Reprinted from the JOURNAL OF THE AMERICAN VETERINARY MEDICAL ASSOCIATION, Vol. 183, No. 11, Pages 1195-1197.

Toxicity and efficacy of ivermectin in chelonians

J. Andrew Teare, DVM, and Mitchell Bush, DVM

SUMMARY

Five red-footed tortoises *(Geochelone carbonaria)* treated for intestinal nematode parasitism with a single IM injection of ivermectin (0.4 mg/kg) were found in a state of extreme paresis or flaccid paralysis. One tortoise recovered normal motor function over the next 7 to 10 days. The remaining tortoises died within 3 days of receiving the ivermectin. The only consistent postmortem finding was a marked fatty change in the liver.

Studies in the red-footed tortoise showed that some paresis will occur with dosages as low as 0.05 mg/kg. At least 3 other species of chelonians were found to be susceptible to ivermectin toxicosis at similar dosages (0.1 mg/kg or less). The leopard tortoise *(Geochelone pardalis)* appeared to be the most susceptible of the species tested, consistently developing mild paresis with a dosage of 0.025 mg/kg. Death occurred with dosages as low as 0.3 mg/kg.

A dosage of 0.05 mg/kg was found to be safe in red-footed tortoises, provided that treatment was not repeated at intervals of less than 7 days. Shedding of nematode larvae and eggs in the feces of parasitized red-footed tortoises was prevented with 2 to 6 weekly IM injections of ivermectin at a dosage of 0.05 mg/kg, but limited necropsy findings indicated that elimination of adult nematodes from the intestines was incomplete.

IVERMECTIN is a mixture of 2 avermectins (22,23 dihydroavermectin B_1a and 22,23 dihydroavermectin B_1b), which are members of a new class of macrocyclic lactones derived from *Streptomyces avermitilis*. This class has broad-spectrum anthelmintic and insecticidal activity. Ivermectin has been used safely in horses, dogs, cattle, swine, sheep and human beings, with efficacy against a variety of adult and larval nematodes, lice, and mites.[1-6] In pigs, dosages of 0.5 mg/kg, IM, have been used without serious adverse side effects.[7] Recently, ivermectin received approval for IM use in horses, with a recommended dosage of 0.2 mg/kg.[a]

This report concerns toxicosis and death associated with the use of ivermectin in red-footed tortoises. In addition, the results of studies to determine the safety and efficacy of ivermectin in 4 species of chelonians are presented.

Case History

Preshipment fecal examination on 5 red-footed tortoises *(Geochelone carbonaria)*, using direct smears and a centrifugation/sugar flotation technique, revealed nematode larvae. The decision to treat the parasitism was based on previous deaths, in red-footed tortoises at the National Zoo, that were associated with severe parasitism and enteritis. We found that ivermectin at a dosage of 0.4 mg/kg was safe in the corn snake *(Elapha guttata)* and Dumerils monitor *(Varanus dumerili)*. Corn snakes also tolerated dosages of 1.0 mg/kg without apparent adverse clinical signs. On the basis of these experiences, a dosage of 0.4 mg ivermectin/kg of body weight was used to treat the tortoises. The following morning, all 5 were reported to be dead.

While the tortoises did appear to be dead on first examination (head and limbs protruding flaccidly, with eyes closed), all were capable of a weak withdrawal response when the toes were squeezed forcefully. Heartbeats were detected in all the tortoises, using a Doppler ultrasound unit.[b]

The least responsive tortoise was euthanatized and necropsied. Two tortoises were placed in a neonatal incubator at 32 C, with an oxygen flow of 1 L/min. The remaining tortoises were kept overnight at 6 C to reduce metabolic activity. All 4 tortoises were less responsive by the next morning, although heartbeats were still detectable. One of the cooled tortoises died within the next 6 hours and the decision was made to treat the remaining 3 tortoises with picrotoxin. Each tortoise received 0.2 mg of picrotoxin in 5 ml of 5% dextrose in saline intracoelomically, then was placed in the neonatal incubator. Two tortoises receiving picrotoxin did not respond and were dead by the next morning.

From the Department of Animal Health, The National Zoo, Rock Creek Park, Washington, DC 20008. Dr. Teare's present address is Henry Doorly Zoo, 10th and Deer Park Blvd, Omaha, NE 68107.

The authors thank Drs. Richard Montali and Julia Langdenberg, Department of Pathology, National Zoo, for assistance with necropsies and histology.

[a]Eqvalan, MSD Agvet, Inc, subsidiary of Merck & Co, Inc, Rahway, NJ.
[b]Model 822 Doppler, Parks Electronics Laboratory, Beaverton, Oregon.

The final tortoise had a marked increase in responsiveness within 6 hours of receiving picrotoxin and began to produce large quantities of mucus from the eyes, nostrils, and mouth. The following morning (day 3 after the ivermectin treatment), this tortoise received 5 ml of 5% dextrose in saline intracoelomically and began to move around within the incubator. Excessive mucus production from the eyes and nostrils continued for 3 to 4 more days, while motor function and strength gradually improved. The animal was considered to be normal by day 10 (after ivermectin treatment).

Pathologic Findings

The only consistent pathologic finding in the 4 dead tortoises was a marked, diffuse fatty change of the liver. On the basis of previous necropsies of red-footed tortoises at the National Zoo, this was an unusual finding. Microscopically, cross sections of a viviparous nematode *(Proatractis sp)* were seen in the colon and cecum of 3 of the tortoises. An associated enteritis was evident in 2 of these 3 animals.

TABLE 1—Dosage trials of ivermectin in 4 species of Chelonia

Species	Dosage (mg/kg)	Frequency of administration	Number of trials*
Red-footed tortoise	0.01	Once daily for 5 days	2[a,b]
	0.05	Single dose	3
	0.05	Repeat at 72 hours	2
	0.075	Single dose	2[a,b]
	0.1	Single dose	2
Leopard tortoise	0.025	Single dose	2[a,b]
	0.05	Single dose	2[a,b]
Eastern box tortoise	0.10	Single dose	2[a,b]
	0.17	Single dose	1[b]
	0.2	Single dose	2[b]
	0.3	Single dose	1[a]
Red-eared turtle	0.15	Single dose	1
	0.30	Single dose	1

*Superscripts within a species indicate that the same animal was used in all trials with the same superscript. A minimum period of 7 days elapsed between trials on the same animal.

Experimental Study

Nine red-footed tortoises, 2 leopard tortoises *(Geochelone pardalis)*, 3 eastern box tortoises *(Terrapene carolina)*, and 2 red-eared turtles *(Chrysemys scripta)* were used to investigate the safety of ivermectin in chelonians. A range of dosages, from 0.01 to 0.3 mg/kg, was tested (Table 1). Animals used in more than 1 trial had at least 7 days between trials, which proved sufficient time for all animals with signs of toxicosis to regain normal function. In general, higher dosage trials were completed after lower dosage trials had been shown to be safe.

Results

A grading system (scale 0 to 5) was developed to summarize the responses to the various dosages (Table 2). Grades 0 to 4 were considered fully reversible, since animals recovered to apparent normal function if no more ivermectin was given. A grade-5 response was usually followed by death in 1 to 3 days. Response to ivermectin was graded at 24 hours after injection and, in general, responses were easily classified into 1 of the 6 categories.

TABLE 2—Grading system used to evaluate behavioral and physical responses to ivermectin in chelonians

Grade	Characteristics
0	Clinically normal animal
1	Mild slowing of reactions; tendency to move less; doesn't eat
2	Mild paresis (based on degree of difficulty in extracting head or limbs from shell); appears "tranquilized"; often found with head and neck out of shell, but capable of full retraction when stimulated
3	Moderate paresis (easier to extract head and limbs from shell); capable of full retraction when stimulated
4	Severe paresis; incapable of full retraction of head or limbs into shell; responds to stimuli, but quickly relaxes when not stimulated
5	Flaccid paralysis or extreme paresis; no response to minor stimuli; may be slight response to painful stimuli

The dosage of 0.01 mg/kg did not induce an effect (grade 0 response) in the red-footed tortoises, even when repeated daily for 5 days. The 0.05 mg/kg dosage induced grade 0 or 1 response in the 5 red-footed tortoises tested. The signs of toxicosis were increased by 1 grade in the 2 tortoises given a repeat dose (0.05 mg/kg) at 72 hours after the 1st dose. The higher dosages of 0.75 and 0.1 mg/kg induced grade 1 to 2 responses and grade 2 to 3 responses, respectively, in the red-footed tortoises. With these dosages, all animals recovered to normal function within 7 days.

The leopard tortoises tended to be more sensitive to ivermectin. The dosage of 0.025 mg/kg induced grade 1 and 2 responses, whereas the 0.05 mg/kg dosage induced grade 2 and 4 responses. Both tortoises recovered to normal within 7 days.

Box tortoises were more resistant to the effects of ivermectin. Only a grade 0 to 1 response was seen with a dosage of 0.1 mg/kg. Even 0.2 mg/kg induced only a grade 3 response in 1 animal and a grade 4 in the other. Box tortoises receiving 0.2 mg/kg or less recovered within 7 days of injection. However, the box tortoise that received 0.3 mg/kg developed a flaccid paralysis (grade 5) and died 2 days after dosing.

The red-eared turtles were more sensitive to ivermectin than were box tortoises, but trials were insufficient to compare responses with the other 2 species studied. A dosage of 0.15 mg/kg resulted in a grade-4 response. The turtle given 0.3 mg/kg developed a grade-5 response and was dead within 36 hours of injection.

Efficacy Study

Four red-footed tortoises passing larval nematodes (*Proatractis* sp) and nematode eggs (*Kalicephalus* sp) in their feces were used to study the efficacy of ivermectin. The tortoises were injected with ivermectin at a dosage of 0.05 mg/kg, once weekly, for either 2 or 6 treatments. Fecal samples were obtained from each animal at the beginning of treatment and 1 month after the last treatment. An additional fecal examination on the tortoises receiving 6 doses was performed after 4 treatments. Both of these tortoises were still passing nematode larvae and eggs at that time. However, all 4 tortoises were negative for parasites on fecal examination at the end of the study.

Following the completion of that study, the remainder (n = 23) of the red-footed tortoises at the National Zoo were given ivermectin weekly for 6 weeks, at a dosage of 0.05 mg/kg. Pretreatment fecal examinations of this group had repeatedly been positive for nematode larvae (*Proatractis* sp) and eggs (*Kalicephalus* sp). A fecal sample obtained after 2 doses of ivermectin was negative for eggs and larvae. However, a female red-footed tortoise that died 6 days after the last ivermectin dose (from causes unrelated to the ivermectin treatment) had numerous adult nematodes (*Proatractis* sp) in the cecum and colon.

Discussion

Ivermectin is unrelated to other anthelmintics on the market and acts directly at gamma-aminobutyric acid (GABA) synapses to stimulate a marked GABA release. The binding apparently is irreversible, although the various avermectin derivatives have different affinities for the site of attachment.[8] Ivermectin also interacts with the GABA-benzodiazepam receptor complex and there is evidence that ivermectin potentiates the effects of diazepam.[9]

In nematodes, GABA is an important inhibitory neurotransmitter and ivermectin kills nematodes by causing paralysis.[10] A good correlation has been shown between stimulation of GABA release by various avermectin derivatives and the activity of the same derivatives in paralyzing the nematode *Caenorhabditis elegans*.[8] Vertebrates also use GABA as an inhibitory neurotransmitter, but in mammals ivermectin apparently is excluded from the CNS by the blood-brain barrier.

The paresis and paralysis seen in chelonians given ivermectin strongly suggests that ivermectin toxicity in chelonians is mediated through inhibitory GABA neurons. There is no evidence to indicate whether this is due to increased permeability of the blood-brain barrier in chelonians or to a higher dependence on peripheral GABA neurons. Picrotoxin has been shown to reverse some of the effects of ivermectin in vitro, but the in vivo response was disappointing, so we cannot recommend picrotoxin as an ivermectin antagonist in chelonians.[10,11]

Safe dosages of ivermectin (inducing little or no paresis) are up to 10 times lower than the recommended dosage for horses. However, susceptibility to ivermectin toxicosis varied considerably among the chelonians tested. Dosages associated with minimal signs of toxicosis ranged from 0.025 mg/kg in leopard tortoises to 0.1 mg/kg in box tortoises. The appropriate dosage of ivermectin must be determined for each chelonian species, although 0.025 mg/kg once weekly would seem to be a safe starting point.

Evidence of cumulative toxicosis was seen in red-footed tortoises given 0.05 mg/kg twice within a 72-hour interval. These animals had greater paresis after the 2nd dosing than in response to the 1st dosing. This was not surprising with a drug that irreversibly binds to synaptic membranes. It may be speculated that recovery from ivermectin toxicosis can be attributed to membrane turnover, thereby eliminating the drug from the synaptic binding sites. Repeat dosing at short intervals would not allow sufficient time to eliminate bound ivermectin and would result in ivermectin accumulating at the synaptic binding sites.

Ivermectin, as an injectable anthelmintic, has the major advantage in chelonians of allowing easy individual treatment for parasitism. With orally administered anthelmintics, individual treatment in a large group, at an appropriate dosage, can be more difficult. The problem of ivermectin toxicosis in chelonians was overcome with low-dosage, weekly treatments and, when the ivermectin was given repeatedly, fecal examinations indicated that it was efficacious. The 1 animal necropsied after treatment had numerous adult nematodes in the cecum and colon. If these nematodes represent survivors of the treatment, then ivermectin was not efficacious at the dosage used. However, the nematodes may have been the result of reinfection, as the tortoises had access to a grass and dirt enclosure during and after the treatment. Thus, more studies must be completed on ivermectin in chelonians before recommendations can be made concerning the efficacy of this drug in chelonians.

References

1. Aziz MA, Diallo S, Diop IM, et al: Efficacy and tolerance of ivermectin in human onchocerciasis. *Lancet ii:* 171–173, 1982.
2. Stewart TB, Marti OG, Hale OM: Efficacy of ivermectin against five genera of swine nematodes and the hog louse, *Haematopinus suis*. *Am J Vet Res* 42:1425–1426, 1981.
3. Yazwinski TA, Hamm D, Williams M, et al: Effectiveness of ivermectin in the treatment of equine *Parascaris equorum* and *Oxyuris equi* infections. *Am J Vet Res* 43:1095, 1982.
4. Yazwinski TA, Pote L, Tilley W, et al: Efficacy of ivermectin against *Sarcoptes scabiei* and *Otodectes cynotis* infestations of dogs. *VM SAC* 76:1749–1751, 1981.
5. Benz GW, Ernst JV: Anthelmintic efficacy of ivermectin against immature gastrointestinal pulmonary nematodes of calves. *Am J Vet Res* 42:2097–2098, 1981.
6. Armour J, Bairden K, Preston JM: Anthelmintic efficacy of ivermectin against naturally occurring gastrointestinal nematodes of sheep. *Vet Rec* 111:80–81, 1982.
7. Stewart TB, Marti OG, McCormick WC: Efficacy of ivermectin against swine kidney worm, *Stephanurus dentatus*. *Am J Vet Res* 42:1427–1428, 1981.
8. Pong S-S, Wang CC, Fritz LC: Studies on the mechanism of action of avermectin B_1a: Stimulation of release of gamma-aminobutyric acid from brain synaptosomes. *J Neurochem* 34:351–358, 1980.
9. Williams M, Yarbrough GG: Enhancement of in vitro binding and some of the pharmacological properties of diazepam by a novel anthelmintic agent, avermectin B_1a. *Eur J Pharmacol* 56:272–276, 1979.
10. Kass IS, Wang CC, Walrond JP, et al: Avermectin B_1a, a paralyzing anthelmintic that affects interneurons and inhibitory motoneurons in *Ascaris*. *Proc Natl Acad Sci USA* 77:6211–6215, 1980.
11. Fritz LC, Wang CC, Gorio A: Avermectin B_1a irreversibly blocks postsynaptic potentials at the lobster neuromuscular junction by reducing muscle membrane resistance. *Proc Natl Acad Sci USA* 76:2062–2066, 1979.