Toxicology of Avermectins and Milbemycins (Macrocylic Lactones) and the Role of P-Glycoprotein in Dogs and Cats

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KEYWORDS

- Macrocylic lactones • Ivermectin • Dogs
- Cats • P-glycoprotein

The macrocyclic lactones (MLs) are parasiticides able to kill a wide variety of arthropods and nematodes. They have a high margin of safety for labeled indications, and ivermectin has become the best selling antiparasitic in the world.[1] Dogs of certain breeds and mixtures of those breeds have a defect in the *ABCB1* gene (formerly *MDR1* gene) that results in a lack of functional P-glycoprotein (P-gp), which leads to accumulation of the MLs in the central nervous system (CNS) and a higher risk of adverse effects when exposed. With toxicosis, CNS signs such as ataxia, lethargy, coma, tremors, seizures, mydriasis, and blindness predominate. In general, the MLs have a long half-life and therefore exposure results in a long duration of illness when overdoses occur. There is no specific antidote for ML toxicosis so the most important part of treatment is good supportive care.

CHEMISTRY OF MACROCYCLIC LACTONES

The MLs (macrolides) include 2 groups: avermectins and milbemycins. The avermectins include abamectin, ivermectin, eprinomectin, doramectin, and selamectin. The milbemycins consist of moxidectin, milbemycin, and nemadectin. These structurally similar compounds are derived from natural compounds produced by soil-dwelling fungi from the genus *Streptomyces.*[1] The natural compound avermectin is composed

PAE was supported by National Institute of Environmental Health Sciences grant K08 ES017045. The authors have nothing to disclose.

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Vet Clin Small Anim 42 (2012) 313–333
doi:10.1016/j.cvsm.2011.12.005 **vetsmall.theclinics.com**
0195-5616/12/$ – see front matter

of 8 closely related compounds: 4 A- and B-components (A_1, A_2, B_1, B_2), each of which further contains 2 homologous a- and b-components, for example, B_{1a} and B_{1b}.[1] Abamectin and ivermectin are both composed of avermectin B_1 components, differing only in the absence of a double bond in ivermectin.[1] Further modification of B_1 produces eprinomectin.[1] Doramectin and selamectin are closely related and contain A_1 and B_1 components, respectively.[2] Moxidectin is produced from the *Streptomyces* fermentation product nemadectin.[2] Milbemycin oxime (milbemycin) is composed of 5-oxime derivatives of milbemycins A_4 and A_3.[1]

MECHANISMS OF TOXICITY AND THE ROLE OF P-GLYCOPROTEIN

Avermectins and milbemycins have minor differences in some substituents, but they share the same general structure that confers on them the ability to bind to chloride channel receptors.[1] One main mechanism by which the MLs exert their effect is by binding ligand-gated chloride channels.[2,3] Binding of glutamate-gated chloride channels, which are specific to invertebrates, causes influx of chloride ions into the parasite neurons leading to hyperpolarization, paralysis, and death.[2]

In mammals, MLs bind to gamma-aminobutyric acid type A–gated chloride channels ($GABA_A$ receptors).[4] GABA is the primary inhibitory neurotransmitter in the brain, and postsynaptic binding of GABA to its receptors serves to modulate firing of excitatory neurons, such as glutamatergic neurons. MLs are believed to bind $GABA_A$ receptors at sites different than those where GABA, benzodiazepines, barbiturates, or picrotoxin separately bind.[5] Because $GABA_A$ receptors are only present in the CNS, binding of MLs is prevented by the blood-brain barrier (BBB), as discussed later. However, in overdoses, enough ML permeates the BBB that binding to $GABA_A$ receptors, as well as to glycine- and voltage-gated chloride channels, occurs.[3,6] Subsequent chloride influx causes hyperpolarization and decreased firing of the excitatory neurons that express these chloride receptors and channels, leading to clinical signs. Of interest, avermectins actually may reduce GABA effects at lower concentrations, resulting in signs such as tremoring (excitatory signs), and then start to enhance GABA effects as concentrations at the receptor increase, causing a progression of signs to ataxia and CNS depression (inhibitory effects).[3] So avermectins may have stimulatory CNS effects (tremors) at lower concentrations but inhibitory effects (ataxia, depression) at higher concentrations.

Permeability glycoprotein (P-gp) is a transmembrane efflux protein that influences the pharmacokinetics of many of its substrates, including MLs, by actively transporting absorbed substrates back across a variety of cell membranes in the body.[7] P-gp, which is a member of the ATP-binding cassette (ABC) superfamily of transporters, is found in all mammalian species[8] and is well distributed throughout the tissues of dogs[9] and cats.[10] It is characteristically located along the apical border of cell types that serve a barrier function (eg, enterocytes, bile canalicular cells, renal tubular cells, and endothelial cells), so P-gp can be viewed as having a protective function because it limits entry of substrates into internal compartments.[11]

P-gp is important in limiting the entry of MLs and other xenobiotics into the CNS.[12] The BBB regulates entry of endogenous substances and xenobiotics from the circulation into the brain. Tight junctions between endothelial cells prevent paracellular diffusion of substances into the CNS. Also, endothelial cells in the brain are specialized in that they lack pinocytotic vacuoles and fenestrations in their plasma membranes, thus making the BBB selectively permeable.[13] Substances that enter the brain must either diffuse through the endothelial cells or be actively transported into the endothelial cells by uptake transporters.[14] As substances enter the endothelial cells in the brain, they are potentially subject to being extruded back across the apical

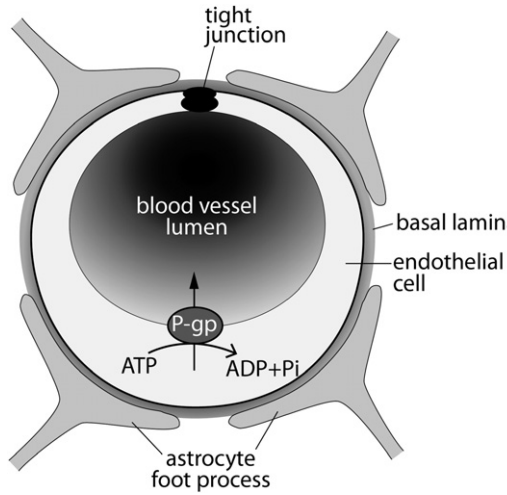

Fig. 1. P-glycoprotein (P-gp) is a component of the blood brain barrier. P-gp actively transports substrates entering CNS endothelial cells back into the systemic circulation, thus preventing entry of substrates, such as ivermectin, into the parenchyma of the brain. Information from Refs.[49,59,82–84] (*Adapted from* Linnet K, Ejsing TB. A review on the impact of P-glycoprotein on the penetration of drugs into the brain. Focus on psychotropic drugs. Eur Neuropsychopharmacol 2008;18:159; with permission.)

membrane by P-gp and other efflux proteins,[13,14] as shown in **Fig. 1**. Further components of the BBB include the basal lamina on the abluminal side of the endothelial cells and the foot processes of the glial astrocytes.[13]

The *ABCB1* gene (formerly called *MDR1*) codes for P-gp in vertebrates[8] and has been sequenced in dogs.[15] In some dog breeds there is a genetic defect in P-gp: a 4–base pair deletion in the *ABCB1* gene (*ABCB1-1*Δ) results in production of an extremely truncated, nonfunctional P-gp.[15] Having the *ABCB1-1*Δ mutation can result in accumulation of P-gp substrates in the brain that would normally be removed by P-gp,[12] so the BBB is compromised and becomes permeable to P-gp substrates, including MLs. In dogs with this defect, treatment with doses of MLs above those used for heartworm prevention may result in accumulation of the drug in the CNS, resulting in neurologic effects. Adverse neurologic effects can also occur in animals without the gene defect when overdoses of MLs are administered, in which case saturation of the transport capacity of P-gp likely occurs. Dogs may be homozygous or heterozygous for the defect, with homozygous dogs being at greater risk of developing toxicosis from ML exposure.[7]

EXPOSURE SOURCES, FORMULATIONS, AND THERAPEUTIC AND TOXIC DOSAGES

Because the MLs are commonly used as parasiticides in many species, they are available in a wide array of formulations.[1] Some of the most common small animal veterinary products include tablets with ivermectin, moxidectin, or milbemycin and topical products with selamectin that are used for heartworm prevention. Ivermectin, moxidectin, milbemycin, and doramectin are also used off-label for various indications including as a heartworm microfilaricide and for treating demodectic and sarcoptic mange as well as other ecto- and endoparasites.[16] Dogs and cats may also be exposed to large animal products either accidentally or by intentional

administration. Many formulations intended for large animals are concentrated so it is easy for accidental overdoses to occur.

Signs of intoxication with MLs generally are related to the CNS. Neurologic depression, ataxia, mydriasis, blindness, tremors, and hypersalivation all may be seen and, as signs progress, an animal may become comatose. Seizures may also occur. The blindness is typically temporary and has been associated with retinal edema and electroretinogram abnormalities in the case of ivermectin.[17] The signs seen are similar in both dogs and cats for all the MLs. Depending on the dose and the breed involved and due to the long half-life of these agents, toxicosis may persist for days to weeks.

Formulations, labeled and off-label therapeutic dosage ranges, and documented "safe" versus toxic dosages for specific MLs are discussed next. When possible, distinctions are made between dosages that affect dogs with or without the *ABCB1-1Δ* gene defect. **Table 1** provides a summary of the information in this section.

Ivermectin

Ivermectin is available in numerous forms for large animal applications including injectable liquid, oral bolus, pour-on, paste, and feed pre-mix. It is available as chewable tablets for heartworm prevention in small animals. Ivermectin is also produced as a 3-mg tablet (Stromectrol) for humans indicated for treatment of gastrointestinal (GI) strongyloidiasis in the United States and for onchocerciasis and strongyloidiasis in other countries. Many of the large animal formulations are of relatively high concentration, from 1% to 1.87% (10–18.7 mg/mL), so it is easy for accidental overdose to occur either from miscalculation of a dosage when using these products off-label, from accidental exposure to the remnants in a discarded tube of equine dewormer, or from blobs of dewormer that fall from a horse's mouth during deworming. Exposure to concentrated (eg, ivermectin 1.87%) MLs eliminated in the dung of treated large animals is also a potential source of exposure (ASPCA Animal Poison Control Center [APCC], Urbana, IL, unpublished information, 2011). In one study, ivermectin concentrations in horse dung were monitored after horses were treated with a manufacturer's recommended therapeutic dosage.[18] Peak ivermectin levels of 2.4 mg/kg of dung were measured 2.5 days after exposure. To place this concentration in perspective, a 27.3 kg (60 lb) collie homozygous for *ABCB1-1Δ* would need to ingest 1.1 kg (2.4 lb) of dung to attain a dosage of 0.1 mg/kg of ivermectin, which is mildly toxic in sensitive collies.[19] In contrast, tablets for heartworm prevention range from 0.068 to 0.272 mg of ivermectin per tablet so toxicosis is rare even when small animals ingest several of these pills.

Ivermectin is used for heartworm prevention at dosages of 0.006 to 0.012 mg/kg in dogs and 0.024 mg/kg in cats. It is also used off-label in dogs as a microfilaricide at 0.05 to 0.2 mg/kg and to treat ectoparasites at 0.3 to 0.6 mg/kg.[16]

Clinical signs have been reported in breeds with a history of ivermectin sensitivity at dosages ranging from 0.08 to 0.34 mg/kg.[20–22] However, none of these dogs were tested for the *ABCB1* gene deletion. In breeds considered to be normal in their response to ivermectin, mild clinical signs have been documented at dosages starting from 0.2 mg/kg,[22] with more severe signs developing at dosages of 1 to 2.5 mg/kg or greater.[22,23] Some of the dogs reported to show signs at relatively low ivermectin dosages in one retrospective study[22] were German shepherds. A small percentage of this breed does carry the *ABCB1* gene defect,[24] which might partly explain the presence of signs in "normal" dogs at relatively low dosages. It is important to emphasize that problems are not expected with standard heartworm preventative dosages even in *ABCB1-1Δ* dogs. Ivermectin-sensitive collies were treated with 10

Table 1
Therapeutic, nontoxic, and toxic dosages of macrocyclic lactones in both normal and sensitive dogs and in cats

Agent	Formulations	Therapeutic Dosages (Labeled and Off-Label) (mg/kg)	Acute, Subacute or Chronic Dosages Published as Safe (mg/kg)	Toxic Dosages ML Sensitive Dogs (mg/kg)	Acute Toxic Dosage Normal Dog/Cat (mg/kg)	References
Ivermectin	Tablets, oral liquid, oral paste, feed premix, injectable, topical, otic	0.006–0.6 PO D 0.024 PO C 0.2–0.4 SC D, C	0.5 PO daily × 12 weeks[a] D 0.06 PO Collies 0.2–1.33[a] PO or SC C 0.72 PO C	0.1–0.4[b] PO 0.2–0.25[b] SC	0.2–2.5 PO D 0.3 SC C	16,22,25 26,27,32 69,85,87
Selamectin	Topical	6 topical D, C	6 PO D, C[c] 40 topical Collies 72–114 topical D 236–367 topical C	5 PO[d]	None found	16,88,93
Moxidectin	Tablets, oral drench, injectable, topical	0.003 PO D 0.17 sustained release SC D 2.5 topical D 1 topical C	1.15 PO daily × 1 year D 0.09 PO Collies 0.85 SC D, Collies	1 PO[e]	1.9–2.8 PO D 1 PO C[f]	2,16,28 89,90,94 95,96
Doramectin	Injectable, pour-on	0.6 SC D, C	0.5–1 PO daily × 91 days D 0.2 SC C	0.2[g]–0.7 SC	None found	16,37,38 86,91
Milbemycin	Tablets	0.5–2 PO D 2 PO C	10 PO Collies 10 PO C	5–10[g] PO 0.8 PO × 2 days 1.5 PO × 13 days	None found	16,33,34 92

Abbreviations: C, cat; collies, ivermectin-sensitive collies; D, dog; PO, orally; SC, subcutaneously.
[a] It should be noted that some animals are also reported to have problems at this dosage.
[b] Many of the collies in these reports were not tested for the *ABCB1-1Δ* gene defect.
[c] Cats exhibited drooling and intermittent vomiting with oral dosing.
[d] One collie was ataxic after this dosage in the safety studies, but others tolerated up to 15 mg/kg PO.
[e] Administered as a product containing 2.5% moxidectin and 10% imidacloprid.
[f] Generally only mild signs seen.
[g] Collies at these dosages were not tested for the *ABCB1-1Δ* gene defect.

times the heartworm preventative dosage (0.06 mg/kg) without signs developing.[25] However, when ivermectin is used at higher dosages as a microfilaricide or for demodicosis, problems can easily occur in patients with the *ABCB1* gene deletion, and sometimes even in dogs with normal *ABCB1* genotype. Clinical signs have developed following oral dosages as low as 0.1 mg/kg in ivermectin-sensitive collies.[19] In overdoses, the most frequent clinical signs reported in dogs were lethargy, ataxia, hypersalivation, tremors, mydriasis, blindness, and bradycardia.[22,26] Coma, seizures, and death have been seen in severely affected animals. Similar signs have been seen in cats[27] (ASPCA APCC, unpublished information, 2011), although miosis rather than mydriasis was noted in one case report.[27]

Moxidectin

Moxidectin is available in many forms including injectable, pour-on, and oral drench for ruminants and horses. It is available as a topical preparation, a subcutaneous (SC) injection and a monthly tablet for heartworm prevention in small animals. As with ivermectin, moxidectin products intended for use in horses and ruminants are of relatively high concentration (0.5%–2% or 5–20 mg/mL), so small animals may be exposed to high doses from relatively small amounts of these products. Also similar to ivermectin, horse dung could be another potential source of moxidectin exposure for dogs, with peak moxidectin concentrations of 2.6 mg/kg of horse dung measured 2.5 days after horses were treated with a manufacturer's recommended therapeutic dosage.[18]

Moxidectin is used in dogs for heartworm prevention orally at 0.003 mg/kg monthly and in sustained-release SC injection at 0.17 mg/kg every 6 months. It is also used topically in dogs at 2.5 mg/kg and in cats at 1 mg/kg monthly for heartworm prevention.[16]

At oral dosages of 1.9 to 2.8 mg/kg, adverse effects have been documented in dogs with normal P-gp genotype.[28] Signs in dogs exposed to equine moxidectin dewormers include ataxia, tremors, seizures, hyperthermia, tachycardia, blindness, hypersalivation, bradycardia, coma, and respiratory depression.[28–31]

Selamectin

Selamectin is available as a topical formulation for dogs and cats that is labeled for prevention of heartworm and for killing fleas and ear mites at a minimum dosage of 6 mg/kg, with concentrations of 60 and 120 mg/mL. It is also used at the same dosages to treat sarcoptic mange and tick infestation in dogs and hookworms and ascarids in cats.[16] Because it is not available as a more concentrated form, overdose is less likely. The most common clinical signs following selamectin exposure include vomiting, drooling, retching, licking of lips, lethargy, agitation, anorexia, and ataxia (ASPCA APCC, unpublished information, 2011). Many of these signs likely result from inadvertent oral exposure or administration.

Abamectin (Avermectin B_1)

Abamectin is generally used in products used to control ants, cockroaches, mites, and other insects. Sometimes abamectin products are labeled as containing avermectin B_1. These are usually found in the form of plastic traps ("baits") for ants and cockroaches, insect spikes, granules, or liquids intended to be sprayed for outdoor and indoor use. The liquids range in concentration from 0.15% to 2%. Generally, the ant/cockroach traps contain between 0.01% and 0.05% of abamectin. A typical ant "bait" weighs about 1.6 to 2 g, giving a range of 0.16 to 1 mg of abamectin per trap;

thus it is rare to see significant signs with exposure. Subchronic studies in several species (dogs, rats, rabbits, and mice) suggest that abamectin and ivermectin have a similar degree of toxicity and that abamectin is marginally more toxic than ivermectin.[32] Dogs are primarily exposed to these insecticide products because some contain attractants, such as peanut butter, which are intended to lure insects but are also appealing to dogs. The clinical signs most commonly reported to the APCC following abamectin exposure are vomiting, ataxia, hypersalivation, lethargy, mydriasis, and diarrhea (ASPCA APCC, unpublished information, 2011). Many of the clinical signs are also likely related to the inert ingredients that can cause mild GI upset.

Milbemycin

Milbemycin is available as an oral chewable tablet (2.3–27 mg) for heartworm prevention in dogs and cats as well as a 0.1% otic solution for treating ear mites.[16] It is not available in a more concentrated dosage form, so overdoses are relatively rare.

The therapeutic dosages of milbemycin for heartworm prevention are 0.5 mg/kg in dogs and 2 mg/kg in cats. Mild clinical signs of ataxia, hypersalivation, mydriasis, and lethargy have been documented in ivermectin-sensitive dogs dosed at 5 to 10 mg/kg.[33] In a separate report, two *ABCB1*-defective dogs developed mild signs (ataxia) after being dosed repeatedly with milbemycin for demodicosis; one dog received 0.8 mg/kg 2 days in a row and the other dog received 1.5 mg/kg daily for 13 days before developing signs.[34] Mild clinical signs have been reported to develop in normal dogs at 10 to 20 mg/kg and in both cats and in dogs with suspected *ABCB1* gene deletions at greater than 5 to 10 mg/kg (ASPCA APCC, unpublished information, 2011). The most common clinical signs reported include ataxia, tremors, lethargy, vomiting, mydriasis, disorientation, and hypersalivation.

Doramectin, Eprinomectin, and Nemadectin

Doramectin is available as an injectable formulation (10 mg/mL) for ruminants and pigs as well as a pour-on for cattle (5 mg/mL).[2] Doramectin has been used off-label to treat demodicosis in dogs and cats at 0.6 mg/kg SC once weekly.[16] Eprinomectin is available as a pour-on for cattle (5 mg/mL).[2] Eprinomectin has been used experimentally to treat *Toxocara canis* at 0.1 mg/kg in dogs,[35] while nemadectin has been used at 0.2 to 0.6 mg/kg in dogs to treat GI helminths.[36] Side effects were not seen in either study. Further information about eprinomectin and nemadectin use in small animals could not be located.

Exposure of small animals to these products occurs less frequently than to some of the more common MLs, but these products are of high concentration so it is plausible that accidental exposure could result in toxicosis. Two case reports regarding dogs exposed to doramectin give us an idea of what clinical signs can be seen. One report involved a collie given 0.2 mg/kg of doramectin SC,[37] while the other involved 2 white Swiss shepherds exposed to 0.7 mg/kg doramectin SC.[38] The dogs in the latter report were confirmed to have the *ABCB1* gene defect, while the collie was assumed to have the gene defect. Clinical signs included blindness, restlessness, CNS depression, recumbency, hypersalivation, tremors, tachypnea, ataxia, head pressing, disorientation, lack of menace response, and bradycardia. Clinical signs from eprinomectin or nemadectin overdose in animals with normal P-gp are expected to be similar, but it is uncertain at what dosage signs would emerge.

TOXICOKINETICS OF MACROCYCLIC LACTONES AND THE ROLE OF P-GLYCOPROTEIN

In general, the MLs have relatively fast oral absorption but a much more gradual absorption rate after SC injection.[39] They also are all highly fat soluble, have a large volume of distribution, and accumulate in fat tissue resulting in a long elimination half-life.[1,40] The authors were unable to locate specific information about metabolism and amounts of drug or metabolites eliminated in bile and urine in the dog or cat. Data from species where this information is known indicate that generally large percentages of MLs are eliminated in the bile with the degree of metabolism varying among the different compounds.[1] Studies in large animal species[1] and humans[41] suggest that enterohepatic circulation, by which xenobiotics are eliminated in the bile and then reabsorbed from the gut, occurs with MLs. However differences in product formulation can alter pharmacokinetic parameters significantly even for the same agent.[39,42]

In dogs, it takes about 4 hours for orally administered ivermectin to reach maximum plasma levels (t_{max} = 4 hours).[43,44] Subcutaneous absorption is slower, with t_{max} being 32 to 36 hours in dogs[43,45] and about 28 hours in cats.[40] The elimination half-life after oral administration of ivermectin to dogs is 3.3 days,[43,44] while after SC administration, the half-life is 3.2 days in dogs[43] and 3.4 days in cats.[40] One study evaluated the differences in pharmacokinetic parameters after SC injection of the same dosage of 7 different ivermectin preparations in dogs.[45] The maximum plasma concentrations ranged from 26.5 to 49.6 ng/mL and the area under the curve ranged from 2523 to 4956 ng • h/mL. The area under the curve, which reflects bioavailability, is a measure of the amount of free drug that reaches systemic circulation.[45] These significant differences illustrate the influence of formulation on pharmacokinetic parameters.

Moxidectin is absorbed faster than ivermectin following oral administration, with a t_{max} of 2 to 3 hours in dogs.[44,46] Moxidectin is highly bioavailable after oral dosing: about 90% of the drug is absorbed in dogs.[47] Reported elimination half-lives in dogs vary from 13.9 to 25.9 days.[44,46,47] This variability is associated with body condition. More obese dogs had a higher volume of distribution,[46,47] resulting in indirectly prolonged elimination due to distribution of the lipophilic drug into their relatively larger fat compartment.

Selamectin is used in dogs and cats topically. With dermal exposure, peak blood levels are reached in 72 hours in dogs and 15 hours in cats; if given orally, t_{max} is 8 hours in dogs and 7 hours in cats.[48] The elimination half-life in dogs is 11.1 days after dermal exposure and 1.9 days with oral exposure. In cats, the half-life is 8.25 days after dermal exposure and 1.1 days after oral exposure.[48] Selamectin is much more bioavailable in cats than in dogs after dermal applications: 72% bioavailability in cats versus 4.4% in dogs.[48] However, it is not known how much of this difference is due to grooming behavior (and therefore oral absorption) in cats. Oral bioavailability of selamectin was 109% in cats and 62% in dogs,[48] which, especially in cats, suggests enterohepatic circulation of selamectin.

Doramectin reaches peak blood levels in 2 hours after oral dosing and 1.4 days after subcutaneous administration in dogs, while the half-life in dogs is 3 to 3.7 days.[43] Kinetic information in small animals could not be located for eprinomectin and nemadectin.

P-gp potentially both limits drug absorption, by moving substrates out of enterocytes and back into the intestinal tract, as well as enhances drug elimination, by depositing substrates into the bile, intestine, and renal tubules.[7,49] Several factors can affect the ability of P-gp to alter the kinetics of MLs. One factor is the affinities of the

MLs for P-gp, with MLs that have higher affinities being more readily transported at lower concentrations. Ivermectin, abamectin, doramectin, and eprinomectin all have higher affinities for P-gp compared to selamectin and moxidectin.[50]. The concentration of MLs presented to transporters is another potentially important factor. P-gp substrates can often stimulate their own transport at lower concentrations while inhibiting transport at higher concentrations,[51] so as levels of an ML crossing the cellular apical border increase, P-gp may become less able to effectively transport it back across the plasma membrane.

Unfortunately, the ability of P-gp to alter the pharmacokinetics of MLs has not been closely examined. One way to evaluate for this is to compare kinetic parameters of MLs between dogs with and without the *ABCB1-1*Δ gene defect. Ivermectin plasma levels did not differ between normal and ivermectin-sensitive collies administered 0.1 mg/kg ivermectin orally,[52] but 0.1 mg/kg may be too small a dose for pharmacokinetic differences to be evident. It has been demonstrated that dogs with the *ABCB1* defect are impaired in the ability to eliminate P-gp substrates into the bile[53] but do not appear to have enhanced intestinal absorption of P-gp substrates.[54] However, MLs were not evaluated in the latter two studies.

SENSITIVE POPULATIONS

Dogs with the ABCB1-1Δ *Gene Defect*

The *ABCB1-1*Δ mutation is typically seen in herding type breeds, primarily collies as well as Shetland sheepdogs and Australian shepherds; in addition, it has been detected in longhaired whippets, old English sheepdogs, silken windhounds, white Swiss shepherds, German shepherds, and some mixes of these breeds.[38,55]

Dogs can be easily tested for the gene defect.[7] However, it is difficult to know whether the frequencies of the gene defect in populations of dogs that are tested is representative of the general population since there may be bias in submitting samples (eg, dogs may be more likely to be tested after an ML-related toxicosis develops or if they are related to dogs known to have the *ABCB1* defect). Mealey and colleagues[24] found that of 5368 client-owned dogs, the breeds with the highest frequency of the *ABCB1-1*Δ mutation were collies and Australian shepherds: of 1424 collies tested, 35% were homozygous and 42% were heterozygous, and of 1421 Australian shepherds tested, 10% were homozygous and 37% were heterozygous. In miniature Australian shepherds, silken windhounds, and longhaired whippets, between 30% and 60% of the dogs tested had one or both copies of the gene defect. In border collies, German shepherds, herding breed mixes, old English sheepdogs, Shetland sheepdogs, and other mixed breeds, less than 15% of dogs had one or both copies of the gene defect. They also tested 659 purebred dogs of other breeds with none having the gene defect. In a smaller study of dogs in Australia, higher rates of the gene defect in collies and Australian shepherds were seen compared to rates in the United States.[55] Interestingly, in both of these studies, it was rare (about 1% frequency) to find the *ABCB1* mutation in border collies. However, a recent report of an *ABCB1* mutation that differs from the *ABCB1-1*Δ mutation in an ivermectin-sensitive border collie[56] demonstrates that other gene defects can produce the ivermectin-sensitive phenotype. Thus, just because a dog does not have the *ABCB1-1*Δ genotype does not mean that it is absolutely certain that it will tolerate higher dosages of MLs.

Animals Treated with Other P-Glycoprotein Substrates

Chronic administration of MLs for demodicosis has resulted in toxicosis in dogs of breeds in which the *ABCB1-1*Δ mutation has not been documented[57] (ASPCA APCC,

unpublished information, 2011), suggesting that factors other than genetics might play a role in the development of ML toxicosis. These dogs developed signs such as ataxia, lethargy, and tremors after administration of extra-label doses of ivermectin, moxidectin, or milbemycin for periods ranging from days to weeks. Bissonnette and colleagues[57] had 28 of these dogs genotyped and found that 27 were normal while one was heterozygous for the *ABCB1-1Δ* gene mutation. Of these dogs, 10 were on other medications that also are P-gp substrates. Acquired P-gp dysfunction due to drug interactions may make animals more susceptible to ML toxicosis.[7] It also may be possible that in these dogs there were other, as yet unidentified, mutations that may impair P-gp function.

Mechanisms by which other P-gp substrates can potentially cause elevated levels of MLs in the brain or plasma include competing with MLs for transport by P-gp and inhibiting P-gp function. These effects can be concentration dependent where a substrate can become an inhibitor as its concentrations at the transporter rise.[51] **Table 2** lists several medications that are known P-gp substrates or inhibitors. In many cases it is not known if an interaction between MLs and these drugs will occur, so this list should be taken as a guideline for when caution should be exercised when co-administering avermectins or milbemycins with the listed medications. However, combining a P-gp substrate with a P-gp inhibitor is more likely to be problematic than treating a patient with 2 P-gp substrates. Two commonly used veterinary drugs that are P-gp inhibitors (as well as substrates) and that interact unfavorably with MLs in dogs, as discussed next, illustrate this principle.

The antifungal drug ketoconazole can cause problems when administered concurrently with ivermectin. Hugnet and colleagues[58] reported that administration of ketoconazole to dogs over a period spanning from 5 days before through 5 days after ivermectin administration resulted in higher plasma concentrations and longer residence time of ivermectin than in dogs treated with ivermectin alone. Ketoconazole is an inhibitor of P-gp, which may result in decreased elimination of ivermectin from the CNS as well as decreased biliary excretion of ivermectin.

When ivermectin and the insecticide spinosad were co-administered, signs of ivermectin toxicosis sometimes developed at dosages not typically expected to cause problems.[59] One study determined that ivermectin pharmacokinetics were altered when ivermectin was given with spinosad: maximum plasma concentrations and area under the curve of ivermectin were increased while clearance was decreased compared to dogs given ivermectin alone.[59] It was determined that spinosad is a substrate and inhibitor of human P-gp, prompting the authors to hypothesize that this inhibition is responsible for the increased risk of ivermectin toxicosis when spinosad is co-administered in dogs.[59] A different study assessed the effects of co-administration of spinosad and milbemycin in collies with the *ABCB1-1Δ* mutation.[60] Up to 10 times the heartworm preventative dose of milbemycin along with spinosad at either 3 or 5 times the labeled therapeutic dose did not result in signs of milbemycin toxicosis. It is interesting to speculate whether milbemycin has a relatively poorer affinity for P-gp, as does moxidectin,[50] compared to ivermectin, which might explain the difference between the 2 studies. However the authors cannot locate information regarding the affinity of milbemycin for P-gp in the literature.

Neonatal and Elderly Dogs and Cats

An important question is whether very young dogs and cats have an immature BBB that would make them more susceptible to ML toxicosis. However, studies that would directly address this question could not be located. Tight junctions between endothelial cells in the brain, which begin forming in conjunction with the development of

Table 2
P-glycoprotein substrates and inhibitors

P-gp Substrates	P-gp Inhibitors
Antibiotics	
Erythromycin	Erythromycin
Tetracycline	Clarithromycin
Doxycycline	
Antifungals	
Ketoconazole	Ketoconazole
Itraconazole	Itraconazole
Antidepressants	
Paroxetine	Paroxetine
Venlafaxine	Fluoxetine
Amitriptyline	St. John's wort
Chemotherapeutics	
Vinblastine	
Vincristine	
Doxorubicin	
Actinomycin D	
Mitoxantrone	
Etoposide	
Docetaxel	
Cardiac drugs	
Digoxin	Amiodarone
Diltiazem	Quinidine
Verapamil	Verapamil
	Carvediol
	Nicardipine
Opioids	
Loperamide	Methadone
Morphine	Pentazocine
Steroid hormones	
Dexamethasone	
Triamcinolone	
Hydrocortisone	
Aldosterone	
Methylprenisolone	
Proton pump inhibitors	
	Omeprazole
	Esomeprazole
	Lansoprazole
	Pantoprazole

(*continued on next page*)

Table 2
(continued)

P-gp Substrates	P-gp Inhibitors
Miscellaneous agents	
Cyclosporine	Cyclosporine
Phenothiazines	Chlorpromazine
Spinosad	Spinosad
Cimetidine	
Fexofenadine	

Data from Refs. [49,59,82–84]

blood vessels in the fetal brain, are vital for sealing the BBB.[61] Evidence suggests that tight junctions exist in the brain prenatally in dogs, with further modifications occurring between 6 days prior to birth and 3 days postpartum.[62] In the authors' opinion, adequate P-gp expression in the endothelial cells is the other important component necessary for the BBB to be able to prevent MLs from accumulating in the brain. Information on P-gp expression in fetal or neonatal dogs or cats could not be located in the literature. In other species, times when there are marked increases in P-gp expression range from, at the earliest, during fetal development in humans[63] to, at the latest, post-natal days 16 to 21 in mice.[64] Given that times for increased P-gp expression are not expected to vary greatly beyond the times seen in other mammalian species, it is best to avoid ML exposure in neonatal dogs and cats. However, the authors speculate that sensitivity to MLs diminishes by weaning, if not sooner, in dogs and cats.

Aging also significantly affects the BBB. Brain P-gp expression is significantly decreased in aged dogs, with a 72% decrease occurring in expression in dogs over 8.3 years of age compared to dogs less than 3 years of age.[65] It is not known if this change is significant in reducing elimination of MLs from the central nervous system, but it does suggest that older patients could be more susceptible to ML toxicosis than adults.

Obese and Malnourished Animals

An animal's nutritional plane and body condition may also impact both the likelihood of ML toxicosis developing and the duration of treatment needed when toxicosis occurs. In moxidectin pharmacokinetic studies, obese dogs, which have a relatively larger volume of distribution, had a significantly longer elimination half-life for moxidectin.[46,47] It is not known if this difference is clinically significant, but it suggests that obese patients may require a longer duration of treatment after overdose due to the longer length of time needed for MLs to redistribute out of the fat compartment. Conversely, obesity could have a protective effect by basically providing more body volume for a given ML dose to distribute into, thus lowering plasma and tissue concentrations. So it is difficult to predict which effect might have more impact in ML toxicosis. A case report describing 3 rottweilers that ingested moxidectin noted that, of the 3, the obese dog received the lowest dose but had the most severe signs.[28] Two of the 3 dogs in the study (including the obese dog) were negative for the *ABCB1-1*Δ gene defect, while the third dog's sample was not adequate for testing.

But what if a patient is malnourished? In vitro binding studies in dogs have shown that ivermectin binds extensively to plasma albumin and lipoproteins.[42] In a severely undernourished or hypoalbuminemic patient, it is possible that a higher free drug concentration could develop resulting in more severe clinical signs. For now, the influence of body condition on ML toxicosis remains speculative, but it should be considered.

TREATMENT

There are no specific antidotes for ML toxicosis. Appropriate decontamination and good supportive care are the cornerstones of treatment. Some patients need to be hospitalized for several days, so it is important that animal owners are advised up front regarding this possibility. However, with commitment to treatment, it is possible for even severely affected animals to make a complete recovery.

Decontamination

Inducing emesis may be considered if oral exposure was recent and the animal is asymptomatic. There are no established criteria for when emesis should be induced or avoided with ML ingestion. Rather, several factors must be considered. Liquid or paste formulations of MLs are anticipated to empty from the stomach rather quickly compared to solid formulations,[66] although mixing with recently ingested food may slow the emptying of nonsolid formulations.[67] Also, inducing emesis will not only delay the administration of activated charcoal, which is likely to be of greater benefit in reducing absorption of MLs than emesis, but will also make it more likely that subsequently administered activated charcoal will be vomited. Additionally, care must be taken to avoid aspiration if neurologic signs have already developed,[68] so emesis should not be induced in patients who are already showing signs such as tremors, seizures, or CNS depression. Ultimately, the decision to induce emesis is best determined on a case-by-case basis, but a rule-of-thumb is to induce emesis if ingestion was within the past 30 to 60 minutes. Emesis could also be considered beyond 1 hour post-ingestion in circumstances such as the consumption of a large meal prior to oral ML exposure.

An initial dose of activated charcoal is likely to be of benefit if given within the first 4 hours of ingestion, given what is known regarding the absorption rate of MLs. Administering repeated doses of activated charcoal as frequently as every 8 hours for 2 days has been advised for ivermectin toxicosis,[22,69] although the efficacy of activated charcoal in treating overdoses of MLs has not been established. Whether a substance undergoes enterohepatic circulation is a key factor in whether repeated doses of activated charcoal are beneficial in enhancing elimination.[67] Since there is evidence that MLs are enterohepatically circulated, it is reasonable to consider repeated doses of activated charcoal in small animal patients regardless of the route of exposure. However, this recommendation caries some caveats. As with emesis, the risk of aspiration can be higher when administering charcoal in a symptomatic patient, especially a comatose patient, so this should not be attempted in a patient with an absent gag reflex. Intubation may offer some degree of airway protection during charcoal administration, but it does not completely remove risk of aspiration.[70] Other complications of activated charcoal administration to consider include hypernatremia and hypermagnesemia, likely due to the loss of free water osmotically drawn into the GI lumen.[67] These electrolyte disturbances are considered infrequent in humans, with an incidence of 6% and 3.1%, respectively, in one study,[71] but the incidence of either has not been reported in small animal patients. An additional consideration is that dogs with the *ABCB1-1*Δ gene defect may have minimal biliary

elimination of P-gp substrates due to nonfunctional P-gp.[53] Therefore, repeated doses of activated charcoal may not be of much benefit in these animals, although this has not been proven. Because the amounts of MLs eliminated in bile in canines have not been evaluated, this would be an excellent avenue for further research that would help better answer questions about the role of repeated administration of activated charcoal in both wild-type and P-gp–defective dogs. In summary, decisions on the frequency of administration of activated charcoal are also best decided on a case-by-case basis, with the authors urging caution and moderation. An initial dose of charcoal given within 4 hours of exposure is strongly advised, provided that marked CNS signs are not present. Subsequent doses administered every 8 hours may be of some benefit, more so in animals with a normal *ABCB1* genotype. Risks of hypernatremia and aspiration should always be kept in mind whenever activated charcoal is used.

Supportive and Symptomatic Care

Fluid therapy, good nursing care of the recumbent animal, and thermoregulation are essential for these patients.[72] If respiratory depression develops, patients may require oxygen, intubation, and positive pressure ventilation. Nutritional support may also be needed. If bradycardia develops, a preanesthetic dose of atropine or glycopyrrolate may be given.

Treatment of tremors or seizures resulting from ML toxicosis is a challenging topic, with the uncertainty of which drugs to use being the main question. In clinical case reports, administration of diazepam either seemed to be of no benefit[30] or resulted in improvement of CNS stimulation soon followed by worsening of CNS depression.[26,28,29] This led Hopper and colleagues[26] to suggest that diazepam be avoided in favor of other suitable drugs such as barbiturates or propofol. Yet a progression of signs from tremors or seizures to severe CNS depression describes a typical clinical course as concentrations of MLs rise in the brain. It is likely that an onset of CNS depression would have occurred regardless of whether diazepam was given. While benzodiazepines such as diazepam can potentiate GABAergic effects, so can barbiturates and propofol, which both bind $GABA_A$ receptors, albeit at different sites than benzodiazepines and MLs.[5] Moreover, an experimental study in rodents suggests that ivermectin worsens the CNS effects caused by barbiturates.[6] The present state of knowledge is that there are several different binding sites on $GABA_A$ receptors, each of which binds different types of xenobiotics. The different binding sites interact allosterically, with binding of a compound to one site influencing the likelihood of different compounds binding to other sites—all of which then influence opening of the channel in the receptor and subsequent chloride influx.[4,73] Assessment of allosteric relationships in the $GABA_A$ receptor can be very challenging,[5] and the relationships between MLs and drugs that bind $GABA_A$ receptors have not been well investigated. Until these allosteric relationships are better established, it is the authors' opinion that diazepam, barbiturates, or propfolol may be cautiously used to attempt to control tremors or seizures.

Specific Therapies

Intravenous lipid emulsion therapy has been suggested to be a treatment that may shorten the duration of clinical signs of ML toxicosis. Lipid therapy was used to treat moxidectin intoxication in a 16-week-old Jack Russell terrier.[29] The dog recovered quickly compared to other reported cases of moxidectin toxicosis, but the amount of moxidectin the puppy ingested is unknown, so it is difficult to draw firm conclusions. Lipid therapy has also been successfully used in a border collie that ingested up to 6

mg/kg of ivermectin paste.[74] The authors demonstrated decreasing blood levels of ivermectin and a relatively rapid improvement in clinical signs in this case with use of lipid therapy. The dog in this case report was found to not have the *ABCB1-1Δ* gene mutation, which may be why the therapy appeared effective: the ability of P-gp to clear ivermectin from the CNS and the circulation was intact in this patient. When lipid therapy was administered several hours after ivermectin exposure in 3 dogs homozygous for the P-gp gene defect, lipid therapy failed to improve stupor or coma.[75] Speculatively, these dogs may have had higher CNS levels or been impaired in the elimination of ivermectin due to nonfunctional P-gp, resulting in the lack of efficacy of lipid therapy.[75] The use of intravenous lipid therapy to treat macrocyclic lactone toxicosis has not been reported in feline patients, but lipid therapy has been used to successfully treat lidocaine toxicosis in a cat.[76]

It is hypothesized that the lipids act as a "sink" and draw lipophilic xenobiotics into the plasma lipid phase, thus removing the harmful agent from the target tissues[29] and increasing the likelihood for more rapid elimination. Although moxidectin is likely the best candidate for this therapy due to its very high lipid solubility, all of the MLs are lipophilic so lipid therapy is potentially beneficial in treating toxicity from any of the avermectins or milbemycins. On one hand, it is important to emphasize that effectiveness and safety of this treatment in reducing the duration of clinical signs or improving outcome with acute toxicosis in clinical patients has not been proven in human[77] or veterinary patients. On the other hand, thus far, adverse effects of lipid therapy have not been reported in case reports and experimental studies where lipid emulsions were administered on a short-term basis.[78] Yet the APCC has received several reports of cases where hyperlipemic serum was noted in dogs after receiving lipid emulsion treatment. Two cases of hemolysis were also reported (ASPCA APCC, unpublished data, 2011). Consider intravenous lipid therapy if pronounced CNS signs, such as severe stupor, coma, or seizures, emerge. The APCC recommends using a 20% lipid solution starting with a 1.5 mL/kg bolus followed by a constant rate infusion (CRI) of 0.25 mL/kg/min for 30 to 60 minutes. This may be repeated every 4 hours as long as serum is not lipemic but should be discontinued if a positive response is not seen after 3 treatments. This protocol is based on the human literature where dose ranges include boluses of 1 to 3 mL/kg and CRIs of 0.2 to 0.5 mL/kg/min for up to 6 hours, with a 1.5 mL/kg bolus and a 0.25 to 0.5 mL/kg/min CRI for 30 to 60 minutes being the most commonly used.[77]

Physostigmine can cause short-term improvement in patients severely affected by MLs. Administration of physostigmine resulted in 30 to 90 minutes of improvement in moderate to severe CNS depression resulting from ivermectin-sensitive collies being administered 0.2 mg/kg ivermectin.[19] Physostigmine is a cholinergic drug that causes increased amounts of acetylcholine to accumulate at the synapse. Acetylcholine modulates inhibitory GABAergic and excitatory glutamatergic neuronal firing,[79] the net result of which may result in an improvement of clinical signs. Physostigmine is best used either to give an owner visual reassurance that the patient can still recover or to try to arouse a patient enough to encourage it to eat and drink. Frequent administration is not recommended as the effects are very temporary and significant cholinergic effects including drooling, urination, and diarrhea, as well as tremors and seizures, may be seen.[26]

Flumazenil is a $GABA_A$ antagonist that appeared to reverse the effects of ivermectin in an experimental model of drug interactions in rodents.[6,80] However, the use of flumazenil to treat ML toxicosis has not been evaluated clinically. Flumazenil is an antagonist at the benzodiazepine binding site, rather than at the GABA binding site, on $GABA_A$ receptors,[73] so flumazenil prevents benzodiazepines from binding $GABA_A$

receptors rather than directly influencing the effect of GABA. If flumazenil interacts with ML binding sites in an allosteric manner to reduce the effect of ML binding, then flumazenil would be of benefit, but it is unknown if this occurs. If it were beneficial, then it would serve a similar purpose to physostigmine: to improve clinical signs, but only transiently since flumazenil has a short time of effect.[81] Reported dosages for flumazenil in dogs range from 0.04 to 0.25 mg/kg IV.[81] Starting at the low end of the dosage range is advised, especially since flumazenil can potentially cause seizures at higher dosages through its effect as a benzodiazepine antagonist.[81]

DIAGNOSTICS

Genotyping in dogs to determine if the *ABCB1-1Δ* gene mutation is present can be performed through the Veterinary Clinical Pharmacology Laboratory at Washington State University College of Veterinary Medicine (http://www.vetmed.wsu.edu/depts-VCPL/) using either blood or cells from a cheek swab. Ideally dogs should be tested prior to using any dose of an ML higher than one for heartworm prevention, especially if the dog is a breed or breed mix of those known to carry the gene defect.

Plasma or stomach contents can be submitted to a veterinary diagnostic laboratory to test for levels of MLs in an effort to document exposure. Response to physostigmine can also be suggestive of ML intoxication if exposure is uncertain.[72] For post-mortem testing, samples to submit include frozen brain, liver, and fat.[72]

OUTCOME

The prognosis may be guarded to good depending on the exposure dose and agent involved. Severely affected dogs may require long-term care, which may be a financial burden for some owners. Depending on the dose and half-life of agent involved, recovery can take days to weeks. Reportedly one dog recovered completely after being comatose for 7 weeks.[69] After recovery, long-term sequelae are not expected.[72] Sedation and blindness seem to the longest lasting signs, but even blindness is not expected to be permanent as most dogs seem to recover visual ability (ASPCA APCC, unpublished information, 2011). Two dogs with documented retinal edema did recover well with only residual retinal scarring.[17]

SUMMARY

Drugs in the avermectin and milbemycin classes have a wide margin of safety between therapeutic and toxic dosages when administered to companion animals at their labeled dosages and dosing frequency. Toxicosis becomes more likely when higher, extra-label dosages are administered to dogs with the *ABCB1-1Δ* gene mutation or when companion animals are inadvertently exposed to, or iatrogenically overdosed with, concentrated ML-containing products intended for large animal use. Drug interactions between MLs and other P-gp substrates, such as spinosad or ketoconazole, might also result in ML toxicosis. Once clinical signs develop, recovery can take days to weeks due to extensive distribution of MLs in the body and their slow elimination. Decontamination measures instituted soon after exposure and good supportive care are the aspects of treatment that are most likely to favorably influence outcome. Intravenous lipid emulsion therapy has been suggested to be a beneficial treatment of ML toxicosis. However controlled clinical trials are lacking, and questions remain as to whether dogs with defective P-gp are a subpopulation in which lipid therapy is effective.

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