J. Toxicol. Pub. Health
Vol. 17, Supplement, pp. 79-82, 2001

Effect of Dosage Level of Carcinogen and *Clonorchis sinensis* Infestation on Cholangiocellular Carcinoma Induction in Hamsters

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ABSTRACT *: The infection of liver flukes, Clonorchis sinensis (CS) and Opisthorchis viverrini (OV), has been known as a risk factor to induce cholangiocellular carcinoma (CCC) in human living in the endemic area, providing promoting effect on the liver initiated by chemical carcinogens. The present study evaluated the relationship between the dosage level of dimethylnitrosamine (DMN) and the infection load of CS in the neoplastic development by histopathological examination of the treated hamsters. To evaluate the effects of DMN, different doses of DMN ranging from 0 to 25 ppm were administered to hamsters with 20 CS metacercariea. For the risk assessment of the infection load, 0, 5, 15, 50 CS metacercariae were respectively infected with 12 ppm DMN. The mortality was closely related to the infection load rather than the concentration of DMN. The infection of CS clearly promoted the induction of CCC even at dose level of 6 ppm DMN. Only five metacercariae were enough to promote CCC induction at the concentration of 12 ppm DMN.*

Key Words *: Cholangiocellular carcinoma, Clonorchis sinensis, Hamster*

I. INTRODUCTION

Clonorchis (*C.*) *sinensis* is a liver fluke that dwells in the bile duct of human and animal liver (Carpenter, 1998; Rim, 1990). The infection with *C. sinensis* results from eating raw fish contaminated with metacercariae of *C. sinensis*. *C. sinensis* infection and still remains as one of the major public health problem in Korea (Rim, 1990). *C. sinensis*, when infected, cause cholangitis, biliary adenomatous hyperplasia, bile duct obstruction and subsequently cholangiofibrosis (Carpenter, 1998). *C. sinensis* has also been proven to be risk factor of cholangiocellular carcinoma (CCC) (Belamaric, 1973; Kim *et al.*, 1989; Parkin *et al.*, 1993). High incidence of CCC has been reported from people living in liver fluke endemic area (Parkin *et al.*, 1993; Rim, 1990).

C. sinensis infection when combined with chemical treatment results in bile duct dysplasia followed by development of CCC in hamster (Lee *et al.*, 1993). *C. sinensis* itself is not a complete carcinogen but known to act as a promoter like *H. pylori* in gastric cancer. Since no CCC was found in hamsters given either parasite or chemical carcinogen alone it also support the idea that there is a definite synergism between two factors for tumor development.

The present study was initiated to investigate the inter-relationship between parasite burden and carcinogen-dose level on neoplastic development in hamster CCC model.

II. MATERIALS AND METHODS

1. Preparation of *C. sinensis* metacercariae

The metacercaria of *C. sinensis* were collected from the flesh of fresh water fish (*Pseudorasbora parva*) captured in Nakdong River Basin, an endemic area of liver fluke infection in Korea as previously described (Yoon *et al.*, 2000). Briefly, the whole flesh of the fish was digested with artificial gastric juice (0.6% pepsin in 0.7% HCl, pH 2.0), filtered, selected under

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stereomicroscope and kept in the refrigerator until infection.

2. Animals and Experimental Procedures

Male, Syrian golden hamsters were housed in temperature and humidity controlled rearing system (Daejong, Co., Korea) with a 12 hr dark/light illumination cycle. Hamsters aged 5-6 weeks were used 1 week after acclimatization to the housing environment.

A total of 150 hamster were divided into 2 group. Group 1 was given 0, 6, 12, 25 ppm DMN, respectively with same number (20) of *C. sinensis* metacercariae. Group 2 was given 0, 5, 15, and 50 metacercaria, respectively with same concentration (12 ppm) of DMN. Twenty untreated hamsters severed as negative control. Each hamster in the experimental group was injected with assigned numbers of *C. sinensis* orally followed by administration of given concentration of dimethylnitrosamine in their drinking water for 28 days.

Hamsters found dead or moribund were included in the effective numbers. They were euthanized and complete postmortem examination was performed. After 15 week post initiation of the exposure, the livers and other parenchymal organs of the rest animals were fixed in 10% neutral phosphate buffered formalin, routinely processed and stained with hematoxylin and eosin (H&E) for histopathological examination.

III. RESULTS

Microscopically, the hepatic changes were classified as choloagiocellular carcinoma, cholangioma, chlangiofibrosis, cholangitis or cystic lesion. Often these lesions coexisted in the same hamster. Cholangiocarcinoma was well to poorly differentiated on histopathology. The neoplastic nodules which were very invasive on growth consists of glandular structures that is lined by one to multiple layers of anaplastic and pleomorphic tumor cells (Fig. 1). Mitotic figures were frequently observed. Different degrees of desmoplastic reaction and inflammation were often associated with the neoplasia. In case of cholangioma, the neoplastic foci were composed of well differentiated cuboidal shape epithelium that form glandular structures and the neoplastic mass was well-demarcated from the surrounding parenchymal structure (Fig. 2). In cholangimatous area that is surrounded by extensive fibrosis is defined as cholangiofibrosis (Fig. 3). In addition, cystic structures lined by flat epithelial cells were also

Fig. 1. Cholangiocarcinoma induced by DMN treatment and CS infection. Cholangiocarcinoma consisted of irregular ductular structures formed by anaplastic cells. Mitotic figures are common. H&E, X100.

Fig. 2. Cholangioma induced by DMN treatment and CS infection. Cuboidal neoplastic cells form well differentiated ductular structures with a mild desmoplastic reaction. H&E, X200.

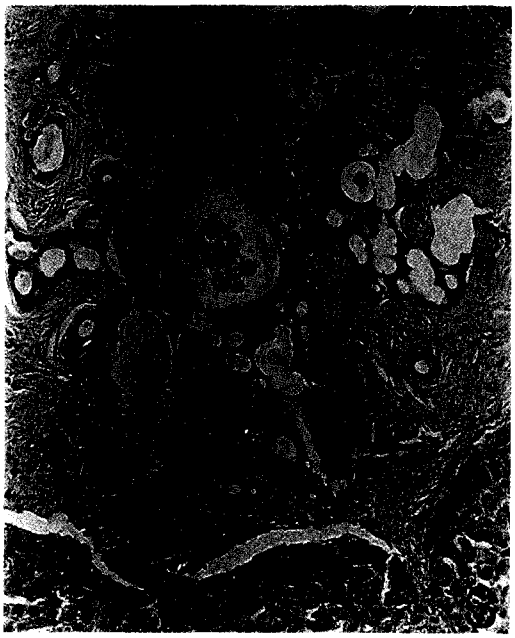

Fig. 3. Cholangiofibrosis induced by DMN treatment and CS infection. Note abundant collagenous connective tissues surrounding the bile ducts. H&E, X200.

Fig. 4. Cystic lesion induced by DMN treatment and CS infection. Note markedly dilated cystic structures lined by flatten epithelial cells. H&E, X200.

occasionally found (Fig. 4).

C. sinensis infection alone only results in chronic cholangitis but did not developed any neoplastic lesion regardless of the concentration of DMN. However, when *C. sinensis* infection was combined with DMN, the incidence of tumor development gradually increase in the DMN dose-dependent manner ranging from 10 to 50 to 90%. Especially, when 25 ppm DMN was given with *C. sinenesis*, 70% out of 90% tumor was malignant in nature. Cholangiofibrosis or cystic lesion also was seen only in the DMN treated hamsters.

In group 2, where the hamsters were treated with different numbers of *C. sinensis* with same DMN level (12 ppm), the tumor incidence ranges from 52.5 to 60 to 75%. Only 1 cholangiocarcinoma (10%) was seen in the hamster treated with DMN alone without *C. sinensis* infection.

IV. DISCUSSION

The results of this study revealed that chemical carcinogen and *C. sinensis* act synergistically on cholangiocellulargenesis in the hamster model. It was clear from the present examination that tumor yield increase in parallel to the degree of parasite burden. The presence of parasite as low as 5 metacercariae was capable of promoting the neoplastic development significantly. Correlation between morbidity and degree of parasite infection was reported in a human field study.

The mortality was affected more by DMN concentration than by *C. sinensis* load. When the hamsters were infected with different numbers of metacercaria with 12 ppm DMN, no hamster died before the experiment was terminated (mortality : 0%) when they were given 5 metacercaria. However, the hamsters start to die beginning 7 and 9 week post initiation when they were given 15 and 50 metacercariae, respectively. Therefore the mortality is due to overwhelming inflammatory reaction following *C. sinensis* infection.

Our study suggested that *C. sinensis* contributes as strong promoter. It has been speculated that many factors such as secretory or excretory products from the worms or egg or oxygen radicals, nitrogen radicals and growth factors secreted from the infiltrated inflammatory cells following *C. sinensis* infection might play important role in collagnicarcinogenesis (Oshima and Bartsch, 1994; Oshima *et al.*, 1994; Srivatanakul *et al.*, 1991). Further studies are in need to elucidate the regulatory mechanisms involved in the development of cholangiocellular carcinoma in hamster model.

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