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Research Note

Influence of Formulation on Efficacy of PF1022A in Rats Infected with Adult *Angiostrongylus cantonensis*

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In our previous studies for examination of the *in vivo* effects of anthelmintics against *Angiostrongylus cantonensis* and/or *A. costaricensis*, we used propylene glycol as a vehicle for dissolving avermectin B_{1a} (Ishii *et al.*, 1983), 30% glycerol for suspending milbemycin D (Terada *et al.*, 1987), and olive oil or cremophor EL, a derivative of castor oil and ethylene oxide, for suspending mebendazole (Terada *et al.*, 1993b; Tungtrongchtr *et al.*, 1993). In such a study using *A. costaricensis* in mice, it was recently reported that the difference in formulation of PF1022A showed various results on drug efficacy and that the formulation as oral cream showed the strongest effects among the four formulations examined (Terada *et al.*, 1993a). To make sure that formulation is one of the important factors influencing drug efficacy, this time we compared the effects of PF1022A suspended in cremophor EL and formed as oral cream and n-oral cream, a newly modified formulation, against adult *A. cantonensis* in rats.

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Male Wistar rats aged 5 weeks were used as the final host. Four groups of five or six rats each were inoculated with 20 *A. cantonensis* infective third-stage larvae by the method described by Kachi *et al.* (1994a). Beginning at 8 weeks post-infection (PI), after confirmation of the onset of output of first-stage larvae in feces from each rat, two groups were treated with five successive oral doses of PF1022A at 10 mg/kg/day in the oral cream or n-oral cream, respectively. The third infected group was treated similarly with PF1022A suspended in cremophor EL. In addition, one group with infection and one group without infection were given only the vehicle for the n-oral cream and served as non-treated and non-infected controls, respectively.

The first-stage larval count in host feces and host body weights were monitored until 18 weeks PI. All rats were killed at 18 weeks PI by an overdose of diethyl ether and then dissected. The number of worms recovered, the number of first-stage larvae detected per gram (dry weight) of feces (LPG) and the distribution of first-stage larvae and eggs in lung tissues were examined as parameters in the worm. The relative weight expressed in grams per 100 g body weight of the heart-lungs and spleen, and pathologic changes in lung tissues as determined by visual or microscopic observation were examined as parameters in the host. Significant differences be-

tween the values obtained were analyzed by Student's *t*-test.

By five successive oral treatment of PF1022A in the oral cream the first-stage larvae in rat feces disappeared completely at 2 weeks after treatment and the larval disappearance persisted until dissection of the rats at 18 weeks PI. On the other hand, in the group treated with PF1022A in the n-oral cream, a few larvae appeared again 2 weeks after complete disappearance and they were observed during the experimental period. When rats were similarly treated with PF1022A suspended in cremophor EL, no complete larval disappearance was observed, and small amounts of larvae were observed in the rat feces during the experimental period (Fig. 1).

Table 1 shows the results on various parameters in host rats and worms. At dissection at 18 weeks PI, no eggs were detected from all rats treated with PF1022A in the oral cream. Many eggs with various stages were, however, found in the lung tissues of all rats in the non-treated control and other treated groups, except one rat each in the n-oral cream and cremophor EL groups, respectively.

The mean values for worm recovery was 16.0 worms in the non-treated control group. In the oral cream group, almost complete reduction in the number of female worms was observed, but the number of male worms decreased by only 13%, resulting in a 53% reduction in the total worm count. However, in the n-oral cream and cremophor EL groups, a lesser reduction of 63% and 39% in the female worm count was observed and little influence on the male worm count, resulting in 37% and 12% reduction in the total worm count, respectively.

As compared with values obtained in the non-infected control animals, there were significant changes in body weight and the relative weight of spleen and heart-lungs in the non-treated control. Regarding all these parameters, the oral cream group showed values significantly different from the non-treated control. In other two treated groups significant change was observed only in the relative weight of the heart-lungs.

With respect to microscopic pathologic changes in the lung tissues, the degree of lesion formation was smaller in the treated groups, especially in the oral cream group. The microscopically determined changes agreed well with the visually observed pathologic changes, and all values obtained in the treated groups were significantly lower than the 3.1 score recorded for the non-treated group.

To treat the diseases caused by tissue-dwelling parasites it is essential for anthelmintic drugs to be absorbed from host intestinal tract and be distributed into sites of action. Recently, various devices in drug formulation and drug delivery system have

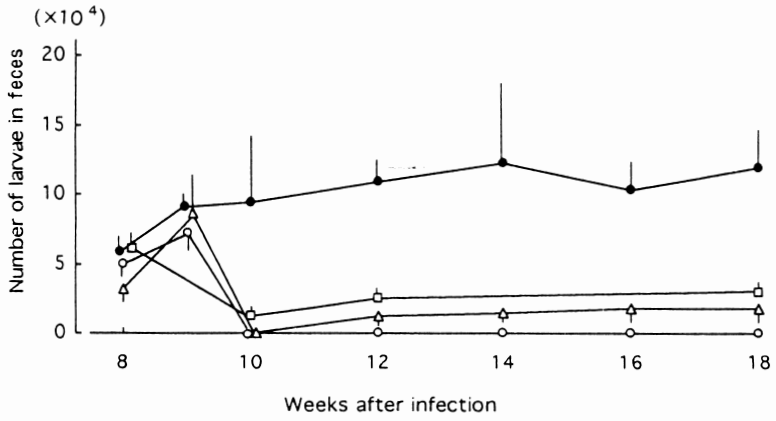

Fig. 1 Changes of number of the first-stage larvae per gram feces (LPG) per rat infected with *Angiostrongylus cantonensis*. Results were calculated from LPG examined for 5 or 6 rats in each group and represent as mean±SE (vertical bar). Five successive oral doses of PF1022A at 10 mg/kg/day in various formulations were given from 8 weeks post-infection to rats. —●—, Non-treated control; —○—, PF1022A emulsified in the oral cream; —△—, PF1022A emulsified in the n-oral cream; —□—, PF1022A suspended in cremophor EL.

Table 1 Influence of formulation on efficacy of PF1022A in rats infected with adult *Angiostrongylus cantonensis*

Parameter	Non-treated	10mg/kg×5 oral cream	10mg/kg×5 n-oral cream	10mg/kg×5 cromophor EL	Non-infected
Number of rats	5	6	6	5	6
Body weight (g)	323.5±7.6	351.3±8.6*	341.2±7.4	337.1±7.9	355.9±7.5*
Relative wet weight (g/100g body weight)					
Spleen	0.29±0.01	0.24±0.01†	0.26±0.01	0.28±0.01	0.20±0.01†
Heart-lungs	1.95±0.15	1.20±0.02†	1.31±0.05†	1.42±0.07*	0.93±0.02†
Degree of nodule in the lung tissues					
+++	5/5	0/6	3/6	3/5	0/6
++	0/5	2/6	1/6	2/5	0/6
+	0/5	4/6	1/6	0/5	0/6
–	0/5	0/6	1/6	0/5	6/6
Visual pathologic changes of the lung tissues	3.1±0.1	0.9±0.2†	1.4±0.3†	1.9±0.3†	–
No. of worms recovered					
Female	7.6±0.9	0.3±0.2†	2.8±0.6†	4.6±1.1	–
Male	8.4±1.3	7.3±1.5	7.8±0.6	9.4±1.2	–
Total	16.0±1.1	7.5±1.3†	10.1±0.4†	14.0±1.5	–
1st larvae in feces					
Detected	5/5	0/6	5/6	5/5	–
Not detected	0/5	6/6	1/6	0/5	–
Eggs in host lungs					
Many eggs with various stages including larvae	5/5	0/6	5/6	4/5	–
Many developing eggs without larvae	0/5	0/6	0/6	0/5	–
Few eggs without development	0/5	0/6	0/6	0/5	–
Not detected	0/5	6/6	1/6	1/5	–

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

been carried out by many investigators to enhance absorption and distribution of drugs or to prolong duration of drug action. For example, the better efficacy of albendazole was observed when the drug was administered as controlled release capsules (Venning, 1991), slow release capsules (Rhodes, 1993) and encapsulated liposomes (New *et al.*, 1994). Another investigators examined the influence of various formulations of anthelmintic drugs such as a micellar formulation of ivermectin (French *et al.*, 1983), a paste formulation of morantel-trichlorfon (Drudge *et al.*, 1984), a loaded form of tetramisole into zeolite (Shaker *et al.*, 1992) and formulations of moxidectin named as 'injectable' and 'pour on' (Whang *et al.*, 1994).

The n-oral cream has the same component as the oral cream, but the former has a different physical state from the latter. 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, we thought it might be more absorptive and effective when administered to animals. Five successive doses of PF1022A in the n-oral cream and oral cream were little effective against *A. cantonensis* staying in the central nervous system of rats (Kachi *et al.*, 1994a), and thereby the influence of the formulation could not be examined in this model. From the results of the above experiment, however, it was confirmed and concluded that PF1022A does not pass through the blood-brain barrier (Akyol *et al.*, 1993; Kachi *et al.*, 1994a; Cheng *et al.*, 1995). Kachi *et al.* (1994b) have reported that PF1022A in the oral cream was conspicuously effective against adult *A. cantonensis* in the pulmonary arteries of rats. Thus, in this study we compared the effects of PF1022A prepared as emulsified formulations, the oral cream and n-oral cream, and suspended type in cremophor EL against adult *A. cantonensis* in rats, and we could observe a remarkable influence of formulation. But contrary to our expectation, the strongest effects were shown not in PF1022A formed as the n-oral cream but in the oral cream and PF1022A suspended in cremophor EL showed the weakest effects.

To prepare the drugs for administration to experimental animals, traditional methods such as dissolving or suspending drugs are undoubtedly easier than the methods for preparing formulations such as the oral cream. However, present results suggest that it is reasonable to devise the formulation of drugs or drug delivery system in future studies for screening and examining the anthelmintic *in vivo* efficacy of drugs.

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