Parasitol Res (2005) 97:S76–S80
DOI 10.1007/s00436-005-1448-x

Imidacloprid plus moxidectin topical solution for the prevention of heartworm disease (*Dirofilaria immitis*) in dogs

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Abstract

A topically applied formulation containing 10% imidacloprid+2.5% moxidectin (Advocate®/Advantage multi®) has been developed for monthly application to dogs for the prevention of canine heartworm (HW) disease caused by *Dirofilaria immitis*; and for the treatment and control of flea infestations, mite infestations, and intestinal nematode infections. The efficacy of this formulation to prevent canine HW disease was confirmed at three study locations which included the use of 88 purpose-bred beagles 6–8 months of age. Two of these studies also evaluated the effects of post-treatment water exposure or shampooing on product performance. Each dog was infected with 50 third-stage *D. immitis* larvae on test days –30 to –45. Dogs were blocked according to gender and body weight on test day –1. Topically applied test articles were administered once on test day 0 as follows: 10% imidacloprid+2.5% moxidectin (52 dogs); 2.5% moxidectin mono solution (eight dogs); 10% imidacloprid mono solution (16 dogs); and placebo solution (12 dogs). Treatment dosages were applied to provide a minimum of 10 mg/kg imidacloprid and/or 2.5 mg/kg moxidectin. Subgroups of dogs were exposed to water to simulate swimming/rain exposure at designated post-treatment intervals. Additional dogs were shampooed at 90 min, 4 h, or 24 h post-treatment. All dogs were necropsied 110–119 days post-treatment for recovery of adult *D. immitis*. No adult *D. immitis* were recovered at necropsy from any of the dogs receiving 10% imidacloprid+2.5% moxidectin or 2.5% moxidectin mono solution, demonstrating 100% efficacy for prevention of *D. immitis* infection. A total of 701 adult *D. immitis* were recovered at necropsy from dogs receiving 10% imidacloprid mono solution or placebo (range of 11–40 *D. immitis*/dog). The efficacy of 10% imidacloprid+2.5% moxidectin treatment for the prevention of HW disease was not decreased when dogs were shampooed as early as 90 min post-treatment, or when dogs immersed in water 5 times post-treatment at weekly intervals.

Introduction

A combined formulation of 10% w/v imidacloprid+2.5% w/v moxidectin spot-on for dermal application to dogs (Advocate®/Advantage multi®) has been developed by Bayer HealthCare, Animal Health. The combination product has been formulated using the insecticide, imidacloprid, and the macrolide anthelmintic, moxidectin. It is intended for monthly application to provide treatment and control of flea infestations, mite infestations, intestinal nematodes, and for the prevention of canine heartworm (HW) disease due to *Dirofilaria immitis* (Fourie et al. 2003; Hellmann et al. 2003; von Samson-Himmelstjerna et al. 2003). Imidacloprid is already marketed worldwide as Advantage® (10% w/v imidacloprid=9.8% w/w), a spot-on treatment for the control of fleas on cats and dogs (Arther et al. 1997; Hopkins et al. 1996). For small-animal veterinary medicine, moxidectin has been marketed for some time as an oral canine HW monthly prophylactic treatment (Proheart®, Fort Dodge, Animal Health), and more recently as an injectable slow-release formulation (Lok et al. 2001). The formulation of 10%

imidacloprid+2.5% moxidectin spot-on is produced by replacing a proportion of the pharmaceutically inactive component of the Advantage formulation with moxidectin. With this additional component, Bayer has extended the Advantage spectrum of activity to include activity against mites and intestinal nematodes, as well as the prevention of HW disease.

Materials and methods

Three well-controlled efficacy studies were conducted to establish efficacy of imidacloprid+moxidectin the prevention of HW disease. These studies included the use of 88 purpose-bred dogs, 6–8 months of age. Common protocol procedures were followed for all three studies. The dogs were held in individual pens or runs indoors, in temperature/ humidity-controlled facilities free of mosquitoes. All dogs were handled similarly, and with due regard for their welfare. Care and housing of the animals was in full compliance with applicable Federal guidelines. The studies were conducted in accordance with the US Food and Drug Administration and Center for Veterinary Medicine guidelines (1997). The studies included the use of pre-filled unit dose applicator tubes designed to deliver a minimum of 0.1ml/kg test article. Test articles were applied topically to the skin after parting the hair along the dorsal midline between the shoulder blades. Test articles were applied to one spot for dogs weighing <9.1 kg, and to three or four sites for dogs >9.1 kg. On the day of treatment, each dog was examined for any adverse reactions to treatment at 1, 2, 3, 4, 6, 8, 12, 18, and 24 h post-treatment. The dogs were then examined once or twice daily for the remainder of the study.

Two studies evaluated the effects of post-treatment water exposure and shampooing on product performance. Site-specific details are provided.

Heartworm study no. 1

This controlled laboratory study was conducted to evaluate the efficacy and safety of topically applied 10% imidacloprid and 2.5% moxidectin (each alone and in combination) against experimentally induced *D. immitis* infections in dogs. A total of 24 beagles (12 males/12 females) were used in the study. On test day –45, each dog was infected with 50 third-stage *D. immitis* larvae. On test day –3, the dogs were blocked according to gender and body weight, and were randomly assigned to the following three treatment groups of eight dogs each: 10% imidacloprid+2.5% moxidectin (group 1), 2.5% moxidectin only (group 2), 10% imidacloprid only (Advantage®, Bayer AG, Leverkusen, Germany) (group 3). On test day 0 (45 days following experimental infections with *D. immitis*), the dogs were treated topically with the appropriate test article at a minimum dose equal to 0.1 ml/kg body weight. Study animals were maintained until test day 119 (164 days post-infection). At that time, they were euthanized and necropsied for recovery of adult HWs within the abdominal and thoracic cavities, heart, lungs, and connecting vascular system.

Heartworm study no. 2

A second controlled laboratory study was conducted to further evaluate the safety and efficacy of topically applied 10% imidacloprid+2.5% moxidectin against experimentally induced *D. immitis* infections in dogs, and to evaluate the effects of post-treatment water exposure and /or shampooing on product performance. A total of 40 purpose-bred beagles (20 males /20 females) were used in the study. On test day –30, each dog was infected with 50 third-stage *D. immitis* larvae. On test day –1, dogs were blocked according to gender and body weight and were then randomly assigned to five treatment groups of eight dogs each. On test day 0 (30 days post-infection), dogs in groups 1–4 were treated topically with 10% imidacloprid+2.5% moxidectin at the minimum dose of 0.1 ml/kg body weight. Dogs in group 5 were treated with an equivalent volume of 10% imidacloprid solution. At 60 min post-treatment, dogs in group 1 were immersed in a tank of clean tepid water. Each dog was entirely immersed for a period of 2 min, apart from the face. The aim of this procedure was to simulate exposure of the dog to water as would occur from heavy rain or swimming. Dogs in group 2 were exposed to water in a similar manner 4 h post-treatment. Water exposure was then repeated for group 2 dogs on test days 7, 14, 21, and 28. Dogs in group 3 were shampooed 4 h post-treatment using a

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commercial grooming shampoo (Allergroom, Virbac). The shampoo was massaged into the wetted coat, lathered freely, and allowed to remain in contact with the skin and hair for 2–3 min before rinsing. Dogs in group 4 were shampooed as described above at 24 h post-treatment. Dogs in group 5 were exposed to water using the same procedure and repeated water exposure schedule as group 2 dogs. Study animals were maintained until test day 110 (140 days post-infection). At that time, they were euthanized and necropsied for the recovery of adult HWs within the abdominal and thoracic cavities, heart, lungs, and connecting vascular system.

Heartworm study no. 3

A third controlled laboratory study was conducted to further evaluate the safety and efficacy of topically applied 10% imidacloprid+2.5% moxidectin against experimentally induced *D. immitis* infections in dogs, and to further evaluate the effects of post-treatment shampooing on product performance. A total of 24 purpose-bred beagles (12 males /12 females) were used in the study. On test day –33, each dog was infected with 50 third-stage *D. immitis* larvae. On test day –1, dogs were blocked according to gender and body weight and were then randomly assigned to four treatment groups of six dogs each. On test day 0 (33 days post-infection), dogs in groups 1 and 2 were treated topically with 10% imidacloprid+2.5% moxidectin at the minimum dose of 0.1 ml/kg body weight. Dogs in groups 3 and 4 were treated with an equivalent volume of placebo solution (solvent vehicle without active ingredient). Dogs in groups 2 and 4 were then shampooed 90 min post-treatment using Allergroom shampoo as described above.

Study animals were maintained until test day 113 or 114 (146 or 147 days post-infection). At that time, they were euthanized and necropsied for recovery of adult HWs within the abdominal and thoracic cavities, heart, lungs, and connecting vascular system.

Percent HW efficacy for the three studies was calculated based on recovery of adult HWs at necropsy, using the following formula:

$$\% \text{ Efficacy} = [(N_2 - N_1)/N_2] \times 100$$

where N_1 = geometric mean HW count of the treated group, and N_2 = mean HW count of the control group.

Results

All dogs remained in good clinical health from the time of treatment through all post-treatment observations. No post-treatment adverse clinical events were observed for dogs in any of the treatment groups.

The dose schedule followed for the three studies is displayed in Table 1. The pre-filled unit dose applicators and dose banding were designed to provide a target dose of 10 mg/kg imidacloprid+2.5 mg/kg moxidectin for dogs treated with the combined formulation.

A total of 52 dogs were treated with the combined formulation. For HW study no. 1, eight dogs each in groups 2 and 3 received the singular active ingredients of at least 2.5 mg/kg moxidectin and 10 mg/kg imidacloprid, respectively. For HW study no. 2, eight dogs received the singular active ingredient of at least 10 mg/kg imidacloprid.

A summary of the treatment groups for the three combined studies is given in Table 2. The control dogs in

Table 1. Test article dose schedule for heartworm (HW) treatment efficacy studies

Dog Body weight (kg)	Test article[a] Applicator tube size (ml)
Up to 4.5	0.4
4.6-9.0	1.0
9.1-25	2.5
25.1-45	4.0

[a]Test articles included 10% imidacloprid+2.5% moxidectin (Advocate/Advantage Multi), 2.5% moxidectin mono solution, 10% imidacloprid mono solution (Advantage), and placebo (vehicle without active ingredient)

Table 2. Summary of treatment groups designed to evaluate the efficacy of topically applied imidacloprid and moxidectin for prevention of HW disease in dogs

	No. of dogs treated with each test article			
HW efficacy study no.	10% Imidacloprid +2.5% moxidectin	2.5% Moxidectin mono solution	10% Imidacloprid mono solution	Placebo
1	8	8	8	—
2	32[a]	—	8[b]	—
3	12[c]	—	—	12[c]

[a]Included eight dogs exposed to water 60min post-treatment; eight dogs exposed to water 4h and 7, 14, 21, and 28 days post-treatment; eight dogs shampooed 4h post-treatment; and eight dogs shampooed 24h post-treatment
[b]Dogs were exposed to water 4h and 7, 14, 21, and 28 days post-treatment
[c]Included six dogs shampooed 90min post-treatment

HW studies 1 and 2 were treated with 10% imidacloprid singular solution, because a previous study indicated that imidacloprid has no activity against the development of HW infections in dogs (Arther et al. 2002).

A summary of the efficacy of the topically applied test articles for the prevention of development of adult *D. immitis* infections in dogs is given in Table 3 and Fig. 1.

Table 3. Efficacy of topically applied test articles for prevention of the development of adult HW in dogs

Test article	No. dogs treated	Geometric mean no. adult HW recovered at necropsy	Percent efficacy
10% Imidacloprid +2.5% Moxidectin	52	0.0	100
2.5% Moxidectin	8	0.0	100
10% Imidacloprid	16	28.6	18.5
Placebo	12	35.1	—

No adult HWs were recovered at necropsy from any of the dogs treated topically with 10% imidacloprid+2.5% moxidectin, or from dogs treated with the singular active ingredient of 2.5% moxidectin. Efficacy against development of adult HW infection was 100%. Post-treatment shampooing conducted as early as 90 min post-treatment, water exposure at 60 min post-treatment, as well as five repeated exposures to water beginning at 4 h post-treatment did not adversely affect the efficacy of the treatment against HW.

Geometric means of 28.6 and 35.1 adult *D. immitis* were recovered at necropsy from the dogs treated with 10%

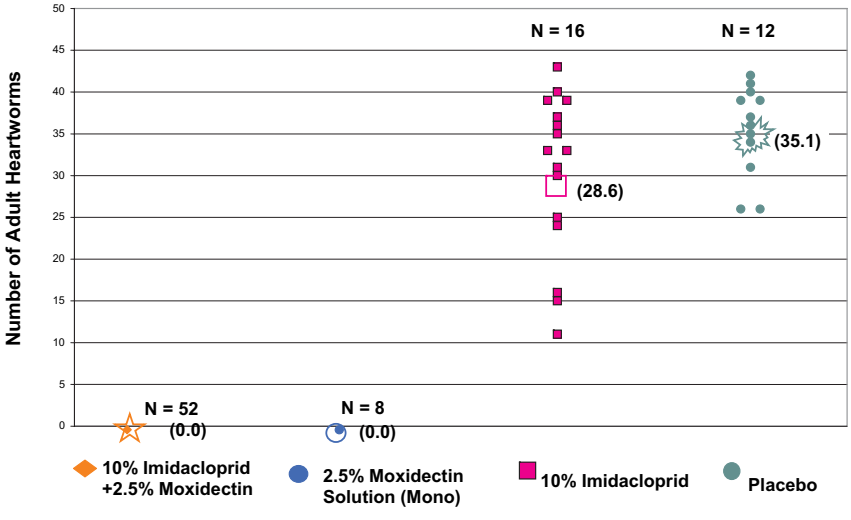

Fig. 1. Summary of recovery of adult heartworms at necropsy (geometric means)

imidacloprid singular solution or placebo, respectively. Imidacloprid alone provided little or no activity against the development of HW in dogs, but did not interfere with the activity of moxidectin in the combined formulation to prevent the development of adult HWs.

Discussion

The results of these studies demonstrate that monthly treatment of dogs with 10% imidacloprid+2.5% moxidectin solution, with a dosage volume of 0.1 ml/kg to provide at least 10 mg/kg imidacloprid+2.5% mg/kg body weight moxidectin, is 100% efficacious for the prevention of HW disease in dogs due to *D. immitis*. The efficacy of the treatment against HW is not diminished when dogs are repeatedly exposed to water or after they are shampooed.

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