Effects of Amorphous and Polymorphs of PF1022A, a New Antinematode Drug, on *Angiostrongylus costaricensis* in Mice

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ABSTRACT—To enhance the bioavailability of PF1022A (cyclo(D-lactyl-L-*N*-methylleucyl-D-3-phenyllactyl-L-*N*-methylleucyl-D-lactyl-L-*N*-methylleucyl-D-3-phenyllactyl-L-*N*-methylleucyl)), a newly developed antinematode drug, we examined whether the new drug has polymorphism or not. First, four forms of PF1022A, designated as form α, form I, form II and form III of PF1022A, were prepared. By examining physicochemical properties of these forms by various methods including X-ray powder diffractometry and differential scanning calorimetry, it became apparent that PF1022A had one amorphous (form α) and three crystalline polymorphic forms, form I, form II and form III. Secondly, a dissolution study was carried out, and form α and form III were found to have higher solubility than form I and form II. Thirdly, anti-larval effects of the 4 forms of PF1022A on tissue-dwelling nematode, *Angiostrongylus costaricensis*, in mice were compared when given orally for 5 successive days at 10 or 40 mg/kg/day. Significant effects were observed in almost all parameters in host mice and worms in the groups treated with form α or form III, each at 40 mg/kg, but form I and form II had little effect. The present results suggest that PF1022A has polymorphism and that the form α and form III were more effective against tissue-dwelling nematodes than the form I and form II when given orally.

Keywords: PF1022A, Anthelmintic, Polymorph, Amorphous, *Angiostrongylus costaricensis*

Development of new antinematode drugs is an urgent matter because many nematode worms have become resistant against traditional anthelmintic drugs including benzimidazole derivatives and macrocyclic lactone derivatives (1, 2). PF1022A (cyclo(D-lactyl-L-*N*-methylleucyl-D-3-phenyllactyl-L-*N*-methylleucyl-D-lactyl-L-*N*-methylleucyl-D-3-phenyllactyl-L-*N*-methylleucyl)) is a new antinematode anthelmintic developed in Japan, which has a unique cyclic depsipeptide structure (Fig. 1) (3). The drug was effective against various intestinal nematodes in vivo (3, 4). It is an epoch-making discovery that PF1022A paralyzes nematode worms synergistically by stimulating the GABAergic mechanism and inhibiting the cholinergic mechanism (5). Much attention has been paid to determining the safety of PF1022A, by assessing its medical lethal doses (LD_{50}) (1), and it has also been determined that little of the drug can pass through the blood-brain barrier (6-8).

Using our experimental models for studying drug effects on tissue-dwelling nematodes, we have showed that PF1022A was effective on adult and larval stages of tissue-dwelling nematodes (4, 9). To increase the efficacy of drugs against tissue-dwelling parasites, improvement in drug absorption from the intestinal tract of host animals, which is related closely to higher bioavailabil-

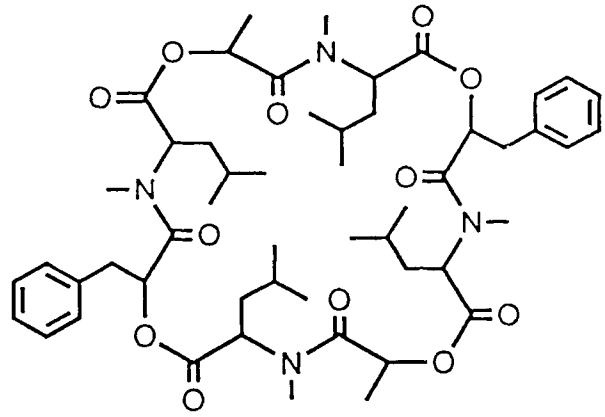

Fig. 1. Chemical structure of PF1022A.

ity, is very important. As one of such strategies, we devised a drug formulation in our previous studies (9–11), and we showed that the emulsified formulation, the oral cream, had the strongest effects (9). However, our formulation may have some practical limitations for use in extensive PF1022A field studies because of some problems such as difficulty in preparation and in administration, instability in storage, and toxicity of additives in preparing the formulation.

Therefore, to improve the bioavailability of PF1022A, we tried another strategy in the present study. We prepared 4 forms of PF1022A and examined whether it has polymorphism. Because PF1022A was found to have one amorphous and three polymorphic forms, we compared the anti-larval effects of these 4 forms on *Angiostrongylus costaricensis* in mice, one of our experimental models for studying drug effects on tissue-dwelling nematodes.

MATERIALS AND METHODS

Materials

PF1022A bulk sample was supplied from Meiji Seika Kaisha, Ltd. (Tokyo), and other drugs used were obtained from the following sources: sodium laurylsulfate (SLS) for in vitro use, KH_2PO_4, NaOH and solvents used (Junsei Chemical Co., Tokyo); lactose (Gokyo Trading Co., Osaka); SLS for in vivo use (EMAL 0; Kao Corp., Tokyo); hydroxypropylmethylcellulose 2208 (HPMC) (Metolose 90SH-100; Shin-etsu Chemical Co., Tokyo); pepsin (Wako Pure Chemical Ind. Co., Osaka).

Preparation of 4 forms of PF1022A

The amorphous form and crystal forms were prepared as follows. First, PF1022A bulk was dissolved in methanol at 50℃. Excess water was added in the solution while it was being sonicated. This suspension was cooled and filtered. The residue retained on the filter was dried in vacuo. The amorphous form obtained was designated as form α. Secondly, PF1022A bulk was dissolved in ethyl acetate at 50℃ and cooled. This suspension was filtered. The residue remaining on the filter was dried at room temperature in air. The crystal form obtained was called form I. Thirdly, PF1022A bulk was dissolved in methanol at 50℃. One-hundredth volume of water was added to this solution with cooling. While still being cooled, this suspension was filtered. The residue retained on the filter was dried in vacuo. The crystal form obtained was designated as form II. Finally, PF1022A bulk was dissolved in acetone at 50℃. Excess hexane was added to the solution. This suspension was cooled and filtered. The residue on the filter was dried in vacuo. The crystal form obtained was called form III.

Physicochemical properties of 4 forms of PF1022A

All forms obtained from PF1022A bulk were classified by X-ray powder diffraction analysis, thermal analysis, measurement of the melting point, polarized microscopy and measurement of particle size.

X-ray powder diffraction analysis was performed at room temperature with an X-ray powder diffractometer (RINT2000; Rigaku Ind. Corp., Osaka). The operating conditions were as follows: target, Cu; filter, Ni; voltage, 40 kV; current, 40 mA; receiving slit, 0.15 mm; scanning speed, 4° 2θ/min.

Thermal analysis was performed with a differential scanning calorimetry (DSC) (TAC 7/DK; Perkin Elmer Co., Norwalk, CT, USA). The operating conditions in the closed-pan system were as follows: sample weight, 5–10 mg; heating rate, 5℃/min; N_2 gas pressure, 2 kg/cm^2.

Melting point was measured with a thermal system (Thrmo System FP90/82HT; Mettler-Toledo Co., Greifensee, Switzerland) at 1℃/min heating rate.

Polarized microscopic observation was performed with a polarized microscope (D7082; Carl Zeiss Co., Oberkochem, Germany).

Average particle size was measured with a particle size analyzer (SK Laser Micron Sizer PRO-7000; Seisin Enterprise Co., Tokyo).

Dissolution behavior of amorphous and polymorphic PF1022A

The dissolution behavior of each form was studied by the rotating paddle method. The apparatus used was a Riken's Dissolution Tester (Miyamoto Riken Ind. Co., Osaka) as the same as described in The Pharmacopeia of Japan XIII. The dissolution medium was prepared as follows: One hundred and eighteen milliliters of 0.2 M NaOH was added to 250 ml of 0.2 M KH_2PO_4 containing 2 g of SLS, and the mixture was diluted with purified water to 1000 ml. Nine hundred milligrams of 5% of each form mixed with lactose (PF1022A content: 45 mg) was introduced into 900 ml thermostated dissolution medium (40±1℃). The rotation speed of a paddle was adjusted to 200 rpm. The aliquots of samples were withdrawn at different time intervals and filtered through a Milipore filter with 0.45-μm pore size. The concentration of PF1022A dissolved in the filtrate was determined by the HPLC method. A Jasco HPLC system equipped with an 880-PU Pump, 875-UV Detector, 850-AS Sampler, 801-SC System Controller (Japan Spectroscopic Co., Tokyo) and C-R4A Integrator (Shimadzu Corp., Kyoto) was used. The operating conditions were as follows: column, STR ODS-H (150 mm × 4.6 mm i.d.); detection, 217 nm; mobile phase, 75% acetonitrile; flow rate, 1.2 ml/min; injection volume, 10 μl.

Anti-larval effects of amorphous and polymorphic PF1022A on Angiostrongylus costaricensis in mice

Male ddY mice, 5 weeks of age, were used as the final host. Infective third-stage larvae of *Angiostrongylus 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℃ . Each mouse was inoculated orally with 10 infected larvae of *A. costaricensis*.

Each form of PF1022A was mixed with SLS at a ratio of 4 to 1 for oral treatment at 10 mg/kg or at a ratio of 16 to 1 for oral treatment at 40 mg/kg. Then the mixture was suspended in 0.5% HPMC solution just before administration.

Nine infected groups of seven mice each and one group of seven mice without infection were used. Beginning at 6 days post-infection (p.i.), eight infected groups were treated orally with five successive daily doses of PF1022A of each form, form α, form I, form II or form III, at 10 mg/kg/day and 40 mg/kg/day each, respectively. One infected group and one non-infected group were given the vehicle and served as the non-treated and non-infected controls, respectively.

All surviving mice were killed at 40 or 41 days p.i. by a overdose of diethyl ether and dissected. The following parameters in the host were examined: changes in host body weight, number of surviving mice, the relative wet weight (expressed in grams per 100 g body weight) of the intestine and spleen, the relative area (expressed in square millimeters per gram body weight) of the mesenteric node, the hemoglobin content (Hb) and the hematocrit (Ht). The following parameters in the worms were examined: number of worms recovered, worm body length, detection of the first-stage larvae in feces and distribution of the first-stage larvae and eggs in the terminal ileum. All parameters except hematologic values and worm body length were examined according to the methods described by Terada et al. (9). The Hb and Ht values were determined by the CN-methemoglobin method and micromethod with capillary tubes, respectively. The worm body length was estimated from photographs by using a computerized image analyzer (Videoplan; Kontron Co., Munich, Germany). Significant differences between the values obtained were analyzed by Student's *t*-test or Aspin-Welch's *t*-test after doing the *F*-test.

RESULTS

Physicochemical properties of 4 forms of PF1022A

X-ray powder diffraction analysis: The X-ray powder diffraction profile of each form is shown in Fig. 2. Form α did not show a diffraction peak but showed a halo pattern. Form I showed a main diffraction peak at 6.0° (2θ) and another two peaks at 6.2° (2θ) and 6.5° (2θ). Form II showed a main diffraction peak at 14.6° (2θ) and another two peaks at 9.2° (2θ) and 9.9° (2θ). Form III showed a main diffraction peak at 6.3° (2θ) and another two peaks at 7.3° (2θ) and 11.2° (2θ).

Thermal analysis and melting point: The DSC curve of each form is shown in Fig. 3. Form α showed no peaks, and form I showed an endothermic peak at 173℃. Form II showed an endothermic peak at 135℃, and form III showed a broad and weak endothermic peak from 99℃ to 105℃.

Melting points of form α, form I, form II and form III were 100–103℃, 170–173℃, 130–134℃ and 102–105℃, respectively.

Polarized microscopic observation and particle size: Polarized microphotographs of each form are shown in Fig. 4. Form α aggregated with primary particles did not show polarization. Other forms showed polarization. Form I and form III presented needle-like crystals, and form II presented a plate-like crystal.

Average particle sizes of form α, form I, form II and form III were 9.6, 5.3, 29.0 and 5.0 μm, respectively.

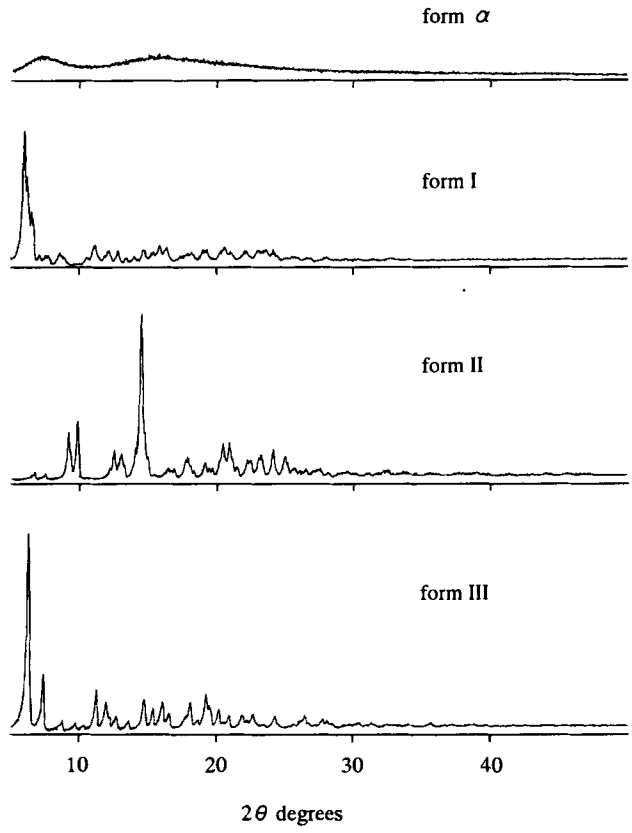

Fig. 2. X-ray diffraction patterns of form α, form I, form II and form III of PF1022A.

Dissolution behavior of amorphous and polymorphic PF1022A

The dissolution curve of each form is shown in Fig. 5. Maximum concentrations of form α, form I, form II and form III were 49.2±0.58, 22.0±0.99, 29.1±0.89 and 49.2±0.03 μg/ml, respectively. Form α, form I and form III dissolved immediately to almost maximum concentration level within 30 min, but form II dissolved gradually and the concentration at 30 min was 9.0±0.18 μg/ml, which was about one-third of the maximum concentration. Though form I dissolved immediately, its maximum concentration was lower than those of form α and form III. Final concentrations of form α, form I, form II and form III of at 24 hr were 49.2±0.58, 14.4±0.48, 29.1±0.89 and 42.6±0.18 μg/ml, respectively.

Anti-larval effects of amorphous and polymorphic PF1022A on Angiostrongylus costaricensis in mice

Parameters in host mice (Table 1): Changes of host body weight are shown in Fig. 6. Mean body weight in the non-infected mice kept increasing during the experiment and was 37.3±1.3 g at dissection. The weight of the non-treated control group gradually decreased after around the 22nd day p.i., and the weights of the surviving mice at dissection was 30.9±0.5 g. The weight of two groups treated with form α and form III at 40 mg/kg kept increasing, and the values at dissection were significantly larger than that of the non-treated control group. The weights of the other groups were gradually decreased after around the 18th to 25th days p.i., and the values at dissection were not significantly different from that of the non-treated control group.

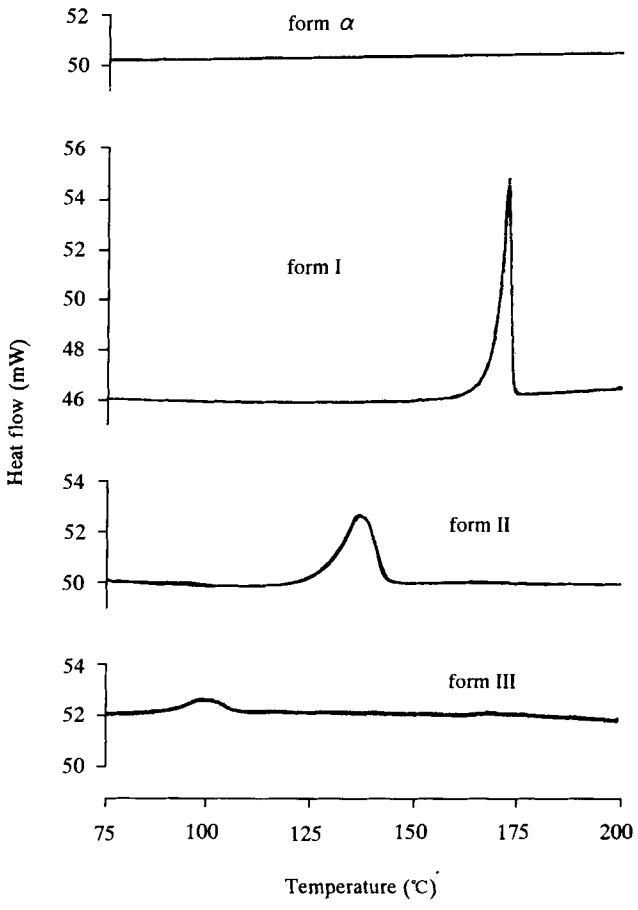

Fig. 3. DSC curves of form α, form I, form II and form III of PF1022A. Sample weight of form α, form I, form II and form III were 5.85, 6.03, 9.88 and 5.69 mg, respectively.

Two mice in the non-treated group died 22, 32 days p.i. In groups treated with form I, two mice treated with 10 mg/kg died 27, 32 days p.i. and two mice treated with 40 mg/kg died 29, 36 days p.i. One mouse treated with form II at 10 mg/kg died 36 days p.i. None of the mice died in the other treated groups.

As compared with the mean value obtained in the non-infected control mice, the relative wet weight of the spleen (g/100 g body weight) increased 5.7 times in the non-treated control. The relative weight of the spleen in the two groups treated with form α at 10 mg/kg and 40 mg/kg significantly decreased compared with that in the non-treated control. But no significant difference in the spleen relative weight was observed between the other treated groups and the non-treated control.

As compared with the mean value obtained in the non-infected control, the relative wet weight of the intestine increased 2.1 times in the non-treated control. The relative weight of the intestine in the two groups treated with form α and form III at 40 mg/kg significantly decreased compared with that in the non-treated control. However, no significant difference in the intestine relative weight was observed between the other treated groups and the non-treated control.

The mean value of the relative area (mm^2/g body weight) of the mesenteric node was 2.79±0.28 in the non-treated control and the value was 8.2 times larger than that in the non-infected control. The relative area of the mesenteric node in the two groups treated with form α and form III at 40 mg/kg significantly decreased compared with that in the non-treated control. However, no significant difference in the relative area of the mesenteric node was observed between the other treated groups and the non-treated control.

As compared with the Hb value of 15.1±0.1 g/dl and the Ht value of 42.9±0.6% measured in the non-infected control, these parameters decreased significantly in the non-treated control. The Hb value in the two groups treated with form α and form III at 40 mg/kg and the Ht value in the group treated with form α at 40 mg/kg significantly increased compared with the respective values

in the non-treated control. However, no significant difference in Hb value and Ht value was observed between the other treated groups and the non-treated control.

Parameters in worms (Table 2): The mean number of worms recovered from the non-treated control group was 6.8 ± 0.4. No worm was recovered from the group treated with form α at 40 mg/kg. Significant reduction in recovered worms was observed in the three groups treated with form III at 10 mg/kg and 40 mg/kg and with form II at 40 mg/kg. In the group treated with form α at 10 mg/kg, the number of worms recovered was reduced but

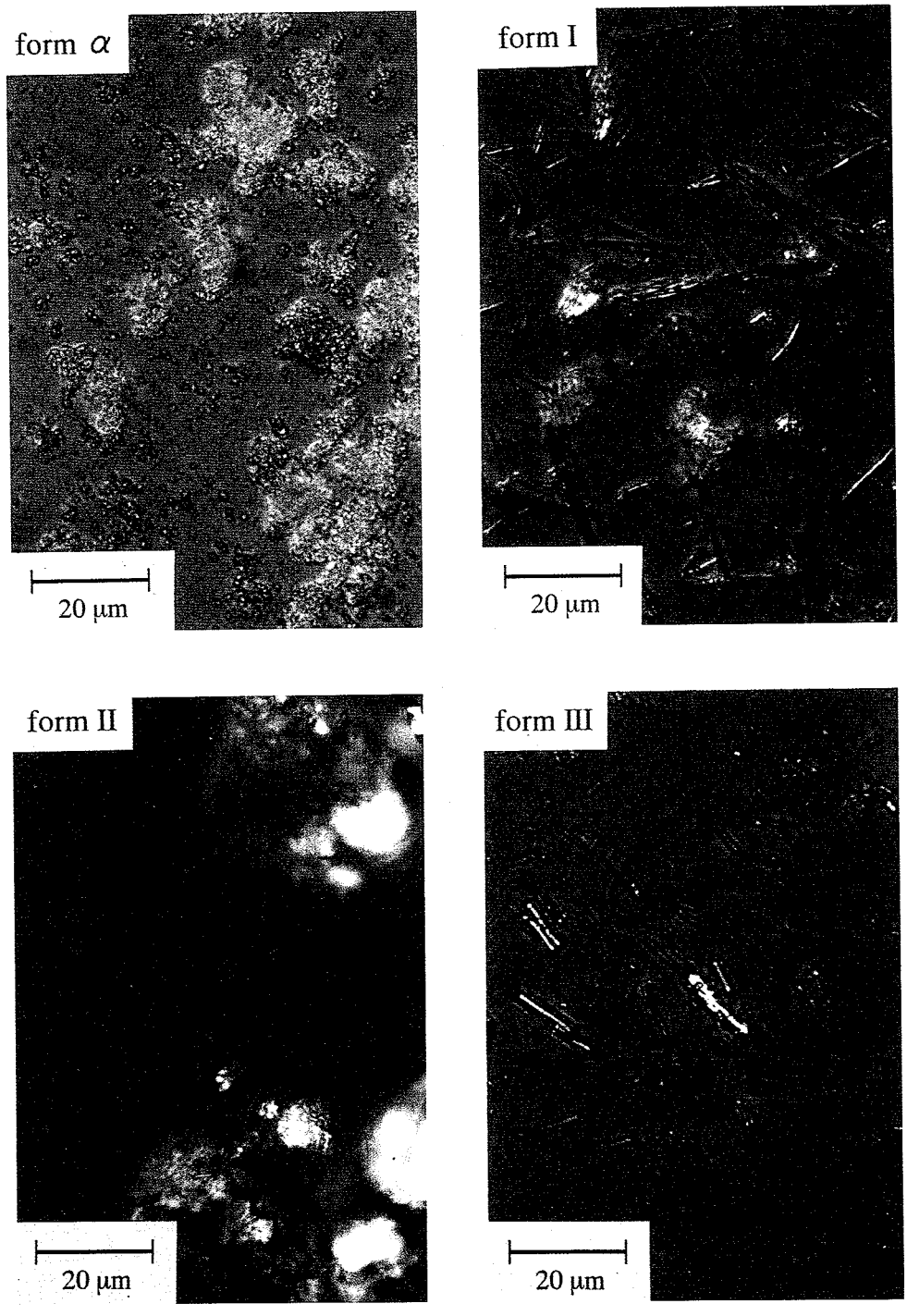

Fig. 4. Photomicrographs of form α, form I, form II and form III of PF1022A.

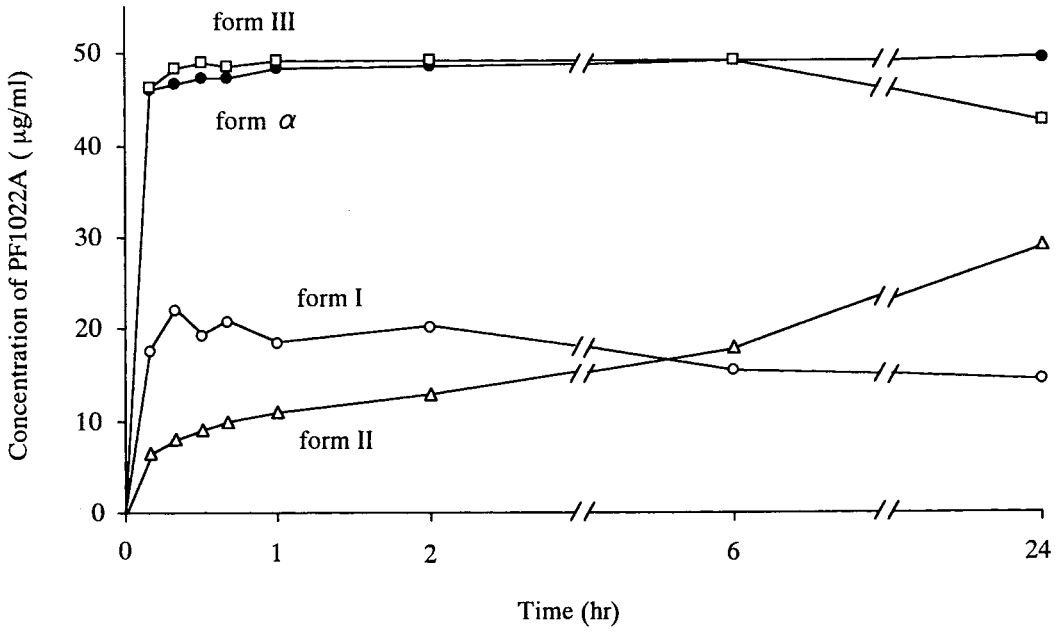

Fig. 5. Dissolution curves of one amorphous form, form α, and three polymorphs, form I, form II and form III of PF1022A in phosphate buffer (pH 6.8) containing 0.2% sodium laurylsulfate at 40℃ by the rotating paddle method. A 900-mg sample of 5% of each form mixed with lactose (PF1022A content: 45 mg) was introduced into 900 ml dissolution medium.

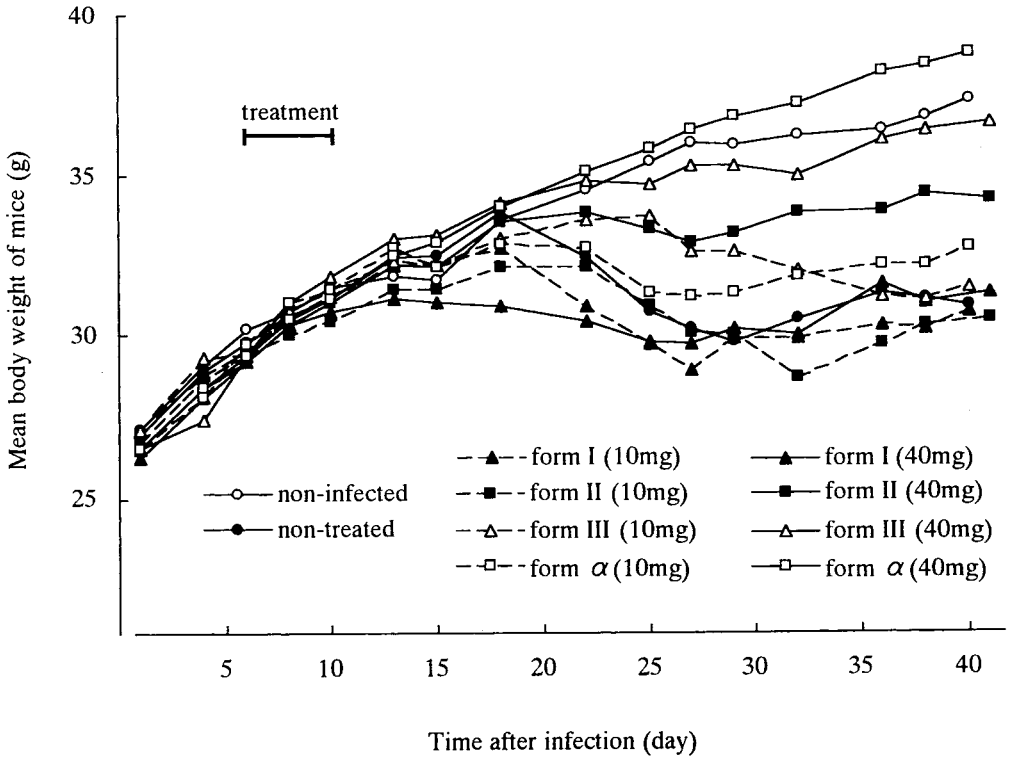

Fig. 6. Changes in the mean body weight of surviving mice treated orally with five successive daily doses of one amorphous form, form α, and three polymorphs form, form I, form II and form III, of PF1022A at 10 mg/kg or 40 mg/kg.

Table 1. Effects of PF1022A orally given at 10 and 40 mg/kg/day for 5 successive days on angiostrongyliasis costaricensis in mice with special reference to polymorphism: parameters in host mice at dissection

Parameters	Non-treated control	Polymorph						Amorphous		Non-infected control
		Form I		Form II		Form III		Form α		
		10 mg/kg	40 mg/kg	10 mg/kg	40 mg/kg	10 mg/kg	40 mg/kg	10 mg/kg	40 mg/kg	
No. of survivals/total	5/7	5/7	5/7	6/7	7/7	7/7	7/7	7/7	7/7	7/7
Body weight (g)	30.9±0.5	30.7±0.5	31.3±0.6	30.5±1.3	34.2±1.2*	31.5±1.1	36.6±1.6*	32.7±1.7	38.8±1.0**	37.3±1.3**
Relative wet weight (g/100 g body weight)										
Spleen	1.83±0.19	1.62±0.25	1.63±0.13	1.58±0.19	1.48±0.22	1.84±0.17	1.19±0.29	1.20±0.18*	0.44±0.06**	0.32±0.02**
Intestine	18.99±1.18	18.69±0.83	20.76±2.81	19.00±1.49	15.79±1.61	16.61±1.38	13.08±1.03**	16.22±1.63	9.88±0.23**	9.21±0.37**
Relative area (mm^2/g body weight) of mesenteric node	2.79±0.28	3.05±0.12	3.06±0.22	2.62±0.20	2.02±0.31	2.33±0.24	1.56±0.34*	2.48±0.38	0.95±0.14**	0.34±0.01**
Hematologic values										
Hb (g/dl)	8.40±0.94	10.33±1.07	8.85±0.66	10.03±0.54	9.83±1.29	7.62±0.98	11.74±0.93*	10.49±1.22	14.74±0.21**	15.11±0.14**
Ht (%)	28.6±2.52	33.2±1.88	29.4±1.71	33.8±1.08	31.6±2.74	26.4±3.06	35.4±1.94	32.4±2.53	41.7±0.56**	42.9±0.64**

Results are presented as the mean±S.E. of surviving mice. Significant difference from the non-treated control: *$P<0.05$, **$P<0.01$.

Table 2. Effects of PF1022A orally given at 10 and 40 mg/kg/day for 5 successive days on angiostrongyliasis costaricensis in mice with special reference to polymorphism: parameters in worms at dissection

Parameters	Non-treated control	Polymorph						Amorphous	
		Form I		Form II		Form III		Form α	
		10 mg/kg	40 mg/kg	10 mg/kg	40 mg/kg	10 mg/kg	40 mg/kg	10 mg/kg	40 mg/kg
No. of worms recovered									
Female	3.6±0.4	3.4±0.2	3.4±0.5	3.7±0.7	2.1±0.6	2.7±0.4	1.3±0.4**	2.7±0.8	0.0±0.0
Male	3.2±0.2	3.4±0.5	3.6±0.7	2.5±0.4	2.1±0.6	1.4±0.4**	0.6±0.3**	2.3±0.6	0.0±0.0
Total	6.8±0.4	6.8±0.4	7.0±0.8	6.2±0.4	4.3±0.8*	4.1±0.6**	1.9±0.6**	5.0±1.1	0.0±0.0
Worm length (mm) (No. measured)									
Female	31.1± 0.56 (15)	30.1± 0.51 (12)	29.2± 0.58*(12)	30.0± 0.67 (12)	30.0± 0.59 (12)	29.0± 0.44**(15)	29.7± 0.94 (6)	28.6± 0.42**(13)	—
Male	19.1± 0.29 (13)	18.3± 0.30 (11)	18.4± 0.31 (12)	17.7± 0.69 (11)	18.2± 0.33 (11)	18.4± 0.35 (9)	16.3± 0.48**(4)	18.1± 0.36* (10)	—
1st larvae in feces									
Detected	5/5	5/5	5/5	6/6	6/7	6/7	3/7	5/7	0/7
Not detected	0/5	0/5	0/5	0/6	1/7	1/7	4/7	2/7	7/7
Eggs in host intestine									
Many eggs with various stages including larvae	5/5	5/5	5/5	6/6	6/7	6/7	3/7	5/7	0/7
Many developing eggs without larvae	0/5	0/5	0/5	0/6	0/7	0/7	0/7	0/7	0/7
Few eggs without development	0/5	0/5	0/5	0/6	0/7	1/7	2/7	0/7	0/7
Not detected	0/5	0/5	0/5	0/6	1/7	0/7	2/7	2/7	7/7

Results are presented as the mean±S.E. of surviving mice. Significant difference from the non-treated control: *$P<0.05$, **$P<0.01$.

not significantly. In the other treated groups, few difference from the non-treated control was observed.

The mean body length of the worms recovered from the non-treated control group was 31.1±0.56 mm for females and 19.1±0.29 mm for males. For female worms, the length of worms from the three groups treated with form α and form III at 10 mg/kg and with form I at 40 mg/kg was significantly shorter than that from the non-treated control. For male worms, the length of the worms from two groups treated with form α at 10 mg/kg and form III at 40 mg/kg was significantly shorter than that from the non-treated control. In the other treated groups, few differences from the non-treated control were observed.

The first-stage larvae were observed in feces of all surviving mice in the non-treated control and in the three groups treated with form I at 10 mg/kg and 40 mg/kg and with form II at 10 mg/kg. No first-stage larvae were detected in the feces of one mouse each in the groups treated with form II at 40 mg/kg and with form III at 10 mg/kg, of four mice in the group treated with form III at 40 mg/kg, of two mice in the group treated with form α at 10 mg/kg, and of all mice in the group treated with form α at 40 mg/kg.

Many eggs with various stages including larvae were observed in the terminal ileum of all mice from which the first-stage larvae were detected in their feces. A few eggs without development were observed in the tissue sections of three mice from which no larvae were detected in their feces, whereas no eggs were observed in the tissue sections of the other such mice.

DISCUSSION

It is urgent matter to develop new antinematode drugs because many nematode species have become resistant against many traditional anthelmintic drugs including benzimidazole, macrocyclic lactones and pyrantel (1, 2).

PF1022A is one of the prospective drugs listed by Conder and Campbell (1). It has a unique cyclic depsipeptide structure (3). Terada showed by using *Angiostrongylus cantonensis* and the isotonic transducer method that PF1022A paralyzed worms synergistically by stimulating the GABAergic mechanism and inhibiting the cholinergic mechanism (5). In addition, PF1022A has been carefully investigated with respect to its safety as judged from the medical lethal doses (LD_{50}) (1) and its very low passage through the blood-brain barrier (6–8).

Indeed it has been shown that orally given PF1022A is strongly effective against various intestinal nematodes in vivo (3, 4). Against tissue-dwelling nematodes, however, the effects of orally given PF1022A were less than those of the drug given intraperitoneally (4, 9). It is suggested that the difference in efficacy is dependent on bioavailability which is influenced by various factors such as doses, drug formulation, route of administration and so on. If the bioavailability of PF1022A is enhanced by devising an appropriate drug delivery system such as drug formulation, the crystal polymorph and so on, the efficacy of the anthelmintic should also increase against tissue-dwelling nematodes. To obtain high efficacy of PF1022A against tissue-dwelling parasites, we devised drug formulations in our previous studies (9–11), and we showed that the emulsified formulation, the oral cream, had the strongest effects (9). In the present study, we tried another strategy using drug polymorphism.

Generally, drugs have an amorphous form and some polymorphic forms, and they have different physicochemical properties. Especially, aqueous solubility has a significant influence on the bioavailability of a drug (12, 13). If PF1022A also has amorphous and polymorphic forms, it may be useful for improving drug bioavailability.

The results of X-ray powder diffraction analysis, thermal analysis and polarized microscopic observation indicated that form α is amorphous, and form I, form II and form III are crystals with different structures. In thermal analysis, the endothermic peaks of form I and form II were observed at 173℃ and 135℃, respectively. Form III showed a broad and weak endothermic peak from 99℃ to 105℃. On the basis of these results, it was suggested that form I is the most stable form and form II is a meta-stable form among the three crystalline forms. Form III was thought to be the more unstable form. Generally, regarding the aqueous solubility, an amorphous form is more favorable than a crystal form, and an unstable form such as form III, which has lower lattice energy than a stable form, is favorable among the polymorphic forms. Indeed, in the present dissolution study of PF1022A, form α, being amorphous, showed the highest concentration (49.2±0.58 μg/ml) and form I, the most stable form, showed the lowest concentration (14.4±0.48 μg/ml) at 24 hr. Among the crystal polymorphic forms, form III showed about a three-fold higher concentration (42.6±0.18 μg/ml) than form I (14.4±0.48 μg/ml) at 24 hr. On the other hand, form α, form I and form III dissolved immediately to their respective maximum concentration, but form II dissolved gradually. This seems partially attributable to the average particle size of form II (29.0 μm), which is larger than the other forms (form α, 9.6 μm; form I, 5.3 μm; form III, 5.0 μm). We thought that form I was the most stable one among the three crystals prepared in this study. However, the concentration of form I in the dissolution test seemed to decrease with time. If a form more stable than form I exists, dissolved form I may transform into the more stable form,

which probably has lower solubility than form I and be precipitated again, however, the existence of such a form was not actually demonstrated in this study. From these results, it has become apparent that the solubilities of form α and form III were higher than those of form I and form II.

It is well known that solubility influences bioavailability of a drug given orally. Thus, we compared the actual effects of the 4 forms of PF1022A on a tissue-dwelling nematode.

As one of the model systems for studying drug effects on tissue-dwelling nematodes, we have established the mouse abdominal angiostrongyliasis model (14, 15) using *Angiostrongylus costaricensis*, a causative agent of human abdominal angiostrongyliasis (16). Cotton rats have been used as an experimental final host of *A. costaricensis* (17), but they are not supplied commercially in Japan for use as an experimental animal. We selected the outbred ddY mouse from various inbred and outbred mice as the in vivo model animal for experimental chemotherapy (18). 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 p.i., worms became matured in the mesenteric arteries. It was also suggested that pathologic changes such as decrease in body weight and decrease in hematologic values are attributable to eggs and/or hatched larvae (14, 15). Using this animal model, we have examined anti-larval effects and effective ways of delivering chemotherapy of some drugs such as mebendazole and PF1022A (9, 19).

Indeed in the present study using the model system of mouse abdominal angiostrongyliasis, form α and form III showed higher efficacy against tissue-dwelling nematodes than form I and form II. Significant effects were observed in almost all parameters in host mice and worms in the groups treated with form α and form III at 40 mg/kg, but little effect was observed in groups treated with form I and form II. Especially, effects were complete in the group treated with form α at 40 mg/kg, and no worms were recovered and no eggs in host tissues and no larvae in feces were observed. All parameters in host mice such as body weight, relative wet weight of the spleen and intestine, and hematologic values recovered to those in non-infected group. On the other hand, in the group treated with form α at 10 mg/kg, significant effects were observed in some parameters such as wet weight of the spleen and worm length, but in the other groups, at 10 mg/kg, almost all parameters showed no difference from those of the non-treated control group. These in vivo effects of the amorphous form and the polymorphic forms of PF1022A against *A. costaricensis* in mice correlated with the in vitro dissolution behavior of each form of the anthelmintic.

Conclusively, the present results indicated that PF1022A had one amorphous form (form α) and three crystal polymorphic forms (form I, form II and form III) and that form α and form III were more effective against tissue-dwelling nematodes than form I and form II when given orally. Therefore, it is strongly suggested that the strategy using drug polymorphism will also enhance bioavailability and efficacy of PF1022A and extend its application as a new drug against tissue-dwelling nematodes that can be substituted for traditional drugs such as benzimidazole derivatives and macrocyclic lactone derivatives.

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