P-gp substrate that is a ligand for CNS receptors has the potential to cause unwanted neurological clinical signs in dogs or cats with P-gp deficiency.

Biliary excretion by P-gp expressed on biliary canalicular cells. An absolute absence of biliary excretion of P-gp substrates has been demonstrated experimentally in dogs homozygous for the MDR1 mutation (P-gp null). A reduction in biliary excretion of roughly 50% relative to normal dogs has been demonstrated in dogs heterozygous for the MDR1 mutation and in dogs with 'acquired' P-gp deficiency. One would expect the same for cats with corresponding causes or P-gp deficiency. This decrease in biliary clearance of P-gp substrates in P-gp deficient dogs and cats increases overall exposure of the patient to P-gp substrate drugs resulting in a greater risk of adverse effects. This has been demonstrated with vincristine, which is significantly more likely to cause neutropenia and thrombocytopenia in P-gp deficient dogs (heterozygous or homozygous for ABCB1-1Δ) than in dogs with normal P-gp function. The primary route of excretion for vincristine is P-gp-mediated biliary excretion of the parent compound. Acquired P-gp deficiency results in similar defects in biliary clearance and enhanced toxicity of the vinca alkaloid vinblastine. A Boston Terrier cross (MDR1 normal/normal genotype) experienced grade 4 gastrointestinal toxicity and myelosuppression, leading to sepsis, cardiac arrest and death after concurrent treatment with the P-gp substrate ketoconazole.

T-lymphocytes Calcineurin inhibitors such as cyclosporine, sirolimus, and tacrolimus, are ligands for intracellular T-cell receptors. Binding triggers a series of events that results in decreased expression of cytokines such as interleukins and interferon-γ. P-gp is normally

expressed on T-cell plasma membranes, limiting intracellular T-cell concentrations of calcineurin inhibitors. P-gp-deficient dogs and cats accumulate higher concentrations of P-gp substrates (e.g., cyclosporine) intracellularly relative to dogs and cats with normal P-gp function, resulting in exaggerated immunosuppression relative to animals with normal P-gp function. This has been documented in a Border Collie (MDR1 mutant/normal) treated with a relatively low dose of cyclosporine. The dog developed secondary infections attributed to excessive immunosuppression despite cyclosporine plasma concentrations at the low end of the therapeutic range. Pharmacodynamic monitoring, however, demonstrated nearly complete suppression of interleukin-2 mRNA synthesis (< 5% of control). Typically, cyclosporine concentrations within the therapeutic range suppress interleukin-2 synthesis in the range of 25-50% of control.

2. P-gp deficiency

There are two ways a dog or cat can experience P-gp deficiency. The first is "intrinsic" P-gp deficiency which is caused by a 4 base-pair deletion mutation in the canine ABCB1 (formerly MDR1) gene for dogs and by a 2 base-pair deletion mutation in the feline ABCB1 (MDR1) gene for cats. Both deletion mutations generate multiple premature stop codons, so animals that inherit 2 mutant alleles have a P-gp-null phenotype and are highly susceptible to adverse reactions caused by P-gp substrates. Heterozygotes (animals that inherit 1 mutant and 1 normal allele) have partial P-gp function and are more susceptible to P-gp-mediated adverse reactions than animals with normal P-gp function (2 copies of the wildtype MDR1 allele) but are less sensitive than animals with 2 mutant alleles. Succinctly, in terms of sensitivity to P-gp substrate drugs, MDR1 mutant/mutant > MDR1 mutant/normal > MDR1 normal/normal. The canine MDR1 mutation occurs most frequently in herding breeds but can occur in mixed breed dogs and has been identified purebred Siberian Huskies and Boxers. The feline MDR1 mutation appears to affect Maine Coons more than any other breed (frequency of ~ 6%) and occurs at a frequency of ~1% in nonpurebred cats.

Phenoconversion is a term used in human pharmacogenetics to explain when an individual's drug metabolism genotype mismatches the observed phenotype for that individual. Phenoconversion is the result of non-genetic factors, often a drug-drug interaction. This phenomenon can occur in dogs and cats with MDR1 normal/normal genotypes. The author prefers to describe this as "acquired" P-gp deficiency instead of "phenoconversion". Acquired P-gp deficiency occurs when an MDR1 normal/normal animal is concurrently treated with two P-gp substrate drugs that then compete for P-gp binding sites. Competition for P-gp-mediated efflux can cause increased CNS penetration, decreased biliary clearance and/or enhanced intracellular lymphocyte concentrations of one or both drugs. Importantly, acquired P-gp deficiency has the potential to affect all dogs and cats and has caused fatal adverse drug reactions in both species.

3. How does P-gp interact with Substrates?

While there are documented species differences in P-gp substrate specificity, there is a great deal of overlap. Table 1 displays the percent differences in amino acids comprising each of the transmembrane domains of canine and human P-gp. The transmembrane domains create P-gp's binding pocket, for which there are >200 substrates. Three

overlapping binding sites (R,H, and P) have been described for human P-gp. The author has shown experimentally, using a competitive fluorescence efflux assay, that canine P-gp functions similarly. Some substrates appear to bind only one of these sites while others overlap with all three sites. The risk for and severity of P-gp-mediated drug-drug interactions may, therefore, depend on the specific binding "position" of P-gp substrates.

Table 1. Percent amino acid (AA) difference between the transmembrane (TM) domain of canine and human P-gp. The transmembrane domains comprise P-gp's binding pocket.

TM Domain	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
% AA Difference	15	4	10	5	3	4	15	11	0	0	10	7

4. How might treatment strategy change if you know a drug is a P-gp substrate

The implications of a drug's P-gp substrate status differ depending on the patient's MDR1 genotype. For dogs and cats with absolute P-gp deficiency, (homozygous for the MDR1 mutation) the P-gp substrate itself can cause toxicity so dose modification (i.e., 50% dose reduction for a drug like vincristine) or selection of an alternative, non-P-gp substrate, medication is generally indicated. For dogs and cats with normal P-gp function (homozygous MDR1 wildtype), treatment with only one P-gp substrate does not require a change in treatment strategy, but if two or more P-gp substrates are concurrently administered, then a dose modification or alternative, non-P-gp substrate may be indicated. The most complex patients to manage with respect to P-gp substrates can be heterozygotes. Heterozygotes treated with a P-gp substrate often require dose reductions, albeit smaller than the dose reductions recommended for dogs or cats with absolute P-gp deficiency because the former has partial P-gp function (i.e., 25% dose reduction for a drug like vincristine). However, if treated concurrently with two or more P-gp substrates, competitive inhibition may overwhelm P-gp efflux resulting in absolute P-gp deficiency and the need for greater dose reductions to avoid an adverse drug event.

5. Assessment of P-gp substrate status

The "ivermectin sensitive" collie model has been used for over two decades to assess P-gp substrate status, particularly of macrocyclic lactones. However, there are numerous disadvantages to this model and other target animal models including ethical considerations and cost. A murine *mdr1* knockout, canine *MDR*1 knock-in mouse model engineered by FDA scientists, offers a slight advantage over target animal models. Cell culture assays are considered sufficient for screening new chemical entities that are candidates for human drugs so should be considered sufficient for new chemical entities that are candidates for canine or feline drugs. The author has generated both a canine P-gp over expressing cell line and a feline P-gp over expressing cell line and has developed assays for identifying drugs as canine or feline P-gp substrates, respectively. These cell lines could be used by manufacturers to determine P-gp substrate status of canine and feline drug candidates for inclusion on the drug label. The availability of this information would help veterinarians prevent serious adverse drug reactions.