ENHANCING EFFECT OF SODIUM TAUROCHOLATE ON N-METHYL-N' -NITRO-N-NITROSOGUANIDINE-INDUCED STOMACH TUMORIGENESIS IN RATS

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Male Wistar rats that had received a low dose of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and sodium taurocholate showed a significantly higher incidence of hyperplastic and neoplastic lesions in the stomach mucosa than did the MNNG-treated controls. The result suggested an enhancing effect of taurocholate in stomach tumorigenesis.

Key words: Stomach cancer — N-Methyl-N'-nitro-N-nitrosoguanidine — Enhancing effect — Taurocholate

It has long been a subject of controversy whether or not the frequency of carcinoma in the remnant stomach following partial gastrectomy for benign lesions is higher than that of stomach carcinoma in the general population. Some authors who reported an increased incidence presented data indicating that the incidence depends on the type of operation performed, i.e., Billroth I or II type of procedure, and these data led them to suggest a role of bile and/or pancreatic juices in carcinogenesis in the remnant stomach.

The tumor-enhancing effect of bile or bile acid was studied mainly in colon carcinogenesis and only occasionally in other organs, such as liver, forestomach and stomach. In this communication, we report that sodium taurocholate, a primary bile acid, enhanced stomach tumorigenesis initiated by oral administration of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG).

Forty-five male Wistar rats (Ishikawa Laboratory Animals, Kashiai, Saitama), 7 weeks old, were divided into 3 groups. Group 1, consisting of 19 rats was given MNNG solution at a concentration of 80 μg/ml for 7 weeks and received control diet plus 0.25% sodium taurocholate for 52 weeks from the 3rd week after the cessation of MNNG. Group 2, consisting of 12 animals, received MNNG solution at the same concentration for the same period as group 1 and was ob-

Fig. 1. Macroscopic pictures of the stomach in rats of the three groups. A, group 1; B, group 2; and C, group 3.
served for 55 weeks as MNNG-treated controls. Group 3 (14 rats), the negative controls, received control diet plus 0.25% sodium taurocholate for 52 weeks without previous administration of MNNG. Statistical analysis was performed by the use of Student’s t-test.

As shown in Fig. 1, stomach mucosa of group 1 rats showed a large number of polypoid lesions which were observed only sporadically in group 2 rats. No remarkable macroscopic changes were detected in the stomach mucosa of group 3 rats. The histology of these polypoid lesions consisted of hyperplasia (Fig. 2A), adenoma (Fig. 2B) and adenocarcinoma (Fig. 2C). The hyperplasia was focal and consisted exclusively of foveolar epithelium, and adenoma was defined as the proliferation of glands showing moderate atypia which did not infiltrate the muscle layer of the stomach wall. When they infiltrated the muscle layer, they were diagnosed as carcinoma. As shown in Fig. 2B, adenoma revealed definite neoplastic features similar to those observed in early stages of human stomach cancer and therefore this lesion was grouped into the category “neoplasia” together with carcinoma. The animals that received MNNG plus sodium taurocholate had a significantly higher incidence of each hyperplastic and neoplastic lesion in the stomach mucosa than did the MNNG-treated controls. The neoplasia in group 1 consisted of five adenomas and two carcinomas. Stomach mucosa of group 3 rats showed no remarkable changes histologically (Table I).

The results of experimental studies on the effects of bile and bile acid in stomach carcinogenesis were inconsistent. Dahm and his co-workers demonstrated a higher in-

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Table I. Incidence of Hyperplastic and Neoplastic Lesions of the Stomach in Rats Treated with MNNG and/or Taurocholate

<table>
<thead>
<tr>
<th>Group (No. of rats)</th>
<th>Treatment</th>
<th>Hyperplasia</th>
<th>Neoplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of rats with lesions</td>
<td>Total No. of lesions (mean ± SD)</td>
</tr>
<tr>
<td>1 (19)</td>
<td>MNNG + taurocholate</td>
<td>19</td>
<td>172 (9.1 ± 3.0)a</td>
</tr>
<tr>
<td>2 (12)</td>
<td>MNNG</td>
<td>6</td>
<td>14 (1.2 ± 1.5)</td>
</tr>
<tr>
<td>3 (14)</td>
<td>Taurocholate</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

a) P<0.001 between groups 1 and 2.  
b) P<0.02 between groups 1 and 2.
TAUROCHOLATE ENHANCES STOMACH TUMORIGENESIS

cidence of remnant stomach cancer using a combination of short loop gastroenteric anastomosis providing a continuous duodenogastric reflux and oral administration of MNNG. On the other hand, Domellöf and his co-workers gave bile acid by gavage 3 times a week throughout the administration period of MNNG and found no significant difference in the frequency of stomach carcinoma compared to the relevant control group. In both experiments, MNNG was given for 7 months at a concentration of 120 μg/ml, which was enough to induce stomach carcinomas without the administration of bile or bile acid. In our study, however, MNNG was administered at a lower concentration (80 μg/ml) for a shorter period (7 weeks), because we wanted to show more clearly the presumed enhancing effect of bile acid on stomach carcinogenesis by reducing the tumor production in MNNG-treated controls. Considering the fact that neither adenoma nor carcinoma was observed in the MNNG-treated controls (group 2), we think that this aim was successfully fulfilled. In a previous experiment by one of the authors (O.K.), administration of MNNG at a concentration of 80 μg/ml for 15 weeks was determined to be the minimal dose of MNNG for the induction of stomach carcinoma, over an observation period of 20–25 weeks. Based on these data, a 7-week administration of MNNG at a concentration of 80 μg/ml is equivalent to nearly half of the minimal dose: no stomach tumor has been reported to be induced by such a low total dose.

The mechanism of the tumor-enhancing effect of bile acid is still unknown. Silverman and Andrews reported that some of the bile salts themselves are not mutagenic in the Ames test. However, when they are included in the Ames assay with suboptimal levels of known carcinogens, there is an increase in mutagenicity. Their results obtained in vitro are compatible with ours obtained in vivo with sodium taurocholate. We did not test other bile salts in this experimental system. Recently, Cohen and his co-workers showed that when MNNG was administered with sodium taurocholate, leiomyosarcomas developed in the stomach. They postulated that the increased permeability permitted the more extensive diffusion of MNNG from the luminal surface into the smooth muscle of the stomach wall.

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Addendum After submission of the present manuscript, Salmon and his co-workers reported a significant enhancement of MNNG-induced gastric tumorigenesis by taurocholic acid mixed with control diet at a concentration of 0.2%.

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