Short Communication

Suppression of Cholangiocarcinoma Development by Aminoguanidine in the Liver Fluke-infested Hamster

Ki-Taek Nam1, Dae-Yong Kim2, Mi-Sun Park2, Dong-Deuk Jang1, Ki-Hwa Yang1, Jeong-Hee Han3, and Byung-Il Yoon3

1National Institute of Toxicological Research, KFDA
2Department of Veterinary Pathology, College of Veterinary Medicine and School of Agricultural Biotechnology, Seoul National University
3Department of Veterinary Medicine, Kangwon National University, Korea

Abstract: Clonorchis sinensis (CS) infection combined with hepatocarcinogen treatment results in marked cholangiocarcinoma (CC) formation in hamsters. Hamsters were kept for 16 weeks with or without 1% aminoguanidine (AG) exposure through drinking water, and were treated with dimethylnitrosamine for 4 weeks, 2 days after being infected orally with 15 metacercariae of CS. Interestingly, of the 11 hamsters not treated with AG, 9 had malignant tumors, 1 had a benign tumor, and 1 was normal; however, of the 10 hamsters treated with AG, 5 had benign tumors, 4 had malignant tumors, and 1 was normal. Based on this result, AG has an inhibitory effect on the progression of CC. Further mechanistic study into the result of this morphological study is warranted. (J Toxicol Pathol 2005; 18: 65–68)

Key words: aminoguanidine, cholangiocarcinoma, Clonorchis sinensis, dimethylnitrosamine, hamster

Clonorchis sinensis (CS) is a liver fluke that dwells in the bile ducts of humans and animals1,2. CS in humans causes cholangitis, bile duct obstruction, and subsequently cholangiobrosis in the liver3,4. It has also been known as an etiological factor of cholangiocarcinoma (CC) in the eastern part of Asia including Korea and Japan2,5,6. With ongoing exposure, repeat infection readily occurs following curative treatment, and cumulative infection may result in significant morbidity and a predisposition to CC7. CS infection combined with hepatocarcinogen treatment results in marked oval cell proliferation and atypical bile duct hyperplasia followed by development of CC in hamsters8–10. Therefore hamsters have been widely used as an animal model for studying human CC.

It has been proposed that the formation of reactive oxygenous and nitrous free radicals as a result of the chronic inflammatory process in response to infections can cause injury to the epithelial cells, contributing to the initiation and promotion of tumor. The examples are H. pylori-associated gastric cancer in humans and mice and H. hepaticus-induced hepatocellular carcinoma in mice11–14. CC in both the human and hamster is also well known to be related to chronic inflammation induced by uncurled liver fluke infection15,16. Nitric oxide (NO) overproduction is associated with the pathogenesis of a variety of disorders including cancer and NO also stimulates tumor angiogenesis17 as well as vascular permeability in solid tumors18. Increased iNOS activity has been observed in patients with chronic gastritis and precancerous lesions as well as gastric adenocarcinoma, and iNOS levels were significantly lowered after their eradication19,20.

Chronic inflammatory diseases of the gastrointestinal tract and liver which are thought to precede cancer include Barrett’s esophagus, ulcerative colitis, H. pylori-associated gastritis and parasitic cholangitis, and all are associated with increased iNOS expression and considerable nitrosative stress21–24. These observations suggest that chronic overexpression of iNOS and the associated NO overproduction contribute to tumorigenesis, making this process an attractive target for chemoprevention strategies.

In the present study, the effect of iNOS amelioration by aminoguanidine (AG) on CC development was investigated using the hamster CC model.

Thirty-one male Syrian golden hamsters weighing 55–60 g were housed five or six to a polycarbonate cage and were maintained in a temperature (21 ± 2°C) and humidity (55 ± 10%) controlled air-filtered rearing system (Daejong Co., Korea) with a 12-hr light/dark cycle. The animals were divided into 3 groups: non-treated control group (10 animals) and two experimental groups, CS+DMN (11 animals) and CS+DMN+AG group (10 animals). Each
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Hamsters in the two experimental groups received 15 ppm dimethylnitrosamine (DMN) in drinking water for 28 days with or without 1% aminoguanidine exposure by drinking water after infestation with 15 metacercariae of CS, then were maintained for 12 weeks. Hamsters in the CS+DMN+AG group received 1% AG exposure to drinking water, which was given *ad libitum* throughout the experimental period. The experimental protocol is summarized in Fig. 1.

All the hamsters were sacrificed under ether anesthesia after 16 weeks of the experiment. After thorough gross examination, the livers were removed, and multiple sections from each lobe were fixed in 10% phosphate buffered neutral formalin. After fixation, the specimens were processed routinely, embedded in paraffin wax, and stained with haematoxylin and eosin (H&E) for histological evaluation of biliary tumor development. Based on histopathological findings, the lesions of liver were respectively classified as normal (no tumor), cholangitis with bile duct hyperplasia, cholangiofibrosis (periportal ductular hyperplasia concomitant with prominent fibroplasia), benign (adenomatous hyperplasia of normal-appearing bile ducts, larger than normal hepatic lobule) and malignant tumor (neoplastic glands invading surrounding hepatic parenchyma)9,25. The incidence of CC was statistically analyzed using the Chi-square test (JMP software package (version 4.0), SAS Institute, Cary, NC). The probability values less than 5% (P<0.05) were considered to be statistically significant.

Biliary tumors, ranging from cholangioma to CC, developed in most hamsters of the two experimental groups with or without AG treatment (Table 1, Fig. 2). It was, however, of great interest that the treatment of AG considerably prevented the development of CC, as shown in Table 1. Extensive dilatation of pre-existing bile ducts due to the presence of worms and prominent hyperplasia of bile duct cells as well as goblet cell metaplasia were also frequently noted in the hamster livers of the experimental groups.

The major purpose of this study was to investigate the outcome of iNOS inhibition on the development of CC in a hamster. Our hypothesis was that blocking iNOS by AG would significantly suppress CC development in the hamster. The results signify that chronic iNOS overexpression and its associated NO production might principally contribute to cholangiocarcinogenesis. These observations suggest the possibility that amelioration of NO production might be a feasible way of chemoprevention.

Ohshima et al. previously reported increased expression of iNOS in the liver fluke-infected hamster liver by immunohistochemistry and proposed that increased iNOS activity played some role in cholangiocarcinogenesis26. Genetic ablation of iNOS resulted in significant decrease of tumor development in the mouse model of *H. pylori*-associated gastric cancer14. When analyzed according to tumor pathology, the incidences of both gastric adenoma and

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**Table 1. Tumor Development in Hamsters Exposed or not Exposed to 1% Aminoguanidine in Drinking Water throughout the Study Period of 16 weeks**

<table>
<thead>
<tr>
<th>Histopathology</th>
<th>Groups</th>
<th>Control</th>
<th>DMN+CS</th>
<th>DMN+CS+AG</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals bearing tumor</td>
<td></td>
<td></td>
<td>10/11 (90.9%)</td>
<td>9/10 (90%)</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td>5/5</td>
<td>0/11 (0.0%)</td>
<td>0/10 (0%)</td>
</tr>
<tr>
<td>Cholangitis with bile duct hyperplasia</td>
<td></td>
<td>0/5</td>
<td>11/11 (100.0%)</td>
<td>10/10 (100%)</td>
</tr>
<tr>
<td>Cholangiofibrosis</td>
<td></td>
<td>0/5</td>
<td>1/11 (9.1%)</td>
<td>1/10 (10%)</td>
</tr>
<tr>
<td>Cholangioma</td>
<td></td>
<td>0/5</td>
<td>1/11 (9.1%)</td>
<td>5/10 (50%)</td>
</tr>
<tr>
<td>Cholangiocarcinoma</td>
<td></td>
<td>0/5</td>
<td>9/11 (81.8%)</td>
<td>4/10 (40%)*</td>
</tr>
</tbody>
</table>

(): % to total number of animals examined.
DMN: dimethylnitrosamine; CS, *Clonorchis sinensis* infection; AG, Aminoguanidine.
*: significantly different from CS+DMN group at P<0.05 (Chi-square test, JMP software package (version 4.0)).
adenocarcinoma were also significantly lower in iNOS-knockout mice compared with iNOS-wild type mice.

In conclusion, the inhibition of iNOS decreased tumor development and progression in CS infected hamsters. The inhibition of iNOS may be a relevant target for suppressing tumor growth and enhancing chemopreventive effects; however, many factors associated with CS infection should also be considered, including secretory or excretory products from the worms and eggs. Further studies are warranted to better elucidate the mechanistic roles of iNOS in cholangiocellular carcinogenesis.

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References