Prophylactic Activity of Ivermectin against *Dirofilaria immitis* Infection in Dogs: Establishment of Effective Dose and Administration Schedule

Isamu OHISHI, Hiromi KATAE[1)], Mineo HAYASAKI, Kazuhide NAKAGAKI, and Yusuke TADA[2)]

Department of Veterinary Internal Medicine, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183, [1)]Ritto Experimental Farm, Veterinary Division, Dainippon Pharmaceutical Co., Ltd, 91 Higashizaka, Ritto-cho, Kurita-gun, Shiga 520–30, and [2)]MSD Japan Co., Ltd, 9–20 Akasaka 1-chome, Minatoku, Tokyo 107, Japan

(Received 22 January 1987/Accepted 19 February 1987)

ABSTRACT. To establish a recommended dose and prophylactic program of ivermectin for preventing *D. immitis* infection, antilarval activity of ivermectin at 3 or 6 μg/kg against one day, 30 and 60 days old larvae was evaluated. The worms were recovered from all dogs given ivermectin at 3 μg/kg one day after inoculation with an average infection ratio of 21.9 %. One male worm was recovered from one of 6 dogs given ivermectin at 3 μg/kg 30 days after inoculation with an extremely low average infection ratio of 0.1%. Out of 6 dogs given ivermectin at 3 μg/kg 60 days after inoculation, 3 animals yielded worms with a low infection ratio of 4.0 %. The results suggest that ivermectin at 3 μg/kg demonstrates incomplete antilarval activity against *D. immitis* larvae of one day, 30 and 60 days old and is not suitable to monthly or bimonthly interval mdication for preventing *D. immitis* infection. Small numbers of the worms were recovered from 5 of 6 dogs given ivermectin at 6 μg/kg one day after inoculation with an average infection ratio of 5.7 %. No worms were recovered from all dogs given ivermectin at 6 μg/kg 30 and 60 days after inoculation. The results show that a single oral dose of ivermectin at 6 μg/kg, though the dose demonstrates incomplete antilarval activity against one day old larvae, would be a recommended dose which can completely kill the larvae which are naturally infected at any time when the dose is given once a month at one month interval during the period from one month after the first infection to one month after the last infection of *D. immitis*.—KEY WORDS: *Dirofilaria immitis*, dog, ivermectin, larvicidal activity.

Jpn. J. Vet. Sci. 49(3): 439–445, 1987

Antilarval activity of ivermectin against developing larvae of *Dirofilaria immitis* was first suggested by Campbell and Blair (1978)[6] with avermectin B_{1a}, a relative compound of ivermectin. Since then, the efficacy of the drug was also shown in experimental studies with dogs and ferrets [1–4,8,11], and semifield trial were conducted under naturally infections conditions with *D. immitis* in dogs [10]. However, results of those studies are not well enough to determine a minimum dose level and administration schedule of ivermectin in preventing the *D. immitis* infection in dogs. We have been working to establish a prophylactic method with ivermectin against *D. immitis* infection in dogs, and in a previous paper, we reported that a minimum single dose of ivermectin orally given to demonstrate its complete efficacy against 30 days old larvae of *D. immitis* was 3 μg/kg of body weight [12].

Based on the data obtained from a previous study, this experimental study was undertaken to evaluate the efficacy of ivermectin against one day, 30 and 60 days old larvae of *D. immitis*. Results of the present study suggest that the effective dose and administration schedule of ivermectin for preventing the *D. immitis* infection in dogs could be logically established. The purpose of this paper is to describe the details of the

study.

MATERIALS AND METHODS

Forty three mongrel dogs, 6 to 7 months old, non-infected dogs with *D. immitis* were used in this study. They were born during April to May period of the non-infectious season with *D. immitis* and kept in a mosquito-proof house. They were randomly divided into 6 groups of 6 dogs. Each group equally consisted of 2 male and 4 female dogs. The dogs were orally administered ivermectin at doses of 3 and 6 μg/kg one day, 30 and 60 days after inoculation. Additional group of 7 dogs served as a control. Body weights of individual test dogs ranged from 6.3 to 16.0 kg; average body weights of the groups closely ranged from 9.3 to 11.0 kg. All dogs were kept in a mosquito-proof house for duration of the study, and fed once a day with commercial dog food. Individual dogs were subcutaneously inoculated in the back with 100 to 148 infectious larvae of *D. immitis* with averages of 111 to 122 larvae per dog of each group. The larvae were freshly harvested from experimentally infected *Aedes togoi* with *D. immitis*.

The dogs were once orally administered ivermectin at 3 and 6 μg/kg one day, 30 and 60 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 treated dog. Control dogs were given at 9.67 mg/kg of pulverized placebo-tablets which were equivalent to tablet weight of 3 μg/kg of ivermectin one day (2 dogs), 30 days (2 dogs) and 60 days (2 dogs) after inoculation. One dog was kept as a non-treated. Body weights of the test dogs at administration of the drug ranged from 6.5 to 19.7 kg, while differences in average body weights of the treated dogs with ivermectin at each administration time were found within 1.0 kg. Larvicidal activity of ivermectin was evaluated in each dog at necropsy on day 147 to 151, average day 149 and 150 for each group when the infected larvae completely migrated to the right ventricle. The dogs were euthanatized with sodium pentobarbital. Immediately, the heart and lungs were removed, and 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 infection ratios, percentages of worms recovered for larvae inoculated, in individual control dogs ranged from 29.9 to 68.3 %, and averaged 46.3 %. The worms were recovered from all dogs given ivermectin at 3 μg/kg one day after inoculation, and the average infection ratio of the group was 21.9 %, while the ratios of individual dogs widely ranged from 2.8 to 46.9 %. Out of them, two dogs showed lower infection ratios 2.8 and 6.7 %, respectively. Only one male worm was recovered from one of 6 dogs given ivermectin at 3 μg/kg 30 days after inoculation, and no worms were recovered from the rest dogs. The infection ratios of individual dogs ranged from 0 to 0.8 % with an extremely low average of 0.1 %. No worms were recovered from 3 of 6 dogs given ivermectin at 3 μg/kg 60 days after inoculation, but one to 25 worms recovered from the rest dogs. The infection ratio of individual dogs ranged from 0 to 19.4 % with a low average of 4.0 %. The statistically significant differences in the average infection ratios of the 3 μg/kg treated three groups and the control group were found. Especially, the difference between the 30th day or 60th day treated

Table 1. Larvicidal effects of ivermectin against the developing stages of *Dirofilaria immitis* in experimentally infected dogs

Group	No. dogs	Inoculum sizes	Days after inoculation	No. worms recovered	% recovery		% efficacy
					Min.–Max.	Mean±SE	
Control (Placebo)	7	112	149	51.9	29.9–68.3	46.3±5.55	—
3 μg/kg 1[a)]	6	111	149	25.0	2.8–46.9	21.9±6.58[b)]	52.7
3 μg/kg 30[a)]	6	118	149	0.2	0 – 0.8	0.1±0.13[c)]	99.8
3 μg/kg 60[a)]	6	112	149	5.0	0 –19.4	4.0±3.14[c)]	91.4
6 μg/kg 1[a)]	6	113	149	6.8	0 –14.2	5.7±2.16[c)]	87.7
6 μg/kg 30[a)]	6	115	150	0		0	100
6 μg/kg 60[a)]	6	122	150	0		0	100

a) Medicated on 1, 30 and 60 days postinoculation.
b) Significantly different from the control with *t*-test at a) $P<0.05$ or b) $P<0.001$.

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

Group	No. dogs	Days after inoculation	Sex ratio (Female/Male)	Worm length (cm)			
				Female		Male	
				No. worms	Mean±SE	No. worms	Mean±SE
Control (Placebo)	7	149	1.21 (198/164)	190	16.9±0.19	156	13.4±0.10
3 μg/kg 1 day	6	149	0.76[a)] (65/ 85)	64	17.1±0.28	83	13.5±0.13
3 μg/kg 60 days	3	149	0.76 (13/ 17)	13	10.1±0.48[b)]	17	8.5±0.36[b)]
6 μg/kg 1 day	5	149	0.77 (17/ 22)	15	15.7±0.55[b)]	20	11.9±0.22[b)]

a) Significantly different from the control at $P<0.05$, using x^2-test.
b) Significantly different from the control at $P<0.001$, using *t*-test.

group and the control group was markedly significant ($P<0.001$). Ivermectin at 3 μg/kg showed 99.8 (almost complete), 91.4 and 52.7 (lowest) % of efficacies against 30 and 60 days, and one day old larvae of *D. immitis*, respectively.

The worms were recovered from the right ventricle and pulmonary arteries in 5 of 6 dogs given ivermectin at 6 μg/kg one day after inoculation. Numbers of the worms recovered were small (ranging from one to 19 with an average of 6.8 worms). The infection ratios of individual dogs ranged from 0 to 14.2 % with an average of 5.7 %. The infection ratio was significantly different from that of the control ($P<0.001$). No worms were recovered from dogs given ivermectin at 6 μg/kg 30 and 60 days after inoculation, and the infection ratios of both groups were 0 %. Ivermectin at 6 μg/kg was incompletely effective against one day old larvae of *D. immitis* (87.7 %), while the drug at the same dose was completely effective against 30 and 60 days old larvae (100 %). 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 the dogs treated with ivermectin at 3 μg/kg one day or 60 days after inoculation, and at 6 μg/kg one day after inoculation. The average sex ratio in the control group was found to be 1.21. The average sex ratios in the treated groups closely ranged from 0.76 to 0.77. The average sex ratio value of any treated group was lower than that of the control group, and the number of female worms was smaller than that of male worms. However, the average sex ratio of only one treated group at 3 μg/kg one day after inoculation was found to be significantly different from that of the control ($P<0.05$). The average body lengths of worms recovered from the control and treated groups are show in Table 2. The average body lengths of female and male worms recovered from the group given ivermectin at 3 μg/kg one day after inoculation were almost equal to those of the control, and no significant difference in the average body lengths of the worms between them was observed. The average body lengths of the female and male worms recovered from the groups given ivermectin at 3 μg/kg 60 days after inoculation and at 6 μg/kg one day after inoculation were significantly shorter than those of the worms recovered from the control group ($P< 0.001$). No adverse reactions associated with administration of ivermectin were observed.

DISCUSSION

To prevent *D. immitis* infection, larvae of developing stages at intermediate locations should be killed before the larvae infected by mosquitos migrate to the right ventricle. When a periodical, monthly or bimonthly, treatment is adopted for prevention of *D. immitis* infection, a drug for the treatment should be widely effective against developing larvae of one day to 2 months old. On the other hand, few researchers except Blair and Campbell (1980)[3] and Blair et al.(1982)[4] studied if ivermectin can be effective against developing larvae of one day to 2 months old. They showed that a single oral administration of ivermectin at 50 μg/kg was completely effective against developing larvae of one day, 30 and 60 days old. In a previous report [12], the authors showed that a single oral dose of ivermectin to demonstrate complete efficacy against 30 days old larvae was 3 μg/kg of a minimum. Based on the results of the previous study, this study was undertaken to evaluate if ivermectin at 3 or 6 μg/kg is effective against developing larvae of one day to 2 months old in experimentally infected dogs. In the present study, a single oral dose of ivermectin at 3 μg/kg allowed the dogs to receive the infection of *D. immitis*, though the infection ratios of the groups treated with the drug one day, 30 or 60 days after inoculation were lower than that of the control group. The worms were recovered from all dogs at 3 μg/kg of ivermectin one day after inoculation with a relatively high average of 21.9 %. The worms were recovered from half of the dogs given ivermectin at 3 μg/kg 60 days after inoculation. However, the number of the recovered worms of the 60th day treated group was smaller and the larvicidal activity of ivermectin in the group is considered to be higher than that of the 1st-day treated group. In the present study, ivermictin at 3 μg/kg 30 days after inoculation which had showed complete larvicidal activity in the previous study [12] allowed one dog to yield one worm and showed incomplete larvicidal activity. Results of the previous study is being considered. the infection ratio of the group in the present study seems to be extremely low. Results of the present study reveal that ivermectin at 3 μg/kg one day. 30 or 60 days after inoculation is found not to be enough to prevent the establishment of the infection in dogs. It is considered,

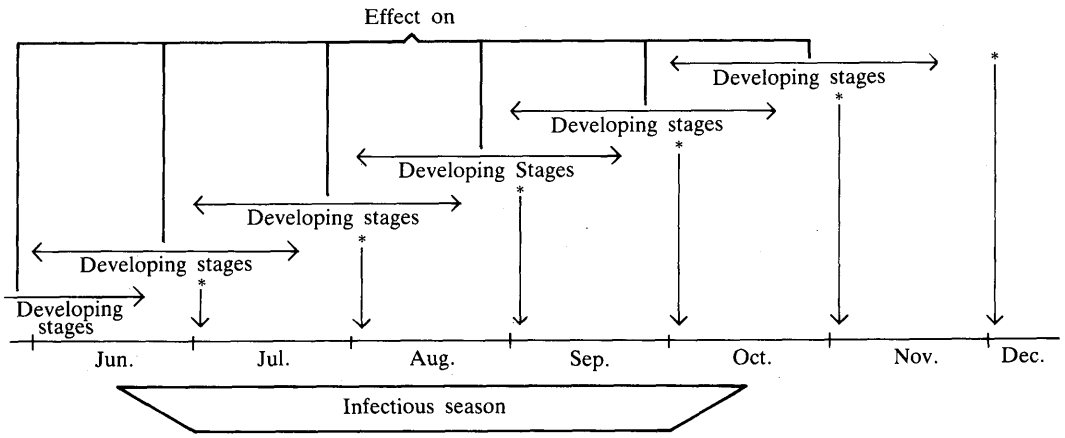

Fig. 1. Larvicidal effect of intermittent medication of ivermectin on the developing stages of *Dirofilaria immitis* infected naturally throgh the infectious season.
*: Oral administration of ivermectin at 6 μg/kg B.W.

therefore, that the dose of ivermectin at 3 μg/kg is insufficient for prophylactic treatment of canine dirofilariasis with monthly or bimonthly single oral administration of the drug. McCall et al. (1983)[10] reported that monthly single oral dose of ivermectin at 1 or 3.3 μg/kg showed sufficient prophylactic activity against canine dirofilariasis in a semifield study. Results of the previous [12] and present studies suggest that the theory that monthly dose of ivermectin at 1 or 3.3 μg/kg is completely effective in suppressing the infection could not be accepted.

Ivermectin at 6 μg/kg one day after inoculation allowed 5 of 6 dogs to yield worms with a low infection ratio and was incompletely effective in preventing the establishment of the infection, while ivermectin at 6 μg/kg 30 or 60 days after inoculation did not allow all dogs to yield any worms and was 100 % effective against the infection. The results of the present study indicate that administration of ivermectin at 6 μg/kg is incompletely effective against the L_3 stage of larvae, immediately after inoculation, but completely effective against the L_4, late L_4 and early L_5 stage of the larvae, up to 2 months old. It is considered, therefore, that the larvae would have been in the L_3 stage, immediately after infection and might have escaped being killed by the first monthly administration of ivermectin at 6 μg/kg can be perfectly killed by the secondary monthly administration of the drug as they grow to 30 days old at the administration. It is our thought that under natural infection of *D. immitis*, as shown in Fig. 1, single oral administration of ivermectin at 6 μg/kg at monthly intervals during the period from one month after the first infection of *D. immitis* larvae to one month after the last infection of the larvae, can completely kill the larvae, whenever they are infected during the period, to prevent the establishment of the infection.

The results of the present study prove that different developing stages at intermediate locations are differently sensitive to ivermectin. The sensitivities of the larvae in the L_3 stage, immediatery after inoculation, the late L_4 to early L_5 stage, 60 days after inoculation, and the L_4 stage, 30 days after inoculation, to ivermectin are found to be the weakest, higher and the highest, respectively. It is also known that the larvae in each developing stage showed different sensitivity to levamisole[9].

The previous report indicated that the

larvicidal activity of ivermectin was more potent to the females than the males based the sex ratio of the worms migrating to the right ventricle [12]. The present study also shows that administration of ivermectin causes the low sex ratio value, and ivermectin is more effective against the females than the males. In the previous paper [12], it was shown that administration of ivermectin at 0.5 to 2 μg/kg 30 days after inoculation caused a decrease in average body lengths of the immature worms recovered from the right ventricle and pulmonary arteries of the treated dogs. The present study also reveals that administration of ivermectin at 3 μg/kg one day after inoculation causes no decrease in the body lengths of the worms, but administration of the drug at 6 μg/kg one day after inoculation or at 3 μg/kg 60 days after inoculation shows a decrease in the body lengths of the worms. These results suggest that the restraint effect of ivermectin on the body lengths depends on the growth stages of the worm; ivermectin has marked restraint effect on growth of the 30 days old larva (L_4-stage), less effect on that of the 60 days old larva (late L_4 or early L_5-stage) and least effect on that of the one day old larva (L_3-stage). Same observations that the activity of ivermectin depends on individual growth stage of the worm are found in its larvicidal activity and seem to be a common phenomenon caused by ivermectin.

As reported in the previous paper [12], the results of the present study show that no ectopic parasitism is found in the body cavities, and ivermectin exerts no action on stimulating the ectopic parasitism. The fact that a minimum oral toxic dose ivermectin in dogs except for collie breed is over 2.5 mg/kg is being considered [5,7], it is reasonable that oral single dose of ivermectin at 3 or 6 μg/kg causes no toxic findings in dogs in the present study. The present study shows that ivermectin at 6 μg/kg would be a recommended dose for prevention of *D. immitis* infection in field conditions. It is clear, therefore, that the recommended dose of 6 μg/kg is one four hundred sixteenth of the minimum toxic dose with wide safety margin provided, and causes no adverse effect on dogs.

REFERENCES

1. Blair, L.S., and Campbell, W.C. 1978. Trial of avermectin B_{1a}, mebendazole and melarsoprol against pre-cardiac *Dirofilaria immitis* in the ferret (*Mustela putorius furo*). *J. Parasitol.* 64:1032–1034.
2. Blair, L.S., and Campbell, W.C. 1980. suppression of maturation of *Dirofilaria immitis* in *Mustela putorius furo* by single dose of ivermectin. *J. Parasitol.* 66: 691–692.
3. Blair,L.S., and Campbell, W.C. 1980. Efficacy of ivermectin against *Dirofilaria immitis* larvae in dogs 31, 60 and 90 days after infection. *Am. J. Vet. Res.* 41: 2108.
4. Blair, L.S., Williams, E., and Ewanciw, D.V. 1982. Efficacy of ivermectin against third-stage *Dirofilaria immitis* larvae in ferrets and dogs. *Res. Vet. Sci.* 33: 386–387.
5. Campbell, W.C., and Benz, G.W. 1984. Ivermectin: A review of efficacy and safety. *J. Vet. Pharmacol. Therap.* 7: 1–16.
6. Campbell, W.C., and Blair, L.S. 1978. Efficacy of avermectins against *Dirofilaria immitis* in dogs. *J. Helminthol.* 52: 308–310.
7. Campbell, W.C., Fisher, M.H., Staplay, E.O., Albers-Schönberg, G., and Jacob, T.A. 1983. A potent new antiparasitic agent. Science. 221: 823–825.
8. Egerton, J.R., Birnbaum, J., Blair, L.S., Chabala, J.C., Conroy, J., Fisher, M.H., Mrozik,H., Ostlind, D.A., Wilkins, C.A., and Campbell, W.C. 1980. 22, 23-dihydroavermectin B_1, a new broad-spectrum antiparasitic agent. *Br. Vet. J.* 136: 88–97.
9. Hayasaki, M., Nakagaki, k., and Ohishi, I. 1984. Larvicidal effects of the short-term medication of levamisole hydrochloride to developing stages of *Dirofilaria immitis* in infected dogs. *Jpn J. Parasitol.* 33: 573–576. (in Japanes)
10. McCall, J.W., Cowgil, L.M., Plue, R.F., and Evans, T. 1983. Prevention of natural acquistion of heartworm infection in dogs by monthly treatment with ivermectin. pp. 150–152. *In*: Proc. Heartworm Symp. '83 (Morgan, H.C. *et al.* eds.), Vet. Med. Pub. Co., Kansas.
11. McCall, J.W., Lindemann, B.A., and Porter,

C.A. 1981. Prophylactic activity of avermectins against experimentally induced *Dirofilaria immitis* infection in dogs. pp.126–130. *In*: Proc. Heartworm Symp. '80 (Morgan, H.C. *et al.* eds.), Vet. Med. Pub. Co., Kansas.

12. Ohishi, I., Katae, H., Hayasaki, M., and Tada, Y. 1987. Prophylactic activity of ivermectin against *Dirofilaria immitis* infection in dogs: Larvicidal activity of ivermectin against *D. immitis* larvae 30 days after infection. *Jpn. J. Vet. Sci.* 49: 117–122.

要　　約

犬糸状虫感染に対するivermectinの予防効果；有効投薬量と投与計画の確立：大石　勇・片江宏巳[1)]・早崎峯夫・中垣和英・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票東試験場，[2)]日本MSD）――犬糸状虫感染予防に用いるivermectinの有効投与量と投与計画を決定するため，感染後1，30，60日の幼虫に対するivermectin 3，6 μg/kg投与の抗幼虫効果を検討した。3 μg/kgでは，感染後1日の投与で全例が感染し，平均感染率21.9%であった。30日後の投与では1/6頭に雄虫1匹が検出され平均感染率は0.1%であり，60日後投与では3/6頭に虫が検出され，平均感染率は4.0%であった。この成績から，3 μg/kgの投与では感染後1，30，60日の幼虫に対して殺虫効果が不確実であり，1，2カ月間隔での予防投薬には不適であることがわかった。6 μg/kgを感染後1日に投与すると，5/6頭に少数の虫が検出され，平均感染率5.7%であった。しかし，30，60日後投与の両群には虫は検出されなかった。この成績から，6 μg/kg 1回経口投与は感染後1日の幼虫に対して効果が不確実であるが，この投与量を感染開始後1カ月から終了後1カ月の期間，1ヵ月間隔で月に1回投与すれば，隨時自然感染する幼虫は完全に殺滅され，確実な予防効果が得られると推定された。