Absorption and efficacy of a spot-on combination containing emodepside plus praziquantel in reptiles

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SUMMARY

Oral treatment of nematodiasis in reptiles can be very challenging, especially in stressed, uncooperative or dangerous species. A combination of emodepside and praziquantel (Profender®, Bayer) for topical application might be a better treatment option. A pharmacokinetic study was performed with 12 healthy adult specimens from different reptile species with different types of skin. Blood was collected before treatment and at 3 intervals (5, 24 and 48 hours) after treatment with topical emodepside/praziquantel at a dosage of 0.56 ml per kg bodyweight (bw), corresponding to 12 mg emodepside and 48 mg praziquantel per kg bw. Serum from 20 untreated specimens was collected and pooled as baseline data. The results showed that both actives penetrate reptile skin, as seen in the cat, and can be found in the serum at different levels depending on the species. Reptiles with a thicker integument showed relatively low concentrations for both praziquantel and emodepside. In addition, a dose titration study was performed to establish an effective dose, depending on the type and thickness of the integument. A dose of 4 drops per 100 g (1.12 ml per kg bw, 24 mg emodepside and 96 mg praziquantel per kg bw) was found to be effective in thick-skinned reptiles.

Keywords: Exotic pets, reptiles, parasitology, nematodes, anthelmintics, emodepside, praziquantel.

RÉSUMÉ

Absorption percutanée et efficacité de l'association emodepside-praziquantel en solution spot-on chez les reptiles

Le traitement antiparasitaire par voie orale de reptiles peut être particulièrement difficile, notamment sur des animaux stressés, non coopératifs ou appartenant à des espèces dangereuses. Une combinaison d'emodepside et de praziquantel (Profender®, Bayer) destiné à une application cutanée peut représenter une solution thérapeutique intéressante. Une etude pharmacocinétique a été conduite sur 12 specimens adultes appartenant à différentes espèces de reptiles et présentant différents types de peau. Un prélèvement de sang a été réalisé avant le traitement et à 3 intervalles (5, 24 et 48 heures) après l'application du mélange actif à la dose de 0.56 ml/kg de poids vif, ce qui correspond à 12 mg d'emodepside et 48 mg/kg de praziquantel par kg de poids vif. Le sang de 20 specimens non traités a été prélevé et analysé pour servir de ligne de base. Les resultants ont montré que les deux principes actifs pénètrent à travers la peau des reptiles et peuvent être retrouvés dans le serum à différents niveaux en fonction des espèces. Les reptiles à tegument epais présentent des concentrations relativement faibles des deux principes actifs. De plus, un essai a été conduit pour déterminer la dose active en fonction du type et de l'épaisseur de la peau. Une dose de 4 gouttes pour 100 g (1,12 ml par kg poids vif, 24 mg emodepside et 96 mg de praziquantel par kg poids vif) semble efficace chez les reptiles à peau épaisse.

Mots clés : Animaux de compagnie exotiques, parasitologie, nematodes, anthelmintique, emodepside, praziquantel.

Introduction

Intestinal helminths, pentastomids, and intestinal and blood protozoa are common endoparasites of captive or wild reptiles. Reptiles may serve as definitive, intermediate, accidental or paratenic hosts [2,4,5,7,9]. Worms are probably the most commonly encountered parasites in reptiles but their pathogenicity is highly variable. Generally, these parasites are of limited importance in wild reptiles but they may become more pathogenic when present in high concentrations or if their host is weakened by inadequate environmental conditions in captivity (Fig. 1-3). Most deworming protocols for reptiles (suffering from nematodiasis, cestodiasis, trematodiasis or acanthocephalidiasis) originated from experience with the treatment of domestic or companion animals. Over time, some of these compounds were tested and were recommended empirically because of their efficacy and safety. Many references promoting the use of these anthelmintic compounds in reptiles are currently available [2, 3, 4, 5, 6, 7, 8, 9, 10, 13].

Emodepside belongs to a relatively new class of anthelmintics (depsipeptides) which achieve their antiparasitic effect against nematodes by a novel mechanism of action. Emodepside acts at the neuromuscular junction by stimulating presynaptic receptors. It is highly effective against a number of nematodes affecting a wide range of animal species. Studies in sheep, cattle, horses, cats and dogs have shown emodepside to display effective anthelmintic activity against Trichostrongylidae, Ascarididae, Ancylostomatida, Trichocephalidae, Strongylidae and Dictyocaulidae. The spot-on combination product with praziquantel investigated in this study was licensed for cats in 2005.

Praziquantel is a broad-spectrum anthelmintic drug used in human and veterinary medicine; it ensures reliable control of trematode infestations [blood fluke, liver fluke (except

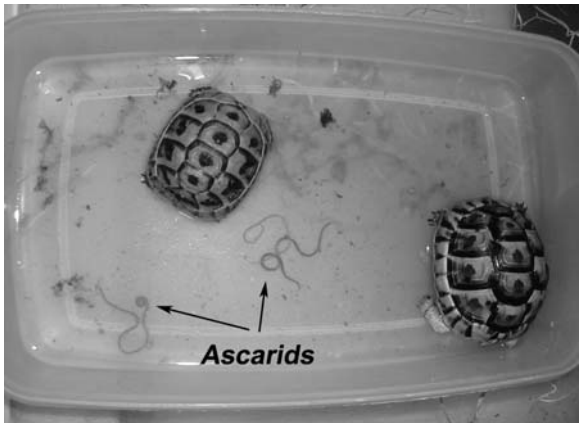

FIGURE 1 : Ascarididae in the faeces of juvenile spur-thighed tortoises after treatment (*Testudo graeca*).

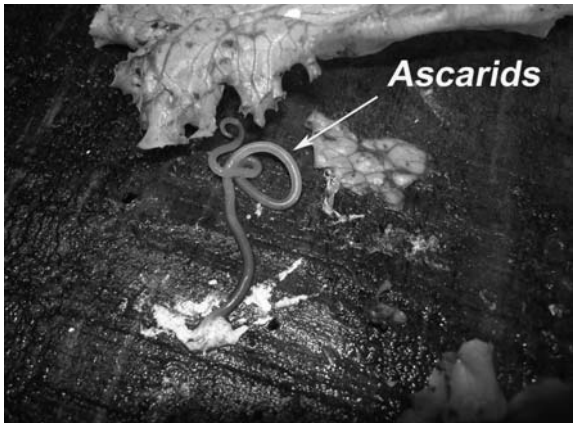

FIGURE 2 : Ascarididae in the faeces of Hermann's tortoises after treatment (*Eurotestudo hermanni*).

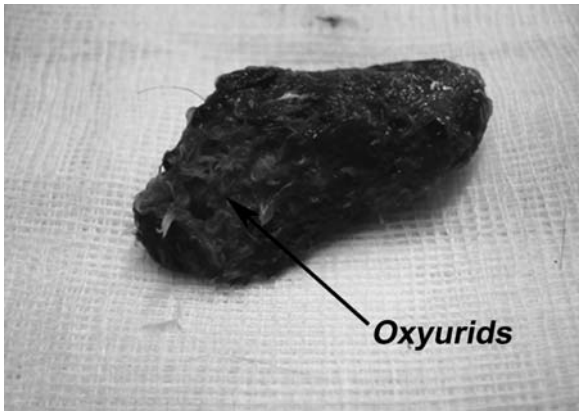

FIGURE 3 : Oxyuridae in the faeces of a green iguana after treatment (*Iguana iguana*).

Fasciola hepatica) and lung fluke] and all stages of intestinal growth in cestodes.

In a previously published report, positive results were seen after topical administration of the combination emodepside-praziquantel (Profender®, Bayer) in a wide range of reptiles (snakes, aquatic turtles, agamids, varans and geckos) found to be harbouring high concentrations of nematodes belonging to the families Oxyridae, Ascarididae, Strongylidae, Trychostrongylidae and Capillaridae [11].

Because of the thick stratum corneum of the epidermis of reptiles, it was necessary to administer four times the dosage of Profender® used in cats, i.e. approximately 0.56 ml for an animal weighing 1 kg [11]. Dosages 15, 30 and 50 times higher than those recommended for cats were administered to a variety of reptile species (snakes, geckos, and anolids) with no visible adverse effects [11].

Materials and Methods

PHARMACOKINETIC STUDY

12 healthy, adult, non-gravid reptiles from 8 different species (3 green iguanas, 1 Argentinian black and white tegu, 1 ball python, 1 corn snake, 2 savannah monitors, 1 Hermann's tortoise, 1 spur-thighed tortoise, 1 red-eared slider) were studied (Table 1). The animals were kept at 30 – 32°C for the entire study period. Blood was collected before and after spot-on treatment at a dosage of 0.56 ml/kg bw at the following times: 0h (before treatment), 5h, 24h and 48h post treatment. Blood samples were heparinised, centrifuged and frozen (-14 to -18°C). Serum from 20 untreated turtles and tortoises (control animals) was collected and pooled to obtain baseline data. The samples were analysed for emodepside and praziquantel concentrations. The plasma was deproteinised by mixing it 1 + 9 v/v with acetonitrile with subsequent centrifugation. The quantitative determination was performed by HPLC with detection by tandem mass spectrometry. The limit of quantitation was 2 µg/L for both analytes.

DOSE TITRATION STUDY

Reptiles of various species harbouring nematodes from different families (Oxyuridae, Ascarididae, Rhabditidae, Strongylidae) were included in the study. Faeces from these reptiles (adult and juvenile) produced positive results in examinations for faecal parasites, either by direct faecal smear or using a flotation technique. Results were recorded semiquantitatively as the number of eggs per microscopic field. All animals were treated with a single dose of the spot-on solution.

One group of 16 reptiles (group 1) was treated at a dosage of 0.56 ml per kg bw (Table 2), a second group of 20 animals (group 2) with a dosage of 1.12 ml per kg bw (Table 3). The product was applied on Day 0 to areas where the epidermis is relatively thin or on the animals' backs (prefemoral and gular fossa in turtles and tortoises, between the scales of the gular region or above the cordal spine in snakes, on the neck or under the armpits or the skin folds in lizards) (Figures 4-6). The treated animals were kept in dry conditions at approximately 30°C for the entire study (15 days). Semiquantitative faecal examinations were performed on Day 0+7 and on Day 0+15. All reptiles were kept on newspaper, which was replaced after every defecation to avoid reinfestation after treatment.

Results and discussions

PHARMACOKINETIC STUDY

Concentrations of emodepside [µg/L] in animal no.											
	Green iguana (Iguana iguana)			**Ball python** (Python regius)	**Argentine black and white tegu** (Tupinambis merianae)	**Corn snake** (Elaphe guttata)	**Savannah monitor** (Varanus exanthematicus)		**Hermann's tortoise** (Eurotestudo hermanni)	**Spur-thighed tortoise** (Testudo graeca)	**Red-eared slider** (Trachemys scripta elegans)
Sampling time	**1**	**2**	**3**	**4**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
0h	< LoQ	< LoQ	< LoQ	< LoQ	< LoQ	< LoQ	< LoQ	37.6	<LoQ	8.45	< LoQ
5h	< LoQ	3.09	2.71	8.22	181	17.1	3.20	23.9	2.27	15.4	< LoQ
24h	< LoQ	< LoQ	21.3	7.56	70.0	13.1	2.35	3.95	5.99	29.3	< LoQ
48h	< LoQ	2.16	< LoQ	< LoQ	78.0	10.1	5.6	< LoQ	4.05	10.6	< LoQ
Concentrations of praziquantel [µg/L] in animal no.											
	Green iguana (Iguana iguana)			**Ball python** (Python regius)	**Argentine black and white tegu** (Tupinambis merianae)	**Corn snake** (Elaphe guttata)	**Savannah monitor** (Varanus exanthematicus)		**Hermann's tortoise** (Eurotestudo hermanni)	**Spur-thighed tortoise** (Testudo graeca)	**Red-eared slider** (Trachemys scripta elegans)
Sampling time	**1**	**2**	**3**	**4**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
0h	< LoQ	< LoQ	< LoQ	< LoQ	< LoQ	< LoQ	< LoQ	35.6	< LoQ	14.3	2.80
5h	4.97	16.1	12.9	310	1069	881	74.0	132	26.0	26.0	356
24h	< LoQ	11.6	174	120	546	53.8	20.4	67.2	72.2	75.2	74.0
48h	3.06	18.8	28.6	< LoQ	458	9.57	62.7	16.7	4.21	6.86	58.9

< LoQ =< 2 µg/L

TABLE I : Pharmacokinetic study (emodepside and praziquantel in reptile plasma).

N°	Reptile species	Family of parasites at the beginning of the study (D0)	Weight (in g)	Quantity of Profender in ml	Results on D0+7	Results on D0+15
1	*Eurotestudo hermanni*	Ascarididae + Oxyuridae +++	1025	0.57	+ (Oxyuridae)	+ (Oxyuridae)
2	*Pogona henrylawsoni*	Oxyuridae +++	45	0.02	+	+
3	*Boa constrictor*	Rhabditidae ++	3225	1.806	-	-
4	*Testudo ibera*	Oxyuridae + Ascarididae +++	750	0.42	+ (Oxyuridae)	-
5	*Testudo marokkensis*	Oxyuridae +	995	0.56	-	-
6	*Testudo graeca*	Oxyuridae +++	360	0.2	+	+
7	*Iguana iguana*	Oxyuridae +++	2100	1.17	+	-
8	*Uromastyx acanthinurus*	Oxyuridae ++	320	0.18	+	+
9	*Eurotestudo hermanni*	Oxyuridae +++ Ascarididae +++	1200	0.67	+ (Oxyuridae)	+ (Oxyuridae)
14	*Crotaphytus collaris*	Oxyuridae +++	55	0.03	+	+
15	*Pogona vitticeps*	Oxyuridae +++	245	0.13	+	-
16	*Pogona vitticeps*	Oxyuridae +	230	0.13	+	+

+ < 10 eggs per field; ++ between 10 and 20 eggs per field; +++ more than 20 eggs per field.

TABLE II : Efficacy of Profender® in reptiles at a dosage of 0.56 ml/kg bw.

The results of the pharmacokinetic study showed that both ingredients penetrate the skin and can be found in the serum of the tested reptiles at levels similar to those already reported in cats [14].

However:

1. Serum levels can differ considerably depending on the species.

2. Subjects no. 9 and 11 had positive values for emodepside and praziquantel before treatment. This must have been due to unreported previous treatment of these two animals. These two subjects were probably treated in the pet shop which sold them to the owners selected for our study.

3. The red-eared slider (*Trachemys scripta elegans*) showed no value for emodepside but normal to high values for praziquantel. This subject no. 12 was the only aquatic species in our study and was returned to the water one hour after treat-

N°	Reptile species	Family of parasites at the beginning of the study (D0)	Weight (in g)	Quantity of Profender in ml	Results on D0+7	Results on D0+15
1	Testudo graeca	Ascarididae ++	860	0.96	+	-
2	Iguana iguana	Ascarididae ++ Oxyuridae +++	2200	2.46	-	-
3	Testudo graeca	Oxyuridae +++	450	0.5	+	-
4	Pogona vitticeps	Oxyuridae +++	185	0.2	-	-
5	Python molurus bivittatus	Rhabditidae ++ Ascarididae ++	7255	8.12	+ (rhabditids)	-
6	Testudo marginata	Ascarididae ++	1850	2.0	-	-
7	Eurotestudo hermanni	Oxyuridae ++ Ascarididae +	1423	1.59	+ (Oxyuridae)	-
8	Eurotestudo hermanni	Oxyuridae +++ Ascarididae ++	1142	1.28	+ (Ascarididae)	-
9	Agrionemys horsfieldi	Oxyuridae + Ascarididae +	263	0.29	+ (Oxyuridae)	-
10	Eublepharis macularius	Oxyuridae +	55	0.06	-	-
16	Geochelone pardalis	Oxyuridae ++ Ascarididae ++	32150	36	+	-
17	Eublepharis macularius	Oxyuridae +	39	0.04	-	-
18	Python molurus	Strongylidae +	2424	2.71	+	-
19	Crotaphytus collaris	Oxyuridae +++	61	0.06	+	-
20	Rhacodactylus ciliatus	Oxyuridae +++	36	0.04	+	-

+ < 10 eggs per field; ++ between 10 and 20 eggs per field; +++ more than 20 eggs per field.

TABLE III : Efficacy of Profender® in reptiles at a dosage of 1.12 ml/kg bw.

FIGURE 4 : Application of Profender® spot-on solution to the integument of a green iguana (*Iguana iguana*).

FIGURE 5 : Application of Profender® spot-on solution to the integument of a spur-thighed tortoise (*Testudo graeca*).

ment. As emodepside is expected to increase later than praziquantel in reptiles, this absence of emodepside in the serum shows that aquatic reptiles should not be returned to their aquarium for 48 hours after treatment. Otherwise the nematodicidal compound cannot penetrate the skin properly.

4. In many of the studied reptiles whose integument is particularly thick (terrestrial tortoises, green iguanas, ball pythons, savannah monitors), a higher dosage may be needed to achieve therapeutic levels (they showed either relatively low values for both praziquantel and emodepside or a nearly zero value for emodepside (< 2 mcg/L) 48 hours post administration).

5. Surprisingly, the tegu (*Tupinambis merianae*) showed high values for both compounds despite having the same type of thick integument as iguanas or tortoises. This particular species may be very sensitive to this medication. Further investigations should be carried out to verify this hypothesis.

DOSE TITRATION STUDY

The results for group 1 showed that a dosage of 0.56 ml per kg bw is only partially effective at the second control 14 days after treatment, particularly if the animal is infested by large numbers of pinworms or ascarids, and especially in reptiles whose integument is thick (such as terrestrial tortoises) (Table 2). No treatment-related adverse effects were observed in any animal. Group 2 treated at a dosage of 1.12 ml/kg bw showed complete cessation of egg shedding at the second control 14 days after treatment, even terrestrial tortoises – whose integument is particularly thick – which were massively infested with Oxyuridae or Ascarididae (Table 3). Again, no treatment-related adverse effects were observed in the specimens treated at this higher dosage (Mehlhorn, 2005). These results correlate with another clinical study performed on a much larger number of reptiles (Brames, 2008).

On the basis of these pharmacokinetic and efficacy studies, the dosage of 1.12 ml/kg bw (4 drops/100 g bw) is recommended. Aquatic species must be kept in a dry place for 48 hours following spot-on application. This new treatment method and product may find a wide range of applications in herpetological medicine: spot-on administration is relatively simple and less traumatic, and it reduces the stress generated by repeated orogastric intubation in "shy" lizards and turtles. Even though these pharmacokinetic and clinical studies are not statistically significant in terms of demonstrating efficacy, this combination is effective against the most common internal parasites encountered in these exotic species.

Despite these initial encouraging results, caution should be exercised in using this medication. Further research needs to be undertaken in a wide range of reptile species to verify the safety and efficacy of this promising drug, especially in sick animals.

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