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Turnover of biliary epithelial cells in *Clonorchis sinensis* infected rats

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Abstract: We performed bromodeoxyuridine (BrdU) staining to observe the proliferation pattern of epithelial cells on the biliary mucosa in *Clonorchis sinensis* infection. Albino rats were infected with 100 metacercariae each and their livers were processed for histopathological observation after BrdU injection. Five to six sites in the liver of a rat were selected for paraffin section, and stained immunohistochemically to visualize BrdU incorporating cells. The flukes were mainly in the common bile duct and right or left hepatic bile ducts. The proportion of stained epithelial cells in the infected bile ducts where the worms were found on the section was 2.9-10.2% at 1 week after infection, 7.3-12.8% at 2 weeks, 7.3-13.4% at 5 weeks, and 8.4-14.8% at 15 weeks while in the non-infected ducts 0 to 2.7% cells were stained. The stained cells were mainly at the base of the mucosal layer. It is suggested that mucosal epithelial cells of the bile ducts infected with *C. sinensis* become hyperplastic mainly by direct and local stimulation of the worms.

Key words: *Clonorchis sinensis*, rat, biliary epithelial cells, turnover rate, bromodeoxyuridine

INTRODUCTION

Clonorchis sinensis is the most prevalent human parasitic helminth by stool examination recently in Korea (Kim *et al.*, 1984; Min *et al.*, 1986; Chai, 1990; Kim *et al.*, 1990). It crawls into the intrahepatic bile ducts and induces severe hyperplasia of epithelial cells and metaplasia of mucopolysaccharide-producing cells in the mucosa (Lee *et al.*, 1978; Song *et al.*, 1989; Hong *et al.*, 1990). It is also known to be related with cholangiocarcinoma (Chung and Lee, 1976; Flavell, 1981; Kim, 1984; Lee *et al.*, 1993). Some of the changes of the biliary mucosa were observed to remain even after praziquantel treatment (Lee *et al.*, 1987 & 1988).

However, we still have little information on the mechanism of the hyperplasia. Up until now, the pathogenetic mode of action of *C. sinensis* has been regarded as mechanical and chemical (Seo, 1978), however, further details on the factors still remain unknown. Furthermore, few data show the relation between the site of host reaction and worm location. The worm must make friction or sucking stimulation on the mucosa and induce marked exfoliation of the epithelial cells.

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Additionally, any chemical stimulation should be anticipated. The worm must secrete its various metabolites which can influence the host cells in any way of methods. Some of them are immunogens and some are chemical reagents.

Recently, several staining methods have been developed to visualize dividing cells. Incorporation of bromodeoxyuridine (BrdU) is one of them, which stains the cells in S phase of division (Risio *et al.*, 1988). The staining of the mucosal cells of *C. sinensis* infected bile ducts can show the pattern of cell turnover on the hyperplastic mucosa. The pattern may give some information on the nature of the tissue reaction in clonorchiasis.

MATERIALS AND METHODS

1. Infection of *C. sinensis* to rats

Sprague-Dawley rats were infected with the metacercariae of *C. sinensis* which were collected by peptic digestion of the freshwater fish, *Pseudorasbora parva*. Each animal was introduced with 100 metacercariae into the stomach via a gavage needle. The rats were bred in an animal quarter with commercial rat diet and pipe water. A part of the infected rats were orally introduced with praziquantel 100 mg/kg/day for 3 days, 8 weeks after infection for the treated groups. The treated rats were kept for 1 or 4 weeks.

2. Injection of BrdU and preparation of the liver

The rats were intraperitoneally injected with 10 mg/kg BrdU (Sigma Co., USA) 2 hours before sacrifice. Each group of 1, 2, 5, and 15 weeks after infection consisted of 5 rats. The rats were sacrificed by cervical dislocation, and their livers were removed and fixed in Carnoy solution. The fixed livers were divided by the lobe, and peripheral and central part of the median, left, and caudate lobes were sampled at same location. The selected liver fragments were processed for paraffin embedding and microtome section in 5 μm thickness.

3. Staining of the liver sections

The liver sections were stained with anti-BrdU mouse polyclonal antibodies (1:100 dilution) after deparaffination and hydration. Biotinylated anti-mouse horse serum (Cappel Co., U.S.A.), 1:100 diluted, was applied as a secondary antibody. The sections were further stained with peroxidase conjugated avidin, and the substrate was diaminobenzidine (DAB) for color reaction. Hematoxylin was used for counter staining. The stained sections were observed microscopically, and the brown stained cells were counted in the sectioned ducts. The proportion of stained cells over total number of epithelial cells was obtained in the examined ducts of each group. The findings were analyzed by the location of *C. sinensis*.

RESULTS

1. Location of the worms

The worms were found in the intrahepatic bile ducts of all the median, left, and caudate lobes. We didn't choose the right lobe for the experiment. In the first week the worms were found in the proximal (central) ducts of the 3 lobes but not in the distal (peripheral) ducts. However, the worms were in both distal and proximal ducts in the second week, and again they were only in the proximal ducts in the 5th and 15th weeks (Fig. 1).

2. Control animals

The intrahepatic ducts were very narrow and only less than 1.0% of the cells were incorporating BrdU in the control group (Fig. 2 & Table 1).

3. One week after infection (1 w)

Proximal ducts of the median, left, and caudate lobes contained the worms. Although the proximal ducts with worms showed 0% to 4.8%, mean 3.5% staining rates in the median lobe, the infected ducts of the left or caudate lobes stained more cells of 5.7% and 9.5% respectively. The distal ducts without worms stained about 2%, which were slightly higher rates than those of the control (Table 1).

4. Two weeks after infection (2 w)

The worms were found also in the distal ducts of the median or left lobes. In the median lobe, the infected proximal ducts showed 3.3% to 15.3% (mean 11.0%) stained cells (Fig. 3)

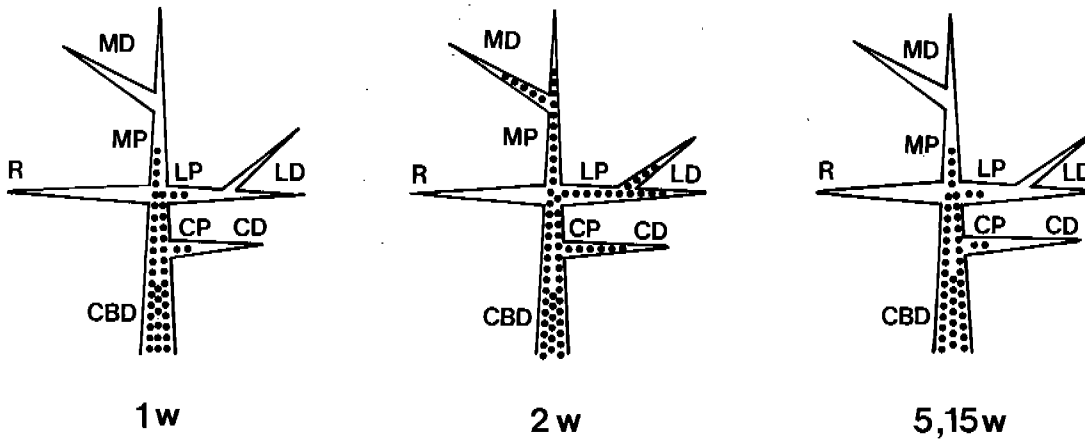

Fig. 1. Distribution of *C. sinensis* in the schematically illustrated ducts of the rats. The dotted ducts were infected by the worms. The worms were only in the proximal ducts of individual lobes at the first week, and then migrated further into the distal ducts at the second week. At 5 or 15 weeks after infection the worms were found in the proximal ducts but not in the distal ducts. The right lateral lobe (R) was not examined. CBD: common bile duct, CP: caudate lobe proximal duct, CD: caudate lobe distal duct, LP: left lateral lobe proximal duct, LD: left lateral lobe distal duct, MP: median lobe proximal duct, MD: median lobe distal ducts, R: right lateral lobe (excluded in this examination).

Table 1. Proportion (%) of BrdU incorporating cells on the biliary mucosa and location of the worms in the liver of rats infected by *C. sinensis*

Lobe/Ducts	Groups[a]						
	Control	1 w	2 w	5 w	15 w	T1w	T4w
Median lobe							
proximal, with worms[b]	—	3.5	11.0	13.2	11.3	6.3	9.5
proximal, no worms	1.0	—	—	—	—	—	—
distal, with worms	—	—	8.3	—	—	—	—
distal, no worms	0	2.0	1.0	0.6	2.1	0	0
Left lat. lobe							
proximal, with worms[b]	—	5.7	11.3	9.4	8.5	5.5	7.0
proximal, no worms	0.6	—	—	—	—	0	0
distal, with worms	—	—	6.2	—	—	—	—
distal, no worms[b]	0.4	2.3	1.0	0.4	0.8	0	0
Caudate lobe							
with worms	—	9.5	7.3	13.8	14.6	—	—
no worms	0.4	1.2	1.4	1.7	0.6	0	0
Extrahepatic							
with worms[b]	—	—	—	10.1	9.0	8.9	7.0
no worms[c]	—	—	—	1.7	0	0	0

[a]Groups: The 1w, 2w, 5w and 15w groups are 5 rats 1, 2, 5 and 15 weeks after infection respectively. T1w and T4w rats were treated with praziquantel 8 weeks after infection, and 1 and 4 weeks after treatment respectively. [b]Ducts with dilatation and periductal fibrosis in T1w and T4w. [c]Ducts without dilatation and periductal fibrosis in T1w and T4w

while infected distal ducts had 7.6% to 9.6% (mean 8.3%) stained cells. The stained cell proportions in the infected proximal ducts were 2.7% to 16.4% in the left and caudate lobes, but the rates in the infected distal ducts of the left lobe were 3.9% to 7.7% (mean 6.2%).

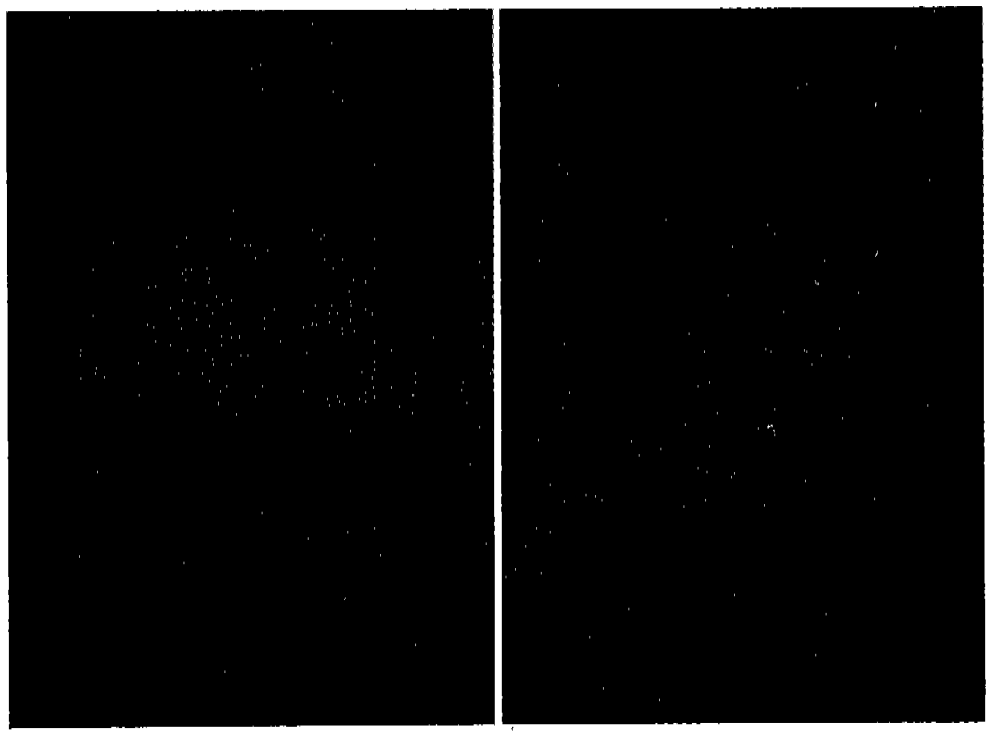

Fig. 2. A proximal intrahepatic bile duct in the median lobe was narrow and lined by monolayer of epithelial cells. No cells were incorporating BrdU. Original magnification, × 200. **Fig. 3.** An infected proximal intrahepatic bile duct in the median lobe by *C. sinensis* was markedly dilated and showed severe hyperplasia of epithelial cells 2 weeks after infection. Numerous cells(cells with brown nuclei) were incorporating BrdU at the base of the hyperplastic epithelial layer. Original magnification, ×200.

The staining rate of the non-infected distal or proximal ducts were in range of the control (Table 1).

5. Five weeks after infection (5 w)

The infected ducts were all in proximal location in the biliary tree. The stained cells were 5.6% to 16.8% (mean 13.2%) among the epithelial cells in the median lobe, 5.3% to 10.4% in the left lobe, and 11.6% to 15.7% in the caudate lobe. In the infected extrahepatic bile ducts, 4.6% to 13.4% cells (mean 10.1%) were stained, but in the ducts without worms were stained only 0% to 3.1% cells in the 3 lobes (Table 1).

6. Fifteen weeks after infection (15 w)

The proportions of stained cells in the infected ducts were 10.3% to 14.7% in the median lobe, 7.3% to 9.8% in the left lobe, 12.2% to 16.9% in the caudate lobe, and 3.9% to 11.0% in the extrahepatic duct. Contrary to these, the proportions in the ducts without worms were not high, 0% to 3.7% (Table 1).

7. Treated groups (T 1w & T 4w)

In the treated rats, no worms were found. The ducts with dilatation and hyperplasia were regarded as infected ducts. They showed 5.5% to 8.9% positive cells 1 week after treatment, and 7.0% to 9.5% cells were positive 4 weeks after treatment (Table 1).

DISCUSSION

The BrdU incorporating cells are over 10% among the biliary epithelial cells in the ducts with *C. sinensis* after 5 weeks of infection. The proportion is higher than that of hepatocellular carcinoma of low grade and similar with grade III to IV cancers (Tarao *et al.*, 1991). The high proportion of stained epithelial cells means that the cells are exfoliated in large scale and thus rapid division of the cells is essential to

the tissue in clonorchiasis. However, the cells actually proliferate much more than the loss, and therefore the mucosal layer undergoes severe hyperplasia. At the first week the cells already showed increased proliferation, and the proportion of stained cells already reached a plateau 2 weeks after infection. The persistently high proportion in the ducts 15 weeks after infection suggests that the hyperplasia is still progressive during the first 15 weeks after infection.

The proportion of dividing cells decreased after deworming by chemotherapy with a pattern of slow slope. The cell percentage decreased a little 1 week after treatment, but the proportion was higher than that of the control. It was still sustained high 4 weeks after treatment. The cell or tissue keeps the memory of stimulation by *C. sinensis* quite long even after deworming. The metaplasia of mucus secreting cells or adenomatous hyperplasia was still found to remain 6 months after treatment (Hong *et al.*, 1990). Therefore the remaining hyperplasia is not a simple residue of the histopathological changes. For a certain period after elimination of the worms, probably more than 6 months, the cells must keep the activity of higher proliferation than control. At present no data for the nature of the cell memory are available.

The cells of the ducts with worms were stained in significantly high proportions regardless of the location in the liver, but the ducts without worms showed no increase of the stained cells. In the ducts without worms, were stained the cells same as the control level (Table 2). This fact reveals that proliferation of the epithelial cells is provoked by only direct contact with worms. The worms are moving continuously in the duct and devour epithelial cells and blood cells (Rim, 1986). Therefore its suckers and tegument come in intimate contact with the biliary epithelial cells, and the adjacent cells must be influenced greatly by direct mechanical stimulus. However, it is still plausible that the host cells receive other stimuli than the mechanical one, such as immunological or chemical stimuli.

For immunological reactions, several experiments detected antibody reaction to *C. sinensis* (Hahm *et al.*, 1984; Hong, 1987).

Table 2. Proportion (%) of BrdU incorporating cells on the biliary mucosa in the liver of rats infected by *C. sinensis*

Groups	In the ducts	
	with worms[a)]	without worms[b)]
Control	—	0.6%
1 w	6.3%	2.0%
2 w	9.1%	1.0%
5 w	9.4%	0.5%
15 w	11.2%	1.4%
T1 w	6.3%	0
T4 w	7.8%	0

[a)]Ducts with dilatation and periductal fibrosis in T1 w and T4 w. [b)]Ducts without dilatation and periductal fibrosis in T1 w and T4 w. *The proportion between the two duct groups are significantly different by Wilcoxon rank sum test ($s = 300$, $p = 0.02$).

However, the role of host immune response on biliary epithelial cells is still unclear. Although the chemical factors are not proved yet, some chemicals from the worm may be expected to stimulate the cells. However, the present observation proposes only direct and local stimulation on proliferation of the epithelial cells by the chemicals, if any in clonorchiasis. Even in the proximal duct to the worm parasitization which drained mixed bile with worm secreta or eggs, there found very low uptake of BrdU. The finding informs that the mechanical communication between the worm and mucosal cells may make the most potent stimulation on proliferation of epithelial cells and the chemical stimulation by the worm is influencing only the adjacent mucosa if any.

Whether the hyperplastic biliary mucosa is advantageous for *C. sinensis* or not should be a question to be deliberated. The highly proliferated epithelial cells seem to make a good environment for the worm to live in. The lumen of dilated bile ducts becomes highly folded and soft, and thus the worm can keep its position more easily. The mucosal epithelium of bile ducts forms only a tract for bile flow, major role of which is collecting and bounding bile mechanically. The knowledge on the functional aspect of the hyperplastic biliary mucosa is very poor now, but the tissue may

have another physiologic role as well as the thick mechanical barrier. Also the increased turnover of epithelial cells can supply rich nutrition to the worm. Is the internal milieu prepared by the highly proliferated cells fit better for survival of *C. sinensis*? Isn't it a predesigned condition by the worm? Is it only an outcome of inflammation? The defensive role or any nursery function of the severely proliferated mucosal epithelium should be evaluated in the future.

The stained cells were found mainly at the base layer of the mucosa. This is a normal figure of cell turnover in the intestine or other organs with thick layer of epithelium. The present finding can be interpreted as the cell division occurs mostly at the mucosal base and also as the mucosal cells on the upper half layer are fully differentiated. The confined location of dividing cells suggests that the mucosal change in clonorchiasis is different from adenoma or adenocarcinoma which show random distribution of dividing cells.

REFERENCES

Chai JY (1990) Intestinal parasite examination of the Seoul National University Hospital patients (unpublished data).

Chung CS, Lee SK (1976) An epidemiological study of primary liver carcinomas in Busan area with special reference to clonorchiasis. *Korean J Pathol* **10**(1): 33-46.

Flavell DJ (1981) Liver-fluke infection as an etiological factor in bile duct carcinoma of man. *Trans Roy Soc Trop Med Hyg* **27**(4): 787-794.

Hahm JH, Lee JS, Rim HJ (1984) Comparative study on the indirect immunofluorescent antibody test, complement fixation test and ELISA in diagnosis of human clonorchiasis. *Korea Univ Med J* **21**: 177-184 (in Korean).

Hong ST, Huh S, Kho WG, *et al* (1990) Changes of histopathological and serological findings of the liver after treatment in rabbit clonorchiasis. *Seoul J Med* **31**(2): 117-127.

Kim SS, Han MH, Park SK, Lim HS, Hong ST (1990) A survey on the epidemiological factors of clonorchiasis in the Pohang industrial belt along the Hyungsan River, Kyongsangbuk-do. *Korean J Parasit* **28**(4): 213-219.

Kim SS, Lee SY, Lee SK, *et al* (1984) Results of routine parasitologic examination in 22690 cases, 1979. 1.—1983. 12. 31. *Inje Med J* **5** (4): 379-385.

Kim YI (1984) Liver carcinoma and liver fluke infection. *Arzneim-Forsch/Drug Res* **34**(II): 1227-1230.

Lee JH, Rim HJ, Bak UB (1993) Effect of *Clonorchis sinensis* infection and dimethylnitrosamine administration on the induction of cholangiocarcinoma in Syrian golden hamsters. *Korean J Parasit* **31**(1): 21-30.

Lee SH, Chai JY, Yang EC, Yun CK, Hong ST, Lee JB (1988) Observation of liver pathology after praziquantel treatment in experimental *Clonorchis sinensis* infection in guinea pigs. *Seoul J Med* **29**(3): 253-262.

Lee SH, Hong ST, Kim CS, Sohn WM, Chai JY, Lee YS (1987) Histopathological changes of the liver after praziquantel treatment in *Clonorchis sinensis* infected rabbits. *Korean J Parasit* **25**(2): 110-122.

Lee SH, Shim TS, Lee SM, Chi JG (1978) Studies on pathological changes of the liver in albino rats infected with *Clonorchis sinensis*. *Korean J Parasit* **16**(2): 143-155.

Min DY, Ahn MH, Kim KM, Kim CW (1986) Intestinal parasite survey in Seoul by stool examination at Hanyang University Hospital. *Korean J Parasit* **24**(2): 209-212.

Rim HJ (1986) The current pathobiology and chemotherapy of clonorchiasis. *Korean J Parasit* **24**(suppl): 26-39.

Risio M, Coverlizza S, Ferrari A, Candelaresi GL, Rossini FP (1988) Immunohistochemical study of epithelial cell proliferation in hyperplastic polyps, adenomas, and adenocarcinomas of the large bowel. *Gastroenterology* **94**(4): 899-906.

Seo BS (1978) Clinical Parasitology 2nd ed: 237-243. Ilchogak, Seoul (in Korean).

Song GA, Kim JD, Lee DW, *et al* (1989) Histological and histochemical studies on the intrahepatic duct in rabbits experimentally infected with *Clonorchis sinensis*. *Korean J Int Med* **37**(3): 344-355.

Tarao K, Shimizu A, Harada M, *et al* (1991) *In vitro* uptake of bromodeoxyuridine by human hepatocellular carcinoma and its relation to histopathologic findings and biologic behavior. *Cancer* **68**(8): 1789-1794.

=국문초록=

간흡충에 감염된 흰쥐 담관 상피세포의 증식 양상

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간흡충에 감염된 담관은 확장되면서 상피세포의 수가 크게 증식하여 점막층이 두꺼워지고 과증식에 의해 점막의 단면이 유두상으로 관찰된다. Bromodeoxyuridine (BrdU)이 세포가 핵산을 복제할 때에 끼어 들어가는 성질을 이용하여, 이 연구에서는 담관 상피세포 중에서 합성기(S phase)에 있는 세포의 분율을 파악하고자 하였다. 흰쥐에 간흡충의 피낭유충을 100 개씩 경구 감염시키고, 15주까지 간의 각 엽에 있는 담관의 조직절편을 만들어서 다세포군항체와 ABC complex를 이용한 염색을 실시하였다. 그 결과 충체가 들어 있는 담관에서는 감염 1주에 상피세포의 2.9-10.2%가 염색에 양성이었고, 2주에 7.3-12.8%가, 5주에는 7.3-13.4%가, 15주에 8.4-14.8%가 BrdU를 함유하였다. 대조군 흰쥐나 실험군 동물의 감염되지 않은 담관의 경우에는 0-2.7%가 염색에 양성으로 나타났다. 염색된 상피세포는 주로 점막 상피세포층의 기저부에서 관찰되었다. 이 결과에 의하면 간흡충과 직접 접촉하는 담관의 상피세포가 뚜렷하게 증식하고 있어 충체의 어떠한 물리적인 또는 화학적인 자극이 있더라도 가까운 부위에서 주로 작용하고 있다고 판단하였다.

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