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Effects of PF1022A from *Mycelia sterilia* on *Trichinella spiralis* in Mice

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Abstract

PF1022A is a cyclic depsipeptide isolated from *Mycelia sterilia*. The effect of PF1022A was examined by *in vivo* experiments on adults and larvae of *Trichinella spiralis* in mice. Mice were perorally infected with muscle larvae of *T. spiralis*. In the treatment of adults, PF1022A was orally administered to infected mice for 3 successive days from day 3 post infection (PI) with doses of 10, 2.5, or 0.6mg/kg/day. Complete elimination of adult worms from the intestine was achieved at doses of 10 and 2.5mg/kg/day, and 85% of worms were eliminated by 0.6mg/kg/day. When the drug was intraperitoneally administered with a dose of 5mg/kg/day for day 3-5 PI, no significant effect was observed. In the treatment of muscle larvae, PF1022A at a dose of 20mg/kg/day was given intraperitoneally for 5 successive days to infected mice. A marked reduction (85%) of muscle larvae was obtained only when the administration of drug started at day 14 PI, but no reduction was observed when the administration of drug started at day 21 PI. These results indicate that PF1022A has anthelmintic activity to adults and larvae of *T. spiralis*.

Key words: PF1022A, chemotherapy, *Trichinella spiralis*, muscle larvae, 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). This drug has been examined as an anthelmintic drug. For practical use of this drug, it is necessary to accumulate more detailed informations such as the susceptibility of helminth species and stages of their life cycles, dose effect and tissue distribution in the host with relationship to the routes of administration. The effect of PF1022A has been found in some nematode parasites including *Heterakis spumosa* (Terada *et al.*, 1991), *Angiostrongylus cantonensis* (Terada, 1992), *Angiostrongylus costaricensis* (Terada *et al.*, 1993) and *Ascaridia galli* (Sasaki *et al.*, 1992) in *in vitro* or *in vivo* experiments. Most of the studies on PF1022A were performed on parasites of animals.

In the present study, we selected *Trichinella spiralis* infection in mice as a model. *T. spiralis* shares the same life cycle in humans and mice, and has two distinct parasitic stages, namely adult stage in the intestine and larval stage in the muscle of the host. The experiments were carried out to examine the effect of PF1022A on both stages of *T. spiralis* with different routes of administration.

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 contains the solvent component without PF1022A, was also prepared. The drug and vehicle were diluted with saline to appropriate concentrations needed for administration to mice.

Animal

Most of the experiments were performed with 5 week old female ddy mice purchased from Japan SLC (Shizuoka, Japan). In the experiments on dose

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effect of PF1022A, inbred SJL/J mice, purchased from Ohmura Laboratory Animals (Kanagawa, Japan), were used.

Infection

Mice were orally inoculated with muscle larvae of *T. spiralis* (Polish strain) using stomach tube under ether anesthesia. Four hundred or 200 larvae for experiments of adult stage and 100 larvae for larval stage were inoculated, respectively.

Experimental design

In the experiments on dose effect to adult *T. spiralis*, mice were orally administered with PF1022A oral cream at doses of 10, 2.5 or 0.6mg/kg/day for 3 consecutive days starting at day 3 PI, respectively. In the experiments for the effect of vehicle, one group of mice were orally administered at a dose of 2.5mg/kg/day PF1022A, the other group of mice were administered orally with vehicle alone according to the same schedule as the experiments of dose effect. In the experiments for the route of administration, mice were administered with PF1022A oral cream intraperitoneally (ip) at a dose of 5mg/kg/day for 3 days starting at day 3 PI. *T. spiralis* infected mice without administration of PF1022A were used as the control in each experiment. On day 6 PI, mice were killed with ether for the recovery of the adult worms, their small intestines were removed and opened longitudinally with scissors, and then incubated in saline at 37°C for 2–3 hours with occasional rinsing. After incubation, the intestines were removed from the culture tubes. The number of adult worms was counted in the sediment part of the incubated solution under microscope.

In the experiments of muscle larvae of *T. spiralis*, mice were administered ip with injectable solution of PF1022A 20mg/kg/day or vehicle for 5 days starting at day 14 PI, respectively. Other mice were also administered ip with PF1022A 20mg/kg/day or vehicle for 5 days starting at day 21 PI. The control mice received neither PF1022A nor vehicle. At day 28 PI, mice were sacrificed and skinned. After removal of visceral organs, the remaining tissues were cut into small pieces with scissors and digested in 200 ml of 0.5% pepsin at 37°C for 2–4hr until the pieces of muscle were not detectable. The larvae were collected by sedimentation in tubes and suspended in 2ml of saline. The number of larvae was counted under microscope in five samples from each suspension (Watanabe *et al.*, 1988). All data were statistically analyzed by Student's t-test.

Results

Effect of PF1022A on T. spiralis *adults*

Representative result on dose effect of PF1022A

Table 1 Effect of PF1022A on *T. spiralis* adults

Exp.	Route of administration	No. of mice	Dose of PF1022A (mg/kg/day)	No. of adults/mouse (mean±SD)
1	Peroral	5	10.0	1±2*
	Peroral	5	2.5	0±0*
	Peroral	4	0.6	20±23*
	Peroral	5	0	138±27
2	Peroral	5	2.5	0±0*
	Peroral	5	0	97±14
	Peroral	5	Vehicle	102±23
3	Intraperitoneal	5	5.0	12±12
	Intraperitoneal	5	0	9±11

Mice were orally infected with 400 muscle larvae in Exp. 1 and 2 or 200 in Exp. 3. These mice were administered for 3 days with PF1022A oral cream from day 3 PI when the larvae had just developed into adult stage. Mice were killed to recover adult worms on day 6 PI when the number of adults had reached a maximum in the intestine of mouse.

*Significant difference from non-treated control ($P<0.01$ or $P<0.001$).

on *T. spiralis* adult was shown in Table 1. Nearly complete elimination of adult worms was achieved in mice administered orally with 10mg/kg/day and 2.5mg/kg/day of PF1022A. In these groups, no worm was found in four out of five mice. Five adults were harbored in one mouse from 10mg/kg group and one adult in one mouse from 2.5mg/kg group. In mice administered with PF1022A 0.6mg/kg, 20 adult worms were recovered. However, the number of worms in mice with PF1022A 0.6mg/kg was significantly lower than that of the control. The cure rate of 0.6mg/kg group was 85%. PF1022A at a dose of 0.6mg/kg had effect to adult worms but not satisfactory. To confirm the antinematode activity of PF1022A, the effect of vehicle was examined (Table 1). The number of worms recovered from mice with vehicle was not significantly different from that of the control mice, excluding the involvement of vehicle to eliminate *T. spiralis* adults.

Route of administration was examined in Exp. 3 (Table 1). PF1022A was administered through intraperitoneal route with oral cream as used in oral route. No obvious effect was observed even at a dose of 5mg/kg. These results suggest that administration of PF1022A for the elimination of adult *T. spiralis* is preferred to oral route and is not recommended through intraperitoneal route.

Effect of PF1022A on T. spiralis *larvae*

Effect on *T. spiralis* larvae was determined by collection of muscle larvae from mice on day 28 PI (Table 2). In the case of PF1022A administration for 5 days with 20mg/kg/day starting at day 14 PI, a marked decrease of larvae (85%) was observed. The number of larvae recovered from this group was significantly lower than that of non treated control. Whereas, administration of PF1022A did not result in complete elimination of muscle larvae. On the other hand, in mice with administration of drug starting at day 21 PI at the same dose (20mg/kg/day) and route (ip), no reduction of recovered larvae was observed. As the control of these results, effect of vehicle was examined. The number of larvae from mice administered ip with vehicle for 5 days starting either at day 14 PI or day 21 PI was comparable to the control. Therefore, no treating activity of vehicle was obtained.

Discussion

Efficacy of PF1022A has been reported in many nematodes, especially in the treatment of intestinal parasites in wide range of hosts such as *Ascaridia galli* in chickens (Sasaki *et al.*, 1992), *Toxocara canis* and *T. cati* in dogs and cats (Fukashe *et al.*, 1990), *Haemonchus contortus* and *Ostertagia ostertagi* in cows and horses (Kurosawa *et al.*, 1992). As the treatment of tissue nematode, effect of PF1022A has also been found in *A. costaricensis* (Terada *et al.*, 1993) and *A. cantonensis* (Kachi *et al.*, 1994). The present experiments demonstrated that susceptibility of PF1022A was expanded to adult and larval stages of *T. spiralis*.

Concerning adult *T. spiralis* in the intestine of mouse, the administration of PF1022A by oral route resulted in a complete elimination of worms from the intestine at a dose of 2.5mg/kg and a marked treatment at 0.6mg/kg. However, intraperitoneal

Table 2 Effect of PF1022A on *T. spiralis* larvae

No. of mice	Administration of PF1022A			No. of larvae/mouse (mean±SD)
5	0			3692±687
5	Vehicle	day	14–18	3864±2187
4	20mg/kg/day	day	14–18	565±131*
5	Vehicle	day	21–25	4412±1337
5	20mg/kg/day	day	21–25	3548±2470

In this experiment, treated groups were administered with injectable solution of PF1022A. Mice were killed to collect muscle larvae on day 28.

*Significant difference from non-treated control ($P<0.001$).

administration of the drug resulted in no effect even in a dose of 5mg/kg which was two times higher than the dose achieved complete treatment by oral route. Therefore, oral route is superior to intraperitoneal one in the treatment of adult *T. spiralis* by PF1022A. The results of oral administration may attribute to direct action of the drug to the worms dwelling in the intestine of the host. In intraperitoneal route, as the drug may be distributed to the tissues of the host or may not be released into the intestine, the concentration of drug in the intestinal lumen becomes low. The striking effect of PF1022A on intestinal nematode *Ascaridia galli* in chickens by oral administration has also been reported by Sasaki *et al.* (1992). 24% of the worms were expelled at a dose of 0.5mg/kg, and nearly 100% of the worms were eliminated at a dose of 2.0mg/kg. These results also confirmed the superiority of oral administration of PF1022A in the treatment of lumen dwelling nematodes. For the treatment of intestinal parasites, it may be a preferable choice to give the drug by oral route because of its convenience and easiness.

The study of PF1022A on tissue dwelling nematodes was relatively limited. It has been demonstrated that PF1022A had effect on developing larvae of *A. costaricensis* parasiting in the mesenteric arteries of mouse (Terada *et al.*, 1993). On the effect of PF1022A on *T. spiralis* larvae, the positive result was obtained according to the timing of administration of the drug. Treating effect was observed only when the administration of the drug started at day 14 PI, but not at day 21 PI. The sensitivity of *T. spiralis* larvae to PF1022A had changed during the lapse between 14 days and 21 days PI. Although we do not know the exact mechanism of such changes, it may be corresponding to changes in the structure or biochemical mechanism of larvae during their development and in surrounding host tissues. It is known that after infection with *T. spiralis* larvae, the worms become mature in the intestine of the host and begin to produce larvae on day 5 PI. The larvae migrate via the blood to the striated muscle and are surrounded by a thick cyst after about 20 days PI (Waklin, 1988). It might be possible that PF1022A could not reach to *T. spiralis* larvae surrounded by a thick cysts which was formed by 3 weeks PI. Therefore, the larvae within the cysts could escape from the attack of the drug and survive. Akyol *et al.* (1993) also reported poor effect of PF1022A in the treatment of *A. cantonensis* in the brain of rats and attributed their result to that the drug could not pass through the blood-brain barrier.

Even when PF1022A was administered ip from day 14 PI at a dose of 20mg/kg/day for 5 days, only partial effect on *T. spiralis* larvae was obtained. In the treatment of developing larvae of *A. costaricensis* a complete effect was achieved by intraperitoneal administration with PF1022A 0.625mg/kg (Terada *et al.*, 1993). The different sensitivity to the drug between these species of nematode larvae was striking. It may be explained that *A. costaricensis* larvae dwell in the mesenteric arteries of the host where the drug can be maintained in a certain concentration being high enough to act on the parasite. On the other hand, high concentration of drug may not be obtained in the muscle where *T. spiralis* larvae are harboring. It is also possible that susceptibility to PF1022A is different between these nematodes.

Finally, Terada (1992) demonstrated that the anthelmintic activity of PF1022A induced the paralysis of nematoda by stimulating the gabergic mechanism and inhibiting the cholinergic mechanism of the worms. It has been proposed that gabergic mechanism plays a role as the inhibitory neurotransmitter and cholinergic mechanism as the primary excitatory neurotransmitter in the body wall muscle of nematodes (Geary *et al.*, 1992). This may illustrate the major antinematode effect of PF1022A.

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