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Effects of PF1022A on *Nippostrongylus brasiliensis* in Rats and *Hymenolepis nana* in Mice

MING WANG[1)], NAOHIRO WATANABE[1)], TOMOKO SHOMURA[2)] AND HIROSHI OHTOMO[1)]

[1)]*Department of Tropical Medicine, Jikei University School of Medicine, 3-25-8 Nishishinbashi, Minato-ku, Tokyo 105, Japan.*
[2)]*Pharmaceutical Research Center, Meiji Seika Ltd., 760 Morooka-cho, Kohoku-ku, Yokohama 222, Japan.*
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Abstract

PF1022A is recently developed as an anthelmintic drug with a structure of cyclic depsipeptide isolated from *Mycelia sterilia*. Effects of PF1022A were examined in rats infected with *Nippostrongylus brasiliensis* and in mice infected with *Hymenolepis nana*. When PF1022A was orally administered for 3 successive days from day 5 post infection (PI) to adults of *N. brasiliensis* in the intestine, almost complete elimination of adult worms was observed at the doses of 2.5 mg/kg/day or higher. To test the effect on developing larvae in the lung, PF1022A was intraperitoneally administered for 2 days from day 1 PI. No effect was found even at a dose of 10 mg/kg/day. In the case of *H. nana*, PF1022A was orally administered at a dose of 10 mg/kg/day for 2 days from day 5 or day 10 PI to cysticercoids or adult worms, respectively. Similar number of worms was recovered from mice with and without administration of PF1022A. These results suggest that PF1022A is effective against adult *N. brasiliensis* of intestinal phase but not against the larva of tissue phase in rats, and that PF1022A can not eliminate the cysticercoid and the adult of *H. nana* from the intestine of mice.

Key words: PF1022A; chemotherapy; *Nippostrongylus brasiliensis*; *Hymenolepis nana*; infection.

Introduction

PF1022A is a cyclic depsipeptide consisting of four L-N-methyl leucins, two D-phenyl lactic acids and two D-lactic acids which is isolated from *Mycelia sterilia* (Takagi *et al.* 1991, Sasaki *et al.* 1992). It has been reported that PF1022A is effective against various nematodes such as adult *Angiostrongylus cantonensis* (Terada, 1992, Kachi *et al.*, 1995) *in vitro*, *Toxocara canis* and *Toxocara cati* in dogs and cats (Fukashe *et al.*, 1990), *Heterakis spumosa* (Takagi *et al.*, 1991) and *Ascaridia galli* (Sasaki *et al.*, 1992) in chickens, *Haemonchus contortus* and *Osteragia ostertagi* in cows and horses (Kurosawa *et al.*, 1992) and larvae of *Angiostrongylus costaricensis* (Terada *et al.*, 1993), *Trichinella spiralis* (Wang *et al.*, 1994) in mice and *A. cantonensis* in rats (Kachi *et al.*, 1995). Some nematode parasites migrate through the lung in their larval stages. Although this period is generally short, harmful damage can be induced. Little has been known about the effect of PF1022A on their lung migrating larvae. *Nippostrongylus brasiliensis* was selected to examine the effect of PF1022A because infected larvae migrate to the lung before they develop into adults in the intestine. Moreover, the effect of PF1022A to the adult of *N. brasiliensis* in the intestine was also examined, because intestinal nematodes had not been tested in rats.

For the application of this drug in the treatment of parasitic diseases, it may be beneficial to examine the anthelmintic activity of PF1022A on parasites other than nematodes. *Hymenolepis nana* infection in mice is a useful model to determine the susceptibility of cestodes to PF1022A.

Correspondence: Naohiro Watanabe
王　鳴[1], 渡辺直熙[1], 庄村知子[2], 大友弘士[1] ([1]東京慈恵会医科大学熱帯医学教室, [2]明治製菓薬品総合研究所)
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Materials and Methods

Drug

PF1022A was prepared at Meiji Seika Ltd.: 0.125% formulation of emulsified type designated as the oral cream, 1% formulation of injectable solution for intraperitoneal administration. Vehicle which contained the solvent component without PF1022A, was also prepared. The drug and vehicle were diluted with saline to appropriate concentrations before administration to animals in each experiment.

Animals and infection

Female Wistar rats, obtained from Ishikawa Laboratory Animals (Saitama, Japan), were used as the host of *N. brasiliensis*. Cultivation of eggs to third stage larvae and passage of *N. brasiliensis* were performed according to the method described by Weinstein and Jones (1956). This strain of *N. brasiliensis* has been maintained in our laboratory for 20 years. Rats were infected subcutaneously with 400 or 500 third-stage larvae of *N. brasiliensis* (Watanabe *et al.*, 1989).

Female ddy mice aged 5 weeks, purchased from Japan SLC (Shizuoka, Japan), were used as the host of *H. nana*. The eggs were obtained by crushing the gravid proglottids of *H. nana* on a metal mesh. After washing with PBS (pH 7.4), the egg suspension was stirred with glass beads for five min to break the eggshells. The number of shell-free eggs was counted under a microscope. Each mouse was perorally inoculated with 100 shell-free eggs in 0.2 ml PBS by stomach tube under anesthesia with diethyl ether (Watanabe *et al.*, 1994).

Experimental design

According to the study of *N. brasiliensis* by Halay (1962), most of the infected larvae have migrated to the lung about 15 hr PI and stay there for a period to grow and undergo the third molt. By about 59 hr PI, most of them have migrated via the respiratory tree, esophagus and stomach to the intestine. Around 6 days PI, the larvae become mature adults in the intestine of the host. In the experiments testing effects of PF1022A on *N. basiliensis* in rats, 3 groups of 5 rats each were infected with 500 third stage larvae (Experiment 1). To test the effect of PF1022A on lung phase of *N. brasiliensis*, one group was intraperitoneally (ip) administered with PF1022A in injectable solution at a dose of 10 mg/kg/day for 2 days from day 1 PI. For adult worms in the intestine, another group of rats were orally administered with PF1022A in oral cream at a dose of 5 mg/kg/day for 3 days from day 5 PI. Remaining group without any administration was served as the control. In the experiment 2, 4 groups of 5 rats each were infected with 400 third stage larvae to examine dose effect of PF1022A on adult *N. brasiliensis*. Two groups were orally given 2.5 mg or 0.6 mg/kg/day of PF1022A for 3 days from day 5 PI. One group of rats was similarly administered with vehicle corresponding to the concentration of 2.5 mg/kg PF1022A. Remaining group without any administration was served as the control. For the recovery of adult worms, rats were killed with diethyl ether at day 8 PI, the small intestines were removed and opened, the number of adult worms was directly counted under a microscope.

In the experiments examining effects of PF1022A on *H. nana* in mice, two groups of 4 infected mice were orally administered with PF1022A in oral cream at a dose of 10 mg/kg/day for 2 days from day 5 or day 10 PI, respectively. Another group of 4 mice was orally administered with corresponding concentration of vehicle for 4 days of day 5, 6, 10 and 11 PI. Remaining group of 4 mice was used as the control without any administration. All mice were killed at day 14 PI. After the small intestines were opened, the segments of the intestines were put into the water in Petri dishes. The recovery of adult *H. nana* was determined by counting the number of scoleces of the worms (Isaak *et al.*, 1977).

Data were statistically analyzed by Student's *t*-test.

Results

Effects of PF1022A on N. brasiliensis *in rats*

Effects of PF1022A were examined on the larva in the lung and the adult in the intestine (Table 1, Exp. 1). Infected rats were administered ip with PF1022A at a dose of 10 mg/kg/day for 2 days from day 1 PI when *N. brasiliensis* had migrated to the lung. If PF1022A could act on the larvae in the lung, the larvae would be disrupted further development

Table 1 Effects of PF1022A on larval and adult *Nippostrongylus brasiliensis* in rats

Exp.	Administration of PF1022A			No. of adults/rat (Mean±SD)
	Dose (mg/kg/day)	Timing	Route	
1	5	day 5–7 PI	peroral*	0±0‡
	10	day 1–2 PI	intraperitoneal†	376±56
	0			399±40
2	2.5	day 5–7 PI	peroral*	1±2‡
	0.6	day 5–7 PI	peroral*	232±42
	0			234±30
	Vehicle	day 5–7 PI	peroral*	221±51

All groups of 5 rats each were infected with 500 (Exp. 1) or 400 (Exp. 2) third stage larvae, and killed at day 8 PI.
*Cream solution was administered; †Injectable solution was administered; ‡Significant difference from non-treated control, $P<0.001$.

and could not reach the intestine of the rat. The number of adult worms recovered at day 8 PI from the drug administered rats (376±56) was not significantly different from that of the control (399±40). This result suggests that PF1022A can not affect *N. brasiliensis* larvae in the lung in this experiment.

In contrast, PF1022A was orally administered to infected rats at a dose of 5 mg/kg/day for 3 days from day 5 PI, when *N. brasiliensis* larvae had developed to the adult and harbored in the intestine of the rat, no adult worm was found in rats at day 8 PI. Comparing with large number of adult worms in the control, a complete treatment to the adult worm was achieved by PF1022A. The effective dose of PF1022A was also examined (Table 1, Exp. 2). In rats administered orally with PF1022A of 2.5 mg/kg/day for 3 days from day 5 PI, almost complete elimination of adult worms was observed. Four worms were detected from only one out of 5 rats. However, in rats with a dose of 0.6 mg/kg/day, the number of worms harbored in these rats (232±42) was comparable to the control (234±30). The number of worms in rats with vehicle administration was not significantly different from that of non-treated control.

Effects of PF1022A on H. nana *in mice*

One group of infected mice was administered orally with PF1022A at 10 mg/kg/day for 2 days from day 5 PI when the eggs of *H. nana* had developed into cysticercoids in the intestinal mucosa. Another group of mice was administered similarly for 2 days from day 10 PI when cysticercoids had become mature adults and dwelled in the lumen of intestine. The effects of PF1022A were determined at day 14 PI by counting scoleces of *H. nana* in the intestine of mice. As shown in Table 2, no significant difference in worm recovery was found among these groups of mice. These results indicated that neither the cysticercoid nor the adulthood of *H. nana* was sensitive to PF1022A.

Table 2 Effects of PF1022A on larval and adult *Hymenolepis nana* in mice

Administration of PF1022A*		No. of adults/mouse (Mean±SD)
Dose (mg/kg/day)	Timing	
0		27±9
10	day 5–6	24±8
10	day 10–11	31±10
Vehicle	day 5–6 & 10–11	27±9

All groups of 4 mice each were infected with 100 eggs, and killed at day 14 PI.
*PF1022A was given in oral cream.

Discussion

In the experiments on *N. brasiliensis* in rats, PF1022A exerted strong antinematode activity on

the adult worms in the infected rats with oral administration at a dose of 2.5 mg/kg/day. This result confirmed the previous reports showing efficacy of PF1022A in the treatment of several species of intestinal nematodes in various animal hosts. Each nematode had different sensitivity to the drug when it was used with lower doses. As found in the present experiments, the effect was not obvious at a dose of 0.6 mg/kg to *N. brasiliensis* in rats. However, when PF1022A was orally administered with 0.5 mg/kg for *Ascaridia galli* in chickens or with 0.6 mg/kg for *Trichinella spiralis* in mice, the cure rate was 24% (Sasaki *et al.* 1992) and 85% (Wang *et al.* 1994), respectively. This difference might be caused by the variation of uptake and/or sensitivity to the drug in each nematode. With higher doses such as 2.0–2.5 mg/kg, complete elimination of these three nematodes from the hosts was achieved. The results implicated that PF1022A was applicable for treatment of intestinal nematodes with higher doses.

Comparing with adults of intestinal nematodes, the effect of PF1022A on developing larvae in the tissue was uncertain. Generally larval stages of nematodes seem to have lower sensitivity to PF1022A. In the present experiments, no effect could be observed on *N. brasiliensis* larve in the lung. Intraperitoneal injection with present formulation of PF1022A may not reach at high enough concentration for affecting the larvae in local tissues. It is also possible that the larvae of *N. brasiliensis* could not accept the drug by their morphological structure or biochemical systems. Terada (1993) reported that complete elimination of larvae of *Angiostrongylus costaricensis* was achieved by 5 successive doses of PF1022A at 10 mg/kg/day po or 0.625 mg/kg/day ip. This satisfactory result may be attributed to longer treatment or to the contact of the drug with high concentration to *A. costaricensis* larvae in the mesenteric arteries of mouse. The same treatment was also effective against larval and adult *A. cantonensis* in rats (Kachi *et al.* 1995). Wang *et al.* (1994) demonstrated that partial effect was obtained on muscle larvae of *T. spiralis* with 3 successive daily doses of PF1022A such a high dose of 20 mg/kg via intraperitoneal route. Relatively low concentration of the drug in the muscle might be responsible for the compromised treatment of muscle larvae. Therefore, the effect of PF1022A in the treatment of adults and larvae of nematodes may be determined by the concentration of the drug in the intestine and tissues of the host. It has been shown *in vitro* study by Terada (1992) that PF1022A exhibited its anthelmintic activity by attacking directly to *A. cantonensis* in the form of original compound.

The experiment testing effects of PF1022A on *H. nana* in mice was conducted to extend the effect of PF1022A on cestodes dwelling in the intestine. Moreover, it has been searching for anticestode drugs without destruction of gravid proglottids to avoid the autoinfection by the leakage of the eggs in the case of *Taenia solium* in humans. Unfortunately, PF1022A had no anticestode activity on *H. nana* in our experiment.

The different susceptibility between nematodes and cestodes to PF1022A may be explained by regulatory mechanisms of neuro-muscular systems of these parasites. It has been demonstrated that neuro-transmitters are acetylcholine as a inhibitor and serotonin as a stimulator in cestodes (Sukhdeo and Mettrick, 1987), and γ-aminobutyric acid (GABA) as a inhibitor and acetylcholine as a stimulator in nematodes (Geary *et al.* 1992). Terada (1992) reported that PF1022A induces paralysis in nematodes by synergistically stimulating the gabergic mechanism and inhibiting the cholinergic mechanism. As gabergic mechanism is found only in nematodes, a stimulation of gabergic mechanism by PF1022A may be a critical factor to exert different anthelmintic activity on nematodes and cestodes. Alternatively, it is also possible that cholinergic mechanism of nematodes is more susceptible to PF1022A than that of cestodes. However, latter possibility is unlikely from the experimental results that no effect of PF1022A with higher dose was obtained on *H. nana* in mice.

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