Prophylactic Activity of Ivermectin against *Dirofilaria immitis* Infection in Dogs: Larvicidal Activity of Ivermectin against *D. immitis* Larvae 30 Days after Infection

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ABSTRACT. Larvicidal activity of ivermectin against *Dirofilaria immitis* larvae of 30 days old was evaluated. A single oral dose of ivermectin to demonstrate complete larvicidal activity was 3 μg/kg of body weight or more. Administrations of ivermectin at 0.5, 1 and 2 μg/kg dose-relatedly showed incomplete antilarval activities. Administration of ivermectin at 0.5, 1 and 2 μg/kg 30 days after infection caused lower levels of the sex ratios (female/male) than that of the control showing definite decrease in numbers of female worms. The average body lengths of both female and male worms recovered from dogs receiving ivermectin at 0.5, 1 and 2 μg/kg, except for that of female worms at 1 μg/kg, were significantly shorter than that of the worms recovered from the control dogs.—KEY WORDS: *Dirofilaria immitis*, dog, ivermectin, larvicidal activity.

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The avermectins are antiparasitic agents which are maclocyclic lactones produced by *Streptomyces avermitilis*, and a complex of eight chemically related components [5, 16]. Of these, avermectin B_{1a} was found to exhibit high potent anthelmintic activity [12]. After further studies, 22,23-dihydroavermectin B_1 was selected from a series of avermectin B_1 component derivatives as a more effective compound on the basis of its wider antiparasitic efficacy and its better safety profile [10]. Ivermectin contains no less than 80% of 22,23-dihydroavermectin B_{1a} and no more than 20% of 22,23-dihydroavermectin B_{1b} [11]. Ivermectin is known as an extraordinally potent anthelmintic agent; it shows superior, wide antiparasitic activity against many nematodes in various animals, and furthermore against many arthropods [6, 9].

Potency of the avermectins against *Dirofilaria immitis* was first reported by Egerton (1976) [8]; oral treatment of a crude concentrate derived from *S. avermitilis* was followed by suppression of microfilaremia. Through the succeeding studies, it is shown that ivermectin has superior efficacy against microfilariae and larvae but not against adult worms [6, 7, 9]. Larvicidal activity of the ivermectin was first suggested by Campbell and Blair (1978) [7] with avermectin B_{1a}. Since then, the efficacy was also shown with basic studies in dogs and ferrets [1–4, 10, 15], and semifield trials conducted under naturally infectious conditions with *D. immitis* in dogs [14]. The results obtained from the above studies suggest that monthly oral administration of ivermectin may prevent dogs from the natural acquistion of *D. immitis* infection. Since the results are not well enough to determine the minimum dose level and treatment schedule with ivermectin in preventing *D. immitis* infection, a further study is seemed to be justified. This study was undertaken to determine the minimum oral dose required

Table 1. Larvicidal effects of ivermectin against the developing stage of *Dirofilaria immitis* in dogs administrated at 30 days postinoculation

Group	No. dogs	Inoculum sizes	Days after inoculation	No. worms recovered	% recovery		% efficacy
					Min.−Max.	Mean±SE	
Control (Placebo)	5	106	151	57.8	3.0−95.1	54.7±15.44	—
0.5 μg/kg	4	111	152	32.0	0 −45.7	29.2±10.11[a)]	46.6
1.0 μg/kg	3	110	153	15.7	5.8−25.7	14.1± 5.97[a)]	74.2
2.0 μg/kg	5	108	151	14.2	6.9−25.7	13.4± 3.32[a)]	75.5
3.0 μg/kg	5	110	151	0		0	100
5.0 μg/kg	5	111	151	0		0	100

a) No significant difference from the control at $P>0.05$ by *t*-test.

to prevent worm infection in dogs experimentally infected with *D. immitis*.

MATERIALS AND METHODS

Twenty seven mongrel dogs, three to 6 months (average 4.3 months) old non-infected dogs with *D. immitis* were used in this study. ivermectin was orally administered at doses of 0.5, 1, 2, 3 and 5 μg/kg to groups of 4, 3, 5, 5 and 5 dogs, respectively. Additional group of 5 dogs served as a control. Control and 1 μg/kg groups consisted of females only, and other groups included one male each. During the study, the dogs were kept in a mosquito-proof house to prevent from natural infection of *D. immitis*. They were fed once a day with commercial dog food.

The dogs were subcutaneously inoculated in the back with 101 to 120 infective larvae of *D. immitis* per dog, an average of 106 to 111 larvae per group. The larvae were freshly harvested from experimentally infected *Aedes togoi*. A single oral administration of ivermectin was made at 30 days after inoculation. Tablets containing 23 μg of ivermectin per each were pulverized and placed in gelatin capsules to give the prescribed dose of ivermectin to each dog in medicated groups. Placebo tablets at 9.67 mg/kg which was equivalent to tablet weight of 3 μg of ivermectin were given to control dogs in a similar manner. Body weights at the administration of the drug ranged from 2.4 to 9.7 kg; average body weights of the groups, except for 3.6 kg of 0,5 μg/kg group, ranged from 5.9 to 6.5 kg. Larvicidal activity of ivermectin was evaluated in each dog at necropsy on day 150 to 153, average day 151 to 153 for each group when the infected larvae completely migrated to the right ventricle. The dogs were euthanatized with sodium pentobarbital. Immediately, the right ventricle and pulmonary arteries were dissected and examined for the worms. The worms in the thoracic and abdominal cavities were also examined.

RESULTS

Table 1 shows numbers of immature worms recovered from the right ventricle and pulmonary arteries of the dogs at necropsy. The worms were found in dogs of the control and treated with ivermectin at 0.5, 1 and 2 μg/kg. No worms were found in dogs treated with ivermectin at 3 and 5 μg/kg. The infection ratios, percentages of worm recovered for larvae inoculated, in individual control dogs ranged from 3.0 to 95.1%, and averaged 54.7%. The ratios in individual dogs given ivermectin at 0.5 μg/

Table 2. Sex ratios and body length of worms recovered from experimentally infected dogs medicated with ivermectin at 30 days postinoculation

Group	No. dogs	Days after inoculation	Sex ratio (Female/Male)	Worm length (cm)			
				Male		Female	
				No. worms	Mean±SE	No. worms	Mean±SE
Control (Placebo)	5	151	1.61 (176/107)	109	12.3±0.12	166	15.8±0.17
0.5 μg/kg	3	152	1.12 (66/ 59)	57	10.6±0.18[b)]	63	13.2±0.27[b)]
1.0 μg/kg	3	153	0.53[a)] (16/ 30)	30	11.1±0.20[b)]	11	14.5±0.65
2.0 μg/kg	5	151	0.21[a)] (12/ 57)	56	10.3±0.29[b)]	9	11.4±0.79[b)]

a, b) Significant difference from the control at $P<0.001$ by X^2-test (a) and t-test (b).

kg ranged from 0 to 45.7%, and averaged 29.2%. The ratios at 1 μg/kg ranged from 5.8 to 25.7%, and averaged 14.1%, and at 2 μg/kg ranged from 6.9 to 25.7%, and averaged 13.4%. The infection ratios in the treated groups dose-relatedly decreased, but no statistical difference was found between the treated groups and the control. The infection ratios at 3 and 5 μg/kg showed 0% with no worms found. This indicated that there was a dose-related increase in efficacy of ivermectin against larvae, and its complete efficacy was found in the treated group at 3 μg/kg or more. No ectopic parasitism of worms was observed in the abdominal and thoracic cavities of the treated and control dogs.

Table 2 shows the sex ratios (female/male) of immature worms recovered from the control dogs, and dogs treated with ivermectin at 0.5, 1 and 2 μg/kg. The sex ratio in the control group was 1.61. The sex ratios in the treated groups ranged from 1.12 to 0.21, and the ratio decreased as the dose increased. A statistically significant difference was found between the groups receiving ivermectin at 1 and 2 μg/kg and the control group, and the numbers of the female worms recovered from the treated groups were lower than the male worms. The average body lengths of worms recovered from the control and treated groups were shown in Table 2. Those of the male worms recovered from the groups given ivermectin at 0.5, 1 and 2 μg/kg were shorter than the control at 9.7 to 16.2% levels and those of female worms recovered from the treated were also shorter than the control at 8.2 to 27.8% levels. Except for the average body length of female worms treated at 1 μg/kg, those of both the male and female worms recovered from the treated groups were significantly shorter than the control. However, no dose-relations in the average body lengths were found in the treated groups. No adverse reactions associated with administration of ivermectin were observed.

DISCUSSION

Campbell and Blair (1978) [7], and other workers [1–4, 10, 15] conducted basic studies with experimental infection of *D. immitis* in dogs and ferrets to evaluate efficacy of ivermectin against developing stages of the larvae. The studies were conducted to evaluate the efficacy of ivermectin against

Table 3. Efficacy of ivermectin against the developing stages of *Dirofilaria immitis* in the dog

Developing stage		Dose of ivermectin (μg/kg B.W.)							References
		3	12.5	50	100	200	400	1,000	
One-day old (L_3)	Single, oral			○[a]					Blair *et al.* (1982)[4]
One-month old (L_4)	Single, oral			○					Blair & Campbell (1980)[3]
	Single, oral	○	○	○		○	○		McCall *et al.* (1981)[15]
Two-months old (L_4 or ealy L_5)	5 days, oral					○			Campbell & Blair (1978)[7]
	Single, oral							○	Campbell & Blair (1978)[7]
	Single, oral			○					Blair & Campbell (1980)[3]
	5 days, oral					○			Egerton *et al.* (1980)[11]
Three-months old (L_5)	Single, oral			△[b]					Blair & Campbell (1980)[3]
	Single, oral			△	△	△			McCall *et al.* (1981)[15]

a) Complete efficacy.
b) Incmplete efficacy.

each developing stage of larvae in experimentally infected dogs, and the results of the studies were summarized in Table 3. According to Orihel (1961) [19], larvae of *D. immitis* soon after inoculation are at the L_3 stage, 1-month old worms are at the L_4 stage, 2-months old worms are at the late L_4 and/or early L_5 stage, and 3-months old worms are at the L_5 stage. Thus, it appears that developing larvae of every stage can be found in dogs naturally infected with *D. immitis*. Therefore, efficacy of ivermectin against the larvae of every stage should be evaluated to establish an administration schedule of the drug for prevention of *D. immitis* infection. To prevent *D. immitis* infection, larvae at intermediate location should be killed before the larvae migrate to the right ventricle or during a period of 70 to 85 days after infection. To ensure prevention of the infection, the larvae at the L_3 to early L_5 stage, during a period of 2 months after infection, should be killed. The previous reports were discussed on this point of view. Blair and Campbell (1980) [3] and McCall *et al.* (1981) [15] reported that administration of ivermectin 3 months after infection showed incomplete efficacy against the larvae. It seems that some of larvae could have migrated to the right ventricle 3 months after infection, and ivermectin fails to show complete efficacy against the larvae at this stage. Table 3 indicates that a single oral administration of ivermectin at 50 μg/kg immediately, 1 month, and 2 months after infection showed complete efficacy against larvae [3, 4] and ivermectin at 3 and 12.5 μg/kg 1 month after infection demonstrated complete efficacy against larvae at the stage [15]. However, it is not clear if ivermectin at these doses is effective against larvae immediately, and 2 months after infection. The number of inoculated larvae for the studies conducted by Blair and Campbell (1980) [3], McCall *et al.* (1981) [15], and Blair *et al.* (1982) [4] may be too small to evaluate efficacy of ivermectin against canine dirofilariasis under natural infections, based on the fact that in an area in Japan where incidences of *D. immitis* were found at 59.1%, numbers of the worm recovered from dogs which had passed one infectious season ranged from 1 to 58 worms (averaged 11.1 worms), and

13.5% of the dogs harboured over 21 of the worms [17]. It is thought in highly infected areas, over 100 infective larvae per dog should be inoculated for basic studies to appropriately evaluate preventive efficacy of ivermectin against the infection. It is considered, therefore, that the previous studies failed to establish optimal dose and dose-interval of ivermectin to demonstrate its sufficient preventive efficacy against the naturally acquired infections.

Referring to the data by McCall *et al.* (1981) [15] tht a single oral administration of ivermectin at 3 μg/kg was effective against 1-month old larvae, we conducted the study using five different doses of ivermectin 0.5, 1, 2, 3, and 5 μg/kg, and inoculum sizes of over 100 infectious larvae to evaluate its efficacy against 1-month old larvae. The results indicated that dose-related decrease in numbers of larvae migrating to the right ventricle was observed and no worms were found in dogs receiving ivermectin at 3 μ/kg or more indicating that the minimum effective dose is 3 μg/kg. The conclusion of the study supports that of McCall *et al.* (1981) [15]. Further studies should be conducted to evaluate if ivermectin at 3 μg/kg is also effective against L_3, late L_4 and early L_5 stages of larvae.

The present results were that the sex ratios of immature worms recovered from the right ventricle and pulmonary arteries of the dogs receiving ivermectin decreased as dose increased, and those of the worms recovered from the dogs receiving ivermectin at 1 and 2 μg/kg were significantly different from the control. This clearly indicates that the number of female worms recovered from the treated dogs was smaller than that of male worms. It is reported that the sex ratio of adult worms recovered naturally infected dogs was 1.04 or 1.16 and the number of female worms was almost equal or slightly larger than that of male worms [17, 18]. Less females in terms of the sex ratios were recovered from dogs receiving ivermectin at 1 and 2 μg/kg than that from naturally infected dogs. It seems likely that the female is more sensitive to ivermectin than the male.

The average body length of worms recovered from the control dogs was almost equal to that of worms of same age reported elsewhere [13]. However, the average body lengths of worms recovered from the dogs receiving ivermectin 0.5, 1 and 2 μg/kg, except for that of female worms at 1 μg/kg, were significantly shorter than those of worms recovered from the control dogs. This suggests that ivermectin had inhibitory effect on growth of larvae. The results of the present study show that no ectopic parasitism of the worms was found in the abdominal and thoracic cavities of the treated dogs, and ivermectin exerts no action on stimulating the ectopic parasitism of the worms. Previous reports [6, 8] suggest that a minimum oral toxic dose of ivermectin in dogs was 2.5 mg/kg except for collies. Ivermectin at extremely low doses of 0.5 to 5 μg/kg caused no abnormal findings in dogs in the present study.

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要　約

犬糸状虫感染に対する ivermectin の予防効果：感染後30日虫齢の幼虫に対する ivermectin の殺虫効果：大石勇・片江宏己[1)]・早崎峯夫・多田融右[2)]（東京農工大学農学部家畜内科学教室，[1)]大日本製薬動物薬品部栗東試験場，[2)]日本 MSD）—— Ivermectin の犬糸状虫幼虫に対する殺虫効果を，感染後30日虫齢の幼虫について検討した。Ivermectin の1回経口投与によって幼虫に対し確実な殺虫効果がある投与量は 3μg/kg 以上であり，0.5，1，2μg/kg 投与量でも幼虫に対して用量依存性に不完全な殺虫効果が認められた。Ivermectin 0.5，1，2μg/kg を30日虫齢の幼虫に投与すると，検出虫の性比（雌虫/雄虫）は対照群より小さく，明らかに雌虫の減少が認められた。また，検出虫の平均体長も投薬群では 1μg/kg 群の雌虫を除き，雌・雄虫ともに対照群より有意に短小であった。