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Effects of PF1022A on *Angiostrongylus cantonensis* Staying in the Central Nervous System of Rats and Mice

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Abstract

Using rat angiostrongyliasis cantonensis it has been reported by Akyol *et al.* (1993) that PF1022A, a new gabergic anti-nematode drug, did not seem to affect worms staying in the brain due to little distribution of the drug in the tissue. Effects of PF1022A on the worms staying in the central nervous system (CNS) of rats and mice were reexamined. First, effects of PF1022A on young adult *A. cantonensis* in rats were examined using the n-oral cream, a different formulation from the oral cream or oral solution used by Akyol *et al.* (1993). Regarding some parameters including worm recovery and pathologic changes in host, little effect of PF1022A was observed when given 5 successive doses of 20 mg/kg/day orally and 2.5 mg/kg/day intraperitoneally from 11 days after 20 larval infection. Second, we examined effects of PF1022A on larval *A. cantonensis* in mice. From some parameters in host such as number of mice which died, change in body weight, and relative spleen and brain weight, five successive oral doses of mebendazole (10 mg/kg/day), a known effective control drug, were effective. Whereas no effects were observed in the group treated with 5 successive oral doses of PF1022A in the oral cream at 20 mg/kg/day from 6 days after 50 larval infection. These results suggest more strongly that PF1022A does not pass through the blood-brain barrier.

Key words: PF1022A, *Angiostrongylus cantonensis*, worms staying in the CNS, the blood-brain barrier

Introduction

Using rat angiostrongyliasis cantonensis it has been reported by Akyol *et al.* (1993) that PF1022A, a new gabergic anti-nematode drug, did not seem to affect worms staying in the brain due to poor distribution of the drug in the tissue. From the standpoint of adverse side effects of gabergic anthelmintic, this finding is epoch-making because most gabergic anthelmintics are known to penetrate the blood-brain barrier of host, relating to their adverse side effects (Akyol *et al.*, 1993).

To make sure the epoch-making finding, in this study we examined again the effects of PF1022A on the nematode worms staying in the central nervous system (CNS) of rats using a different formulation of the drug, n-oral cream from the oral cream and oral solution used by Akyol *et al.* (1993). Additionally, the effects were examined using another experimental model, murine angiostrongyliasis cantonensis.

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Materials and Methods

1. Materials

Male Wistar rats and ddY mice of 5-weeks-old were used as final hosts and were inoculated with the infective third-stage larvae of *A. cantonensis* (Hawaiian strain). The larvae were obtained from experimentally-infected *Biomphalaria glabrata* snail (Puerto Rican strain), by artificial digestion using 0.04% pepsin solution in 0.7% HCl for 30 min at 37°C. Each animal was inoculated orally with infective larvae using a metal catheter. PF1022A was donated from Meiji Seika Kaisha. In this study, a 2.5% formulation of emulsified type designated as the oral cream and the n-oral cream, a new formulation, was used. The formulation named as the n-oral cream had the same composition as the oral cream with small changes in their ratio. Both were diluted to 10 or 40 times with distilled water or sterilized

saline before oral and intraperitoneal treatments, respectively, as described by Terada *et al.* (1993). Mebendazole was donated by Janssen Pharmaceutica, and suspended with 2% cremophor EL (Sigma).

2. Experimental design

Experiment I

Effects of PF1022A given orally or intraperitoneally against young adult *A. cantonensis* in rats were examined. According to Akyol *et al.* (1993), four groups consisting of 5 rats were inoculated with 20 infective larvae of *A. cantonensis*. All treated groups were given 5 successive daily doses from 11 to 15 days post-infection (PI).

Two groups were treated orally with 20 mg/kg of PF1022A in the oral cream and the n-oral cream. One group was treated intraperitoneally with 2.5 mg/kg of PF1022A in the n-oral cream. Another infected group and one group without infection received only the vehicle for the n-oral cream and served as non-treated and non-infected controls, respectively.

All rats were dissected 45 days PI under over anesthesia with diethyl ether and adult worms were recovered from the lungs and heart. Number of worms recovered, relative weight of heart and lungs and visual pathologic changes of the lung tissues were examined. Pathologic changes were visually observed in each lobule and the degree of lesion was estimated as 1/5, 1/4, 1/3, 1/2 and 1/1, and total values were obtained by summing each value of five lobules. Significance of difference in the mean values was statistically analyzed by Student's *t*-test.

Experiment II

Effects of PF1022A on larval *A. cantonensis* in mice were examined. According to Hayashi (1987), three groups consisting of 10 mice were inoculated with 50 infective larvae of *A. cantonensis*, and additional one group consisting of 5 mice was used without infection as non-infected control. Two infected groups were treated orally with 5 successive daily doses of mebendazole (10 mg/kg) and PF1022A in the oral cream (20 mg/kg), respectively, from 6 to 10 days PI. Another infected group used as non-treated control and non-infected control received only the vehicle (2% cremophor EL). Body weight, clinical signs and number of surviving host animals in all groups were monitored until 28 days PI. All surviving mice were dissected 28 days PI under over anesthesia with diethyl ether and relative spleen and brain weight, hemoglobin content (Hb) and hematocrit (Ht) were examined at dissection. Significance of difference in the mean values was statistically analyzed by Student's *t*-test.

Results

Effects of PF1022A on young adult A. cantonensis *in rats (Experiment I): Table 1*

The mean total recovery of worms was 17.2 in the non-treated control group. Regarding total worms, females and males, the recovery in any treated groups was not significantly different compared with the non-treated control.

When compared with body weight and the relative heart and lung weight in the non-infected control, there were significant differences in values from animals in the non-treated control group. Regarding the relative weight and visual pathologic changes, few changes were observed among 4 groups including non-treated and treated groups, though body weights were significantly bigger in all treated groups except the group treated orally with PF1022A in the n-oral cream.

Effects of PF1022A on larval A. cantonensis *in mice (Experiment II): Fig. 1 and Table 2*

Host body weight of non-treated control group started to decrease from around 12 days PI, but later the surviving mice regained. Various clinical signs such as depression and bristled hair were seen in all mice at 12 days PI, and one of them showed wryneck and claudication. Four mice died after these signs became severe, but others were restored later. There was no death and no reduction in body weight in animals treated with 5 successive oral doses of mebendazole (10 mg/kg/day). However, animals treated with 5 successive oral doses of PF1022A (20 mg/kg/day) showed similar changes in all of these parameters to the non-treated control group.

When compared with remarkable changes in body weight between non-infected and non-treated controls in Fig. 1, there were rather slight but significant changes between these two controls regarding

Table 1 Effects of PF1022A on young adult *Angiostrongylus cantonensis* in rats (Experiment I)

Parameters	Non-treated control	p.o. 20mg/kg×5 oral cream	p.o. 20mg/kg×5 n-oral cream	i.p. 2.5mg/kg×5 n-oral cream	Non-infected control
Number of rats	5	5	5	5	5
Body weight (g)	268.0±6.6	312.6±7.5†	292.6±11.1	298.4±7.7*	295.5±9.9*
Relative wet weight of heart-lungs (g/100g body weight)	1.97±0.27	1.72±0.06	2.28±0.35	1.73±0.07	0.83±0.01*
Visual pathologic changes in the lung tissues	3.3±0.2	2.6±0.3	3.4±0.1	2.7±0.2	–
No. of worms recovered					
Female	8.8±0.6	9.8±0.6	9.0±1.1	8.6±0.5	
Male	8.4±0.9	8.8±1.0	8.8±1.0	8.2±0.6	–
Total	17.2±0.9	18.6±0.5	17.8±0.7	16.8±0.7	–

Results are represented as mean±SE.
Significant difference from non-treated control: *P<0.05, †P<0.01.

relative brain weight, Ht and Hb values in Table 2. In all values except the relative spleen weight, treated animals with mebendazole showed similar results to the non-infected control. In the values of the relative spleen and brain weight the PF1022A group was similar to the non-treated control group, but in values of Ht and Hb the treated group was similar to the non-infected control.

Discussion

Since Lämmler and Weidner (1975) many studies on anthelmintic effects of drugs against *Angiostrongylus cantonensis* in rats have been carried out. In addition, the mouse has also been shown to be a useful model for examining drug effects on human angiostrongyliasis (John and Martinez, 1975; Maki and Yanagisawa, 1983; Hayashi, 1987). Thus, the experimental angiostrongyliasis is thought to be available as a model not only for testing drug effects on human angiostrongyliasis but also for examining anthemintic effects on tissue-dwelling nematodes.

In our studies examining anthelmintic effects of gabergic drugs using rat and murine angiostrongyliasis we have observed that some drugs such as avermectin B_{1a} (Ishii *et al.*, 1983), ivermectin (Ishii *et al.*, 1985) and milbemycin D (Terada *et al.*, 1987) had killing action on larval or young adult worms of *A. cantonensis* staying in the CNS of host animals.

In addition, avermectin B_{1a} previously administered to mice enhanced some of the pharmacologic actions of diazepam affecting in the brain (Williams and Yarbrough, 1979). Campbell (1987) detected a higher level of ivermectin than usual concentrations in the brain of collie dogs that showed most severe toxic signs after treatment. All of these reports suggest penetration of these gabergic anthelmintics into the CNS through the blood-brain barrier.

Using rat angiostrongyliasis cantonensis it has been reported by Akyol *et al.* (1993) that PF1022A in the formulation designated as the oral cream and oral solution did not seem to affect worms staying in the brain due to poor distribution of the drug in the tissue.

To make sure the epoch-making finding we examined again the effects of PF1022A using a different formulation of the drug, n-oral cream and using another experimental model, murine angiostrongyliasis cantonensis.

In the experiment I using rat angiostrongyliasis

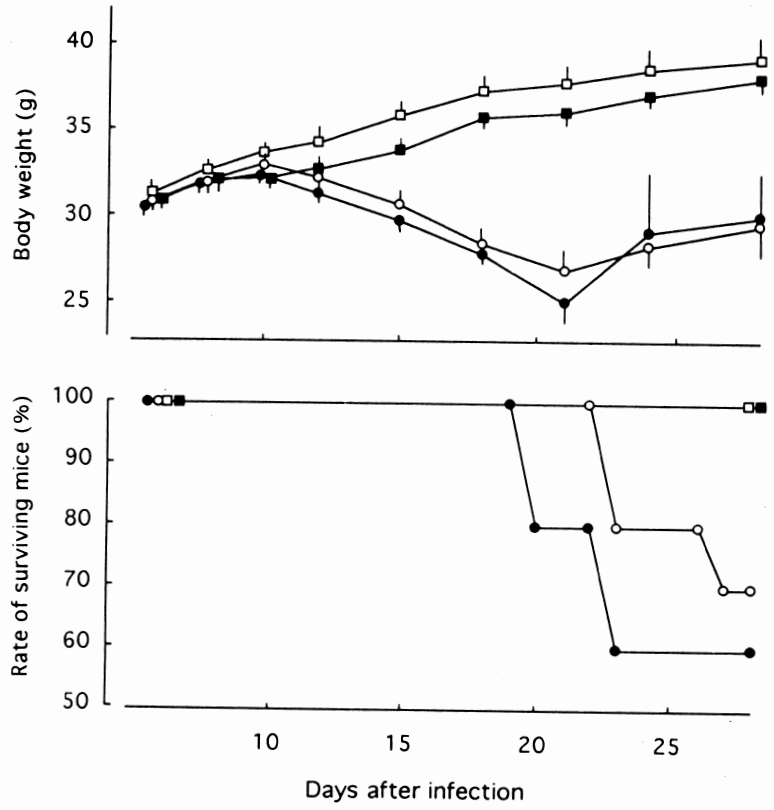

Fig. 1 Effects of 5 successive oral doses of PF1022A or mebendazole on body weight (upper) and rate of surviving host (lower) in murine angiostrongyliasis cantonensis. All mice were inoculated with 50 infective larvae and each group consisted of 10 mice. Non-infected control consisted of 5 mice. Drugs were given 6–10 days PI.
□ : Non-infected control, ● : Non-treated control, ○ : PF1022A at 20 mg/kg/day, ■ : Mebendazole at 10 mg/kg/day.

Table 2 Effects of PF1022A and mebendazole on larval *Angiostrongylus cantonensis* in mice (Experiment II)

Parameters	Non-treated control	PF1022A 20mg/kg×5 p.o.	Mebendazole 10mg/kg×5 p.o.	Non-infected control
Number of mice (surviving/used)	6/10	7/10	10/10	5/5
Relative wet weight (g/100g body weight)				
Brain	1.53±0.08	1.59±0.10	1.19±0.04†	1.19±0.03†
Spleen	0.30±0.04	0.26±0.03	0.32±0.02	0.27±0.03
Ht (%)	42.12±0.87	46.84±0.75†	45.42±0.30*	48.08±0.75†
Hb (g/dl)	14.15±0.26	15.67±0.17†	15.01±0.16†	15.54±0.19†

Results are represented as mean±SE.
Significant difference from non-treated control: *P<0.05, †P<0.01.

cantonensis, effects of a new formulation named the n-oral cream were examined. The formulation has the same composition as the oral cream, but the new one has a different physical state from the oral cream. Compared with the oral cream which is creamy in the formulation, the n-oral cream is liquid crystal and consists of finer particles of PF1022A. As the new formulation is more stable, there may be a possibility that it is more absorptive and effective when administered to animals. However, 5 successive doses of PF1022A at 20 mg/kg/day in the oral cream and n-oral cream had little effect on worms staying in the CNS of rats.

In rat angiostrongyliasis cantonensis it is well known that worms can be matured after migrating into the pulmonary arteries. On the other hand, if mice and man are infected with *A. cantonensis*, worms can not become matured. In murine angiostrongylia-sis cantonensis it is reported that almost all worms reach the brain within 48 hr after infection (Mackerras and Sandars, 1955; Wallace and Rosen, 1969; Alicata and Jindrak, 1970). In the experiment II, 5 successive oral doses of PF1022A at 20 mg/kg/day were little effective, though 5 successive doses of mebendazole at 10 mg/kg/day were markedly effective when orally given from 6 days PI as reported by Hayashi (1987).

On the other hand, it has been shown that 5 successive doses of PF1022A orally given at 5 or 10 mg/kg/day had killing action on adult *A. cantonensis* in the pulmonary arteries and also on larvae migrating into the CNS of rats (Kachi *et al.*, 1995). Therefore, the results obtained in the present studies suggest that PF1022A has little effect on *A. cantonensis* staying in the CNS of host because of little penetration through the blood-brain barrier.

Recently, Cheng *et al.* (1995) have got similar conclusion from studies on effects of PF1022A on the CNS functions of rats and mice. By them the sleeping time and food intake of animals were not affected by intravenus injection of PF1022A, but significantly influenced by intracerebroventricular injection of the drug.

Conclusively, it is apparent that PF1022A has few adverse side effects related to the passage of the blood-brain barrier.

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