

Correlation of sonographic findings with histopathological changes of the bile ducts in rabbits infected with *Clonorchis sinensis*

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Abstract: Clonorchiasis is an important parasitic disease of humans in Korea. The present study intended to compare sonographic findings with histopathological changes in experimental clonorchiasis. Eighteen New Zealand white rabbits were infected with metacercariae of *Clonorchis sinensis*, and examined 4, 10, and 22 weeks post-infection (PI). Four infected rabbits were treated with praziquantel 10 weeks PI and were examined 12 weeks after treatment. Sonography revealed mild to severe dilatation of the intrahepatic ducts (IHDD) and slightly increased periductal echoes in 12 out of 14 rabbits at 4 weeks PI, and all of the animals after 10 and 22 weeks PI and 12 weeks after treatment. The histopathological lesions were duct dilatation, mucosal hyperplasia, and periductal fibrosis, which progressed from 4 weeks to 22 weeks PI and even in treated rabbits. The dilated intrahepatic ducts over 1 mm diameter were detected by sonography. The present results indicate that sonographic findings are well correlated with histopathological lesions in rabbit clonorchiasis except for early phase of light burden of infection. The sonography has a limitation in discriminating residual sequelae of the ducts after praziquantel treatment.

Key words: *Clonorchis sinensis*, rabbit, sonography, pathology

INTRODUCTION

Clonorchis sinensis is the most prevalent parasitic helminth of humans in Korea (Seo *et al.*, 1981; Rim, 1986; MHSA & KAH, 1993). The fluke lives in the bile duct, and induces dilatation of the duct, hyperplasia of the mucosa, metaplasia, dysplasia or neoplasia of the mucosal epithelium, periductal inflammation and fibrosis, and thickening of

the ductal wall (Lee *et al.*, 1978, 1988, 1993a & b; Song *et al.*, 1989).

The nationwide control of human parasites in Korea switched the target from *Ascaris* to *Clonorchis* in early eighties. However, the nationwide control project of clonorchiasis from 1984 to 1990 was evaluated inefficient (MHSA & KAH, 1992). Although the control was carried out intensively by detection of the infected people with fecal examination and treatment with praziquantel at major endemic areas, the overall egg positive rate of *C. sinensis* is still high as 2.2% (MHSA & KAH, 1993). We interpret that the failure of the

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control is mainly due to difficulties in detection of the infected cases and also reinfection after treatment.

The diagnosis of clonorchiasis is easily made by detection of its characteristic eggs in the feces. However, case detection through fecal examination requires too much labours of both health workers and also subjected people. At present, two ways are executed in the field, visiting homes by the health workers or collecting the subjected people at a meeting. Visiting the individual home is labour intensive or time consuming and practically impossible to cover a large population. Collecting the people at a place is the ordinary way of the field work at present, but that is also not efficient because at least 3 times of attendance are needed for distribution of feces container, collection of feces, and treatment. Recently collection of feces becomes more difficult than before mainly because of low voluntary cooperation of the subjected people.

In this context, development of the better diagnostic tool is necessary to replace the fecal examination in diagnosis of clonorchiasis. Until now, skin test, ELISA, and radiological techniques have been applied in diagnosis of clonorchiasis (Rim, 1986; Chen *et al.*, 1994). The skin test is known to be sensitive but not specific. ELISA is evaluated low in sensitivity and specificity. Radiologic modalities include sonography, CT, and cholangiography. The sonography is known to be effective to visualize dilated intrahepatic bile ducts, and practically more feasible than other radiologic methods. Sonographically, diffuse uniform dilatation of the intrahepatic ducts without evidence of extrahepatic biliary dilatation is interpreted as characteristic findings of clonorchiasis (Kang *et al.*, 1980; Kim *et al.*, 1983 & 1988; Lim *et al.*, 1984, 1989; Ryu *et al.*, 1993).

Praziquantel treatment could not completely decompress the dilated ducts (Lee *et al.*, 1987 & 1988). For further evaluation of sonographic diagnosis of clonorchiasis, it is essential to compare the sonographic findings with pathological lesions including the post-treatment condition. The current study was designed to compare sonographic findings with histopathological lesions in experimental clonorchiasis of rabbits.

MATERIALS AND METHODS

1. Infection of experimental animals

The freshwater fish *Pseudorasbora parva* collected in the Naktonggang (River) were digested in pepsin solution, and the metacercariae of *C. sinensis* were recovered. The metacercariae were introduced into the stomach of rabbits through a tube. The number of rabbits was summarized in Table 1. They were kept in the animal room and fed with commercial pellet diet and piped water. For the treated group, 4 infected rabbits were treated with praziquantel (Distocide® Shinpoong Pharmaceutical Co., Korea) 100 mg/kg for 3 days, 10 weeks after infection. They were examined 12 weeks after treatment.

2. Sonographic examination

The rabbits were prepared for sonography by anesthesia with peritoneal injection of ketamine HCl (Ketalar®, Yuhan Co., Korea) and shaving hairs on the upper abdomen. Sonograms of the liver were obtained with a 10 MHz transducer on commercially available real time scanner (SPA 1000, Dasonics, U.S.A.)

3. Histopathological examination

After sonography, the rabbits were sacrificed by ether anesthesia and bleeding by heart puncture. Their livers were removed and separated of their lobes, and were fixed in Carnoy fixative for a few days. Lobes of the livers were sliced at the same proximal and distal sites, and prepared for paraffin embedding and hematoxylin-eosin staining.

4. Stool examination

The stools of the infected rabbits were microscopically examined for counting the eggs of *C. sinensis* by Stoll's dilution method.

RESULTS

One to 6 rabbits were included in 5 experimental groups (Table 1): control, 4 weeks post-infection (PI), 10 weeks PI, 22 weeks PI, and 12 weeks after treatment (T).

1. Control group

One control rabbit showed no dilatation of the intrahepatic bile ducts with both sonographic and histological observations (Fig. 1; Table 2). No ducts were involved in the

Table 1. Scheme of experimental infection of rabbits with *Clonorchis sinensis*

Groups	No. of MC ^{a)}	No. of rabbits	Rabbit ID
Control		1	
Repeated infection 20 × 3 ^{b)}		6	
4 week		1	20-6
10 week		3	20-2,4,5
22 week		2	20-1,3
Heavy infection	400	8	
4 week		1	400-1
10 week		3	400-2,3,12
22 week		4	400-4,5,6,22
Treated with praziquantel ^{c)}	400	4	400-11,14,15,21
Total		19	

^{a)}MC: Metacercariae of *C. sinensis*; ^{b)}Repeatedly infected 3 times biweekly; ^{c)}Praziquantel regimen; 100 mg/kg/day for 3 days

mucosal hyperplasia, thickening of the wall, and inflammation.

2. Four weeks PI group

Fourteen rabbits were screened by sonography 4 weeks after infection. Twelve rabbits were found positive for intrahepatic duct dilatation (IHDD), and six were also associated with increased periductal echoes (PDE) (Table 2 & Fig. 2A). Two rabbits with no sonographic abnormalities were found to have much less dilated intrahepatic ducts, infected ducts, and very low EPG than the others (Tables 3 & 4).

3. Ten weeks PI group

All of the 15 rabbits showed minimal or moderate degree of IHDD (Tables 2 & 3). Eight rabbits with moderate IHDD were also positive for PDE (Figs. 2B & 3). Floating material in the gallbladder was observed in one rabbit. Histologically the livers showed many dilated intrahepatic ducts, severe periductal fibrosis and inflammation (Fig. 3B). Many dilated ducts over 1 mm diameter were found in 5 rabbits out of 6 (Table 4).

4. Twenty-two weeks PI group

All rabbits showed positive sonographic

Table 2. Sonographic findings of rabbits 4, 10 and 22 weeks after infection with *C. sinensis*

Group	No. of rabbits	No. of rabbits with sonographic findings ^{a)}							
		—	IHDD +	++	+++	PDE	FD	FMGB	ASC
Control	1	1	0	0	0	0	0	0	0
4 weeks									
20 MC ^{b)} × 3 infected	5	2	2	1	0	0	0	0	1
400 MC infect	9	0	1	5	3	6	0	1	0
10 weeks									
20 MC × 3 infected	4	0	2	2	0	2	0	1	0
400 MC infect	11	0	5	6	0	6	0	0	0
22 weeks									
20 MC × 3 infected	2	0	1	1	0	1	0	0	1
400 MC infected	4	0	1	3	0	3	0	0	0
Treated	4	0	1	3	0	3	0	0	0

^{a)}IHDD, intrahepatic duct dilatation, +++ for wider ducts than portal vein, ++ for similar ducts with portal vein, + smaller ducts than portal vein, - for no visualized ducts; PDE, periductal echo; FD, filling defects in IHD; FMGB, floating material in GB; ASC, ascites; ^{b)}MC, metacercariae of *C. sinensis*

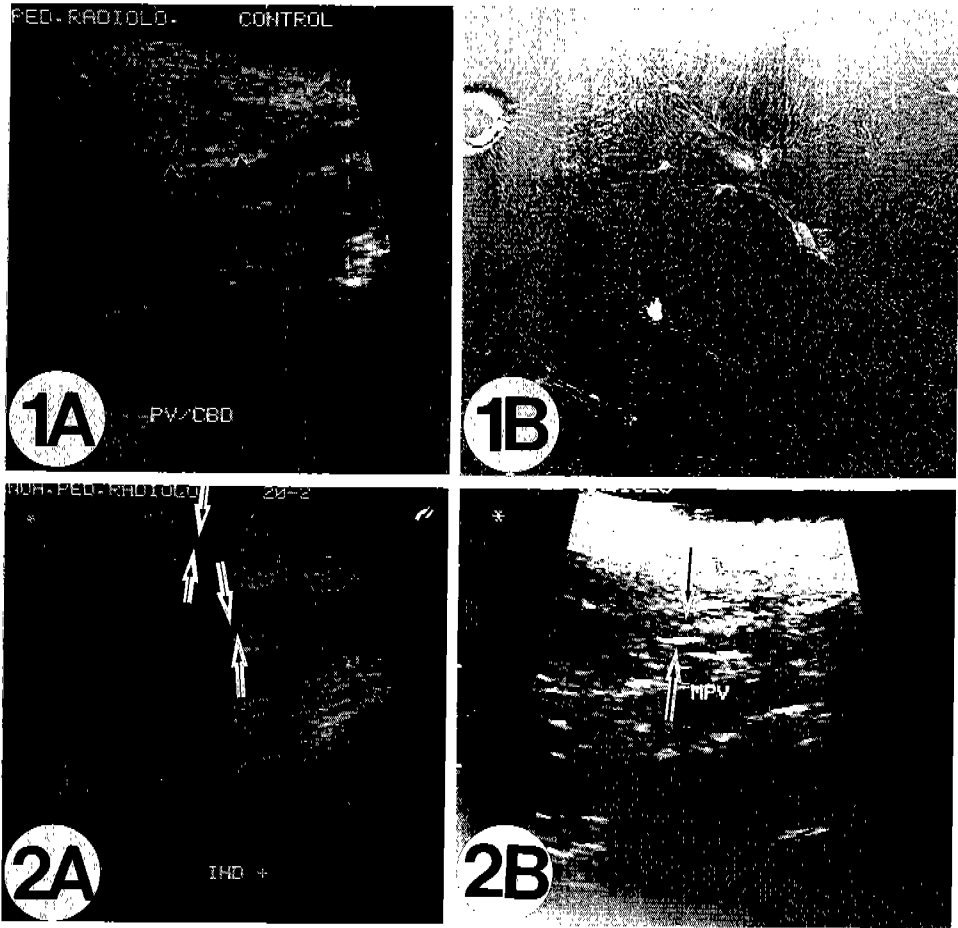


Fig. 1A. Sonogram of a control rabbit liver. The portal vein branches (arrow heads) are visualized but bile ducts are not. **Fig. 1B.** Histopathological observation of the liver of the control rabbit, showing normal architecture of the liver and normal intrahepatic ducts. HE stain, original magnification $\times 60$. **Fig. 2A.** sonogram of the rabbit 20-2 at 4 weeks PI. The portal vein (arrow heads) and bile ducts (arrows) were visualized. **Fig. 2B.** Sonogram of the rabbit 20-2, 10 weeks PI, showing moderate dilatation of the intrahepatic ducts (arrows) along the portal vein and increased periductal echoes.

findings of IHDD, and 4 rabbits with moderate IHDD also showed PDE (Tables 2 & 3). The histological observation of 3 rabbits showed severe bile duct dilatation, mucosal hyperplasia, and periductal fibrosis (Table 4). They had much more ducts over 1 mm diameter than animals of other groups.

5. Treated group

Twelve weeks after treatment, 4 subjected rabbits still showed IHDD mild to moderate degree (Tables 2 & 3). Three were included for histological observation, but one still harboured flukes in its intrahepatic bile ducts. Completely treated rabbits also showed

positive findings with both sonography and histology (Figs. 4A & 4B).

DISCUSSION

Sonography has been regarded as a good radiological tool for the diagnosis of clonorchiasis (Kim *et al.*, 1983; Morikawa *et al.*, 1988; Lim *et al.*, 1987 & 1989; Ryu *et al.*, 1993), especially in cases of chronic infection with heavy burden of flukes. CT scan is reported to give better comprehensive diagnostic information about the whole biliary trees (Kim *et al.*, 1988; Choi *et al.*, 1988 & 1989; Lim, 1990). The main CT findings are

Table 3. EPG and sonographic findings of rabbits 4, 10 and 22 weeks after infection with *C. sinensis*

Group	Rabbit ID	4 weeks			10 weeks			22 weeks	
		EPG	IHDD	PDE	EPG	IHDD	PDE	IHDD	PDE
Control		—	—	—	—	—	—	—	—
4 weeks	20-6	0	—	—	ND	ND	ND	ND	ND
	400-1	ND	++	+	ND	ND	ND	ND	ND
10 weeks									
	20-2	14900	+	—	13700	+	—	ND	ND
	20-4	800	—	—	1100	++	+	ND	ND
	20-5	5500	+	—	18200	++	+	ND	ND
	400-2	100	+++	—	5200	++	+	ND	ND
	400-3	200	+++	—	4200	+	—	ND	ND
	400-12	0	+++	+	200	++	+	ND	ND
22 Weeks									
	20-1	300	++	—	1200	+	—	+	—
	20-3	400			700			++	+
	400-4	ND	+	—	1500	+	—	++	+
	400-5	2600	++	+	900	++	+	++	+
	400-6	ND	ND	ND	6000	++	+	++	+
	400-22	500	++	—	1900	+	—	++	+
Treated									
	400-11	5100	++	+	4500	++	+	++	+
	400-14	3600	+	—	13600	++	+	++	+
	400-15	6600	++	+	3100	+	—	++	+
	400-21	ND	ND	ND	200	+	—	+	—

Abbreviations: EPG, numbers of eggs per gram of feces; ID, identification number; IHDD, intrahepatic duct dilatation, +++ for wider ducts than portal vein, ++ for similar ducts with portal vein, + smaller ducts than portal vein, — for no visualized duct; PDE, periductal echo; ND, not done.

Table 4. Histopathological findings of rabbits infected with *C. sinensis*

Findings	Rabbit Group				
	Control	4 weeks	10 weeks	22 weeks	Treated
No. of rabbits examined	1	2	6	6	3
Mean number of ducts over 1 mm diameter	0	9	23.7	24.5	30.3
Mean proportion (%) of ducts ^{a)} with					
Intrahepatic duct dilatation	0	2.7	2.5	4.6	5.3
Mucosal hyperplasia	0	5.5	8.2	11.5	15.1
Periductal fibrosis	0	7.2	8.2	17.3	26.7
Periductal inflammation	0	14.4	3.2	4.8	12.3
Ducts with worms	0	2.2	1.9	0.8	0.1

^{a)}Mean number of ducts over total number of bile ducts counted on the liver sections. The liver of a rabbit was sectioned on 7 sites; median lobe proximal and distal, left lobe proximal and distal, right lobe proximal and distal, and caudate lobe.

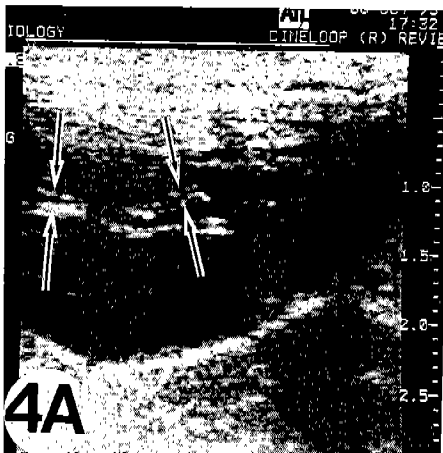
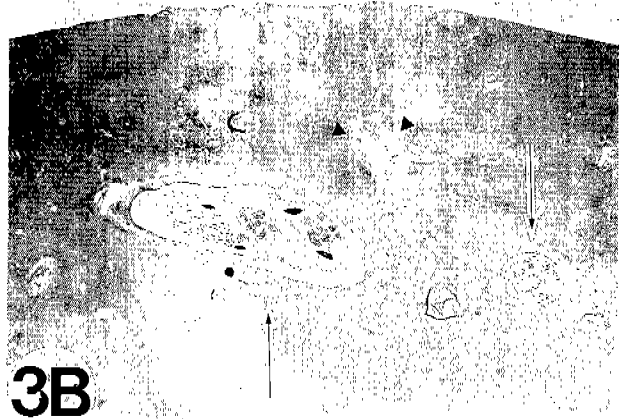
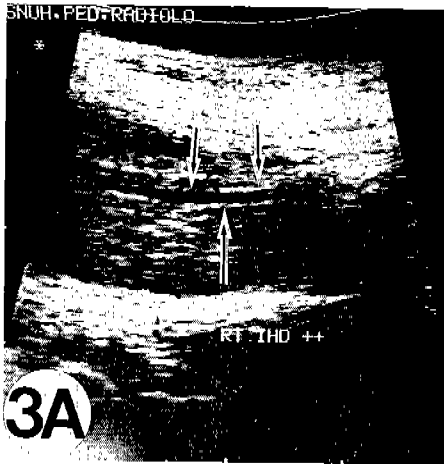


Fig. 3A. Sonogram of the 20-4 rabbit, 10 weeks PI. The ducts (arrows) are dilated moderately with periductal echoes. **Fig. 3B.** Photomicrograph of the rabbit 20-4, 10 weeks PI. The ducts (arrows) infected by flukes are remarkably dilated. Portal vein (arrow heads) is also seen nearby. HE stain, original magnification $\times 15$. **Fig. 4A.** Sonogram of the 400-15 rabbit, 12 weeks after treatment. The ducts (arrows) are still dilated moderately with increased periductal echoes. **Fig. 4B.** Photomicrograph of the rabbit 400-15, 12 weeks after treatment. The ducts (arrows) are still dilated. Portal vein (arrow heads) is also seen nearby. HE stain, original magnification $\times 15$.

dilatation of the intrahepatic ducts and thickening of the wall. However, they are too expensive to apply in screening many people. Furthermore it is not feasible in the field.

Various sonographic findings of clonorchiasis were already known (Lim *et al.*, 1987 & 1989). The findings are dilatation of the peripheral intrahepatic ducts, increased periductal echoes, thickening of bile duct wall, double contours of bile ducts, floating materials in the gallbladder, and some filling defects.

The present results suggest that diffuse dilatation of the intrahepatic ducts is the only

pathognomonic finding among the sonographic findings. Other sonographic findings were observed only from a few infected rabbits of this study, and also from only some human cases (Lim *et al.*, 1989). Periductal echoes may represent thick fibrosis around the ducts, and hyperplasia of the mucosa may induce two different sonographic densities in the duct wall. Sonographic filling defects or floating materials were suggested as masses of the flukes (Lim *et al.*, 1989). Only two rabbits were positive for floating materials in the gallbladder, but the worms were not found at necropsy. The sectioned gallbladder revealed

only eosinophilic material in the gallbladder. The material detected by the sonography might be the worms which had been passed out after sonography or masses of desquamated cells.

The known sonographic findings are readily detectable in the chronically infected hosts with heavy burden of worms because the pathological changes of the ducts are severe enough in that situation (Rim, 1986). However, the rabbit with low burden of worms showed no signs of clonorchiasis by sonography at 4 weeks PI but became positive at 10 weeks PI for intrahepatic duct dilatation. The biliary epithelial cells of all infected rabbits showed proliferative changes even in the early phase of infection, 1 week PI (Hong *et al.*, 1993). The discrepancy might be explained that dilatation of the duct was still mild and localized only around the infection site although the duct was recognized pathologically. According to our results, 13 (86.7%) of 15 infected rabbits were sonographically positive at 4 weeks PI and all rabbits showed positive findings 10 weeks PI or later. The present data prove that sonography is quite sensitive in diagnosis of chronic clonorchiasis. However, still more data are required to estimate the sensitivity in diagnosis of acute clonorchiasis of lighter worm burden. It should be evaluated whether sonography can pick out the host which is infected with only one or two flukes.

The present results also reveal that intrahepatic ducts enlarged over 1 mm diameter are visualized by sonography. The ducts larger than 2.5 mm are visualized as moderate dilatation. The involved ducts showed no predilection in distribution by lobes.

The ducts 12 weeks after treatment still showed positive findings by sonography. As Lee *et al.* (1987) described, the infected bile ducts were not completely recovered after praziquantel treatment. The ducts were still severely dilated by histopathology even after removing the worms and also found positive by sonography. From this finding, the limitation in sonographic diagnosis of clonorchiasis arises. Further researches are required to differentiate the sonographic findings of active infection from residual lesion.

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=국문초록=

간흡충에 감염된 토끼 담관의 초음파 소견과 조직병리학적 병변의 비교

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간흡충은 현재 우리 나라에서 가장 중요한 인체 기생충으로, 감염된 간내 담관이 확장되고 벽이 비후되므로 초음파로 이 소견을 관찰하여 간흡충증을 추정진단할 수 있다. 감염기간과 치료에 따른 초음파상을 조직학적인 병변과 비교 관찰하여 초음파상의 진단적인 가치를 정립하고자 이 연구를 수행하였다. 집토끼 18마리에 간흡충의 피낭유충을 20개씩 3회 반복 감염시키거나 400개씩 1회 감염시켜서, 감염 후 4, 10, 22주에 EPG 검사와 초음파를 관찰하고 일부를 도살하여 조직병리학적 소견을 관찰하였다. 또한 400개의 피낭유충을 감염시킨 토끼 4마리를 감염 후 10주에 프라지판텔로 치료하고 치료 후 12주에 관찰하였다. 간흡충란 정량검사서에서 4주에 EPG 0-14900의 범위 에 있었으며, 10주 후에는 16마리 모두에서 충란을 검출하여 EPG가 200-18200에 있었다. 감염 후 4주에 초음파로 관찰한 14마리 중에 12마리에서 간내담관의 확장이 있고, 담관주위 에코는 담관확장이 중등도 이상 심한 개체 6마리에서만 관찰하였다. 감염 후 10주와 22주, 또한 치료 12주 후에 관찰한 모든 개체에서 간내 담관의 확장 소견이 있었고 일부에서 담관주위 에코를 확인하였다. 조직학적인 소견도 감염 4주 후부터 담관이 확장되고 점막이 증식하며, 담관 주위 염증과 섬유화가 관찰되었고, 시간이 경과하면서 더욱 심하게 진행하였다. 치료 후에도 이러한 병변이 잔존하였다. 이러한 병변은 초음파 소견과 잘 일치하였다. 토끼의 각 간엽에 충체가 고르게 분포하였고 말단 담관에까지도 충체가 들어 있었다. 그러나 경감염 초기에는 초음파의 담관 확장 소견이 없는 개체도 있었다. 여러 가지 초음파 소견 중에서 간내 담관의 확장만이 진단적인 의미를 가지는 소견 이고 10주 이상 감염된 모든 개체에서 관찰할 수 있었다. 그러나 치료 후 잔존 병변을 구분하는 데 에 한계가 있어 이 부분에 대한 연구가 더 필요하다고 생각한다.

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