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Pharmacokinetics of metaflumizone in the plasma and hair of cats following topical application

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Abstract

Controlled laboratory studies have shown that a novel spot-on formulation containing 20% (w/v) metaflumizone (ProMeris[®] for Cats, Fort Dodge Animal Health, Overland Park, KS) is effective for the treatment and control of fleas on cats. Two studies were conducted to determine the distribution of metaflumizone in the plasma and hair of cats following treatment at the minimum recommended dose of 40 mg/kg. Six purpose-bred cats, three males and three females, were used in each study. Plasma or hair samples were collected from each cat just prior to dosing and periodically through 56 days after treatment. Samples were analyzed by HPLC methods validated for the determination of metaflumizone. Metaflumizone concentrations in plasma were below the method limit of quantification (<50 ng/ml) in all samples but one, and were frequently not detectable (<1.1 ng/ml). Plasma collected 3 days post-treatment from one cat had a metaflumizone concentration of 57.8 ng/ml. The frequency of measurable levels of metaflumizone in the plasma was too low to allow the calculation of pharmacokinetic parameters. Analysis of hair samples indicated that metaflumizone was widely distributed in the hair coat of the cat within 1 day after administration, reaching maximum concentrations within 1 or 2 days post-treatment. Low but quantifiable levels were still present at the end of the 56-day study. Data from the present studies indicate that the ectoparasitic activity is due to exposure of the parasites to metaflumizone on the surface of the host (skin and hair), not to exposure via the circulatory system of the host.

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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 cats (Salgado and Hayashi, [this volume](#); Takagi et al., [this volume](#)). A novel spot-on formulation containing 20% (w/v) metaflumizone (ProMeris[®] for Cats, Fort Dodge Animal Health, Overland Park, KS) has been

developed to provide a product for flea control on cats (Sabnis and Zupan, [this volume](#)). Controlled laboratory studies have shown that metaflumizone is effective for the treatment of existing infestations of fleas on cats, and provides control against reinfestation for up to 8 weeks when administered to provide a minimum of 40 mg metaflumizone/kg body weight (Holzmer et al., [this volume](#)).

The objectives of the current studies were to determine the distribution of metaflumizone in the plasma (Study 1) and hair (Study 2) of cats following treatment with metaflumizone at the minimum recommended dose.

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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 cats, three males and three females, were used in each study. Domestic short hair cats were used in Study 1. The cats weighed an average of 2.3 kg (range of 1.9–2.9 kg) and were 4.5 months of age (4.0–5.0 months) at the start of the trial. European breed cats were used in Study 2, with an average body weight of 4.09 kg (range of 2.9–4.9 kg) and an average age of 12.3 months (10.5–14.9 months). Each cat was identified with uniquely numbered electronic device placed subcutaneously in the neck. All cats 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 cats were housed indoors in stainless steel cages with slatted floors. The cages were cleaned at least every 2 days. The temperature in the animal room ranged from 17 to 21 °C and the humidity ranged from 32% to 80% during the course of the two studies.

The animals were fed a pelleted diet [SDS F(E), Special Diet Services] and had access to tap water *ad libitum*.

2.2. Treatment administration

Metaflumizone was administered on day 0 at the rate of 0.2 ml/kg to each cat based on individual body weights to provide 40 mg/kg of metaflumizone. Treatment application was made using disposable syringes. The formulation was applied to a single spot on the back of the neck at the base of the skull 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 cats throughout both studies to prevent oral intake of the product.

The cats were observed approximately 4 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 3 ml blood samples were collected from each cat 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 (BD Biosciences) containing lithium heparin. The blood was centrifuged at 2000 g for 10 min at a temperature of approximately 4 °C and the plasma harvested and divided into two 0.6 ml tubes. Any remaining plasma was transferred to a third tube. The plasma specimens were frozen and stored in the dark at approximately –20 °C until analysis.

2.3.2. Study 2

Approximately 0.3 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 four different sites on each cat using electric clippers. Areas sampled were the middle of the back, lumbar/tail region, right thorax and left thorax. 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 was analyzed by high performance liquid chromatography (HPLC) following extraction from the sample matrix. Quantification in the unknown samples was based on interpolation from a standard calibration curve.

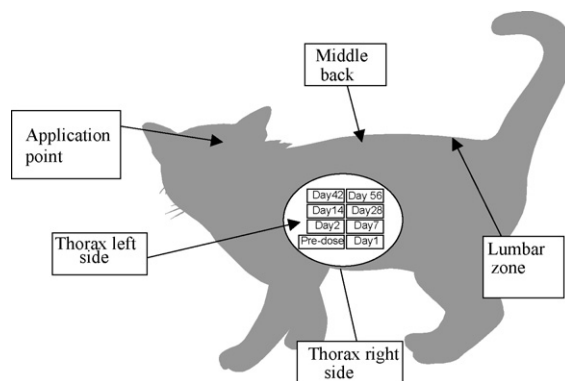


Fig. 1. Site of product application and hair sampling for Study 2.

2.4.1. Study 1

For analysis of plasma, 20 μl acetonitrile/water (50/50 v/v) was added to 0.5 ml of plasma and vortexed. This mixture was loaded onto a Solid Phase Extraction cartridge (Oasis HLB 3 centimeter cube) 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 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. The HPLC mobile phase consisted of acetonitrile/methanol/formic acid (0.1%) in the following ratios: 0–15 min, 30/10/60; 15.01–28 min, 52/10/38; 28.01–33 min 30/10/60. The flow rate was 0.5 ml throughout the run. A Zorbax SB-C18 (150 mm \times 3.0 mm, 3.5 μm ; Agilent) column was used with a Zorbax SB-CD18 4-Pack (4.6 mm \times 12.5 mm, 5 μm ; Interchin) analytical guard column on-line. The column oven temperature was approximately 50 °C. The detector wavelength was 284 nm.

2.4.2. Study 2

Metaflumizone was 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 of acetonitrile was added and the tube mixed for 10 min with a reciprocal shaker at ambient temperature. The samples were centrifuged at 6000 \times 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, 500 μl of *n*-heptane was added and the samples were vortexed again, then centrifuged at 5000 \times g for 5 min at approximately 5 °C. The aqueous phase was separated and transferred to an HPLC vial for analysis. The autosampler temperature was also maintained at approximately 5 °C to minimize compound degradation. The isocratic gradient consisted of acetonitrile/methanol/formic acid (0.1%) (49/3/48 v/v) with a flow rate of 0.5 ml. A Zorbax SB-C18 (150 mm \times 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.

3. Results

3.1. Validation of analytical methods

The analytical methods for both plasma and hair were demonstrated to be valid for the determination of metaflumizone. The mean extraction recovery of fortified control samples was 86.7% from plasma and 87.7% from hair. Based on replicate analysis of these samples, the methods met the requirements for both intra-day and inter-day accuracy (results within $\pm 15\%$ of theoretical) and precision (coefficient of variation $\leq 15\%$). For plasma the linear range was 50–1000 ng/ml, with a limit of quantification (LOQ) of 50 ng/ml and limit of detection (LOD) of 1.1 ng/ml for metaflumizone. For the analysis of hair, the linear range was 0.1–5.0 $\mu\text{g/g}$ (100–5000 ng/g) with a LOQ of 0.1 $\mu\text{g/g}$ and LOD of 0.016 $\mu\text{g/g}$.

3.2. Study 1

The actual dose rates in this study averaged 41.5 mg/kg for metaflumizone, based on the weight of material applied and the assayed concentration of the compound in the formulation. Metaflumizone was not detectable in the plasma samples collected pretreatment. Metaflumizone concentrations in post-treatment plasma samples were below the method LOQ (< 50 ng/ml) in all samples but one and were frequently not detectable (< 1.1 ng/ml). Plasma collected 3 days post-treatment from one cat had a metaflumizone concentration of 57.8 ng/ml (Table 1). The frequency of measurable levels of metaflumizone in the plasma was too low to allow the calculation of pharmacokinetic parameters.

3.3. Study 2

The actual dose rates in this study averaged 41.1 mg/kg for metaflumizone, based on the weight of material applied and the assayed concentration of metaflumizone in the formulation. Mean levels of metaflumizone at each sampling site and time are provided in Table 2 and shown graphically in Fig. 2. Metaflumizone distributed rapidly away from the application site with measurable levels at all sampling sites 1 day after treatment. Concentrations in the hair peaked within 2 days of treatment at all locations, and then declined in a generally linear fashion throughout the remainder of the study. Levels in the hair were quite similar among the sampling sites. Metaflumizone levels were quantifiable in all cats at all sampling sites on day 56.

Table 1
Levels of metaflumizone (ng/ml) in the plasma of cats following administration of a topical formulation of metaflumizone

Sampling time (day)	Males			Females		
	Cat no. 145219	Cat no. 776247	Cat no. 852495	Cat no. 770409	Cat no. 794178	Cat no. 951574
0 (5) ^a	ND ^b	ND	ND	ND	ND	BLQ ^c
0 (10)	ND	ND	ND	ND	ND	ND
1	BLQ	ND	ND	ND	BLQ	ND
2	BLQ	ND	BLQ	ND	BLQ	BLQ
3	57.8	BLQ	BLQ	ND	ND	BLQ
5	BLQ	BLQ	BLQ	ND	BLQ	ND
7	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
10	BLQ	BLQ	BLQ	ND	BLQ	BLQ
14	BLQ	BLQ	BLQ	BLQ	BLQ	ND
21	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
28	BLQ	BLQ	BLQ	BLQ	BLQ	ND
42	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
56	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ

^a Samples were collected 5 and 10 h after dosing on the day of treatment.

^b Not detected (below the LOD: 1.1 ng/ml).

^c Below the limit of quantification (<50 ng/ml R-28153).

Table 2
Mean and standard deviation (µg/g, n = 6) for levels of metaflumizone in cat hair after administration of a topical formulation of metaflumizone

Time of sampling	Sampled zones	Mean	S.D.
Day 1	Lumbar zone	142.8	79.9
	Left side	107.4	42.2
	Right side	169.4	150.3
	Middle back zone	46.9	21.8
Day 2	Lumbar zone	103.1	40.1
	Left side	108.5	53.5
	Right side	106.3	81.3
Day 7	Middle back zone	85.4	88.5
	Lumbar zone	83.8	56.2
	Left side	62.8	45.8
Day 14	Right side	79.9	82.7
	Middle back zone	57.0	50.6
	Lumbar zone	57.9	91.9
Day 28	Left side	33.3	11.3
	Right side	43.4	35.7
	Middle back zone	31.1	19.4
	Lumbar zone	24.4	24.5
Day 42	Left side	15.0	8.7
	Right side	26.9	19.7
	Middle back zone	22.4	17.1
Day 56	Lumbar zone	9.5	9.8
	Left side	10.0	3.8
	Right side	12.4	9.8
	Middle back zone	10.3	8.8
Day 56	Lumbar zone	6.4	3.6
	Left side	6.5	4.3
	Right side	8.7	6.2
	Middle back zone	7.9	6.4

3.4. Health observations

No adverse clinical signs related to treatment were noted in any cat throughout the study and none of the cats required medical treatment.

4. Discussion

These pharmacokinetic studies show that the levels of metaflumizone on the surface of the cat were at least three orders of magnitude greater than those in the plasma (concentrations of mg/kg or ppm on the hair vs. ng/ml or ppb in the plasma). The data indicate that there was essentially no absorption of the compound into the blood stream when the product was applied topically as a spot-on. However, metaflumizone was rapidly and uniformly distributed throughout the surface of the body

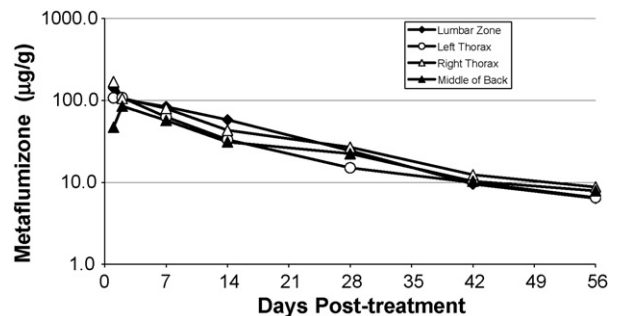


Fig. 2. Mean levels of metaflumizone (µg/g) in cat hair by sampling day.

in the hair and probably on the skin. Similar spreading has been reported for imidacloprid applied as a spot-on to cats (Krämer and Menke, 2001), with compound distributed in the hair over the body within 12 h of treatment. Only minimal levels of imidacloprid were found in the blood, consistent with the findings reported here for metaflumizone. In a similar fashion, fipronil has been reported to translocate broadly through the hair coat of cats 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.

5. Conclusions

Clinical efficacy studies have shown that metaflumizone is effective in eliminating existing infestations of fleas on cats and preventing reinfestation of fleas for several weeks following treatment. Data from the present studies indicate that the ectoparasitic activity of metaflumizone is due to exposure of the parasites to metaflumizone on the surface of the host

(skin and hair), not to exposure via the circulatory system of the host.

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