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**veterinary
parasitology**

Veterinary Parasitology xxx (2007) xxx–xxx

www.elsevier.com/locate/vetpar

Pharmacokinetics of metaflumizone and amitraz in the plasma and hair of dogs following topical application

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Abstract

Controlled laboratory studies have shown that a metaflumizone plus amitraz combination (ProMeris[®]/ProMeris Duo[®] for Dogs, Fort Dodge Animal Health, Overland Park, KS) applied topically is effective for the treatment and control of fleas and ticks on dogs. Two studies were conducted to determine the distribution of both metaflumizone and amitraz in the plasma and hair of dogs following treatment at the minimum recommended dose of approximately 20 mg/kg of each active ingredient. Six purpose-bred, adult Beagle dogs were used in each study. Plasma or hair samples were collected from each dog just prior to dosing and periodically through 56 days after treatment. Samples were analyzed by HPLC methods validated for the simultaneous determination of metaflumizone and amitraz. Amitraz was detectable (>3.2 ng/ml) but not quantifiable (<50 ng/ml) in only two plasma samples, collected 1 and 2 days post-treatment from different dogs. Metaflumizone concentrations in plasma were generally detectable (>1.0 ng/ml) but not quantifiable (<50 ng/ml). Measurable levels were found in one dog 7 days post-treatment, increasing to a maximum of four dogs at 42 days after dosing, with a metaflumizone range of 59–138 ng/ml. Analysis of hair samples indicated that both metaflumizone and amitraz were widely distributed at basically similar levels in the hair within 1-day after administration, reaching maximum concentrations between 2 and 7 days post-treatment. Low but quantifiable levels of both compounds were still present on hair at the end of the 56-day study. These studies indicate that the ectoparasitic activity is due to exposure of the parasites to metaflumizone and amitraz on the surface of the host (hair and/or skin), not to exposure via the circulatory system of the host. © 2007 Published by Elsevier B.V.

Keywords: ProMeris[®]; Metaflumizone; Amitraz; Spot-on; Pharmacokinetics; Dogs

1. Introduction

Metaflumizone is a novel insecticide in the semicarbazone class of chemistry with potent activity against fleas *in vitro*, and *in vivo* via topical application to dogs (Salgado and Hayashi, *this volume*; Takagi et al., *this volume*). Metaflumizone has been combined with the formamidine acaricide amitraz in a novel spot-

on formulation (ProMeris[®]/ProMeris Duo[®] for Dogs, Fort Dodge Animal Health, Overland Park, KS, USA) containing 15% (w/v) of each compound to provide a product for flea and tick control on dogs (Sabnis and Zupan, *this volume*). Controlled laboratory studies have shown that the product is effective for the treatment of existing infestations of fleas and ticks on dogs, and provides control against reinfestation for 4–8 weeks when administered to provide a minimum of 20 mg/kg body weight of each active ingredient (Rugg and Hair, *this volume*; Rugg et al., *this volume*).

The objectives of the current studies were to determine the distribution of both metaflumizone and

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amitraz in the plasma (Study 1) and hair (Study 2) of dogs following treatment with metaflumizone plus amitraz at the minimum recommended dose.

2. Materials and methods

The studies were conducted in accordance with the Organization for Economic Cooperation and Development Principles of Good Laboratory Practice (OECD, 2005).

2.1. Test system

Six adult Beagle dogs, three males and three females, were used in each study. In Study 1, the dogs weighed an average of 9.5 kg (range of 8.6–9.9 kg) and were 14.1 months of age (13.2–15.2 months) at the start of the trial. Body weights averaged 9.1 kg (range of 8.2–9.8 kg) and the age averaged 15 months (14.1–15.9 months) for the dogs in Study 2. In both studies, the animals were individually identified with uniquely numbered electronic devices placed subcutaneously in the neck. All dogs were healthy at the time of treatment. The animals were acclimated to the test facilities for at least 7 days prior to the start of each study. The dogs were housed indoors in stainless steel cages with slatted floors. The cages were cleaned daily. The temperature in the animal room ranged from 17 °C to 20 °C and the humidity ranged from 43% to 90% during the two studies.

Each dog was offered approximately 300 g of a commercial, pelleted dog food (Harlan Teklad 2021 Dog Maintenance) each day and had access to tap water *ad libitum*.

2.2. Treatment administration

Metaflumizone plus amitraz was administered on Day 0 at the rate of 0.134 ml/kg to each dog based on individual body weights to provide 20 mg/kg of each metaflumizone and amitraz. Treatment application was made using disposable syringes. The formulation was applied to a single spot-on the midline between the shoulder blades by parting the hair, placing the tip of the syringe on the skin and depositing the material on the skin. Care was taken to avoid run off. Syringes were weighed before and after application to determine the exact dose. Elizabethan collars were kept on the dogs throughout the studies to prevent oral intake of the product.

The dogs were observed approximately 4 h and 8 h after treatment and daily throughout the study for any

adverse clinical signs that might be related to treatment. General health observations were made daily throughout the acclimation and study periods.

2.3. Sample collection

2.3.1. Study 1

Approximately 10 ml blood samples were collected from each dog just prior to treatment, approximately 5 and 10 h following treatment, and 1, 2, 3, 5, 7, 10, 14, 21, 28, 42 and 56 days after dosing. The samples were taken by direct venipuncture of the jugular vein into vacutainer tubes containing lithium heparin (BD Biosciences). The blood was centrifuged at $2000 \times g$ for 10 min at a temperature of approximately 4 °C and the plasma harvested and divided into three 1.5 ml tubes. The plasma specimens were frozen and stored in the dark at approximately –20 °C until analysis.

2.3.2. Study 2

Approximately 0.5 g hair samples were collected just prior to treatment and 1, 2, 7, 14, 28, 42 and 56 days after dosing. Samples were collected from five different sites on each dog using electric clippers. Areas samples were the neck (cranial to the application site), middle of the back, lumbar/tail region and the right and left thorax (Fig. 1). Samples were collected in such a manner that the initial sample was most distal from the application site with succeeding samples closer to the application site so that sample collection would not interfere with the migration of material away from the application site (Fig. 1). The samples were placed in individually labeled containers and stored at approximately –20 °C until analysis.

2.4. Sample analysis

Metaflumizone and amitraz were analyzed simultaneously using high performance liquid chromatography (HPLC) following extraction from the sample matrix. Quantification of compounds in the unknown samples is based on interpolation from a standard calibration curve.

2.4.1. Study 1

For analysis of plasma, acetonitrile (20 μ l) was added to 0.5 ml of plasma and vortexed. This mixture was loaded onto a solid phase extraction cartridge (Oasis HLB 3 cc) previously conditioned with 1 ml methanol and 2 ml water. The loaded cartridge was washed with 2 ml of water followed by 1 ml of water/methanol (90/10, v/v). The compounds were eluted with

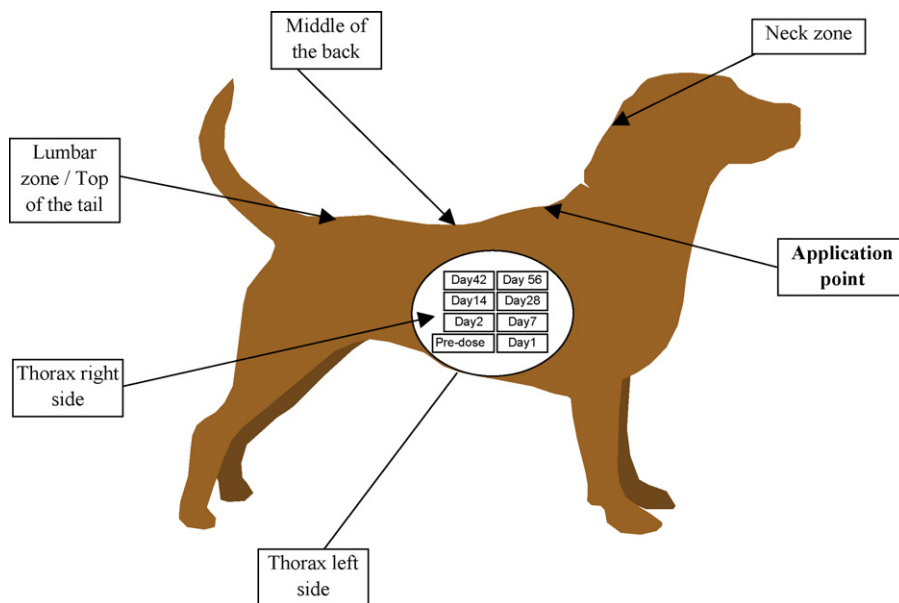


Fig. 1. Site of product application and hair sampling for Study 2.

3 ml acetonitrile and the elute evaporated to dryness under a stream of nitrogen at approximately 50 °C. The dry residue was reconstituted with 0.2 ml of water/acetonitrile (50/50, v/v), vortexed and ultrasonicated to ensure dissolution, centrifuged at 13,000 rpm for 5 min at approximately 5 °C and the supernatant transferred into an HPLC vial for quantification. The autosampler temperature was also maintained at approximately 5 °C to minimize compound degradation. The HPLC mobile phase consisted of acetonitrile/methanol/formic acid (0.1%) in the following ratios: 0–15 min, 30/10/60; 15–28 min, 52/10/38; 28–33 min, 30/10/60. The flow rate was 0.5 ml throughout the run. A Zorbax SB-C18 (150 mm × 3.0 mm, 3.5 μm; Agilent) column was used with an A-102X, 0.5 μm (CIL) filter on-line. The column oven temperature was approximately 50 °C. The detector wavelength was 284 nm.

2.4.2. Study 2

Metaflumizone and amitraz were extracted from hair samples by adding 0.1 ml of water/acetonitrile (50/50, v/v) to 0.1 g of hair in a 10 ml glass tube. After vortexing for a few seconds, 4 ml acetonitrile was added and the tube mixed for 10 min with a reciprocal shaker at ambient temperature. The samples were centrifuged at 6000 × g for 5 min at approximately 5 °C. The supernatant was separated and centrifuged again under the same conditions. The supernatant was evaporated to dryness under stream of nitrogen at approximately 50 °C and reconstituted using 0.3 ml water/acetonitrile (50/50, v/v). After vortexing for a few seconds, the

samples were centrifuged at 13,000 rpm for 5 min at approximately 5 °C and the supernatant transferred to an HPLC vial for analysis. The autosampler temperature was also maintained at approximately 5 °C to minimize compound degradation. The HPLC mobile phase consisted of acetonitrile/methanol/formic acid (0.1%) (49/3/48, v/v/v) with a flow rate of 0.5 ml. The remaining HPLC conditions were as for the plasma analysis in Study 1.

3. Results

3.1. Validation of analytical methods

The analytical methods for both plasma and hair were demonstrated to be valid for the simultaneous determination of metaflumizone and amitraz. The mean extraction recovery of metaflumizone and amitraz was 76.7% and 82.9% from plasma and 100% and 56% from hair, respectively. Based on replicate analysis of fortified control samples, the methods met the requirements for both intra- and inter-day accuracy (results within ±15% of theoretical) and precision (coefficient of variation ≤15%). For plasma the linear range was 50–1000 ng/ml, with a limit of quantification (LOQ) of 50 ng/ml for both compounds and limits of detection (LOD) of 1.1 ng/ml for metaflumizone and 3.2 ng/ml for amitraz. For the analysis of hair, the linear range was 0.1–5.0 μg/g (100–5000 ng/g) with a LOQ of 0.1 μg/g for both compounds and LODs of 0.016 μg/g for metaflumizone and 0.068 μg/g for amitraz.

Table 1

Levels of metaflumizone (ng/ml) in the plasma of dogs following administration of a topical formulation of metaflumizone plus amitraz

Sampling time (Day)	Males			Females		
	Dog no. 808497	Dog no. 994238	Dog no. 150311	Dog no. 792605	Dog no. 982915	Dog no. 146658
0 (5 h) ^a	ND ^b	BLQ ^c	BLQ	ND	ND	ND
0 (10 h)	ND	BLQ	BLQ	BLQ	BLQ	ND
1	BLQ	BLQ	BLQ	BLQ	BLQ	ND
2	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
3	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
5	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
7	BLQ	BLQ	55.4	BLQ	BLQ	BLQ
10	BLQ	BLQ	92.3	BLQ	BLQ	BLQ
14	BLQ	BLQ	63.1	BLQ	BLQ	BLQ
21	BLQ	51.8	71.5	BLQ	BLQ	BLQ
28	67.2	55.4	103.2	BLQ	BLQ	BLQ
42	84.3	58.8	138.3	BLQ	66.3	BLQ
56	BLQ	BLQ	55.6	BLQ	BLQ	BLQ

^a Samples were collected 5 h and 10 h after dosing on the day of treatment.^b Not detected (below the LOD: 1.1 ng/ml).^c BLQ: below the lower limit of quantification (<50 ng/ml metaflumizone).

3.2. Study 1

The actual dose rates in this study averaged 21.5 mg/kg and 22.1 mg/kg for metaflumizone and amitraz, respectively, based on the weight of material applied and the assayed concentration of the two compounds in the formulation. Neither metaflumizone nor amitraz were detectable in the plasma samples collected pretreatment. The levels of metaflumizone in the plasma of dogs after treatment are shown in Table 1. Metaflumizone was detectable (>1.1 ng/ml), but generally not quantifiable (<50 ng/ml) until 28 (three of six dogs) and 42 days (four of six dogs) post-treatment. One dog also had measurable levels at 7, 10 and 14 days and two dogs at 21 days. Levels in the plasma were generally below 100 ng/ml, exceeding this concentration for only one dog for samples collected 28 and 42 days post-treatment. There was a trend for males to have higher levels than females.

Amitraz was essentially not detectable (<3.2 ng/ml) in the plasma over the 56-day study. Only 2 samples (out of the total of 84) had detectable, but not quantifiable levels (<50 ng/ml): one dog at 24 h post-treatment and another at 48 h post-treatment.

The levels of both metaflumizone and amitraz were too low in plasma in this study to allow the calculation of standard pharmacokinetic parameters.

3.3. Study 2

The actual dose rates in this study averaged 21.5 mg/kg and 22.1 mg/kg for metaflumizone and amitraz,

respectively, based on the weight of material applied and the assayed concentration of the two compounds in the formulation. Mean levels of metaflumizone and amitraz at each sampling site and time are provided in Table 2 and shown in Figs. 2 and 3 for the two compounds, respectively. Both compounds migrated rapidly away from the application site with measurable levels at all sampling sites 1-day after treatment. Concentrations in the hair peaked in the mid-back region within 2 days of treatment and at all sites generally by 7 days after dosing, with small increases noted on Day 14 for both compounds at the top of the neck and for metaflumizone at the lumbar region. Concentrations of both compounds declined in a generally linear fashion throughout the remainder of the study, but were quantifiable in all dogs at all sampling sites on Day 56.

3.4. Health observations

No adverse clinical signs related to treatment were noted in any dog throughout the study. Irritation around the base of the neck and ears due to the use of the Elizabethan collars required periodic treatment of several animals from Day 17 to the end of the studies.

4. Discussion

These pharmacokinetic studies show that the levels of both metaflumizone and amitraz on the surface of the dog were at least three orders of magnitude greater than those in the plasma (concentrations of µg/g or ppm on

Table 2

Mean ($\mu\text{g/g}$, $n = 6$) and standard deviation (S.D.) levels of metaflumizone and amitraz in dog hair after administration of a topical formulation of metaflumizone plus amitraz

Time of sampling	Sampled zones	Metaflumizone		Amitraz	
		Mean	S.D.	Mean	S.D.
Day 1	Lumbar zone	62.4	61.6	63.9	51.3
	Left side	49.7	21.6	44.4	19.8
	Right side	122.4	176.4	153.2	247.7
	Middle back zone	122.3	70.8	109.3	59.8
	Top of the neck	19.2	17.5	27.1	28.6
Day 2	Lumbar zone	71.3	30.1	68.3	21.5
	Left side	163.3	73.7	194.5	121.3
	Right side	165.6	51.2	190.3	63.2
	Middle back zone	367.3	423.6	512.7	819.0
	Top of the neck	33.1	13.3	37.9	22.2
Day 7	Lumbar zone	124.2	56.7	125.7	72.4
	Left side	219.5	90.2	221.3	103.0
	Right side	270.6	173.8	266.3	153.0
	Middle back zone	362.4	74.0	510.8	403.1
	Top of the neck	51.0	19.7	51.8	20.8
Day 14	Lumbar zone	148.6	70.4	113.8	61.5
	Left side	199.1	127.3	155.7	123.0
	Right side	272.3	158.1	204.1	150.8
	Middle back zone	347.9	71.3	429.6	280.4
	Top of the neck	55.7	33.1	68.6	76.8
Day 28	Lumbar zone	62.5	34.5	33.6	22.7
	Left side	125.0	96.1	111.2	168.7
	Right side	111.6	81.5	55.2	56.6
	Middle back zone	236.1	161.8	138.1	117.9
	Top of the neck	21.2	11.6	11.1	7.5
Day 42	Lumbar zone	32.9	44.0	14.2	20.7
	Left side	34.1	39.2	16.1	21.3
	Right side	62.3	48.2	21.0	28.4
	Middle back zone	177.1	92.0	150.0	212.2
	Top of the neck	29.0	45.4	4.8	3.9
Day 56	Lumbar zone	16.2	20.9	5.5	10.1
	Left side	88.9	99.1	12.2	23.9
	Right side	32.1	35.7	2.4	2.8
	Middle back zone	113.5	120.9	18.8	32.3
	Top of the neck	4.2	5.5	1.6	2.9

the hair versus ng/g or ppb in the plasma). The data indicate that there was essentially no absorption of either compound into the blood stream when the product was applied topically as a spot-on. However, both metaflumizone and amitraz spread rapidly throughout the surface of the body in the hair and probably on the skin. Similar spreading has been reported for imidacloprid applied as a spot-on to dogs (Krämer and Menke, 2001), with compound distributed in the hair over the body within 1-day of treatment. Only minimal levels of imidacloprid were found in the blood, consistent with the findings reported here for metaflumizone and amitraz. However, it took 14 days for

imidacloprid to reach maximum concentrations on the thorax (21 days on the shoulder), and concentrations declined sharply after 21 days post-treatment. By contrast, maximal levels of metaflumizone and amitraz were found in hair from the thorax within 7 days of dosing with this spot-on formulation, with measurable levels still present 56 days post-treatment. In a similar fashion, fipronil has been reported to translocate broadly through the hair coat of dogs within 1-day of treatment, with minimal percutaneous absorption (Weil et al., 1997). The migration or spreading of these compounds is likely due to diffusion through the skin oils or sebum.

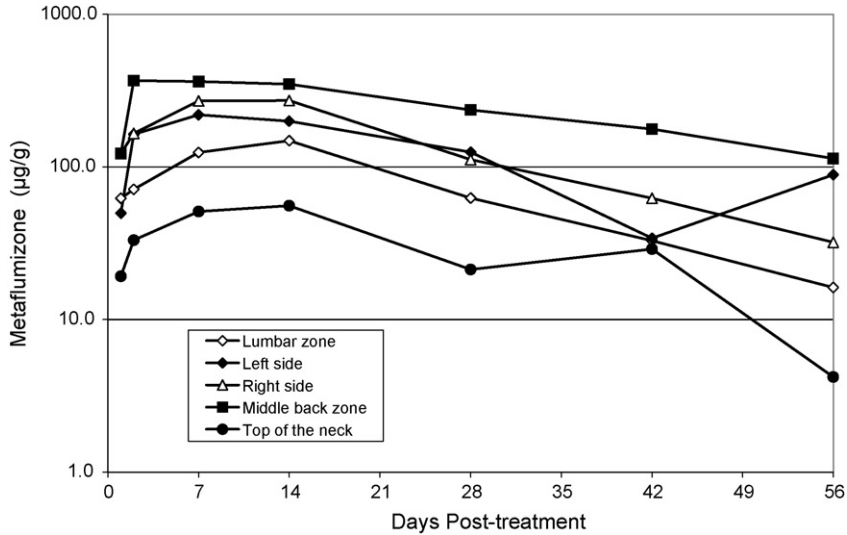


Fig. 2. Mean levels of metaflumizone (µg/g) in dog hair by sampling day.

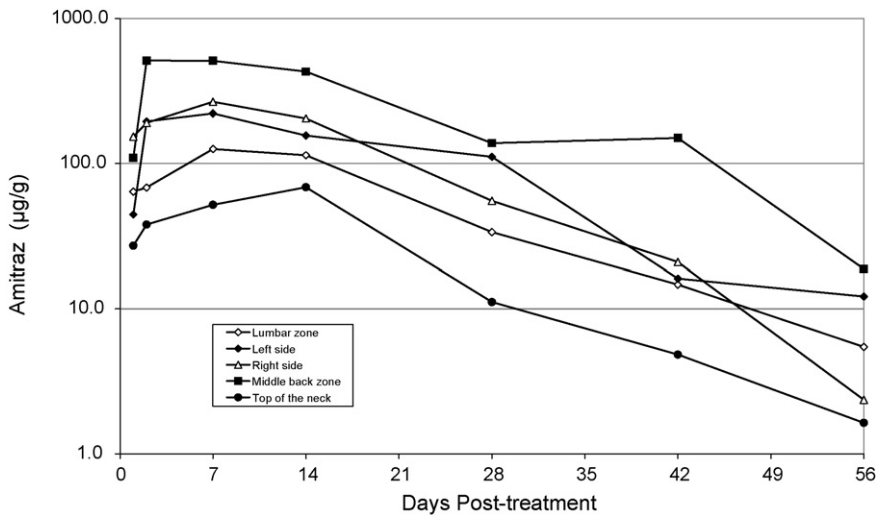


Fig. 3. Mean levels of amitraz (µg/g) in dog hair by sampling day.

5. Conclusions

Clinical efficacy studies have shown that this metaflumizone plus amitraz combination is effective in eliminating existing infestations of fleas and certain species of ticks on dogs and preventing reinfestation of these parasites for several weeks following treatment. Data from the present studies indicate that this ectoparasitic activity is due to exposure of the parasites to metaflumizone and amitraz on the surface of the host (skin and hair), not to exposure via the circulatory system of the host.

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