INDUCTION OF INTESTINAL METAPLASIA IN THE GLANDULAR STOMACH OF RATS BY X-IRRADIATION PRIOR TO ORAL ADMINISTRATION OF N-METHYL-N'-NITRO-N-NITROSOGUANIDINE*1

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CD/CRJ rats were subjected to localized X-irradiation of the stomach and given N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in the drinking water. Rats given MNNG alone and non-treated rats were used as controls. Upon sacrifice at 15 months after the initial MNNG administration, intestinal metaplasia was observed; the histology was of complete type and the incidence was 100% in rats treated with X-rays and MNNG, whereas in rats treated with MNNG alone the intestinal metaplasia was of incomplete type and its incidence was 80%. However, the incidence of gastric cancer in rats treated with MNNG alone was 25%.

Key words: Chemical carcinogenesis — Irradiation — Gastric cancer — Intestinal metaplasia — N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG) — Rat

Human gastric adenocarcinoma of a well differentiated type has been supposed to develop from gastric mucosa via intestinal metaplasia.3,5,6) However, the relationship between intestinal metaplasia and gastric adenocarcinoma has not been fully elucidated, though some experimental work has been reported.1,4,6,7,8) Recently, we have found that intestinal metaplasia with Paneth cells could be induced in the glandular stomach of Wistar rats by localized X-irradiation.11)

This paper describes the induction of intestinal metaplasia in rats by combined localized X-irradiation of the stomach and oral administration of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG).

MATERIALS AND METHODS

Experimental Animals Sixty 5-week-old CD/CRJ (SD) rats (Charles River, Japan Inc., Atsugi) were used in the experiments. They were housed in plastic cages, five in a cage, and kept in an air-conditioned room (24±2°). They were fed with a commercial diet MF (Oriental Yeast, Co., Ltd., Tokyo).

X-irradiation The entire body of the anesthetized rats was shielded with a 0.6 cm thick lead cover with a hole 1.8 cm in diameter in the gastric region.2) They were given 500 rad daily up to a total of 3,000 rad. Exposure factors were as follows: 180 kVp, filter 0.5 Cu + 0.5 Al, focus-to-animal distance 60 cm, tube current 25 mA, half-value layer 1.18 mm Cu, and dose rate 45 R per min.

MNNG Administration MNNG (Aldrich Chemical Co., Inc., Milwaukee, Wis.) was dissolved in deionized water at a concentration of 1 mg/ml as a stock solution. The stock solution was further diluted to 50 mg/liter with tap water just before use, and was given to rats ad libitum. The rats received MNNG for 4 months and then normal tap water.

Experimental Groups Sixty rats were divided into three experimental groups. Group I received localized X-irradiation of the stomach and, with

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an 8-week interval, subsequent administration of MNNG for 4 months. Group II received MNNG alone over the same period as Group I. The control rats in Group III received tap water throughout the experiment.

Examination of Animals Most rats were killed about 15 months after the start of MNNG administration. The stomach was cut along the greater curvature. It was stretched, pinned on cardboard with the mucosal surface upward and fixed in 10% neutralized formaldehyde solution. After fixation, strips were cut perpendicularly to the mucosal surface of the stomach from the lesser curvature and the greater curvature. The sections were stained with hematoxylin and eosin, and by means of the alcian blue (pH 2.5) periodic acid-Schiff reaction (PAS). Alkaline phosphatase activity of paraffin sections of the stomach was tested by the naphthol AS-MX phosphate-fast blue RR staining method of Tomonaga et al.10) Scoring of the crypts on the intestinal metaplastic glands was done by the method reported in a previous paper.11)

Autoradiographic Study To test autoradiographically for newly synthesized DNA, 3H-TdR (28 Ci/mmol, Radiochemical Centre, Amersham, U.K.) at a dose of 1 μCi/g body weight was injected subcutaneously at the 8th week after X-irradiation, or at the 6th week after the last day of MNNG administration. After sacrifice of the animals, the stomach were processed for histological study. The deparaffinized, unstained sections were dipped in Sakura NR-M2 emulsion (Koinshiroku Photo Ind. Co., Ltd., Tokyo) and developed 30 days later for autoradiographic study. Post-staining was done lightly with hematoxylin and eosin. The scoring was done under a microscope equipped with a micrometer. The labeled cell index was expressed as the percentage of labeled cells in 500 counted cells.

RESULTS

Incidence of Adenocarcinoma All the tumors were restricted to the glandular stomach in the pyloric region. Various types of adenocarcinomas were found in 4 out of 16 rats (25.0%) in Group II (Figs. 1 and 2) without metastatic tumor, but none were found in Group I (Table I).

Incidence of Intestinal Metaplasia In Groups I and II, 7 and 3 rats, respectively, died of pneumonia. They were not analyzed because of autolysis of their stomachs.

The numbers of rats with intestinal metaplasia in the stomach are indicated in Table I. Crypts with alcian blue-PAS-positive goblet cells among PAS-positive gastric glands (Type I) were found in 11 out 13 rats (84.6%) in Group I (Fig. 3) and in 12 out of 15 rats (80.0%) in Group II. Intestinal-type crypts without Paneth cells (Type II) were found in 13 out of 13 rats (100%) in Group I, and in 10 out of 15 rats (66.7%) in Group II. Intestinal-type crypts with Paneth cells (Type III) were found in 13 out of 13 rats (100%) in Group I and in 1 out of 15 rats (6.7%) in Group II. The incidence of intestinal-type crypts with Paneth cells in Group I was

Table I. Induction of Intestinal Metaplasia and Adenocarcinoma in the Glandular Stomach of Rats with X-rays and/or MNNG

<table>
<thead>
<tr>
<th>Group No</th>
<th>Treatment</th>
<th>No. of rats examined</th>
<th>Incidence of intestinal metaplasiaa (%)</th>
<th>Incidence of adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X-ray</td>
<td>MNNG</td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>I</td>
<td>+</td>
<td>+</td>
<td>13</td>
<td>11/13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(84.6%)</td>
</tr>
<tr>
<td>II</td>
<td>−</td>
<td>+</td>
<td>16b)</td>
<td>12/15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(80.0%)</td>
</tr>
<tr>
<td>III</td>
<td>−</td>
<td>−</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0)</td>
</tr>
</tbody>
</table>

a) I: Appearance of goblet cells in a normal gastric pit. II: Intestinal-type crypts without Paneth cells. III: Intestinal-type crypts with Paneth cells. IV: Intestinal-type crypts with positive alkaline phosphatase activity.

b) One rat had stomach adenocarcinoma, but was excluded in calculating the incidence of intestinal metaplasia because of mucosal autolysis.

* Significantly higher than the corresponding Group II by Student's t-test (P<0.001).
Fig. 1. Well differentiated adenocarcinoma in the pyloric gland mucosa (Group II). Hematoxylin and eosin. × 40.

Fig. 2. Appearance of adenocarcinoma in the pyloric gland without associated metaplastic glands (Group II). Hematoxylin and eosin. × 40.
Fig. 3. Metaplastic glands with goblet cells and intestinal-type crypts in the pyloric gland mucosa (Group I). Hematoxylin and eosin. ×100.

Table II. Number of Metaplastic Crypts in the Glandular Stomach of Rats Treated with X-rays and/or MNNG

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatment</th>
<th>No. of rats examined</th>
<th>No. and type of metaplastic crypts per section(^a) (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X-ray</td>
<td>MNNG</td>
<td>I</td>
</tr>
<tr>
<td>I</td>
<td>+</td>
<td>+</td>
<td>13</td>
</tr>
<tr>
<td>II</td>
<td>-</td>
<td>+</td>
<td>15</td>
</tr>
<tr>
<td>III</td>
<td>-</td>
<td>-</td>
<td>20</td>
</tr>
</tbody>
</table>

Matsukura et al.\(^4\)  
Watanabe\(^11\)  

|                |          |                      | 8                  | 3.5 ± 1.1           |

|                |          |                      | 12                 | 9.4 ± 2.7           |

\(^a\) I: Appearance of goblet cells in a normal gastric pit. II: Intestinal-type crypts without Paneth cells. III: Intestinal-type crypts with Paneth cells. A section was obtained from the lesser curvature. Significantly different from Group II: * P<0.05, ** P<0.005.

significantly higher than that in Group II. Intestinal-type crypts with cells positive for alkaline phosphatase activity (Type IV) were detected in the duodenal cuff in 11 out of 11 rats (100%) in Group I and 3 out of 15 rats in Group II (P<0.001, Group I vs. Group II). No intestinal metaplasia were found in Group III (Table I).

Metaplastic glands were most frequently found as foci among pyloric glands but not in the fundic glands region. They were not always located among or near adenocarcinomatous foci. The average numbers (±SE) of crypts with goblet cells in all the strips of the glandular stomach were 2.8±0.5 in Group I and 1.7±0.4 in Group II. Intes-
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Table III. Tritiated Thymidine-labeled Cells in the Pyloric Gland Mucosa

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of cells counted/250 µm²</th>
<th>Labeling index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-ray</td>
<td>MNNG</td>
<td>Total cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>586±54</td>
</tr>
<tr>
<td>+</td>
<td></td>
<td>567±102</td>
</tr>
<tr>
<td>−</td>
<td>+</td>
<td>675±110</td>
</tr>
<tr>
<td>+</td>
<td>−</td>
<td>558±71</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

a) Scoring was done from the glands with intestinal metaplasia.

Fig. 4. Metaplastic glands with numerous labeled cells in the pyloric gland mucosa on the right-hand corner and small numbers of labeled cells in the regular pyloric glands on the left-hand corner. The rat was treated with a combination of X-ray and MNNG. Six weeks after the beginning of MNNG administration. Hematoxylin and eosin. ×100.

Intestinal-type crypts without Paneth cells amounted to 22.8±4.2 in Group I and 1.3±0.4 in Group II (P<0.05). Intestinal-type crypts with Paneth cells amounted to 2.4±0.4 in Group I and 0.07 in Group II. The total numbers of crypts with intestinal metaplasia were 27.6±4.7 in Group I and 3.3±0.6 in Group II (P<0.005) (Table II). Labeling indices were scored in the pyloric gland mucosa of three groups of rats and the glands of a rat with intestinal metaplasia (Table III).

Labeling Index. ³H-TdR was injected intraperitoneally into all the experimental animals. Labeled cells were scored under the light microscope and the results are summarized in Table III. The incidence of labeled cells was 3.86±1.56% in the control group, 4.47±1.00% in the X-ray group, 6.90±2.40% in the MNNG group and 4.48±1.30% in the X-ray and MNNG combined group. There was no significant difference among the 4 groups (Table III). Comparing the labeling indices of
the gastric glands in the differently treated stomachs, we found that the index was greatly increased to 31.3% in the glands with intestinal metaplasia (Fig. 4). The incidence and location of labeled cells in a crypt are tabulated in Fig. 5. The scored crypts were distributed in different areas in the pyloric gland of the stomach and duodenum after different treatments.

Migration of the germinal region in terms of the mode of labeled cell index in the crypt was noted in the groups administered X-rays or MNNG or both (Fig. 5). In the stomach of X-ray-administered rats, the mode of labeled cell index shifted downward in comparison with that of the control. However, it shifted upward in the rats treated with MNNG. The combined treatments with X-ray and MNNG produced a slight upward shifting of the germinal region in the glands either with or without metaplastic crypts.

**DISCUSSION**

Intestinal metaplasia of the glands in the pyloric region of the stomach in rats was induced by the administration of either localized X-irradiation alone or combined X-irradiation and oral administration of MNNG, but no gastric tumor was induced by these treatments. MNNG treatment alone, however, induced tumors in the glandular stomach in 25% of the rats. On giving 50 µg/ml of MNNG for 10 months, Tate-matsu et al. reported that gastric tumors were induced in 45% (12 out of 27 rats). Matsukura et al. found 25% (2 out of 8 rats) induction by 83 µg/ml for 2 months and 62.5% (5 out of 8 rats) at the same dose for 4 months. The latter authors argued that there was no direct sequence from intestinal metaplasia to gastric adenocarcinoma.

In this experiment, intestinal metaplasia of the stomach was induced in 12 out of 15 rats treated with MNNG for 4 months and the average number of intestinal-type crypts were 1.7±0.4 per strip of the glandular stomach. Watanabe reported that the number of intestinal-type crypts was 9.4±2.7 and that Paneth cells appeared in 50% of rats after X-irradiation. Matsukura et al. observed that intestinal metaplasia appeared in 100% of rats treated with MNNG for
16 weeks, but the number of metaplastic crypts was rather small (3.5 ± 1.1). However, Paneth cells and alkaline phosphatase activity were found to be positive in all the animals treated with X-rays and MNNG, and the number of intestinal-type glands induced by the combined treatment was greater than in the case of MNNG treatment alone. Under the present conditions, it can be concluded that the combined X-ray and MNNG treatment induces a high frequency of intestinal metaplasia and accelerates metaplastic grading (Type I to Type IV), but does not induce stomach cancer.

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