기 생 충 학 잡 지 제23권 제 1 호
The Korean Journal of Parasitology
Vol. 23, No. 1, 41-46, June 1985

Studies on Intestinal Trematodes in Korea

XVIII. Effect of Praziquantel in the Treatment of *Fibricola seoulensis* Infection in Albino Rats

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INTRODUCTION

Fibricola seoulensis (Trematoda: Diplostomatidae), a trematode of the rodents in Korea, was first found from the small intestine of the house rats in Seoul (Seo *et al.*, 1964). Medical attention has been drawn to this fluke because it infects man (Seo *et al.*, 1982; Hong *et al.*, 1984). Since some of the people in Korea like to eat the snakes, one of the second intermediate hosts (Hong *et al.*, 1982), further human cases may occur. Therefore, studies on the chemotherapy are greatly needed.

Praziquantel, an isoquinolino-pyrazine, appears to be the most appropriate anthelmintic, and it was reported that the treatment of 15 cases of human fibricoliasis with 20mg/kg of praziquantel was satisfactory (Hong *et al.*, 1984). But further study with animal subjects is necessary, so as to observe the dose-dependent drug efficacy and the worm elimination process from the host intestine. In this respect, the present study was undertaken to observe the therapeutic effects of praziquantel in the treatment of experimental *F. seoulensis* infection in albino rats.

MATERIALS AND METHODS

1. Preparation of the Metacercariae

The metacercariae of *F. seoulensis* were obtained from a total of 70 snakes, *Natrix tigrina lateralis*, which were purchased from a local collector in Hoengseong-gun, Kangweon-do during June-July 1984. The snakes were cut their heads off and their skins stripped through their whole length. After identifying the presence of metacercariae at stomach, peritoneal wall and other visceral organs, these organs were removed and digested for one hour in artificial gastric juice. The metacercariae were grouped into 500 or 1,000 in number so as to prepare the challenging dose.

2. Observation of the Therapeutic Effect of Praziquantel

A total of 51 albino rats (Sprague-Dawley strain) were used in this study. They were divided into two large groups of Experiment 1 & 2, and each rat was orally given with 500 (Experiment 1) or 1,000 (Experiment 2) metacercariae.

Experiment 1: This experiment was performed to observe the dose-dependent therapeutic effect of praziquantel. Total 41 rats were divided into 1 control (Group I) and 4 treatment groups according to the dose of praziquantel; 1mg/kg (Group II), 5mg/kg (Group III), 10mg/kg (Group IV) and 20mg/kg body weight (Group V). The treatment was done 7 days after the infection with the metacercariae. Three days after then (on the 10th postinfection day) the rats of treated as well as untreated control groups were sacrificed by cervical dislocation and examined whether they retain *F. seoulensis* in each segment of their small intestine (duodenum, jejunum and ileo-caecum). The worms were searched for

under stereomicroscope after chilling the opened intestinal loops in a 5°C refrigerator for several hours to relax and detach the worms from the intestinal mucosa.

Experiment 2: This experiment was done to identify a part of the process of worm elimination from the host intestine after the treatment with praziquantel. Under this purpose, the early time-related distribution of *F. seoulensis* in each segment of the small intestine of the rats were observed. Total 10 rats were divided into 5 groups each consisted with 2 rats; 1 control and 4 time interval groups after treatment (with 5 mg/kg praziquantel) till sacrifice: 10, 30, 60 and 120 minutes. The small intestines of the rats were resected, divided into duodenum, jejunum and ileo-caecum, and examined for the worms.

RESULTS

1. The Therapeutic Dose of Praziquantel

From the Experiment 1, it was revealed that praziquantel in 4 kinds of dosages had more or less appreciable effects in the treatment of *F. seoulensis* infection in albino rats (Table 1). Out of 8~9 rats of each dosage group, 5 rats in 1mg/kg group revealed 1~215 worms (427 in total) 3 days after the treatment, while only 1, 1 and 1 rats in 5, 10 and 20mg/kg groups harboured 1~8 worms. On the other hand, all of 8 rats in untreated control group harboured as much as 50~338 worms each (2,052 in total). In other words, the worm recovery rate from control group was 51.3% in average, while those from treated ones were much lower; 10.7 % in 1mg/kg group and 0.03~0.2% in 5, 10 and 20mg/kg groups. Therefore, it is concluded that 5mg/kg single dose of praziquantel is the minimum effective does for the treatment of *F. seoulensis* infection in experimental rats.

When the worms were recovered from the small intestine of the rats, the intestinal segment where the majority of worms were recovered was different in control and treatment groups (Table 2). In control group, the great majority (94.5%) of worms was recovered from the duodenum, where is the normal habitat of this fluke in the rats (Hong, 1982; Hong *et al.*, 1983). In treatment groups, quite comparably, the majority (76.8% in 1mg/kg group, for example) was found from the jejunum or ileo-caecum. In 1mg/kg group, other 23.2% of worms were found to remain in the duodenum, however, it seems to be due to under-dose and incomplete efficacy of the drug. The recovered worms from all of treatment groups were alive at the time of recovery, so that they were considered to have resisted the drug that was given 3 days before. It seems that many other worms must have been expelled out of the rat intestine. In this respect, the early event of worm elimination from the rat intestine was studied.

Table 1. The dose-dependent efficacy of praziquantel in the treatment of *F. seoulensis* infection in albino rats*

Group	Dose of praziquantel (mg/kg)	No. rats with worms/ treated**	No. total worms recovered (%)
I	(control)	8/8	2,052 (51.3)
II	1	5/8	427 (10.7)
III	5	1/8	1 (0.03)
IV	10	1/8	4 (0.1)
V	20	1/9	8 (0.2)

* Each rat was given with 500 metacercariae in number.

** Treated 3 days before (at 1 week of infection age)

Table 2. Distribution of *F. seoulensis* in the small intestine of albino rats 3 days after praziquantel treatment

Group	No. worms (%) recovered from the		
	Duodenum	Jejunum	Ileo-caecum
I*	1,940(94.5)	112 (5.5)	0 (0.0)
II	99(23.2)	324(75.9)	4 (0.9)
III	0	1	0
IV	1	3	0
V	0	8	0

* Control group

2. Descending of the Worms to Lower Portion of the Small Intestine

In two control rats of the Experiment 2, which were infected 28 days before and untre-

Table 3. Time-related distribution of *F. seoulensis* in the intestine of albino rats after treatment with 5 mg/kg praziquantel

Time of recovery after treatment (min.)	No. rats used	Total No. metacerc. given	Total No. worms* recovered	No. of worms (%) recovered from		
				Duodenum	Jejunum	Ileo-caecum
Control group	2	2,000	487	388 (79.7)	99 (20.3)	0 (0.0)
10	2	2,000	342	42 (12.3)	300 (87.7)	0 (0.0)
30	2	2,000	103	30 (29.1)	73 (70.9)	0 (0.0)
60	2	2,000	218	2 (0.9)	74 (33.9)	142 (65.1)
120	2	2,000	28	0 (0.0)	0 (0.0)	28(100.0)

* 4 weeks of infection age

ated, the majority of worms (79.7%) were recovered from the duodenum, and some (20.3 %) were from the jejunum (Table 3). The smaller proportion of the recovered worms from the duodenum than in Experiment 1 is due to the larger infection dose, *i.e.*, 1,000 metacercariae compared with 500 in Experiment 1 (94.5 % from the duodenum, Table 2), and it is considered to be a natural phenomenon (Hong *et al.*, 1983). In 10-minute group, although the number of worms in the small intestine was not significantly decreased by the treatment, the majority (87.7 %) were found from the jejunum (Table 3). After 30 minutes, however, the number of worms in the small intestine was reduced to approximately one-fourth of control group, and the distribution pattern of worms in the intestine was similar to 10-minute group. After 60 minutes, the majority (65.1 %) of worms further descended to the ileo-caecum, so that only 0.9% and 33.9% remained in the duodenum and jejunum respectively. Thereafter, *i.e.*, 120 minutes after the treatment, the worms were no more found from the duodenum and jejunum, and only 28 worms were recovered from the ileo-caecal portion.

The time-related decrease of the worm recovery rate observed in this experiment strongly suggests that many worms should have been pushed down to the large intestine within 30-60 minutes after the treatment. However, searching for the worms practically from the large intestine of the rats was very difficult, because of the solid nature of the stools and the difficulty in microscopic observation.

DISCUSSION

It is interesting that the minimum effective dose of praziquantel to treat *F. seoulensis* infection in albino rats was only 5 mg/kg. So far as the trematode infections are concerned, this dose seems to be the lowest ever tried. The doses for the blood, liver and lung flukes are known to be much higher; 20-75 mg/kg in one to three divided doses for *Schistosoma* sp. (Andrews *et al.*, 1983), 40-75 mg/kg for *Clonorchis sinensis* or *Opisthorchis viverrini* (Rim *et* Yoo, 1979; Soh *et al.*, 1979; Bunnag *et* Harinasuta, 1981; Rim *et al.*, 1981; Lee, 1984; Seo *et al.*, 1983) and 75-150 mg/kg for *Paragonimus westermani* (Rim *et* Chang, 1980; Soh *et al.*, 1981). In comparison, for the treatment of tapeworm infections of the cats and dogs, it was reported that as low as 1 mg/kg dose showed 100 % therapeutic efficacy (Andrews *et al.*, 1983). On the other hand, for human tapeworm infections such as taeniases, diphyllobothriasis and hymenolepiasis, higher doses of 5-15 mg/kg were required (Espejo, 1977; Rim *et al.*, 1979; Groll, 1977; Schenone, 1980).

These differences in the praziquantel dose are seemingly due to the following two factors. The one is the habitat or ecological niche of parasites in their hosts and the other is the difference in the pharmacokinetics by different hosts. The large dose needed to treat *P. westermani* infection, for example, seems to be due to the parasitic location in the lung tissue and the worm capsule of the host tissue origin,

which may take the role for a barrier for the drug to contact with the worms. In this regard, muscular or cerebral cysticercosis in the pigs is treated only by greatly high doses, 500-750 mg/kg in 10-15 divided doses (Andrews *et al.*, 1983), probably because of the cyst wall and/or the blood-brain barrier. Also in case of the liver fluke, the contact of the drug with worms occurs only after it is excreted in the bile. The metabolically transformed praziquantel in the liver was reported to be 400-fold less effective in killing the parasites (Andrews *et al.*, 1983). Therefore, a high dose is needed to treat the liver fluke infection. The relatively lower dose needed to treat schistosomes is due to the rapid enteral absorption of the drug and high cencentration in the blood within one hour (Andrews *et al.*, 1983).

In this context, the low dose of praziquantel needed to treat *F. seoulensis* infection in albino rats seems to be due to the parasitic location in the small intestine, especially the duodenum, where high concentration of the drug is available. Other intestinal trematodes and cestodes in general are also treated with low doses of praziquantel because of the same reason. However, it was indicated by Rim *et al.* (1978) that the efficacy of various anthelmintics in the treatment of *M.* yokogawai infection varied according to the subjects treated, who were different in worm burden. They suggested that if the worm burdens are high many worms would intrude into the crypt and the drug could have a limited contact with the worms, to result in an unsatisfactory cure. In case of *F. seoulensis* infection, however, it seems not probable since the worms are large enough not to intrude their whole body into the crypt in the rat host and their posterior body is always exposed to the luminal side (Lee *et al.*, 1985).

The dosage of praziquantel required in the treatment of the same parasitic infection is also variable by kinds of hosts. In the treatment of *C. sinensis* infection, for example, the dosage of praziquantel to obtain a satisfactory effect was 75 mg/kg in man, while as high as 300 mg/kg in albino rats (Rim *et al.*, 1980 & 1981). Also in the treatment of *Schistosoma mansoni* infection, 95% effective oral dose (ED_{95}) was reported to be as high as 685 mg/kg in mice, while 249mg/kg in hamsters and less than 100mg/kg in monkeys and men (Andrews *et al.*, 1983). This was explained by the fact that the small animals such as the mouse are efficient eliminators of praziquantel through the intestinal tract, compared with the larger animals (Andrews *et al.*, 1983). Therefore, the availability of praziquantel in the gastrointestinal tract is higher in the small animals. For this reason, it should be studied whether the minimum effective dose of praziquantel in the treatment of *F. seoulensis* infection in man is higher than 5 mg/kg.

In the present study, it was observed that *F. seoulensis* worms, after the contact with praziquantel, rapidly descended to the lower part of the small intestine in the rats. If this experiment is continued until 180 minutes or later, nearly no worm is expected to remain in the whole small intestine. Therefore, it is evident that the worm shifting to the lower small intestine should be a process of discharge from the host intestine. It is considered to be due to the dual effects; the drug induced damage on the viability of worms and the continuous peristalsis of the rat intestine. According to Lee (1985), who studied the effect of variable concentrations of praziquantel on *F. seoulensis in vitro*, a tetanic contraction of the worms was observed especially in their anterior body by higher drug concentrations than 0.1 μg/ml and immediate death (no movement) ensued by higher concentrations than 1 μg/ml. Therefore, *in vivo*, it seems that the contracted worms following the contact with the drug are detached from the duodenal villi and pushed downwards by the intestinal peristalsis of the host.

In comparison, in case of the blood and lung flukes, the process of worm elimination from the host body is different. It is initiated by the destruction of the worm tegument and followed by the invasion of the host defense cells such as the eosinophilic granulocytes (Mehlhorn *et*

al., 1981; Chiu *et al.*, 1982). Eventually the whole body of the worms may be thoroughly destroyed, lysed and taken by the host cells. In this respect, the elimination of the intestinal flukes suck as *F. seoulensis* seems to be much simple and more successful than in case of the blood or lung flukes.

SUMMARY

The therapeutic effects of praziquantel on *Fibricola seoulensis* infection were studied by experimental infection of albino rats with the metacercariae obtained from the snakes. Total 51 albino rats were infected each with 500 or 1,000 metacercariae in number through intragastric tubes. One or four weeks later the rats were treated with 1, 5, 10 or 20 mg/kg single dose of prazipuantel and sacrificed after 3 days or 10-120 minutes to search for the worms in their small intestine.

The worm recovery rate at 3 days after the treatment was 10.7 % in 1 mg/kg dose group and 0.03~0.2% in 5, 10 and 20 mg/kg groups, while that of untreated control was 51.3%. The minimum effective dose to treat *F. seoulensis* infection in the rats is considered to be 5 mg/kg in single dose. By observing the distribution pattern of worms in the small intestine after the treatment, dislodgement of the worms from the duodenum, their normal habitat, to the lower portions was recognizable within as early as 10 minutes. The majority of worms was found to have descended to the ileo-caecal portion after 60-120 minutes.

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═國文抄錄═

韓國의 腸吸蟲에 관한 硏究

XVIII. 흰쥐의 *Fibricola seoulensis*감염에 대한 프라지콴텔의 治療效果

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Fibricola seoulensis 감염에 대한 praziquantel의 치료효과를 흰쥐 實驗感染을 통하여 관찰하였다. 被囊幼蟲은 뱀의 내장으로부터 分離한 것을 사용하였고 총 51마리의 흰쥐에 각각 500개 또는 1,000개씩을 감염시켰다. 감염 1주 또는 4주후에 praziquantel 1, 5, 10 또는 20mg/kg 用量을 투여하고 3일후 및 10~120分후에 흰쥐를 희생시킨 다음 小腸으로부터 蟲體를 回收하였다.

投藥 3일후 蟲體回收率은 치료하지 않은 對照群의 51.3%에 비해 매우 낮아 1mg/kg群의 경우 10.7%, 5~20 mg/kg群의 경우 0.03~0.%로 나타났다. 따라서 흰쥐의 *F. seoulensis*감염을 治療하기 위한 praziquantel의 最小有效投與量은 5mg/kg인 것으로 생각되었다. 投藥後 흰쥐 小腸 部位別 蟲體 分布狀況을 관찰함으로써 蟲體가 排出되는 過程의 一部를 확인하고자 한 바 投藥 10分後 이미 많은 蟲體가 正常 寄生部位인 十二指腸에서 空腸으로 밀려내려간 것이 관찰되었고, 60~120分後에는 대부분의 蟲體가 廻腸 및 盲腸에서 回收되었다.