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Effects of PF1022A on Developing Larvae of *Angiostrongylus costaricensis* in Mice, with Special Reference to Route, Dose and Formulation

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Abstract

Effects of 5 successive doses of PF1022A on developing larvae of *Angiostrongylus costaricensis* in mice were examined with special reference to dose, route and formulation. 1) When PF1022A in the oral solution, one of our formulations was administered orally at doses of 2.5, 10 and 40 mg/kg/day, dose-dependent effects were observed regarding all parameters in host mice and in worms, and nearly complete effects were observed by 10 mg/kg. 2) When the drug in the same formulation was given intraperitoneally at doses of 0.625, 2.5 and 10 mg/kg/day, almost complete effects were seen even by 0.625 mg/kg. 3) When PF1022A was administered orally at a dose of 10 mg/kg/day in 4 different formulations; 2 solubilized types in water designated as the oral solution and the injectable solution and one emulsified type in water named as the oral cream, and one dissolved type in olive oil, the best results were obtained in the oral cream group, and the worst effects were in the olive oil group regarding almost all parameters in host mice and in worms. 4) When the drug was administered intraperitoneally at a dose of 2.5 mg/kg/day in the above 4 formulations, remarkable effects were obtained from all formulations with similar differences among groups only on some parameters in host mice. From these results it becomes apparent that PF1022A can be absorbed even from the host intestinal tract, and consequently, the new drug can be used as oral as well as parenteral administration, which makes it a preferable, safe anthelmintic available for various tissue and intestinal nematodes.

Key words: PF1022A, *Angiostrongylus costaricensis,* anti-larval effects.

Introduction

PF1022A is newly isolated from fermentation broth of a strain PF1022 belonging to the order Agonomycetales (Mycelia Sterilia) and has a unique structure of cyclic depsipeptide as anthelmintics (Takagi *et al.*, 1991; Terada, 1992).

It was reported that PF1022A had a superior *in vitro* activity against many nematodes including *Heterakis spumosa* and *Angiostrongylus cantonensis* (Takagi *et al.*, 1991; Terada, 1992). The drug is also known to have an ideal mechanism of action, in that paralysis is caused synergistically by stimulating the gabergic mechanism and inhibiting the cholinergic mechanism in nematode worms (Terada, 1992).

PF1022A has also been reported to have superior *in vivo* effects against intestinal nematodes such as *Ascaridia galli* in chickens, *Toxocara canis* and *T. cati* in dogs, and *Haemonchus contortus* and *Ostertagia ostertagi* in cows and horses (Fukashe *et al.*, 1990; Takagi *et al.*, 1991; Kurosawa *et al.*, 1992). For studying availability of PF1022A it is very important to know whether the anthelmintic has efficacy

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against tissue nematodes *in vivo* after parenteral or oral administration.

We have been studying using angiostrongyliasis costaricensis in mice for examining drug activity and establishing treatment against tissue nematodes. Cotton rats have been used as an experimental final host of *Angiostrongylus costaricensis* (Morera, 1973; Monge *et al.*, 1978; Ubelaker *et al.*, 1981), but they are not supplied commercially in Japan as the experimental animal. In previous studies, the outbred ddY mouse (Japan SLC) was selected from various inbred and outbred mice as the *in vivo* model animal for experimental chemotherapy (Terada *et al.*, 1987; Ishii and Sano, 1989). After selecting parameters favorable to detecting chemotherapeutic effects, we studied the relationship between worm growth and host pathologic changes in mouse infections, and clarified that after 15 days post-infection (PI) worms became matured in the mesenteric arteries. It is suggested that pathologic changes are probably attributable to eggs and/or hatched larvae (Terada *et al.*, 1991a, b). Using this animal model, anti-larval effects of some anthelmintics such as milbemycin D and mebendazole could be detected (Terada *et al.*, 1987, 1989). Thus, in the present study we examined effects of PF1022A on developing larvae of *A. costaricensis* in mice, with special reference to dose, route and formulation.

Materials and Methods

1. Drug

PF1022A (Meiji Seika Kaisha) is essentially water insoluble. For examining *in vivo* effects of the compound, it seems better to solubilize or emulsify PF1022A in water using some agents which solubilize or emulsify water insoluble drugs. PF1022A was solubilized at a concentration of 2.5% with polyoxyethylenehydrogenated caster oil, propyleneglycol fatty acid ester I and water, and we designated the formulation as the oral solution. The drug was solubilized at a concentration of 1% with polyoxyethylenehydrogenated caster oil, N-methyl-2-pyrrolidone and water. With the formulation named as the injectable solution, we can treat animals via intravenous as well as oral routes. PF1022A was also emulsified at a concentration of 2.5% using polyoxyethylenehydrogenated caster oil, propyleneglycol fatty acid ester I, propyleneglycol fatty acid ester II and water, and the formulation was named as the oral cream. These three formulations were diluted 5 to 50 times with distilled water or sterilized saline before oral and intraperitoneal treatment depending on doses. Additionally the compound was dissolved in olive oil and given via two routes, and the group was called as the olive oil group.

2. Infection

Male ddY mice of 5-weeks-old were used as final host. Infective third-stage larvae of *A. costaricensis* (Costa Rican strain) were obtained from experimentally infected snails, *Biomphalaria glabrata* (Puerto Rican strain), by artificial digestion using 0.04% pepsin in 0.7% HCl for 30 min at 37°C. Mice were infected orally with 20 third-stage larvae using a metal catheter.

3. Experimental design

Twelve groups of 6 or 7 mice each were treated with 5 successive doses of PF1022A from 7 to 11 days PI. Six groups were treated orally; three groups with PF1022A in the oral solution at 2.5, 10 and 40 mg/kg/day, and 3 groups with 10 mg/kg/day of PF1022A prepared as the injectable solution and the oral cream, and dissolved in olive oil, respectively. The other six groups were treated intraperitoneally; three groups with PF1022A in the oral solution at 0.625, 2.5 and 10 mg/kg/day, 3 groups with 2.5 mg/kg/day of PF1022A in 3 formulations as described in the latter groups orally treated. Another infected group and one group of 7 mice without infection served as non-treated and non-infected controls, respectively.

Number of worms recovered, worm length, the first-stage larvae in feces and eggs in host ileum were examined as parameters in worms at dissection 37 to 38 days PI under over anesthesia with diethyl ether. Changes in host body weight and death of host were observed after infection, and relative spleen and intestinal weight, hemo-

globin content (Hb) and hematocrit (Ht) were examined at dissection as parameters in host mice. All parameters except hematologic values and worm body length were examined according to the methods described by Terada *et al.* (1991a, b). The Hb and Ht values were determined by the CN-methemoglobin method and micro-method with capillary tubes, respectively (Kanai and Kanai, 1988). The worm body length was estimated from photographs using a computerized image analyzer (Videoplan, Kontron Co., Munich, Federal Republic of Germany). Significant difference of data was analyzed by the Student's *t*-test.

Results

I. Effects of 5 successive doses of PF1022A orally given at various doses

1. Parameters in host mice: Table 1 and Fig. 1A

Mean body weight in the non-infected mice kept increasing during the experiment. The weight in the non-treated control group started to decrease from around the 15th day PI, and the weight of surviving mice at dissection was 29.9 g. The weights of surviving mice in all orally medicated groups were heavier than that of the non-treated control, and the 40 mg/kg group showed a significant difference from the control.

Totally three mice in the non-treated control died 17, 22 and 23 days PI. Four mice in the 2.5 mg/kg group died 19, 21, 24 and 27 days PI, while no mice died in other treated groups.

Compared with the relative spleen weight (g/100 g body weight) at 0.30 in the non-infected control mice, this value increased 4.9 times in the non-treated control. Compared with the non-treated control, all the weights of the spleen in the orally medicated groups decreased, and two groups medicated with 10 and 40 mg/kg showed significant differences. Compared with the relative intestinal weight of 9.31 of the non-infected control animals, this value increased 2.2 times in the non-treated control. The intestinal weight in the medicated groups with 10 and 40 mg/kg significantly decreased compared with that in the non-treated control.

Macroscopic pathologic changes including granulomatous inflammatory changes on the serosal surface of the cecum and ileum were seen in all surviving mice of the non-treated control and 2.5 mg/kg groups. Many petechiae were observed in the serosa of the terminal ileum in 3 out of 7 surviving mice of the 10 mg/kg group, but there were no changes in all surviving mice treated with 40 mg/kg.

Compared with the values of Hb and Ht of 15.2 g/dl and 45.4%, respectively, in the non-infected control animals, these values decreased to 37.2 and 47.8% in the non-treated control. Compared with the non-treated control, both values in the medicated groups with 10 and 40 mg/kg showed restoration with significant differences.

2. Parameters in worms: Table 2

The mean worm recovery was 11.0 from the non-treated mice, and almost same number was recovered from the 2.5 mg/kg group, while in the 10 mg/kg group the worms were reduced to 8.2% recovery and no worms were recovered from the 40 mg/kg group.

The mean body length of the worms recovered from the non-treated control group was 29.0 mm in female and 18.1 mm in male, respectively. The length of recovered females and males in the medicated groups with 2.5 and 10 mg/kg was reduced to about 95 and 89%, respectively, of those in the non-treated control.

The first-stage larvae were observed in feces of all surviving mice in the non-treated control and 2.5 mg/kg group with one exceptional mouse with only female infection. No larvae were seen in other medicated groups.

Eggs in the host terminal ileum were examined. Numerous eggs with various stages including larvae were observed in the tissue sections from all surviving mice in the non-treated control and in 2.5 mg/kg groups with an exceptional mouse having only female worms. A few unfertilized eggs were observed in the tissues from mice with only female worms in the 2.5 and 10 mg/kg groups, respectively. No eggs were observed in the tissue from animals in the 40 mg/kg group.

Table 1 Effects of PF1022A orally given on angiostrongyliasis costaricensis in mice: parameters in host mice at dissection

Parameters	Non-treated control	2.5 mg/kg the oral solution	10 mg/kg the oral solution	40 mg/kg the oral solution	10 mg/kg the injectable solution	10 mg/kg the oral cream	10 mg/kg olive oil	Non-infected control
No. of surviving mice	4/7	3/7	7/7	7/7	7/7	7/7	5/7	7/7
Body weight (g)	29.9 ±2.7§	33.8 ±1.0§	37.9 ±0.7‡	39.6 ±1.3†	38.3 ±1.8*	41.4 ±1.5†‖	33.3 ±2.8	41.6 ±1.1†
Pathological changes in serosal surface								
Granulomatous changes around eggs	4/4	3/3[1)]	0/7	0/7	1/7	0/7	3/5[2)]	0/7
Petchiae	0/4	0/3	3/7	0/7	1/7	0/7	1/5[2)]	0/7
Not observed	0/4	0/3	4/7	7/7	5/7	7/7	2/5	7/7
Relative wet weight (g/100 g body weight)								
Spleen	1.47±0.23‡	1.37±0.14‡	0.79±0.16*‡	0.36±0.03*	0.69±0.16*‡	0.38±0.03*‖	0.98±0.20‡	0.30±0.02*
Intestine	20.90±1.27§	20.78±1.70‡	10.47±0.75†	9.36±0.34†	10.62±0.96†	9.08±0.10†	16.50±1.77‡¶	9.31±0.24†
Hematologic values								
Hb (g/dl)	5.64±1.25§	6.85±1.67‡	12.76±0.88†‡	14.73±0.19†	12.50±1.65†	15.28±0.23†‖	9.39±2.15	15.16±0.30†
Ht (%)	21.73±3.82§	26.73±5.34	41.01±2.35†	45.31±0.56†	39.77±4.33†	46.93±0.60†‖	31.38±6.07	45.44±0.89†

Results are represented as mean±SE of surviving mice.
Significant difference from non-treated control: *$P<0.05$, †$P<0.01$, Significant difference from non-infected control: ‡$P<0.05$, §$P<0.01$,
Significant difference from 10 mg/kg in the oral solution: ‖$P<0.05$, ¶$P<0.01$.
1) One mouse had slight granulomatous changes around many unfertilized eggs, 2) One mouse had both changes.

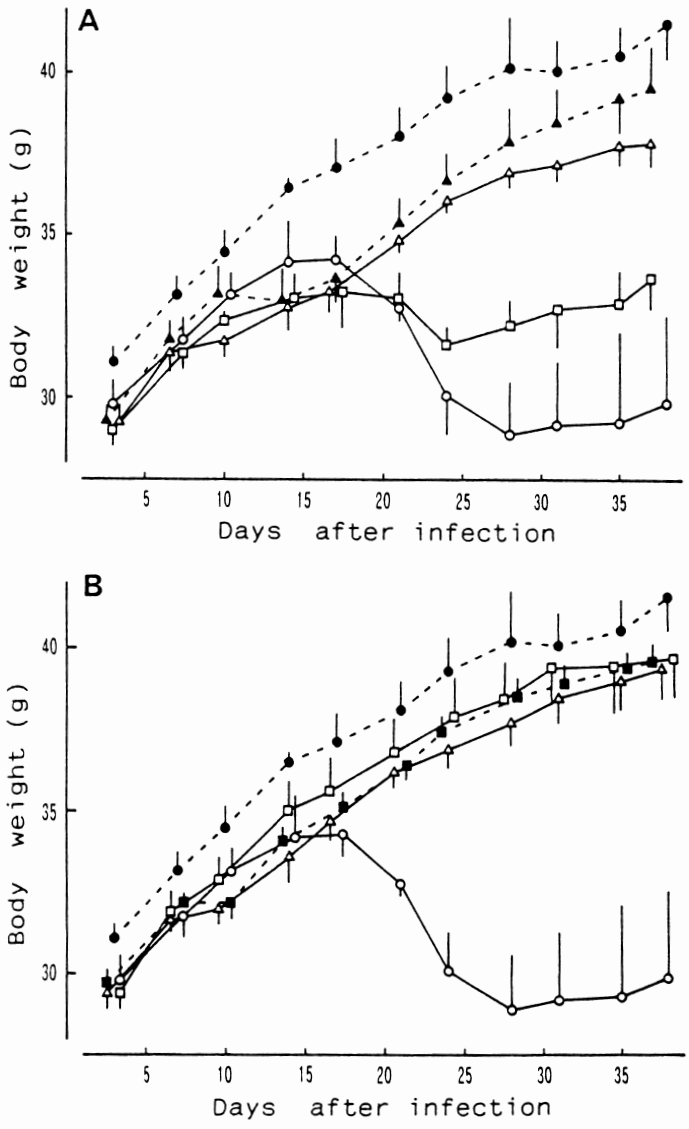

Fig. 1 Effects of 5 successive oral (A) and intraperitoneal (B) doses of PF1022A on body weight of mice infected with *A. costaricensis*. Solubilized formulation of PF1022A designated as the oral solution was used in the experiment. All mice were inoculated with 20 infective larvae and each group consisted of 7 mice. PF1022A was given 7–11 days post-infection.

●- - - -●: Non-infected control,
○——○: Non-treated control,
■- - - -■: PF1022A at 0.625 mg/kg/day,
□——□: PF1022A at 2.5 mg/kg/day,
△——△: PF1022A at 10 mg/kg/day,
▲- - - -▲: PF1022A at 40 mg/kg/day.

Table 2 Effects of PF1022A orally given on angiostrongyliasis costaricensis in mice: parameters in worms at dissection

Parameters	Non-treated control	2.5 mg/kg the oral solution	10 mg/kg the oral solution	40 mg/kg the oral solution	10 mg/kg the injectable solution	10 mg/kg the oral cream	10 mg/kg olive oil
No. of worms recovered	11.0 ±0.8	10.7 ±3.2	0.9 ±0.5†	0.0±0.0†	0.7 ±0.6†	0.0±0.0†	5.0 ±1.3†‡
Worm length (mm)							
Female (No. measured)	29.02±0.36 (11)	27.75±0.86 (7)	25.70±1.06* (6)	–	25.05±0.76 (2)	–	26.76±0.71† (12)
Male (No. measured)	18.08±0.19 (9)	17.17±0.16† (7)	–	–	15.71±1.10† (2)	–	17.00±0.26† (7)
1st larvae in feces							
Detected	4/4	2/3	0/7	0/7	1/7	0/7	0/5
Not detected	0/4	1/3[1)]	7/7	7/7	6/7	7/7	5/5
Eggs in host intestine							
Many eggs with various stages including larvae	4/4	2/3	0/7	0/7	1/7	0/7	3/5
Many developing eggs without larvae	0/4	0/3	0/7	0/7	0/7	0/7	0/5
Few eggs without development	0/4	1/3[1,2)]	3/7[1,2)]	0/7	1/7[1,2)]	0/7	1/5[1,2)]
Not detected	0/4	0/3	4/7	7/7	5/7	7/7	1/5

Results are represented as mean ± SE of surviving mice.
Significant difference from non-treated control: *P<0.05, †P<0.01, Significant difference from 10 mg/kg in the oral solution: ‡P<0.01.
1) Only females were removed, 2) Unfertilized eggs.

Table 3 Effects of PF1022A intraperitoneally given on angiostrongyliasis costaricensis in mice: parameters in host mice at dissection

Parameters	Non-treated control	0.625 mg/kg the oral solution	2.5 mg/kg the oral solution	10 mg/kg the oral solution	2.5 mg/kg the injectable solution	2.5 mg/kg the oral cream	2.5 mg/kg olive oil	Non-infected control
No. of surviving mice	4/7	7/7	7/7	7/7	7/7	7/7	6/6	7/7
Body weight (g)	29.9 ±2.7§	39.6 ±0.6†	39.7 ±1.2†	39.4 ±1.0†	41.2 ±1.2†	39.3 ±1.3†	39.1 ±1.6†	41.6 ±1.1†
Pathological changes in serosal surface								
Granulomatous changes around eggs	4/4	0/7	0/7	0/7	0/7	0/7	0/6	0/7
Petechiae	0/4	0/7	0/7	0/7	0/7	0/7	0/6	0/7
Not observed	0/4	7/7	7/7	7/7	7/7	7/7	6/6	7/7
Relative wet weight (g/100 g body weight)								
Spleen	1.47±0.23‡	0.49±0.07†‡	0.61±0.16*	0.29±0.01*	0.32±0.02*	0.32±0.02*	0.95±0.21‡	0.30±0.02*
Intestine	20.90±1.27§	9.36±0.47†	8.44±0.26†‡	9.56±0.29†	8.30±0.23†‡	8.69±0.13†‡	9.83±0.16†‡‖	9.31±0.24†
Hematologic values								
Hb (g/dl)	5.64±1.25§	14.98±0.45†	13.47±1.47†	14.99±0.25†	15.14±0.27†	15.71±0.25†	12.08±0.41†‡	15.16±0.30†
Ht (%)	21.73±3.82§	46.50±1.08†	41.67±3.56†	45.90±0.78†	45.93±0.62†	47.23±0.68†	39.18±2.71†	45.44±0.89†

Results are represented as mean ± SE of surviving mice.
Significant difference from non-treated control: *$P<0.05$, †$P<0.01$, Significant difference from non-infected control: ‡$P<0.05$, §$P<0.01$,
Significant difference from 2.5 mg/kg in the oral solution: ‖$P<0.01$.

Table 4 Effects of PF1022A intraperitoneally given on angiostrongyliasis costaricensis in mice: parameters in worms at dissection

Parameters	Non-treated control	0.625 mg/kg the oral solution	2.5 mg/kg the oral solution	10 mg/kg the oral solution	2.5 mg/kg the injectable solution	2.5 mg/kg the oral cream	2.5 mg/kg olive oil
No. of worms recovered	11.0 ±0.8	0.1±0.1*	0.0±0.0*	0.0±0.0*	0.0±0.0*	0.0±0.0*	0.0±0.0*
Worm length (mm)							
Female (No. measured)	29.02±0.36 (11)	(22.44)[1)]	–	–	–	–	–
Male (No. measured)	18.08±0.19 (9)	–	–	–	–	–	–
1st larvae in feces							
Detected	4/4	0/7	0/7	0/7	0/7	0/7	0/6
Not detected	0/4	7/7	7/7	7/7	7/7	7/7	6/6
Eggs in host intestine							
Many eggs with various stages including larvae	4/4	0/7	0/7	0/7	0/7	0/7	0/6
Many developing eggs without larvae	0/4	0/7	0/7	0/7	0/7	0/7	0/6
Few eggs without development	0/4	1/7[2)]	0/7	0/7	0/7	0/7	0/6
Not detected	0/4	6/7	7/7	7/7	7/7	7/7	6/6

Results are represented as mean ± SE of surviving mice.
Significant difference from non-treated control: *$P<0.01$.
1) Only one female was recovered, 2) Unfertilized eggs.

II. Effects of 5 successive doses of PF1022A intraperitoneally given at various doses.

1. Parameters in host mice: Table 3 and Fig. 1B

No mice died in all groups treated intraperitoneally with 0.625, 2.5 and 10 mg/kg.

In all parameters in host mice, compared with the non-treated control, almost similar results to the non-infected control were seen in all intraperitoneally treated groups, showing that even a dosage of 0.625 mg/kg had almost complete effects.

2. Parameters in worms: Table 4

Only 1 female was recovered from the 0.625 mg/kg group, while no worm was recovered from other medicated groups. In all the mediated groups, almost complete effects were observed in all parameters in worms.

III. Influence of formulation on effects of 5 successive doses of PF1022A given orally or intraperitoneally.

1. Effects of PF1022A given orally at 10 mg/kg/day: Tables 1, 2 and Fig. 2A

Effects of PF1022A at 10 mg/kg were compared among 2 solubilized and one emulsified types in water and one dissolved type in olive oil. The best results were obtained in the oral cream group from all parameters, some of which being significant from the oral solution group. On the other hand, the olive oil group had the worst results in almost all parameters and significant differences were observed in some such as the relative weight of intestine and the number of the recovered worms.

2. Effects of PF1022A given intraperitoneally at 2.5 mg/kg: Tables 3, 4 and Fig. 2B

When effects of PF1022A at 2.5 mg/kg were compared among above 4 formulations, remarkable effects were seen in all the formulations. Among almost all parameters in host mice and in worms, no differences were observed between the oral solution group and other groups. Remarkable differences were observed only in the relative spleen weight and hematologic values, and the oral cream group showed the best results and the olive oil group the worst effects.

Discussion

To treat some tissue parasitic disease with anthelmintics, firstly we must find some effective drugs which affect directly some site of action in the worms, and *in vitro* experiments are useful for detecting such effects. But, for example, if some drugs are not absorbed from the intestinal tract of host animal, the effective drugs *in vitro* are not always effective *in vivo*. We have pyrantel as one of such drugs directly affecting worm motility *in vitro*. However, it was reported that pyrantel given parenterally showed conspicuous adverse effects on host tissues because the drug acted on the nicotinic receptors in host animals as well as nematodes (Eyre, 1970; Aubry *et al.*, 1970; Terada *et al.*, 1983). Thus, in the case of pyrantel, the poor absorption from host intestine is favorable to the drug action and makes it a superior selective anthelmintic against intestinal nematodes. PF1022A is also known to have a marked action on the motility of nematodes (Takagi *et al.*, 1991; Terada, 1992). It was reported additionally that PF1022A had little toxicity against animals even after parenteral treatment and LD_{50} values for i.p. administration in male ICR mice were to be more than 1000 mg/kg (Takagi *et al.*, 1991). On the other hand, it is known that the drug orally given is effective against *Haemonchus contortus* biting into the mucosae of the host intestine (Kurosawa *et al.*, 1992), suggesting that the drug may be absorbed from the intestinal tract. If PF1022A can be absorbed indeed, it becomes a different case from parentel and we have possibility to use the drug as anthelmintic given orally against various tissue nematodes.

Examining anti-larval effects of PF1022A in the oral solution, one of our formulations, we could detect the efficacy of the new anthelmintic given orally as well as intraperitoneally. From almost complete effects by PF1022A given intraperitoneally at 0.625 mg/kg, it became apparent that the drug had sufficient efficacy on the nematode dwelling in the blood vessel. Though the effects by oral route were about 1/20 compared with intraperitoneal route, PF1022A given orally at 2.5 to 40 mg/kg showed dose-dependent

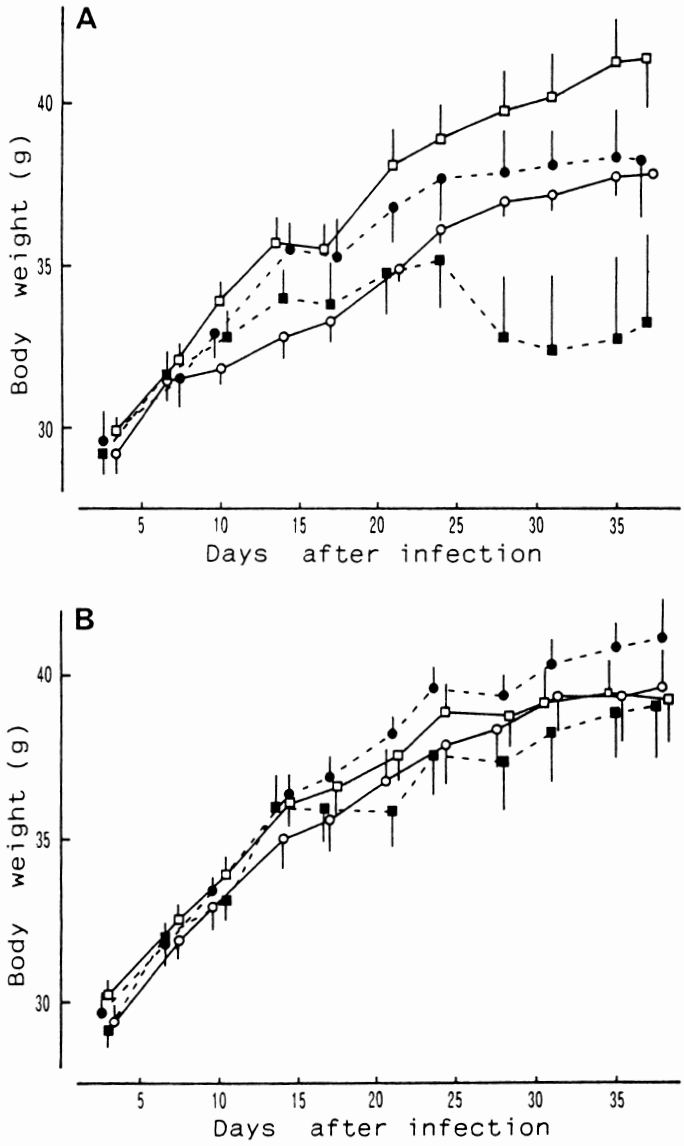

Fig. 2 Effects of 5 successive doses of PF1022A in 4 different formulations on body weight of mice infected with *A. costaricensis*. Except only the olive oil group consisted of 6 mice, other conditions were the same as those shown in Fig. 1. PF1022A was administered orally at 10 mg/kg/day (A) or intraperitoneally at 2.5 mg/kg/day (B) in formulations designated as; ○——○ : The oral solution, ●– – – –● : The injectable solution, □——□ : The oral cream, and ■– – – –■ : Olive oil.

effects, and nearly complete effects were at 10 mg/kg. Thus, we could demonstrate that PF1022A was indeed absorbed from the intestinal tract of mice. In general, oral route is more preferable to parenteral routes from various standpoints such as easiness of actual treatment, safety and economy. Therefore, the present results give PF1022A many possibilities as anthelmintic administered orally against tissue nematodes.

The rate of absorption from the intestinal tracts is known to be influenced by many factors such as the method of administration, solubility and other physical properties of the drug. If the rate can be stimulated by changing formulations of PF1022A, usefulness of the drug will be increased more. In the present study we compared effects of PF1022A in 4 different formulations including 2 solubilized and one emulsified types in water and one dissolve type in olive oil. If PF1022A is absorbed from host intestinal tract as the solubilized form in water, either of solubilized formulations or emulsified one prepared as the oil-in-water type emulsion may be the most effective. On the other hand, if PF1022A may be absorbed via lymphatic system, PF1022A dissolved in olive oil may be the most effective.

When the efficacy of PF1022A orally given at 10 mg/kg was compared among these 4 different formulations, marked differences were observed. From almost all parameters in host mice and in worms, the best results were obtained in the oral cream group and the worst effects in the olive oil group. When PF1022A was given intraperitoneally at 2.5 mg/kg in these 4 formulations, similar differences among groups were observed only in the relative spleen weight and hematologic values in host mice, though no differences were observed among marked effects on other parameters in host and in all parameters in worms. From these results it is suggested that PF1022A in the oral cream is the most preferable and probably produces promising effects against many other tissue nematodes including filariasis, toxocariasis and strongyliasis.

In addition, the new anthelmintic seemed less toxic from the larger LD_{50} values after intraperitoneal as well as oral administration (Takagi *et al.*, 1991) and from the surprising differences in the effective concentrations causing inhibition of the cholinergic mechanism in *A. cantonensis* and frog rectus (Terada, 1992). Therefore, it is quite likely that PF1022A given orally as well as intraperitoneally becomes a superior anthelmintic available for both tissue and intestinal nematodes.

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