기 생 충 학 잡 지 제25권 제1호
The Korean Journal of Parasitology
Vol. 25, No. 1, 24~36, June, 1987

In vitro effect of praziquantel on *Paragonimus westermani* by light and scanning electron microscopic observation

Soon-Hyung Lee, Ho-Jin Park, Sung-Jong Hong, Jong-Yil Chai and Sung-Tae Hong
Department of Parasitology and Institute of Endemic Diseases, College of Medicine
Seoul National University, Seoul 110, Korea

INTRODUCTION

Paragonimus westermani is one of common flukes of human in Korea. For treating its infection, bithionol had been used since Yokogawa *et al.* (1961 a & b) recommended it after their *in vitro* and *in vivo* evaluations. However, too repeated doses and toxicity of bithionol limited its practicability. In 1970s, niclofolan was recognized of its therapeutic efficacy on human paragonimiasis (Rim, 1975; Rim *et al.*, 1976), however, its use was abandoned because of narrow safety margin (Hong *et al.*, 1982). Nowadays, praziquantel is widely used for infections of trematodes or cestodes including paragonimiasis (Thomas *et* Gönnert, 1977; Davis *et al.*, 1979; Rim *et* Chang, 1980; Yokogawa *et al.*, 1980; Rim *et al.*, 1981; Soh *et al.*, 1981).

It is known that the worms exposed to praziquantel reveal immediate contraction followed by relaxation and destruction of tegumental integrity (Andrews *et al.*, 1983). Especially the effect of praziquantel on various trematodes, such as *Schistosoma*, *Dicrocoelium*, *Fasciola*, *Clonorchis*, *Metagonimus*, *Opisthorchis*, *Paragonimus* and *Fibricola* was observed morphologically (Becker *et al.*, 1980; Kim *et al.*, 1982; Mehlhorn *et al.*, 1983; Lee, 1985). Almost all of the worms exposed *in vitro* or *in vivo* were observed to make vacuoles in tegument which ruptured finally. However, any effect of the drug on other tissues or organs than tegument has not been elucidated yet.

Although praziquantel was proved of its killing activity on *P. westermani in vivo*, it had been regarded that the tegument of this fluke was least influenced (Mehlhorn *et al.*, 1983) due to thick tegument with stout spines (Lee *et al.*, 1981; Higo *et* Ishii, 1984; Choi *et* Yoo, 1985). Therefore, it is necessary to observe *in vitro* effect of praziquantel on various organs or tissues including tegument of *P. westermani* to outline the mechanism of action.

This study intended to observe the motility and the destruction process of every organ of *P. westermani* in various concentrations of praziquantel.

MATERIALS AND METHODS

1. Experimental infection and recovery of *P. westermani*

The metacercariae of *P. westermani* were isolated from the crayfish which were collected at Yangyang-gun, Kangwon-do on June 22, 1984. A total of 153 metacercariae was obtained and introduced per os to 2 dogs, 75 and 78 respectively. The dogs were killed after 11 weeks, and total 128 adult flukes were recovered from their lungs. The worms were washed in Tyrode's solution and used for the experiment immediately.

2. Incubation of *P. westermani* in praziquantel solutions

The stock solution of praziquantel was prepared by dissolving praziquantel 500mg in ethanol 17.5ml and diluted with 50ml of water. This stock was serially diluted with Tyrode's solution to make praziquantel concentration of 100μg/ml, 10μg/ml, 1μg/ml, 0.1μg/ml and 0.01μg/ml,

Table 1. Number of *P. westermani* prepared for light microscopy by praziquantel concentration and incubation time

Praziquantel concentration (μg/ml)	No. of worms incubated for						
	15min.	30min.	1hr.	6hrs.	12hrs.	26hrs.	Total
0.01	—	—	4	—	—	4	8
0.1	—	3	4	—	—	—	7
1	—	—	1	—	—	—	1
10	—	4	4	4	4	—	16
100	—	—	4	—	—	—	4
Control	—	—	4	—	—	—	4
Total	0	7	21	4	4	4	40

Table 2. Number of *P. westermani* prepared for scanning electron microscopy by praziquantel concentration and incubation time

Praziquantel concentration (μg/ml)	No. of worms incubated for						
	15min.	30min.	1hr.	6hrs.	12hrs.	26hrs.	Total
0.01	—	—	4	—	—	4	8
0.1	3	3	4	—	—	—	10
1	—	—	4	—	—	—	4
10	4	3	4	4	4	—	19
100	—	—	4	—	—	—	4
Control	—	—	4	—	—	—	4
Total	7	6	24	4	4	4	49

Each solution, 20ml was disposed to 5 petri dishes. Control group was prepared by Tyrode's solution 20ml without praziquantel. Eight active worms were introduced to each petri dish and incubated at 37°C including control solution. Motility and body shape of the worms were observed for 3 minutes through a stereomicroscope 15, 30 minutes, 1, 6, 12 and 26 hours after incubation.

3. Light microscopic observation

Four worms were removed from each group; two of them were fixed under pressure and stained with Semichon's acetocarmine, and other two were fixed in 10% formalin, embedded in paraffin, cut serially in 5μm thickness and stained with hematoxylin and eosin(HE). They were observed with a light microscope. The numbers of worms by group were presented in Table 1.

4. Scanning electron microscopic(SEM) observation

Four worms of each group were washed in 0.1M phosphate buffered saline 3 times after incubation as shown in Table 2. They were fixed in cold 2.5% glutaraldehyde and dehydrated in a freeze dryer. Each worm was mounted on a stub and coated with gold in 200nm thickness. They were observed with ISI-40 or ISI-60 (ISI Korea Ltd.) under 10~30KV.

RESULTS

1. Gross features of worms exposed to praziquantel

The worms of control group incubated in Tyrode's solution without praziquantel showed active movements throughout the experimental procedure of 26 hours. However, the worms in praziquantel solutions showed significant changes in their motility and shape. In 0.01μg/ml concentration, the worms shrank slowly to excrete many eggs from exposure to 30 minutes later. Thereafter, egg excretion decreased steadily up

Table 3. Chronological observations on the activity of *P. westermani* incubated in various concentrations of praziquantel solution

Praziquantel concentration (μg/ml)	No. of worms	Activity of *P. westermani* by incubation time					
		15min.	30min.	1hr.	6hrs.	12hrs.	26hrs.
0.01	8	+	±	±	±	±	±
0.1	8	—	—	—	N	N	N
1	5	—	—	—	N	N	N
10	8	—	—	—	—	—	N
100	8	—	—	—	N	N	N
Control	8	++	++	++	++	++	++

* ++ : very active movement + : active movement ± : slow movement
— : no movement N : not observed

to 26 hours. Although the movement of worms became sluggish and weak, all of them maintained motility until 26 hours of incubation.

In 0.1μg/ml praziquantel solution, the worms shrank also slowly, regurgitated intestinal contents and were immobilized within 10 minutes. In solutions over 1μg/ml concentration, the worms were immobilized and contracted immediately.

In the worms incubated in 10μg/ml solution excretory bladder began to enlarge after 30 minutes, and became larger steadily by 12 hours to result in swelling of posterior body. After an hour incubation, however, the worms began to be relaxed and the anterior part of worms, *i.e.*, between oral and ventral suckers, was elongated. After 12 hours they elongated more and pharynx protruded out through oral sucker. In 100μg/ml praziquantel solution, these changes occurred only 30 minutes after incubation and progressed more quickly. These gross features are summarized in Table 3.

2. Light microscopic findings

A) Control group

The adult worms of *P. westermani* incubated in Tyrode's solution were bean-shaped, 5.8~8.0 mm long and 3.8~4.1mm wide. In sectioned specimens, their tegument consisted of homogenous vesicular syncytium with obliquely inserted spines which were covered with cytoplasmic membranes up to its tip portion, thin basement membrane, circular and longitudinal muscle layer and tegumental cells (Fig. 1). The tegument was thin in median line but thick in lateral. Vitellaria located under the subtegumental layer and were distributed throughout whole body except along the median line. Intestinal ceca showed 3 convolutions with constant diameter. Relatively thin intestinal epithelia were deeply stained with hematoxylin and eosin, and directly connected to parenchymal layers by loose connective tissue (Fig. 2).

A branched ovary was composed of numerous follicles (Fig. 3) and was laid somewhat superficially under the mid-dorsal tegument. Each of ovarian lobes consisted of peripheral germinal layer and medulla, and a number of eggs were formed from the germ cells (Fig. 4). A pair of testes were branched into 4-5 small lobules which were elongated and their margins smooth (Fig. 5). Other parenchymal tissues were relatively loose and the cells showed a variety in size and shape. Cells of Mehlis' gland revealed a centrifugal structure and the oötype lay at its center. Epithelia of the excretory bladder were composed of single layer of cells with irregular margin.

B) Experimental group

1) Tegument: Several vacuoles appeared in the subtegument between oral and ventral suckers of a worm incubated in 0.01 μg/ml praziquantel solution for an hour but the remained portions were still normal. Such vacuolization was more extensive in median and ventral tegument than in lateral and dorsal tegument. Numerous tiny vacuoles were observed in syncytial layer or subtegument between two suckers and around the

genital atrium after an hour in 0.1 μg/ml solution. After 30 minutes incubation in 10μg/ml, it was observed that the postero-lateral syncytium was destroyed by rupture of vacuoles and the spines were retracted within the syncytial layer to be hardly recognizable. After an hour, the vacuolization expanded throughout the whole subtegument but especially severe in the lateral portion, and small vacuoles were forming from the syncytium of dorsal tegument.

After 6-hour incubation, the syncytium between two suckers was destroyed by massive vacuolizations followed by rupture, and even muscle layer became vacuolized (Fig. 6). Also the postero-lateral syncytium was swollen by the vacuoles formed at the base, and a vacuole reaching to the basement membrane was ruptured. After 12-hour incubation, a number of vacuoles were formed in the muscle layer to make muscle fibers separated.

It was observed that the syncytium of a worm, incubated in 100μg/ml praziquantel solution for an hour, was so severely vacuolized and destroyed that their syncytial layer was detached from the basement membrane and a narrow space between spines was exposed. The longitudinal muscle bundle also became loose and the subtegument was severely vacuolized.

2) **Intestine:** In the worms incubated in 0.01 μg/ml praziquantel solution for an hour, intestinal lumen became narrower due to thickened intestinal epithelia and small or medium sized vacuoles appeared in microvilli. Margins of the intestinal wall became irregular. Slight swellings were found at 2-3 loci of the posterior part of intestine of a worm incubated in 0.1μg/ml praziquantel for an hour. They became balloon-like swellings in 1μg/ml 1-hour group and in this case, also the anterior part began to be swollen. In 10μg/ml 1-hour group, such swellings appeared to be more elongated and enlarged, however, the intestinal lumen was still narrower than controls. The balloon formation was found very severe in worms incubated in 10μg/ml praziquantel for 12 hours but still not ruptured (Fig. 7). Vacuoles appeared in connective tissues around the intestine as well as in intestinal villi, in worms of 10μg/ml 30-minute group. According to the increase of drug concentration and incubation time, vacuolization became extensive and diffuse so that the stretched epithelia of balloons were vacuolized (Fig. 8). Eosinophilic substance was observed in the dilatated intestinal lumen of worms incubated for 30 minutes in 10 μg/ml concentration and increased more and more to fill up the swollen intestinal lumen.

3) **Ovary:** In worms incubated in 0.01 μg/ml concentration for 1 hour, digited irregular margin of ovarian branches disappeared and its configuration became almost elliptical. According to the increase of drug concentration to 10 μg/ml the branches were remarkably swollen to be round. Margins of round lobules became blur and indistinctly outlined (Fig. 9). Many vacuoles appeared in the germinal layer of ovary in worms incubated in 0.01 μg/ml concentration for 1 hour. In 0.1 μg/ml and 1-hour incubation group, the vacuoles were increased in size and number, and extended into the medullar portion (Fig. 10). Such vacuolization became more severe according to subsequent increase of the drug concentration and incubation time and the ovary lost its entire configuration in high drug concentrations.

4) **Testis:** In 0.1 μg/ml 1-hour group, terminal portion of testis began to be transformed into small ball-like lobules and the germinal layer was vacuolized. Such ball shaped testicular branches became much more round-up according to increase of the drug concentration (Fig. 11). In 10 μg/ml and 1-hour incubation group, two balls were formed in each branch and their proximal parts stretched to be disintegrated. Disintegration of testicular branches progressed from the proximal part to distal one, the stainability became poor and its size was reduced in 10 μg/ml 12-hour incubation group. From a worm incubated in 100 μg/ml concentration for an hour, two balls, a larger and a smaller ones, were formed and the margin of the larger one became blur and translucent in the median portion. The

vacuolization was also severe in the germinal layer as well as in the medulla (Fig. 12).

5) **Mehlis' gland:** Small vacuoles appeared in the Mehlis' glandular organ near the ovary, of a worm incubated in 0.01 μg/ml concentration for an hour. The size and number of vacuoles increased as the drug concentration was increased. Consequently, higher drug concentrations than 10 μg/ml, the Mehlis' gland was entirely vacuolized and vacuolization reached even to the ootype (Fig. 13).

6) **Excretory bladder:** From all experimental groups, it was observed that epithelia of the excretory bladder were slightly thickened, its rough digitations became shortened and numerous vacuoles appeared (Fig. 14). Eosinophilic material was observed in the lumen of the excretory bladder of a worm incubated in 0.1 μg/ml concentration for 1 hour, and they increased in number according to the increase of drug concentration and incubation time.

3. Scanning electron microscopic findings

A) Control group

The tegument of adult *P. westermani* was covered with cobblestone-like cytoplasmic processes which had pits in their center portion. Between oral and ventral suckers it was more velvety in texture than that of other parts. A number of grouped spines into 4-5 small ones were observed to constitute a band-like zone at base of ventral sucker and they covered whole surface of oral sucker (Fig. 15). The tips of single pointed spines were covered with cytoplasmic membranes (Fig. 16). The spines were densely distributed on the whole tegument except between oral and ventral suckers where they were especially sparse in distribution. Solitary and/or grouped sensory papillae into 2-6 in number, each having a cilium, were distributed most densely around ventral sucker followed by oral sucker but their distribution was sparse on other parts. A few large elevations with a bumpy dome were also observed on the rim of oral and ventral sucker, which were regarded as another type of sensory papillae.

B) Experimental groups

1) **0.01 μg/ml concentration group:** Very small-sized blebs appeared on the tegument between oral and ventral suckers. Spines were retracted within the tegument but sensory papillae were not affected by an hour after incubation. After 26 hours, although the blebs grew slightly in size, the tegument appeared to be nearly intact.

2) **0.1 μg/ml concentration group:** In a worm incubated for 15 minutes, it was observed that numerous small blebs were formed and spines retracted into the syncytium between oral and ventral suckers (Fig. 17). The blebs ruptured to form craters in spine-free zones at the base of two suckers.

After 30-minute incubation, blebs on the tegument between the suckers were increased in size and number, and a few of them were ruptured. In the postero-ventral tegument, several minute blebs were seen of the cytoplasmic membranes covering retracted spines and cytoplasmic processes lost their normal cobblestone-like contour and showed a rough and granular surface. Sensory papillae were not affected by the bleb formation. Several blebs appeared on the dorsal tegument but not ruptured.

After an hour, the bleb formation was extended throughout the whole ventral tegument and large blebs with thin membrane were ruptured (Fig. 18). On the dorsal surface, blebs on the cytoplasmic membranes grew in size but without increase in number, while minute ones on spines were enlarged and increased in number (Fig. 19). The genital atrium was everted outwards revealing many ejected sperms and its tegument was covered with many craters to show honeycomb appearance (Fig. 20).

3) **1 μg/ml concentration group:** Although worms were still round in shape, their oral sucker was slightly protruded anteriorly and the length of the posterior body, from the ventral sucker to posterior end, was longer than that of the anterior one, between oral and ventral suckers. Disintegration of the tegument by crater formation was prominent at the base of two

suckers (Fig. 21). Bleb formation was more remarkable on the antero-ventral tegument than other parts, and the blebs were ruptured so that destruction of the syncytium was accelerated. A few sensory papillae lost their cilium and several blebs were observed at their base portion under the dome (Fig. 22). Dorsal tegument still revealed normal structures except for small blebs formed on the postero-lateral surface.

4) 10 μg/ml concentration group: The length of the posterior body of worms incubated for 15 minutes became the double of the anterior portion. The oral sucker was widened and the pharynx protruded out through the mouth. Numerous blebs on the antero-ventral tegument were minute to medium in size but not ruptured. On the antero-dorsal tegument there appeared small club-shaped blebs with tubular neck part. After 30-minute incubation, the worms were relaxed and elongated so that the ratio of anterior to posterior body length became about 1. Blebs of various sizes were on the antero-ventral tegument and their membranes showed small wrinklings (Fig. 23). Blebs on posterior and lateral teguments were more enlarged than those in worms incubated in lower concentrations of praziquantel and several ruptured ones were transformed into large craters. The dome of sensory papillae was shrunk and their cilia were rooted out.

After an hour, oral sucker protruded slightly and had grape-like grouped blebs and its whole tegument was destroyed by an extensive vacuolization (Fig. 24). On the ventro-lateral tegument, there were many club-shaped blebs with elongated neck (Fig. 25). After 6-hour incubation, worms became pyriform as a result of relaxation between two suckers and expanding of the posterior portion. Large blebs appeared on the whole tegument and a number of large craters were formed on the lateral tegument (Fig. 26).

After 12 hours, elongation of anterior body and crater formation on the lateral tegument progressed remarkably. The everted oral sucker was severely damaged on the whole tegument especially at its base. Blebs and swellings of cytoplasmic processes were also observed at the tegument around the excretory bladder.

5) 100 μg/ml concentration group: Worms incubated for an hour were markedly relaxed but their anterior part was not elongated. The base of two suckers were severely destroyed and numerous large blebs appeared on the postero-ventral tegument. On the dorsal surface, all spines were retracted within the tegument. Large craters were observed on the postero-lateral tegument.

DISCUSSION

The findings of the present study revealed that praziquantel had excellent *in vitro* killing activity on *P. westermani*. Six worms out of 8 incubated in 0.01 μg/ml solution maintained motility during 26 hours of incubation. Contrary to this, all of 8 worms lost motility in 0.1 μg/ml praziquantel. Therefore, ED_{50}(or LD_{50}) of praziquantel on *P. westermani* was between 0.01 μg/ml and 0.1 μg/ml.

The worms exposed to praziquantel showed immediate contraction paralysis and later relaxation. The contraction occurred within 10 minutes in 0.1 μg/ml, however, it occurred immediately after incubation, in solutions over 1 μg/ml. Such rapid contraction was supported by the fact that praziquantel was rapidly absorbed and distributed into parasites(Andrews *et al.*, 1980). This muscle paralysis looks like a functional derangement due to stimulatory blocking because its nature is contraction and occurs instantaneously.

Anterior elongation of the flukes by praziquantel treatment was demonstrated in *Clonorchis sinensis*(Kim *et al.*, 1982), *Opisthorchis viverrini* (Sirisinha *et al.*, 1984), *Schistosoma mansoni* (Mehlhorn *et al.*, 1981) and *Fibricola seoulensis* (Lee, 1985; Seo *et al.*, 1985). This elongation was also found in *P. westermani* about an hour after incubation in this study. This proceeded more rapidly in higher concentration of drug. Such elongation appears to be due to relaxation as a result of cell death after immediate contraction.

Vacuolization or bleb formation in tegument is a well-known effect of praziquantel on various kinds of trematodes or cestodes. By Chiu *et al.* (1982) and Yoon *et al.* (1984) who observed *in vivo* effect of praziquantel on *P. westermani*, vacuolization of tegumental syncytium was originated from vesicles in sensory papillae around suckers. In adult worms, many blebs were made on tegument posterior to ventral sucker, where spines were distributed sparsely. Through disintegrated tegument, numerous host cells invaded to kill and to absorb the worms. As for *in vitro* effect of praziquantel Mehlhorn *et al.* (1983) observed vacuolization in tegument of several flukes, however, *P. westermani* showed only a little vacuolization and preserved tegumental integrity after an hour incubation in 1-100 μg/ml solution. They interpreted it due to thick tegument with compact elastic fibers and spines. This was also found in *Fasciola hepatica*(Becker *et al.*, 1980). However, the worms of the present study revealed numerous vacuoles or blebs in or on tegument, which ruptured finally to disrupt tegumental integrity. Even the living worms in 0.01 μg/ml solution showed vacuoles in their tegument. The difference between the finding of the present experiment and that of Mehlhorn *et al.* (1983) can hardly be explained, however, it can be said that *P. westermani* must undergo vacuolization and eventual disruption of tegument when it is exposed to praziquantel.

In the present study, vacuoles and blebs were found firstly and more at the portion of ventral surface between oral and ventral suckers than any other part. It could be explained that tegument of the portion is relatively thin and is active in metabolism. This part is also primarily responsible for the activity of two suckers in flukes, and especially there are many papillae. Disintegration of this part tegument is regarded as more critical than destruction of other part tegument.

There is still a debate that vacuolization in tegument is no more than a secondary change after death of worms, while many authors regard it as a primary effect of praziquantel. Present study found many vacuoles or blebs in the living worms, and this finding supported vacuolization as a primary process by the drug.

Vacuoles are not essentially terminated bursting out to form crater. *Metagonimus yokogawai* was observed to make vacuoles in tegument by incubation in praziquantel, but without disintegration (Mehlhorn *et al.*, 1983; Lee *et al.*, 1984). This preservation of morphological integrity in spite of vacuolization by drug effect was regarded as due to mechanical support by scale-like spines which covered tegument compactly.

Such vacuolization was also found in other tissues than tegument, i.e. in intestine, ovary, testis, excretory bladder, Mehlis' gland and parenchyme. Lee (1984) found also vacuoles in intestinal epithelium as well as in tegument of *P. westermani* infected in cats after chemotherapy. These findings suggest that the flukes exposed to praziquantel be damaged of all tissues. Especially reproductive organs were disintegrated distinctively. The worms incubated in 0.01 μg/ml praziquantel solution lost the configuration of ovary or testis and their germinal layer was vacuolized although they were still moving. Accordingly, the worms may lose their activity of egg production by praziquantel treatment although they are not expelled from the host. Such phenomenon was observed in *C. sinensis* infection of guinea pigs(Yang, 1986). The flukes remained intact in the liver 4 weeks after praziquantel treatment, but contained few eggs in their uteri. This suppressive activity of praziquantel on oviposition is an important finding. It suggests the possibility of discrepancy between egg negative conversion and worm expulsion after chemotherapy. So called parasitological cure or egg negative conversion does not essentially mean complete deworming. This is a theme for further evaluation.

Although considerable research effort has been invested, the mechanism of action of praziquantel is not defined at the molecular level. Only a few secondary findings were noted. However, immediate contraction induced by praziquantel has been explained on the basis of a change in divalent

cation fluxes, especially calcium. It has been shown that uptake of calcium by schistosomes was increased by praziquantel while that of potassium was reduced. Depletion of external calcium or an excess of magnesium abolished contraction in schistosomes but not in cestodes. Calcium concentration rises actually within muscle cells contracting under the influence of praziquantel (Andrews *et al.*, 1983). Calcium seems to play a role in vacuolization too. Further mechanism is not fully understood yet. Praziquantel has anthelmintic efficacy against trematodes or cestodes but not nematodes. Therefore, its mode of action looks like related with tegumental structure.

SUMMARY

The effect of praziquantel on *P. westermani* exposed *in vitro* was observed by stereomicroscope, light microscope and scanning electron microscope. Following results were found.

1. The worms incubated in 0.01 μg/ml praziquantel were moving after 26-hour incubation. However, all of them were immobilized immediately after incubation in solutions over 0.1 μg/ml concentration.

2. All of the exposed worms showed severe vacuolization not only in tegument but in subtegument, intestine, ovary, testis, Mehlis' gland and excretory bladder.

3. Vacuoles in tegument burst out to form craters. As incubation time went on, tegumental structure was disintegrated severely.

The worms exposed to praziquantel were observed to be immobilized and be vacuolized of all tissues. Disintegration of reproductive organs suggests that praziquantel have suppressive effect on egg production when the flukes are not killed. The drug effects were found more related with incubation time than with drug concentration.

REFERENCES

Andrews, P., Thomas, H. and Weber, H. (1980) The *in vitro* uptake of ^{14}C-praziquantel by cestodes, trematodes and a nematode. *J. Parasit.*, **66**(6):920-925.

Andrews, P., Thomas, H., Pohlke, R. and Seubert, J. (1983) Praziquantel. *Medic. Res. Rev.*, **3**:147-199.

Becker, B., Mehlhorn, H., Andrews, P. and Eckert, J. (1980) Light and electron microscopic studies on the effect of praziquantel on *Schistosoma mansoni*, *Dicrocoelium dendriticum* and *Fasciola hepatica* (trematoda) *in vitro*. *Z. Parasitenkd.*, **83**:113-128.

Chiu, H.S., Kim, S.J. and Rim, H.J. (1982) Electron microscopic studies on the effect of praziquantel to *Paragonimus westermani*. *Korea Univ. Med. J.*, **19**(3):617-630 (in Korean).

Choi, W.Y. and Yoo, J.E. (1985) Ultrastructure of the tegument of adult *Paragonimus westermani*. *Korean J. Parasit.*, **23**(1):111-122 (in Korean).

Davis, A., Biles, J.E. and Ulrich, A.M. (1979) Initial experiences with praziquantel in the treatment of human infections due to *Schistosoma haematobium*. *Bulletin World Hlth. Org.*, **57**(5):773-779.

Higo, H. and Ishii, Y. (1984) Scanning electron microscopy of the newly excysted juveniles *Paragonimus westermani* (Kerbert, 1878) Braun, 1899 (parthenogenetic Type) and *P. miyazakii* Kamo, Nishida, Hatsushika and Tomimura, 1961. *Jpn. J. Parasit.*, **33**(5):421-427.

Hong, S.T., Lee, S.H., Ahn, H.S. and Yun, C.K. (1982) A case of Niclofolan(Bilevon®) intoxication. *Korean J. Parasit.*, **20**(1):49-52.

Kim, S.S., Kim, S.J. and Rim, H.J. (1982) Electron microscopic studies on the effect of praziquantel to *Clonorchis sinensis*. *Korea Univ. Med. J.*, **19**(1):91-105 (in Korean).

Lee, G.J. (1984) Electron microscopic observation on the effects of praziquantel on *Paragonimus westermani*. *J. Catholic Med. College*, **37**(2):433-455 (in Korean).

Lee, S.H. (1985) *In vitro* effects of praziquantel on *Fibricola seoulensis*. *Seoul J. Med.*, **26**(1):41-51.

Lee, S.H., Seo, B.S., Chai, J.Y. and Hong, S.J. (1984) Study on *Metagonimus yokogawai* (Katsurada, 1912) in Korea VII. Electron microscopic observation on the tegumental structure. *Korean J. Parasit.*, **22**(1):1-10 (in Korean).

Lee, S.M., Hong, S.T., Lee, S.H. and Shim, T.S. (1981) Ultrastructural study on the teguments of juvenile and adult *Paragonimus westermani*. *Chung-Ang J. Med.*, **6**(4):579-589 (in Korean).

Mehlhorn, H., Becker, B., Andrews, P., Thomas, H.

and Frenkel, J.K. (1981) *In vivo* and *in vitro* experiments on the effects of praziquantel on *Schistosoma mansoni*. *Arzn.-Forsch./Drug Res.*, **31**(1), 3a:544-554.

Mehlhorn, H., Kojima, S., Rim, H.J., Ruenwangsa, P., Andrews, P., Thomas, H. and Bunnag, B. (1983) Ultrastructural investigations on the effects of praziquantel on human trematodes from Asia: *Clonorchis sinensis*, *Metagonimus yokogawai*, *Opisthorchis viverrini*, *Paragonimus westermani* and *Schistosoma mansoni*. *Anrz.-Forsch./Drug Res.*, **33**(I), 1:91-98.

Rim, H.J. (1975) Studies on the chemotherapy of trematode infections in Korea. *Korea Univ. Med. J.* **12**:425-457.

Rim, H.J. and Chang, Y.S. (1980) Chemotherapeutic effect of niclofolan and praziquantel in the treatment of paragonimiasis. *Korea Univ. Med. J.*, **17**(1): 113-128 (in Korean).

Rim, H.J., Kim, M.S., Ha, J.H. and Chang, D.S. (1976) Experimental chemotherapeutic effects of niclofolan (Bayer 9015, Bilevon) on the animals infected with *Paragonimus westermani* and *P. iloktsuenensis*. *Korean J. Parasit.*, **14**(2):140-146.

Rim, H.J., Lyu, K.S., Lee, J.S. and Joo, K.H. (1981) Clinical evaluation of the therapeutic efficacy of praziquantel (Embay 8440) against *Clonorchis sinensis* infection in man. *Ann. Trop. Med. Parasit.*, **75**(1):27-33.

Seo, B.S., Cha, I.J., Chai, J.Y., Hong, S.J. and Lee, S.H. (1985) Studies on intestinal trematodes in Korea XIX. Light and scanning electron microscopy of *Fibricola seoulensis* collected from albino rats treated with praziquantel. *Korean J. Parasit.*, **23**(1):47-57.

Sirisinha, S., Puengtomwatanakul, S., Sobhon, P., Saitongdee, P., Wangpayabal, P., Miranonde, V., Radomyos, P., Bunnag, D. and Harinasuta, T. (1984) Alterations of the surface tegument of *Opisthorchis viverrini* exposed to praziquantel *in vitro* and *in vivo*. *Southeast Asian J. Trop. Med. Pub. Hlth.*, **15**(1):95-103.

Soh, C.T., Ahn, Y.K., Bae, K.H. and Park, C.Y. (1981) Praziquantel in the treatment of paragonimiasis. *Yonsei Rep. Trop. Med.*, **12**:22-32.

Yokogawa, M., Nimura, M., Hata, H., Kobayashi, M. and Tokita, T. (1980) Efficacy of praziquantel against *Clonorchis* and *Paragonimus* infections in rats and dogs. *Jpn. J. Parasit.*, **29**(6):515-522 (in Japanese).

Yokogawa, M., Yoshimura, H., Sano, M., Okura, T., Tsuji, M., Takizawa, A., Harada, Y. and Kihata, M. (1961a) Chemotherapy of paragonimiasis with bithionol I. Experimental chemotherapy on the animals infected with *Paragonimus westermani* or *P. ohirai*. *Jpn. J. Parasit.*, **10**(2):302-316.

Yokogawa, M., Yoshimura, H., Okura, T., Sano, M. and Tsuji, M. (1961b) Chemotherapy of paragonimiasis with bithionol II. Clinical observations on the treatment of bithionol. *Jpn. J. Parasit.*, **10**(2): 317-327.

Yoon, J.S., Kim, S.J. and Rim, H.J. (1984) Electron microscopic studies on the effect of praziquantel to the young adult worm of *Paragonimus westermani*. *Korea Univ. Med. J.*, **21**(1):113-125 (in Korean).

EXPLANATIONS FOR FIGURES

Fig. 1-14. Photographs of light microscopy

Fig. 1. Tegument of control worms, hematoxylin and eosin(HE) stained, ×200.

Fig. 2. Intestinal epithelium of a control worm, HE stained, ×200.

Fig. 3. A branched ovary(OV) of control worms with digited irregular margins, Semichon's acetocarmine(SA) stained, ×40.

Fig. 4. Ovary(OV) of a control worm. Note the compact germinal layer and medulla, HE stained, ×200.

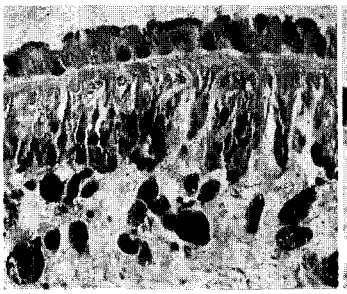
Fig. 5. Testes(T) of control worms elongated with smooth margin, SA stained, ×40.

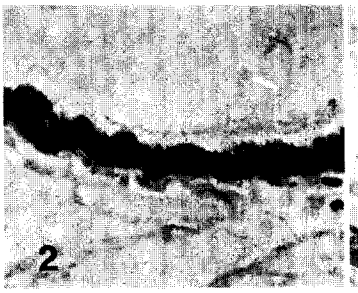
Fig. 6. Antero-ventral tegument of a worm incubated in 10μg/ml praziquantel for 6 hours, with destruction of syncytium, muscle and subtegument by vacuoles(V), HE stained, ×400.

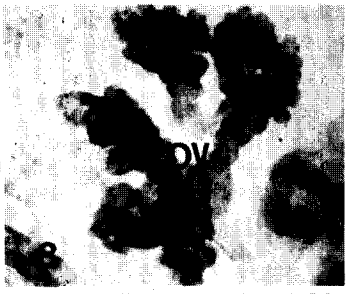
Fig. 7. Intestine of a worm incubated in 10μg/ml praziquantel for 6 hours showing constriction, and balloon (Ba) formation, and rough margin of wall, SA stained, ×40.

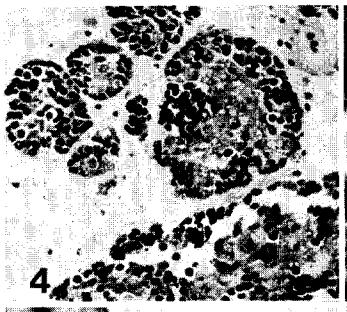
Fig. 8. Intestinal epithelium of a worm incubated in 10μg/ml praziquantel for 1 hour was thickened and vacuolized (arrow heads), HE stained, ×400.

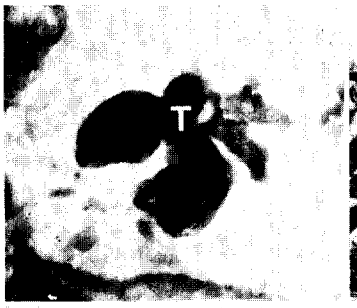
Fig. 9. Ovary(OV) of a worm incubated in 10μg/ml praziquantel for 12 hours showing ball formation at distal part of every branch by constriction and disintegrated of proximal part, HE stained, ×40.



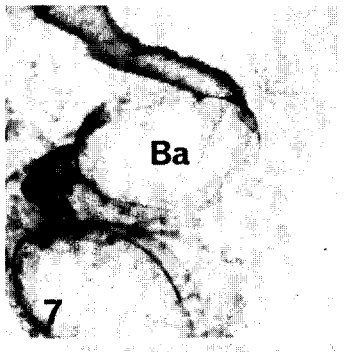

2


OV

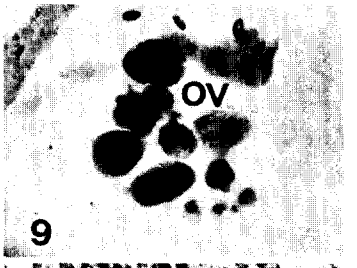

4

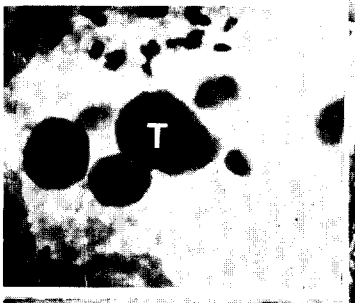

T

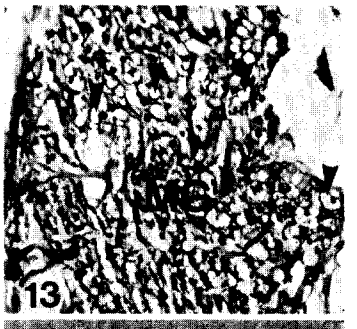

V

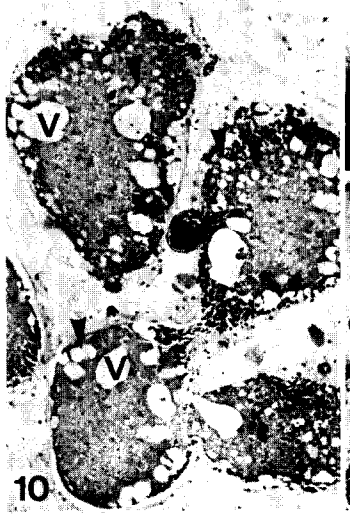

Ba
7

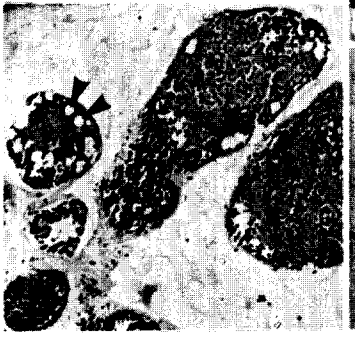

8

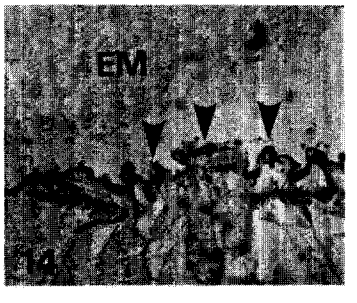

OV
9


T


13


V
V
10




EM
14

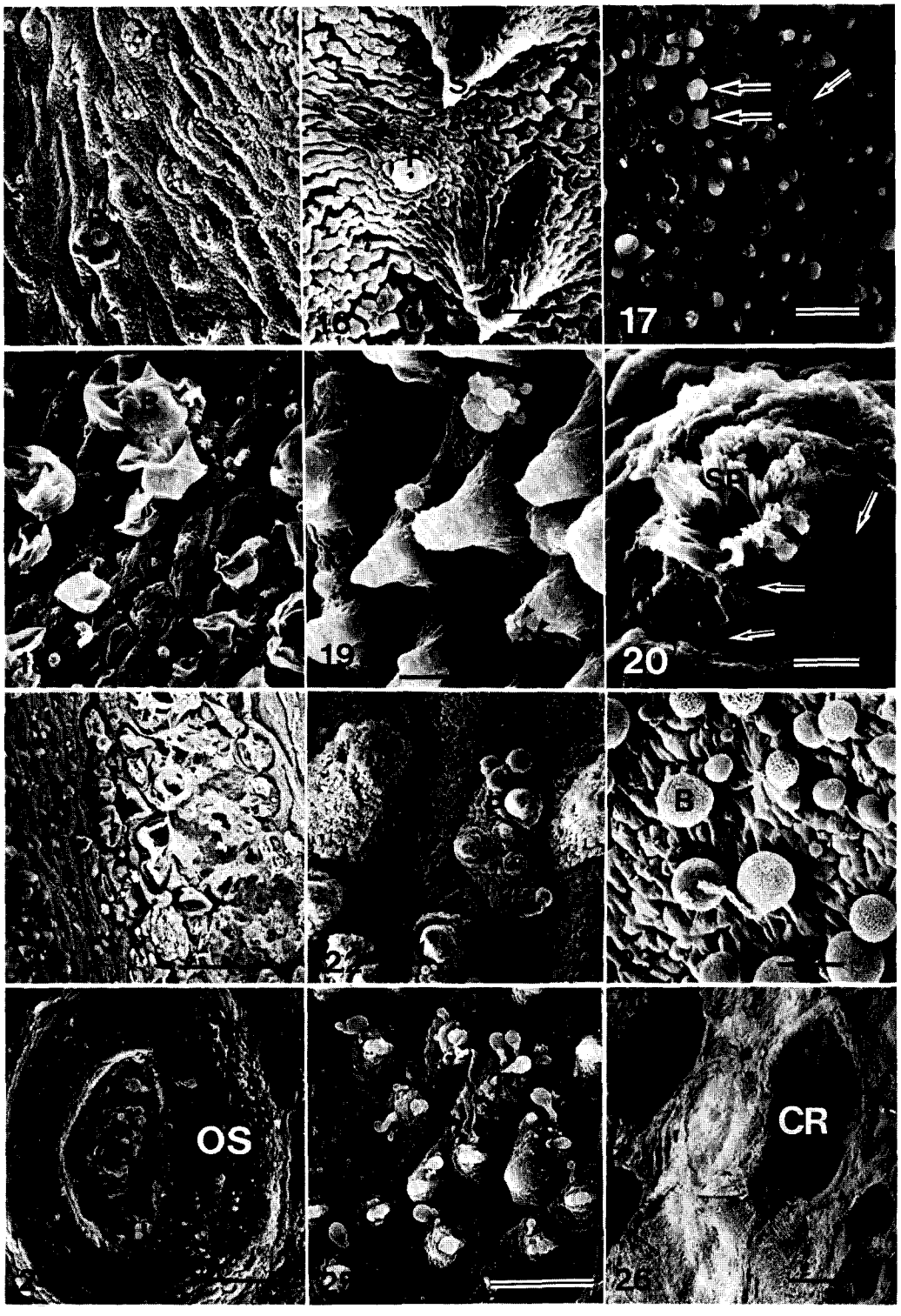

16
17
19
20
SR
B
OS
CR

Fig. 10. Ovary of a worm incubated in 0.1μg/ml praziquantel for 1 hour. Note large(V) and small vacuoles (arrow heads) in medulla, HE stained, ×100.

Fig. 11. Testes(T) of a worm incubated in 1μg/ml praziquantel for 1 hour. Note the single ball formed at the distal part of every testicular branch, HE stained, ×40.

Fig. 12. Testis(T) of a worm incubated in 0.1μg/ml praziquantel for 1 hour showed vacuoles(arrow heads) in periphery, HE stained, ×100.

Fig. 13. Mehlis' gland(MG) in 10μg/ml praziquantel for 6 hours was vacuolized(arrow heads), HE stained, ×200.

Fig. 14. Epithelium of excretory bladder showing shortened digitation(arrow heads) with eosinophilic excretion (EM) incubated in 0.1μg/ml praziquantel for 1 hour, HE stained, ×200.

Fig. 15-26. Photographs of scanning electron microscopy(SEM) after gold coating.

Fig. 15. Ventral surface between oral and ventral suckers of a control worm showing velvety cytoplasmic processes, minute grouped spines(GS), and solitary or grouped papillae with a cilium(P), Bar=20μm, ×1,000.

Fig. 16. Postero-ventral tegument of a control worm. Note the cytoplasmic processes, single tipped spines(S) covered with cytoplasmic membrane and a solitary papilla(P) with a cilium, Bar=5μm, ×3,300.

Fig. 17. Beginning of bleb-formation(arrows) on the tegument between oral and ventral suckers of a worm incubated in 0.1μg/ml for 15 minutes, Bar=20μm, ×600.

Fig. 18. The ruptured blebs(B) and small ones on the anteroventral tegument of a worm incubated in 0.1μg/ml praziquantel for 1 hour, Bar=20μm, ×1,000.

Fig. 19. Minute blebs(arrow heads) on tips and grouped ones on base of spines in postero-ventral tegument of a worm incubated in 0.1μg/ml praziquantel for 1 hour, Bar=10μm, ×2,010.

Fig. 20. Genital atrium of a worm incubated in 0.1μg/ml praziquantel for 1 hour. Note the honeycomb-like craters(arrows) in tegument of dome and sperms(SP) in the genital opening, Bar=20μm, ×980.

Fig. 21. Disintegration of base of oral sucker by the ruptured blebs in a worm incubated in 1μg/ml praziquantel for 1 hour, Bar=20μm, ×990.

Fig. 22. Antero-ventral tegument of a worm incubated in 1μg/ml praziquantel for 1 hour. Note the blebs (arrow heads) around papillae(P) and the autolysis of swollen tegument, Bar=10μm, ×2,000.

Fig. 23. The wrinkled large blebs(B) on the postero-ventral tegument of a worm incubated in 10μg/ml praziquantel for 30 minutes, Bar=30μm, ×500.

Fig. 24. A worm incubated in 10μg/ ml praziquantel for 1 hour, the inner and outer tegument of oral sucker (OS) was destroyed by numerous blebs and a grape-like blebs(GB) appeared in oral cavity, Bar=50μm, ×300.

Fig. 25. A few rubber bulb-like (club-shaped) blebs(RB) which had cylindrically extended tubular proximal part were observed on the ventrolateral tegument of a worm incubated in 10μg/ml praziquantel for 1 hour, Bar=20μm, ×1,010.

Fig. 26. The very large craters(CR) reaching to basement membrane layer and smooth surface disintegrated by the vacuoles formed from syncytium was observed in ventrolateral tegument of a worm incubated in 10μg/ml praziquantel for 6 hours, Bar=20μm, ×720.

=국문초록=

폐흡충에 대한 Praziquantel의 시험관내 작용에 관한 광학 및 주사전자현미경적 관찰

서울대학교 의과대학 기생충학교실 및 풍토병연구소
이순형 · 박호진 · 홍성종 · 채종일 · 홍성태

Praziquantel이 폐흡충에 미치는 영향을 광학 및 주사전자현미경으로 관찰하기 위하여 본 연구를 수행하였다. 가재에서 분리수집한 폐흡충의 피낭유충을 2마리의 개에 경구감염시키고 11주후에 도살하여 폐로부터 128마리의 성충을 회수하였다. Tyrode 용액에 praziquantel을 0.01, 0.1, 1, 10, 100μg/ml 농도로 희석하여 배양액으로 사용하였다. 배양액에 활발히 움직이는 충체를 8마리씩 넣고 37°C에서 배양하면서 15분, 30분, 1시간, 6시간, 12시간, 26시간에 운동성 및 형태학적 변화를 해부현미경으로 관찰하였다. 또한 각 실험군의 충체를 광학 및 주사전자현미경 표본으로 제작하여 관찰하였다.

Praziquantel에 작용된 충체는 즉시 수축하고 점차 이완되었으며 표피와 표피하층의 공포화, 소화관의 수축과 내강의 확장, 소화관벽의 비후, 난소와 고환의 공포화 및 말단의 구형화, Mehlis선의 공포화등이 관찰되었다. 주사전자현미경적 소견으로 소수포의 형성은 구흡반과 복흡반 사이의 표면에서 가장 먼저 관찰되었고 배양시간과 praziquantel의 농도가 증가함에 따라 전체표면으로 확산되었다. 고농도의 배양액에 배양한 충체의 측후방 표피에는 분화구모양의 손상이 관찰되었으며 점차 전체표피로 확산되었다.

Praziquantel의 작용을 받은 폐흡충의 형태학적 변화는 표피뿐만 아니라 소화기관, 생식기관 기타 조직등 전부위에서도 일어나는 것이 관찰되었다. 특히 충체가 살아있는 농도에서도 생식기관이 파괴되어 산란에 지장을 초래할 것으로 예상된다. 이와같은 변화는 praziquantel의 농도보다 배양시간에 보다 더 영향을 받는 것으로 보인다.