

## Observation of Liver Pathology after Praziquantel Treatment in Experimental *Clonorchis sinensis* Infection in Guinea Pigs

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**= Abstract =** The liver of guinea pigs infected experimentally with *Clonorchis sinensis* was grossly and histologically examined after treatment with praziquantel. Total 38 male guinea pigs were infected each with 300-500 metacercariae. Five weeks later praziquantel treatment was done on 28 animals, and repeated if needed. Remaining 10 were used for untreated controls, while 3 others were for uninfected controls. Liver examinations were done at 1, 2, 4 and/or 9 weeks following the treatment.

Compared with uninfected controls, *C. sinensis*-infected guinea pigs grossly revealed remarkably enlarged and swollen liver, with yellowish discolorations at surrounding portions of infected sites. Histologically these livers showed cystic hyperplasia of the bile ducts accompanied by irregular dilatation, glandular and/or papillary hyperplasia of the lining epithelia as well as periductal fibrosis.

The livers of praziquantel-treated animals grossly revealed more of less recovering features later than 2 weeks after the treatment. Histologically, although adenomatous hyperplasia was quite subsided, other bile duct changes such as cystic hyperplasia and fibrosis were persistently retained in most animals until 9 weeks after the treatment. The results suggest that the liver damage induced by *C. sinensis* is persistent for at least a few months after deworming with praziquantel.

**Key words:** *Clonorchis sinensis*, Liver fluke, Praziquantel, Guinea pig, Bile duct

### INTRODUCTION

The liver of man and animals infected with the liver fluke, *Clonorchis sinensis*, shows characteristic pathological features of dilatation and obstruction of bile ducts, portal inflammation and enlargement, proliferation and hyperplasia of duct epithelia in glandular and/or papillary fashion, and periductal fibrosis (Lee *et al.* 1978a & b; Flavell 1981). Furthermore, cholangiocarcinoma is regarded as a possible outcome of clonorchiasis (Hou 1956; Chung and Lee 1976; Kim

1984).

The excellent efficacy of praziquantel in the treatment of *C. sinensis* infection has been known for 10 years (Rim and Lyu 1979; Soh *et al.* 1979; Seo *et al.* 1983; Lee 1984; Rim 1986). The drug, however, acts on worm viability and thus can make worm expulsion from the bile duct, but does not directly act on the tissue recovery. Therefore, it is of clinical importance whether the liver pathology in clonorchiasis is easily repaired after worm expulsion by chemotherapy. Few reports are available on this subject. Recently, in experimental clonorchiasis of rabbits, Lee *et al.* (1987) indicated that liver changes in early stages of infection were re-

Table 1. Gross pathology of guinea pig liver before and after treatment with praziquantel

	Control group	Time after treatment			
		1 week	2 weeks	4 weeks	9 weeks
Enlargement	+++	+	—	—	—
Swelling	+++	—	—	—	—
Rough surface	+++	++	—	—	—
Nodular changes	+++	+	+	+	+
Yellowish discoloration	+++	+++	+++	++	++
Cystic changes	+++	+++	+++	++	++

\*—; no change, +; mild change, ++; moderate change, +++; severe change

versible after praziquantel treatment, however, some of the biliary epithelial changes that occurred in chronic stages of infection were not.

In guinea pigs, the liver damage due to liver fluke infection was reported much severer than that observed in rabbits (Sun and Gibson 1969; Lee *et al.* 1978b). Therefore, it is a question to what extent the damaged liver of guinea pigs would be repaired after deworming. The present study aimed to observe whether the liver pathology in *C. sinensis*-infected guinea pigs is repairable in a few months after praziquantel treatment.

MATERIALS AND METHODS

The metacercariae of *C. sinensis* were collected from 500 artificially digested *Pseudorasbora parva*, which were caught at Nakdong river, Kimhae-City, Kveongsang-nam-do (Province). A total of 28 male guinea pigs (450-600 g) were each given 300-500 metacercariae, while 3 guinea pigs were kept uninfected for use as controls. Five weeks after the infection all of them were found to pass the eggs of *C. sinensis*.

Experiment 1: At 5 weeks after the infection 300 mg/kg praziquantel (100 mg/kg × 3 times, for 1 day) was orally given to each of 12 animals. Six were untreated and used for untreated (infected) controls. At 1, 2 and 4 weeks after the treatment 2 untreated controls and 4 treated guinea pigs were sacrificed under ether anesthesia and their livers were extracted. After gross observation of whole livers, at least 5 paraffin blocks for each liver were prepared from defined areas, cut into 5-7 μm sections, and processed for routine hematoxylin and eosin stains.

The livers of praziquantel-treated guinea pigs did not reveal significant repair up to 4 weeks after the treatment, and in some liver sections a few *C. sinensis* worms were found to be still there. Four of 12 treated animals revealed considerable numbers of eggs in their stool at 1-4 weeks after the treatment. In order to rule out the possibility that the persistent liver pathology was due either to incomplete treatment and retained worms in the bile duct or to relatively short follow-up period, another experiment was performed.

Experiment 2: After 5 weeks of *C. sinensis* infection 8 guinea pigs were given two or three courses of treatment each course with 600 mg/kg praziquantel (300 mg/kg × 3 times, for 2 days) at 5 week interval, until their stools no more revealed *C. sinensis* eggs. At 4 or 9 weeks after the last treatment they were sacrificed, with 2 untreated controls, and their livers were examined.

RESULTS

1. Gross findings of the guinea pig liver

The livers of untreated control (*C. sinensis*-infected) group at 5-9 weeks of infection showed severe pathological features such as remarkably enlarged size (weight not measured), with cystic elevations, swellings and yellowish discolorations at surface areas (Fig. 1 and Table 1). The texture of the liver surface was generally rough and fibrotic.

Comparably, the livers of praziquantel-treated animals showed more or less recovering features (Fig. 2 and Table 1) from the above pathological condition, after one course or 2-3

Table 2. Histopathology of guinea pig liver before and after treatment with praziquantel

	Control group	Time after treatment			
		1 week	2 weeks	4 weeks	9 weeks
Irregular duct dilation	+++	+++	+++	+++	+++
Widening of portal space	+++	+++	+++	+++	++
Glandular hyperplasia	+++	+++	+++	+	+
Cystic hyperplasia	+++	+++	++	++	++
Periductal fibrosis	+++	+++	++	++	++
Papillary hyperplasia	+++	+++	++	++	+
Cell infiltration	+++	+++	++	++	++
Duct epithelial cells atrophy	+++	+++	++	++	++
squamous metaplasia	+++	+++	++	++	++
cribriform formation	+++	+++	++	++	++
mucoid dysplasia	+++	+++	++	++	++

\* +; mild change, ++; moderate change, +++; severe change

courses of treatment. Liver enlargement and/or swelling was no more recognizable later than 1 or 2 weeks post-treatment. The surface texture of the liver became generally smooth. However, nodular elevations, cystic changes and yellowish discolorations were consistently observed over the surface of the liver until 4 weeks (Fig. 2) or 9 weeks post-treatment.

2. Histopathological findings of the guinea pig liver

The livers of untreated control (*C. sinensis*-infected) group, at 5-9 weeks after infection, showed histopathological changes of variable severity, especially in biliary system (Figs. 3-6). Sectioned worms were occasionally demonstrated in bile ducts (Figs. 3 & 4). Major histopathological features were tortuous and irregularly dilated bile ducts with cystic changes in part (Fig. 5) and widened portal space. Egg granulo-mas were focally seen around bile ductules (Fig. 6). In addition, the epithelia of bile ducts showed partial compression atrophy, adenomatous proliferation, thickening and/or papillary growth. Periductal fibrosis was severe, even forming fibrous connection between portal space and accompanied periductal inflammatory cell infiltration (Figs. 3-6).

Most of the above findings were largely unchanged during 1-4 weeks following praziquantel treatment (Figs. 7-13). Nine weeks after the last treatment only glandular proliferation of adenomatous type was remarkably subsided and papillary growth of the duct epithelia became

less manifest (Figs. 14-16 and Table 2).

In one course treatment group, mucous metaplasia of duct epithelia (Fig. 9) and cystic hyperplasia around bile ducts persisted, and were severe in degree at 1 week (Figs. 7-8) and 2-4 weeks post-treatment (Figs. 10-12). Papillary growth of the duct epithelia was also persistent until 4 weeks (Figs. 10 & 12). In a few instances *C. sinensis* worms were found retained in bile ducts.

Also in two or three-course repeated treatment group, the pathological changes of periductal areas have not been recovered until 4 weeks (Fig. 13) or 9 weeks (Figs. 14-16) following the last treatment. No worm was retained in bile ducts except in one guinea pig. At 4 weeks cystic dilatations were still conspicuous around large bile ducts, where no worm was retained in them (Fig. 13). Glandular hyperplasia was remarkably reduced (Fig. 13). Even after 9 weeks, enlargement of bile ducts with periductal fibrosis was still remarkable (Figs. 14-16). Cystic lesions appeared to have regressed in some portions (Figs. 14 & 15), while not in others (Fig. 16).

DISCUSSION

The present study revealed that, although praziquantel treatment was effective to kill and remove *C. sinensis* worms from the bile duct of guinea pigs, their liver pathology was not completely resolved in 9 weeks after the last treatment.

The therapeutic efficacy of praziquantel on *C.*

*sinensis* infection is well-known, both in man and animals (Rim 1986). However, the drug dosage and regimen for animals are known to be different from those required for humans, in part due to differences in the drug absorption rate from the gut (Andrews *et al.* 1983). For example, total 60–90 mg/kg in 2–3 divided dose was as effective to show 91–95% cure rate in human *C. sinensis* infection (Lee 1984), whereas more than 300 mg/kg was required to obtain over 90% cure rate in rats (Rim *et al.* 1980; Yokogawa *et al.* 1980).

In guinea pigs, however, few reports are available on the therapeutic dosage of praziquantel for *C. sinensis* infection. In the present study, total 300 mg/kg treatment was tried first, but only 8 of 12 guinea pigs treated were cured when judged from the disappearance of eggs in their feces. Hence, this regimen appeared to be inadequate for complete cure of *C. sinensis* infection in guinea pigs. Repeated treatment (2 or 3 courses every 5 weeks) with higher dosage (double) was tried next, which brought about excellent deworming efficacy in guinea pigs.

For this reason, in the first experiment of this study, incomplete removal of *C. sinensis* from the bile duct was thought to be an important reason for the persistence of liver pathology. However, it appeared to be not necessarily true. Even after a complete expulsion of worms, the damaged liver did not reveal significant repair up to 9 weeks following two or three courses of treatment.

The persistence of *C. sinensis*-induced liver pathology in guinea pigs, after treatment with praziquantel, seems to be a characteristic feature, being different from other kinds of animals. In rabbits, pathological changes of the liver induced in early stages of infection (within 2 weeks) were recovered by praziquantel treatment, although some of those induced in chronic stages (later than 4 weeks) were not (Lee *et al.* 1987). In rats, complete resolution of liver pathology was recognized in as early as 2 weeks after praziquantel treatment, although in a few animals portal space widening was persistently observed (unpublished observation). No reports are available on the liver pathology resolution in human clonorchiasis.

Reasons for the persistence of liver pathology in *C. sinensis*-infected guinea pigs, after worm

eradication, remain to be elucidated. There are two possibilities for the interpretation of this finding. The one is that the liver pathology in guinea pigs was so severe that complete resolution of the pathology would require longer time. The other is that some pathological changes around bile ducts might be a kind of permanent scar and thus hardly reversible. Further studies are needed to understand biliary pathophysiology of guinea pigs infected with *C. sinensis*.

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= 국문초록 =

간흡충 감염 Guinea pig에 있어서 프라지판텔 투여후 간병변  
치유여부에 대한 관찰

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실험적으로 간흡충을 감염시킨 guinea pig에 프라지판텔을 투여한 다음 간병변 치유여부를 육안적 및 조직학적으로 관찰하였다. 총 28마리의 guinea pig(암컷)에 간흡충 피낭유충을 각각 300-500개씩 투여하였고, 5주후에 20마리에 대하여 프라지판텔을 투여하여 치료하였다. 나머지 8마리는 비투약 대조군으로, 또다른 정상 guinea pig 3마리는 비감염 대조군으로 사용하였다. 1차 투약 또는 2-3회 반복 투약후 1주, 2주, 4주 또는 9주에 각각 간조직을 적출하여 검사하였다.

비감염 대조군과는 달리 간흡충 감염 guinea pig의 경우 육안적으로 간이 커졌었고, 황색 변성 부위와 간표면의 거칠음 등을 보였다. 한편, 조직학적으로 이들 간은 낭상변성(cystic change), 담관주위의 섬유화(periductal fibrosis), 불규칙한 담도 확장(irregular dilation of bile ducts), 선종증식(glandular hyperplasia), 담관상피의 유두상 증식(papillary growth of duct epithelia) 등을 나타내었다.

프라지판텔을 투여하여 간흡충 치료를 시행한 guinea pig에서 간조직의 육안소견은 투약 2주 후부터 다소간 병변이 치유되어 가는 양상을 보였다. 그러나 조직학적으로 관찰한 바 선종증식의 점차적 소멸 이외에는 투약후 9주까지 낭상변성, 담도주위의 섬유화, 담도확장 등 담관주위의 병변이 거의 모든 guinea pig에서 소멸되지 않고 그대로 남아있었다.

이상의 소견으로 보아 간흡충에 의해 초래된 guinea pig의 간병변은 프라지판텔로 충체를 배출한 후에도 최소한 2-3개월까지는 지속되며 따라서 짧은 기간내에 치유되기가 어렵다는 것을 알 수 있었다.

## EXPLANATIONS FOR FIGURES

- Fig. 1. Gross feature of the liver of a guinea pig infected with *C. sinensis*. Untreated control group, 9 weeks after infection. Note enlarged liver and rough texture of surface, with small nodular elevations and cystic lesions (arrows).
- Fig. 2. *Ibid.* Treatment group, 4 weeks after treatment (9 weeks after infection). The liver size nearly regressed but there remained cystic lesions and nodular elevations (arrows).
- Fig. 3. Liver section of an untreated control group, 5 weeks after infection. An adult *C. sinensis* is seen in bile duct. Periductal fibrosis and inflammation are prominent. H-E stain,  $\times 40$ .
- Fig. 4. *Ibid.* Section of another portion. Parenchymal organs of *C. sinensis* such as sucker, intestine, seminal vesicle and uterine tubules containing numerous eggs are seen. H-E stain,  $\times 40$ .
- Fig. 5. *Ibid.* Another guinea pig. Cystic hyperplasia is conspicuous, with occasional papillary growth of the duct epithelia. H-E stain,  $\times 40$ .
- Fig. 6. *Ibid.* A figure showing several eggs of *C. sinensis* entrapped in bile ductules (arrows), probably carried with reflux of bile from large, infected ducts. H-E stain,  $\times 200$ .
- Fig. 7. Praziquantel treatment group, 1 week after treatment. Periductal cystic hyperplasia is still severe and no signs of recovery are recognizable. H-E stain,  $\times 40$ .
- Fig. 8. Magnification of Fig. 7, showing cystic dilatations, periductal fibrosis, and cell infiltration. H-E stain,  $\times 100$ .
- Fig. 9. Treatment group, 1 week after treatment. Magnification of a duct wall. Severe epithelial changes and glandular hyperplasia are characteristically seen. H-E stain,  $\times 200$ .
- Fig. 10. Treatment group, 4 weeks after treatment. Cystic hyperplasia is still severe. H-E stain,  $\times 40$ .
- Fig. 11. *Ibid.* Another animal. The enlarged bile duct has no worm but accompanies cystic hyperplasia and periductal fibrosis. H-E stain,  $\times 40$ .
- Fig. 12. *Ibid.* Magnification of a portion showing papillary growth from the duct epithelia. Cell infiltrations and fibrosis are severe. H-E stain,  $\times 100$ .
- Fig. 13. Repeated treatment group (3 times at 5 week interval), 4 weeks after the last treatment. Pathological changes at periductal areas have not yet been recovered. Cystic dilatations are seen around a large bile duct, where no worm is retained. Glandular hyperplasia became less severe (right side). H-E stain,  $\times 100$ .
- Fig. 14. *Ibid.* Nine weeks after the last treatment. The biliary change is retained just the same as before treatment. H-E stain,  $\times 40$ .
- Fig. 15. *Ibid.* Another guinea pig, 9 weeks after the last treatment. Cystic lesions appear to have regressed, but the enlargement of bile ducts and periductal fibrosis have not been repaired. H-E stain,  $\times 100$ .
- Fig. 16. *Ibid.* Another portion. The size of bile duct became relatively smaller but glandular hyperplasia is still seen. H-E stain,  $\times 100$ .







