Evaluation of the biliary and brain distribution of technetium Tc 99m sestamibi in healthy dogs with the ABCB1 wildtype genotype before and after treatment with spinosad

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Objective—To determine whether the reported drug-drug interaction between the flea medication spinosad and ivermectin is attributable to inhibition of P-glycoprotein by spinosad.

Animals—6 healthy adult dogs with the ABCB1 wildtype genotype.

Procedures—The study was conducted as a prospective, masked, randomized crossover design. Six dogs were allocated to 2 groups; each dog served as its own control animal. Dogs in one of the groups received spinosad at the manufacturer's recommended dose; the other group received no treatment. Forty-eight hours later, scintigraphic imaging of the head and abdomen were performed with the radiolabeled P-glycoprotein substrate methoxy-isobutyl-isonitrile (sestamibi) in both groups of dogs. After a washout period of 60 days, the dogs in each group received the alternate treatment, and scintigraphic imaging again was performed 48 hours later. Gallbladder-to-liver and brain-to-neck musculature ratios of technetium Tc 99m sestamibi were calculated for each dog and compared between treatments.

Results—No significant differences in gallbladder-to-liver or brain-to-neck musculature ratios were found between treatments.

Conclusions and Clinical Relevance—Results provided evidence that spinosad did not inhibit P-glycoprotein function 48 hours after spinosad was administered at the manufacturer's recommended dose. Further investigations will be necessary to elucidate the mechanism of the reported toxic interaction between spinosad and ivermectin. (*Am J Vet Res* 2012;73:814–820)

Since the release of spinosad in late 2007, reports of abnormal neurologic signs in dogs receiving both spinosad and ivermectin (at high, extralabel doses used for treatment of mite infestations) prompted the US FDA to issue a warning regarding the concurrent use of these 2 drugs.[1] Clinical signs observed shortly after administration of spinosad to dogs also receiving ivermectin have included trembling or twitching, salivation or drooling, seizures, ataxia, mydriasis, blindness, and disorientation.[2] For comparison, the most common adverse signs observed after treatment with spinosad alone are vomiting, a decrease in appetite, lethargy, and diarrhea.[2] Because these clinical signs resemble those of ivermectin toxicosis,[3] it is reasonable to hypothesize that the administration of spinosad increases systemic exposure to ivermectin.

Abbreviations

B:M	Brain-to-neck musculature ratio
GABA	γ-Aminobutyric acid
G:L	Gallbladder-to-liver ratio
ROI	Region of interest
^{99m}Tc	Technetium Tc 99m

It is known that there is increased systemic exposure to ivermectin in dogs with impaired P-glycoprotein function.[4] The most commonly known and examined example of this involves Collies with the ABCB1-1Δ (formerly MDR1) mutation that results in the production of a severely truncated, nonfunctional P-glycoprotein molecule.[5] In contrast to dogs with normal P-glycoprotein function, dogs with the ABCB1-1Δ mutation are highly sensitive to the adverse effects of ivermectin and develop signs of toxicosis when they receive the high, extralabel dose often prescribed for treatment of mite infestations.[5] The ABCB1-1Δ polymorphism is

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most prevalent in Collies, but it has also been described in numerous other breeds.[6]

P-glycoprotein is a large membrane-spanning protein of the ATP-binding cassette superfamily of membrane transporters[7]; P-glycoprotein functions as an ATP-dependent efflux pump, and the importance of P-glycoprotein as a membrane transporter has been reported.[8,9] Interest in P-glycoprotein was initially centered on the fact it was found in tumors with multidrug resistance to chemotherapeutic agents.[10,11] Natural expression of P-glycoprotein was subsequently detected within the epithelial cells of organs with excretory or protective functions. Mammalian tissues that express P-glycoprotein include the apical border of intestinal epithelial cells,[12] brain capillary endothelial cells,[13] biliary canalicular cells,[14] renal proximal tubular epithelial cells,[15] placental tissues,[16] and testicular tissues.[17] In dogs, P-glycoprotein has been detected in the liver, kidneys, adrenal glands, colon, and brain capillary endothelial cells.[18]

The presence of P-glycoprotein in the blood-brain barrier and liver is of particular importance with regard to drug disposition in both dogs and humans. A large number of structurally and pharmacologically unrelated drugs are substrates of P-glycoprotein, and the ability to predict that a drug is a substrate on the basis of chemical composition is poor.[19,20] In addition to ivermectin, other drugs commonly used in veterinary medicine (such as vincristine, doxorubicin, loperamide, and digoxin) are also substrates of P-glycoprotein.[21–24] These drugs have low therapeutic indexes and have potentially fatal adverse effects at even mildly increased plasma concentrations.

Dysfunction of P-glycoprotein can result from intrinsic and extrinsic factors. Intrinsic dysfunction is seen in dogs with the ABCB1-1Δ mutation. Extrinsic dysfunction can be induced by administration of certain drugs, such as ketoconazole, which act to inhibit P-glycoprotein function.[4,25] Coadministration of ketoconazole (an antifungal drug) and ivermectin dramatically alters the disposition of ivermectin, which leads to increased systemic exposure to ivermectin.[4]

Ivermectin, a macrolytic lactone of the avermectin family, is an insecticide that induces tonic paralysis in invertebrate organisms by potentiating glutamate-gated or GABA-gated chloride channels of the peripheral nervous system.[26] In most mammals, including dogs, GABA-gated receptors are limited to the CNS, and glutamate-gated chloride channels targeted by ivermectin have not been detected in mammals.[26,27] Ivermectin typically is excluded from the CNS of mammals through the action of the membrane transporter P-glycoprotein; thus, abnormal neurologic signs are not evident in animals receiving recommended doses of ivermectin.[5] However, dogs that have deficient P-glycoprotein function (intrinsic or extrinsic) markedly accumulate increased concentrations of ivermectin within their CNS tissues, which causes abnormal neurologic signs in dogs treated with extralabel doses of ivermectin.[4,5]

Spinosad, a member of the spinosyn class of insecticides, is a tetracyclic macrolide produced naturally by the bacterium *Saccharopolyspora spinosa*.[28,29] Spinosad is marketed as an oral chewable product that is administered monthly for flea prevention in dogs. The mode of action for spinosyns is modulated primarily through binding at unique sites on nicotinic acetylcholine receptors not targeted by other antiparasitics and secondarily through binding to unique sites on GABA-gated receptors. The spinosyns are selective for insect nervous systems, which makes them ideal for use in mammals. Exposure of certain insects to the spinosyns results in neurologic hyperexcitability characterized by involuntary muscle contractions and tremors that lead to prostration, paralysis, and rapid death.[28,29] The safety and efficacy of spinosad have been reported in dogs with the ABCB1 wildtype genotype and ABCB1-1Δ mutation, with doses of up to 5 times the label recommendation administered without incident in dogs with the ABCB1-1Δ mutation.[30] Therefore, although spinosad is unlikely to be a substrate for P-glycoprotein, the role of spinosad as a potential inhibitor of P-glycoprotein has not been investigated.

The radiolabeled pharmaceutical ^{99m}Tc-sestamibi is used primarily for myocardial perfusion imaging in humans.[31] The noncardiac distribution of ^{99m}Tc-sestamibi in dogs has been described.[32] Specifically, ^{99m}Tc-sestamibi is actively secreted by P-glycoprotein into the gallbladder and is excluded from the brain by P-glycoprotein expressed on brain capillary endothelial cells. As a P-glycoprotein substrate, ^{99m}Tc-sestamibi is a sensitive indicator of P-glycoprotein function.[25,33,34] Furthermore, ^{99m}Tc-sestamibi has been successfully used in studies conducted to assess both the intrinsic and extrinsic impairment of P-glycoprotein in dogs. Dogs with the ABCB1-1Δ mutation have significantly greater ^{99m}Tc-sestamibi uptake in the brain, compared with uptake for dogs with the ABCB1 wildtype genotype, which indicates a deficiency of P-glycoprotein function at the blood-brain barrier in dogs with the ABCB1-1Δ mutation.[34] Similarly, a group of dogs with the ABCB1 wildtype genotype had significantly less accumulation of ^{99m}Tc-sestamibi in the gallbladder after treatment with ketoconazole, which indicated the ability of ketoconazole to inhibit P-glycoprotein–mediated biliary excretion in clinically normal dogs.[25]

Understanding the mechanisms for the toxic interaction between spinosad and ivermectin may be helpful in preventing other potentially fatal drug-drug interactions that involve spinosad. Therefore, the objective of the study reported here was to determine whether spinosad inhibits P-glycoprotein function. Our hypothesis was that the distribution of ^{99m}Tc-sestamibi activity would be significantly different in dogs after administration of spinosad. Specifically, we expected that there would be increased accumulation of ^{99m}Tc-sestamibi in brain tissues and decreased accumulation of ^{99m}Tc-sestamibi in the gallbladder of dogs after spinosad administration, compared with results for untreated dogs.

Materials and Methods

Animals—Six healthy adult dogs (4 neutered males and 2 spayed females) owned by employees of the Washington State University Veterinary Teaching Hospital were included in the present study. The dogs had a mean age of 8.7 years (range, 3 to 12 years) and

were assessed as healthy on the basis of results of a physical examination, CBC, serum biochemical analysis (including total serum bilirubin concentration), and urinalysis. In addition, none of the dogs had abnormalities of the biliary tract, as determined via abdominal ultrasonography. All dogs had the ABCB1 wildtype genotype, as determined with a PCR assay at a university laboratory.[a] None of the dogs received medications known to interact with P-glycoprotein[35] within 30 days before undergoing scintigraphic imaging. Informed consent for use of the dogs was obtained from each owner. The Washington State University Institutional Animal Care and Use Committee approved all animal procedures.

Spinosad treatment—The study was conducted as a prospective, masked, randomized crossover design with each dog serving as its own control animal. All 6 dogs were assigned a number. The randomization function in a spreadsheet software program[b] was used to assign 3 dogs to each of 2 groups. Dogs in one of the groups received spinosad[c] at the manufacturer's recommended dose (30 to 60 mg/kg); the dogs in the other group did not receive any treatment (control treatment). Forty-eight hours later, scintigraphic imaging was performed on both groups of dogs. After a 60-day washout period, dogs in each group received the alternate treatment, and scintigraphic imaging was again performed 48 hours later.

One investigator (KLM) administered the oral spinosad preparation. All dogs received the spinosad within 2 hours after consuming a meal; spinosad was mixed with 113.5 mg of baby food to aid in administration. All dogs were closely observed for at least 4 hours after administration of the spinosad, which was selected as a sufficient amount of time to ensure adequate absorption of the spinosad. None of the dogs vomited or regurgitated during that time.

Scintigraphic evaluations—Another investigator (CSM) who was unaware of the treatment administered to each dog performed the imaging and data analysis. A catheter was inserted in a cephalic vein in each dog. All dogs were sedated by administration of a combination of dexmedetomidine hydrochloride[d] (mean dose, 0.016 mg/kg) and hydromorphone hydrochloride (mean dose, 0.1 mg/kg). Dexmedetomidine and hydromorphone were used for sedation because neither of these drugs has been found to interact with P-glycoprotein. Dogs were sedated immediately before IV injection of ^{99m}Tc-sestamibi (mean ± SD dose, 10.2 ± 3.8 MBq/kg); the ^{99m}Tc-sestamibi was obtained from a local distributor.[e] A gamma camera[f] was fitted with a low-energy, all-purpose parallel hole collimator. A 20% window centered at the 140-keV photopeak of ^{99m}Tc was used. A 256 × 256 matrix was used for all acquisitions. Sixty-second static acquisition ventral images of the abdomen and dorsal images of the head and neck were obtained 0, 60, and 120 minutes after IV injection of ^{99m}Tc-sestamibi. These methods were modified from the methods reported in 2 other veterinary studies.[25,34]

Dogs were handled in accordance with Washington State University radiation safety protocols. The dogs were placed in isolation housing after the scintigraphic procedures until their body radioactivity, as measured with a Geiger-Muller survey meter, was ≤ 0.1 mR/h at 1 m.

ROI calculations—Change in activity over time for the liver and gallbladder was determined. An ROI was manually drawn around the liver and around the gallbladder at each time point. Because the liver ROI included the gallbladder activity, net liver activity was obtained by subtracting the gallbladder activity from the gross liver activity by use of the following equation:

Net liver counts/pixel = (gross liver counts – gallbladder counts)/(gross liver pixels – gallbladder pixels)

Mean liver and gallbladder counts per pixel were recorded. Standardized elliptical-shaped ROIs were placed around the brain and neck musculature at each time point, and mean counts per pixel were recorded. The automated image analysis software program included in the gamma camera computer was used to acquire all ROIs. All ROIs were drawn and placed by the same investigator (CSM) to limit variability among ROIs. At all 3 time points, G:L and B:M, as determined via mean counts per pixel, were calculated with spreadsheet software.[b]

Statistical analysis—A paired Student *t* test was used to compare G:L and B:M for the control and spinosad treatments at 120 minutes after IV injection of ^{99m}Tc-sestamibi. The 120-minute time point was used to determine significant differences between treatments on the basis of results of previous studies[25,34] in dogs. Comparisons were performed with commercial statistical software.[g] Values of $P < 0.05$ were considered significant.

Results

All dogs were treated with the appropriate dose of spinosad (mean, 40 mg/kg; range, 32 to 53 mg/kg). No

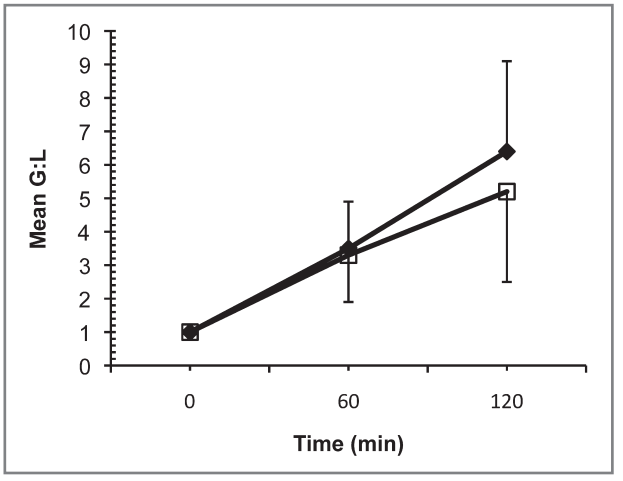

Figure 1—The G:L determined via scintigraphic imaging at 0, 60, and 120 minutes after IV injection of ^{99m}Tc-sestamibi in 6 healthy adult dogs with the ABCB1 wildtype genotype. In accordance with a prospective, masked, randomized crossover design, each dog received a spinosad treatment (white squares) and a control treatment (black diamonds); there was a 60-day washout between treatments. Values reported represent the mean and SD counts per pixel. There was no significant ($P = 0.275$) difference in the mean value for the 6 dogs between the control and spinosad treatments.

significant (P = 0.275) difference in mean G:L activity was detected between the control (mean ± SD, 6.4 ± 2.7; range, 1.7 to 10.9) and spinosad (mean, 5.2 ± 1.3; range, 2.7 to 7.3) treatments at 120 minutes after administration of ^{99m}Tc-sestamibi (**Figure 1**). Scintigraphic images of the abdomen were obtained before and after treatment with spinosad (**Figure 2**). For both treatments, there was no visible difference in G:L activity at time 0, and a G:L of 1:1 was assigned to all dogs for both treatments at this time point. For both treatments, the G:L activity increased at the 60- and 120-minute time points, which was consistent with secretion of ^{99m}Tc-sestamibi into the bile by P-glycoprotein. One dog had a lower G:L for both the control (1.7) and spinosad (2.7) treatments, compared with results for other dogs of the present study. However, that dog had a similar B:M for both the control and spinosad treatments, compared with the B:M of the other dogs. Another dog had a lower G:L activity for the spinosad treatment (5.2) than for the control treatment (10.9), but the G:L for the spinosad treatment in that dog was not different from the mean G:L (5.2) for the spinosad treatment of all dogs in the present study. The B:M activity of that dog was well within the range of B:M values for all other study dogs for both the control and spinosad treatments.

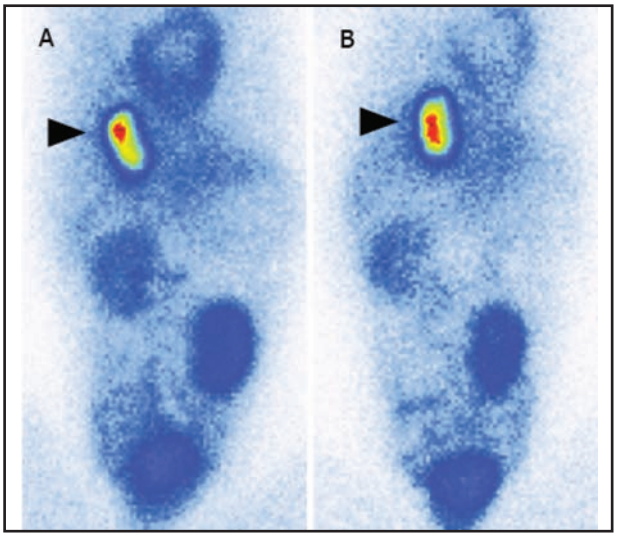

Figure 2—Ventral scintigraphic images of the abdomen of a representative dog 120 minutes after IV injection of ^{99m}Tc-sestamibi for the control (A) and spinosad (B) treatments. Notice the activity of ^{99m}Tc-sestamibi in the gallbladder (arrowhead) is similar for the control and spinosad treatments. The ^{99m}Tc-sestamibi activity is coded on a color spectrum from blue to red, where blue represents the lowest values, yellow represents the intermediate values, and red represents the highest values.

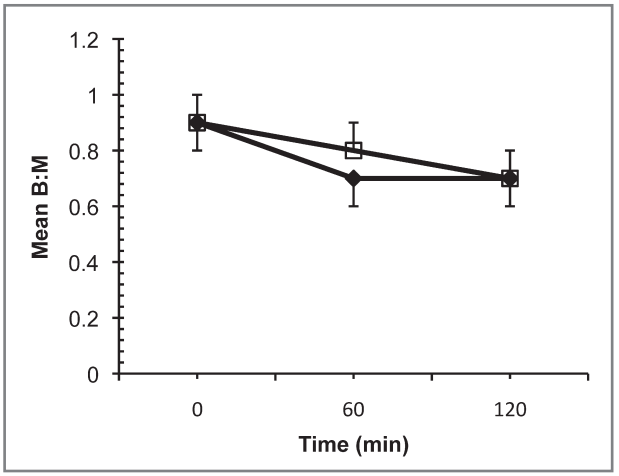

Figure 3—The B:M determined via scintigraphic imaging at 0, 60, and 120 minutes after IV injection of ^{99m}Tc-sestamibi for the spinosad treatment (white squares) and control treatment (black diamonds) for the same 6 healthy adult dogs in Figure 1. Values reported represent the mean and SD counts per pixel. There was no significant (P = 1.000) difference in mean value for the 6 dogs between the control and spinosad treatments.

No significant (P = 1.000) difference in mean B:M activity was detected between control (mean ± SD, 0.7 ± 0.1; range, 0.6 to 0.8) and spinosad (mean, 0.7 ± 0.1; range, 0.6 to 0.8) treatments at 120 minutes after administration of ^{99m}Tc-sestamibi (**Figure 3**). Scintigraphic images of the brain were obtained for the control and spinosad treatments (**Figure 4**). Decreased accumulation of ^{99m}Tc-sestamibi within the brain, compared with the amount that accumulated in the neck musculature, was identified, which was consistent with the P-glycoprotein efflux of ^{99m}Tc-sestamibi at the blood-brain barrier.

Discussion

In the study reported here, we did not detect a significant change in accumulation of ^{99m}Tc-sestamibi in the gallbladder and brain of 6 healthy dogs with the ABCB1 wildtype genotype after treatment with the flea preventative spinosad at the manufacturer's recommended dose. These results provided evidence that the drug-drug interaction described in dogs receiving both spinosad and ivermectin did not result from inhibition of P-glycoprotein by spinosad, as was hypothesized. The importance of this finding lies in the fact that numerous drugs commonly used in veterinary medicine, including digoxin, doxorubicin, loperamide, and vincristine, are P-glycoprotein substrates. Dogs with impaired P-glycoprotein function have increased systemic exposure to these drugs or altered distribution of these drugs and are at increased risk for the harmful adverse effects of these drugs.[21–24] Had spinosad

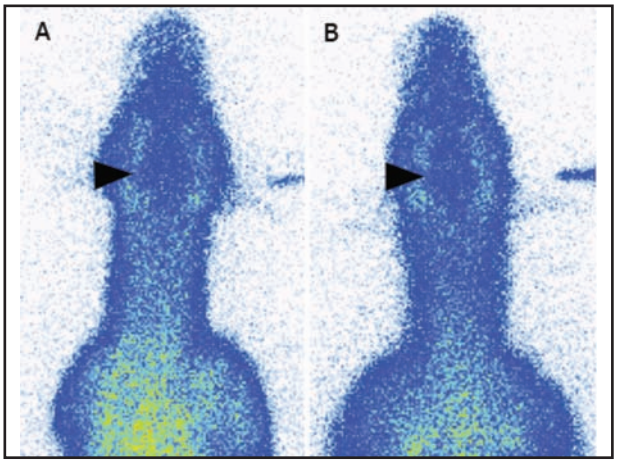

Figure 4—Dorsal scintigraphic images of the brain of the same dog in Figure 2 at 120 minutes after IV injection of ^{99m}Tc-sestamibi for the control (A) and spinosad (B) treatments. Notice the activity of ^{99m}Tc-sestamibi in the brain (arrowhead) is similar for the control and spinosad treatments. *See* Figure 2 for remainder of key.

been found to inhibit P-glycoprotein, a recommendation to limit concurrent use of these medications and spinosad would have been reasonable.

It is known that ^{99m}Tc-sestamibi is a substrate of P-glycoprotein. In a previous study,[25] investigators determined that scintigraphy with ^{99m}Tc-sestamibi provides adequate sensitivity to detect impaired secretion of ^{99m}Tc-sestamibi into the gallbladder of dogs after treatment with the P-glycoprotein inhibitor ketoconazole. Similarly, investigators in another study[34] found that scintigraphy with ^{99m}Tc-sestamibi is adequately sensitive to detect increased B:M activity in dogs with impaired P-glycoprotein function (ABCB1-1Δ mutation), compared with the B:M activity in dogs with normal P-glycoprotein function (ABCB1 wildtype genotype). If spinosad were a P-glycoprotein inhibitor, we should have detected decreased biliary activity or increased brain activity of ^{99m}Tc-sestamibi after treatment with spinosad in the present study. One dog did have a decrease in gallbladder activity for the spinosad treatment (G:L, 5.2), compared with the control treatment (G:L, 10.9), but the G:L for the spinosad treatment in that dog was identical to the mean G:L (5.2) for the spinosad treatment of all dogs. Because the G:L was not significantly below the mean G:L for all dogs, as would be expected with P-glycoprotein suppression, it is unlikely that spinosad inhibited gallbladder secretion of ^{99m}Tc-sestamibi in this particular dog. A more likely explanation would be that this dog had an abnormal increase in G:L activity attributable to unknown causes on the day of the control treatment. Whether dogs have day-to-day variation in P-glycoprotein expression or function, as detected via scintigraphy with ^{99m}Tc-sestamibi, is not currently known. Although it might have been interesting to repeat scintigraphic imaging of this dog for the control treatment and record the G:L, there was not a legitimate reason to exclude results from the original images. Interestingly, there was no significant change in B:M activity of ^{99m}Tc-sestamibi between the control and spinosad treatments in this dog. Thus, it is difficult to conclude that P-glycoprotein was inhibited in this dog.

Another dog had a lower G:L for both the control and spinosad treatments, compared with results for all other dogs. The G:L in this dog actually increased for the spinosad treatment, which is in contrast to results that would be expected if spinosad inhibited P-glycoprotein. The reason for the persistently low G:L in this dog is unknown. However, given the small study population, comment on the normal variation in P-glycoprotein activity as measured by use of ^{99m}Tc-sestamibi between and within dogs with the ABCB1 wildtype genotype is not possible. It would be necessary to obtain scintigraphic images from a much larger population of dogs with the ABCB1 wildtype genotype on multiple days to determine the interdog and intradog variability in P-glycoprotein function as measured with ^{99m}Tc-sestamibi. Although this information would be interesting, it is not considered necessary when these methods are used to detect drug-induced differences in P-glycoprotein activity, as was reported in the present study and other studies.[25,34] Only a small number of dogs were included in the present study because of results from other studies.[25,34] The fact that only a small number of dogs are necessary to detect a significant difference in B:M or G:L activity of ^{99m}Tc-sestamibi makes this a practical technique for use in future investigations.

To the author's knowledge, the present study is the first veterinary study in which ^{99m}Tc-sestamibi was used experimentally to determine whether a drug suppressed P-glycoprotein. Because it is currently unknown whether there is uniform suppression of P-glycoprotein across the various organ systems as a result of exogenous drug administration, we chose to obtain scintigraphic images of both the liver and brain of the dogs in the present study. In previous studies, it was reported that the gallbladder activity of ^{99m}Tc-sestamibi is reduced in dogs with the ABCB1 wildtype genotype that are treated with ketoconazole as well as in dogs with the ABCB1-Δ mutation[25] and that brain activity is increased in dogs with the ABCB1-Δ mutation.[34] However, it is not known whether accumulation of ^{99m}Tc-sestamibi in the brain is similarly increased in dogs with the ABCB1 wildtype genotype that are treated with ketoconazole. Therefore, it is our recommendation that all future studies that involve the use of ^{99m}Tc-sestamibi to investigate potential P-glycoprotein function should include scintigraphic images of both the brain and liver.

Only a limited number of studies have been performed to determine the mechanism involved with the drug-drug interaction between spinosad and ivermectin. Studies[30,36] conducted to investigate the safety of spinosad in clinically normal dogs and dogs with the ABCB1-1Δ mutation have been performed but have failed to directly address the concerns raised by the FDA. In 1 study,[30] it was determined that spinosad can be safely administered to ivermectin-sensitive dogs with the ABCB1-Δ mutation at up to 5 times the label dose (300 mg/kg) with or without the concurrent administration of up to 10 times the label dose of milbemycin (10 mg/kg). Milbemycin is an avermectin similar to ivermectin and has toxic effects similar to those of ivermectin when administered at high, extralabel doses to dogs with the ABCB1-1Δ mutation. It is interesting that none of the ivermectin-sensitive dogs with the ABCB1-1Δ mutation in that study[30] developed signs of avermectin toxicosis when treated with the 10 mg/kg dose of milbemycins; in another study,[37] all of the ivermectin-sensitive dogs (ABCB1 genotype unknown) developed mild transient signs of toxicosis after receiving a 10 mg/kg dose of milbemycin. Regardless, given that toxic effects were not detected in dogs receiving 10 times the label dose of milbemycin and 5 times the label dose of spinosad, it can be concluded that spinosad is unlikely to be a highly competitive substrate for P-glycoprotein and therefore is safe for use in dogs with the ABCB1-1Δ mutation. Unfortunately, that study[30] did not include a cohort of dogs that more accurately reflected the population of dogs reported by the FDA to be at risk[1] (ie, dogs with the ABCB1 wildtype genotype concurrently receiving high doses of ivermectin [often at least 20 times the label dose] and spinosad).

In addition to the small sample size, the present study was limited in that only 1 spinosad dose was included. Treatment with higher-than-recommended doses of spinosad was not included. Given that the reported toxic interactions between spinosad and ivermectin were detected in dogs receiving recommended doses of spinosad, we did not consider that such a treatment was warranted. Additionally, scintigraphic imaging was conducted at only 48 hours after spinosad treatment, and it remains possible that plasma concentrations of spinosad were higher initially. However, because the elimination half-life of spinosad is 10 days, plasma concentrations of spinosad would be only slightly less (approx 90% of the maximum plasma concentration) at the 48-hour time point. Therefore, we are unable to conclude that spinosad did not inhibit P-glycoprotein at earlier time points following spinosad administration, although we considered the possibility of such inhibition to be unlikely.

In the present study, we did not detect a significant difference in the activity of ^{99m}Tc-sestamibi in the brain and gallbladder of 6 dogs with the ABCB1 wildtype genotype after treatment with spinosad, a preventative flea medication administered monthly. This finding provides evidence that the toxic events reported with concurrent use of spinosad and high doses of ivermectin are not a result of P-glycoprotein suppression. Further investigations are necessary to determine whether this toxic interaction between spinosad and ivermectin can be repeated in a controlled environment and to determine the mechanism responsible for the toxicosis.

a. Veterinary Clinical Pharmacology Laboratory, Washington State University, Pullman, Wash.
b. Microsoft Excel, Microsoft Corp, Redmond, Wash.
c. Comfortis, Elanco Animal Health Co, Indianapolis, Ind.
d. Dexdomitor, Pfizer Animal Health, New York, NY.
e. Cardinal Health Inc, Spokane, Wash.
f. Scintron IV, Medical Imaging Electronics America Inc, Elk Grove Village, Ill.
g. Statistix, version 7.0, Analytical Software Inc, Tallahassee, Fla.

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