Induction of Intestinal Metaplasia in Rats by N-Ethyl-N’-nitro-N-nitrosoguanidine but Not by Sodium Hydroxide

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Intestinal metaplasia (IM) in the glandular stomach of male Wistar rats induced by oral administration of N-ethyl-N’-nitro-N-nitrosoguanidine (ENNG) and/or intubation of 0.1N sodium hydroxide (NaOH) was studied as follows. Experiment I, sequential study: Rats in group I were given 100 μg/ml ENNG in drinking water for 12 weeks. Rats in group II were given 5 ml of 0.1N NaOH by gastric intubation once a week for 12 weeks. Group III control rats were given tap water. Rats were killed from week 1 until week 69 sequentially. IM was first found at week 26 in group I and at week 58 in groups II and III, its incidence being significantly higher in group I than in the other two groups (P<0.01), but without any difference between group II and group III. Experiment II, two-stage carcinogenesis: Rats in groups I and II were treated in the same way as in experiment I, while rats in group III were given 100 μg/ml ENNG for 12 weeks, followed by 0.1N NaOH once a week for 12 weeks intragastrically. All rats were killed at week 56. The numbers of metaplastic glands in groups I and III were higher than in group II. Gastric carcinomas were induced in all groups of rats treated with ENNG. The results of these two experiments show that IM is effectively induced by a carcinogen but is not enhanced by regeneration induced by alkaline treatment.

Key words: Intestinal metaplasia — N-Ethyl-N’-nitro-N-nitrosoguanidine — Sodium hydroxide — Regeneration — Rat

Materials and Methods

Male WBN/Kob rats (Ishikawa Laboratory Animals, Fukaya, Saitama) were used. Rats were 7 weeks old and about 130 g in average body weight at the start of experiments. ENNG (Aldrich Chemical Co., Inc., Milwaukee, Wis.) was given to rats at
a concentration of 100 μg/ml in drinking water. Five
milliliters of 0.1N NaOH was intubated into
the stomach of rats under ether anesthesia.

Experiment I: Fifty-four rats were divided into
3 groups randomly. Group I — Eighteen rats were
given ENNG for 12 weeks and thereafter given tap
water. Group II — Eighteen rats were given NaOH
by intubation once a week for 12 weeks and then
given tap water. Group III — Eighteen rats were
given tap water. Two rats from each group were
killed at 1, 4, 18, 26, 34, 43, 50, 58 and 69 weeks
from the start of the experiment.

Experiment II: Thirty-six rats were divided into
3 groups randomly. Group I — Eight rats were
given ENNG solution for 12 weeks and then given
tap water. Group II — Eight rats were given
NaOH by intubation once a week for 12 weeks and
then given tap water. Group III — Twenty rats
were given ENNG solution for 12 weeks followed
by intubation of NaOH once a week for 12 weeks.
They were then given tap water. The experiment
was terminated after 56 weeks.

ENNG was dissolved in deionized water at a
concentration of 500 μg/ml and diluted 5-fold with
tap water just before administration to rats ad
libitum. Rats were maintained on CE-2 basal diet
(CLEA Japan, Inc., Tokyo). Weights of rats were
measured once a month and all rats were autopsied
when they became moribund or at the end of the
experiment. The stomach was opened along the
greater curvature and fixed with 10% formalin.
The fixed stomach was cut along the lesser curva
ture into 8 strips. When an intestinal tumor was
found macroscopically, 2- to 3-mm-wide longi
tudinal strips were made from the tumorous le
sions. The tissue was embedded in paraffin, cut into
sections and stained with hematoxylin and eosin
(H-E) and Alcian Blue-periodic acid Schiff (PAS).
The chi-squared test and Student’s t-test were used
for statistical comparisons of incidence and num
ber, respectively, of IM and cancer between groups.

RESULTS

Experiment I  The body weight showed no
significant differences among the 3 groups
during the experiment. In group I, superficial
ulceration was found at week 1 and focal
ulceration, involving a generative cell zone in
the mucosa, was observed at week 4. In group
II, ulceration was found in the upper one-
third of the pyloric glands, and in the focal
area, a generative cell zone was involved in
the ulceration (Fig. 1a, b). The appearance of
IM and carcinomas was observed se
quentially, and the cumulative incidence and
number of metaplastic glands per rat are
summarized in Figs. 2 and 3, respectively.
Metaplastic glands were diagnosed as previ
ously reported. Briefly, the presence of
Alcian Blue-positive goblet cells was taken as
an indicator of the presence of metaplastic
glands. All of the metaplastic glands in Ex
periments I and II were sparsely located in
the pyloric gland area and showed the
morphological characteristics of incomplete
type IM (Fig. 4a, b). Metaplastic glands
were found in a rat of group I killed in week
26. In groups II and III, no metaplastic glands
were found until week 58. Group I showed a
significantly higher cumulative incidence of
IM than group II or III (P<0.01) at 69
weeks. The number of metaplastic glands per
rat was also higher in group I than in group II
or III.

Carcinomas and sarcomas were induced
only in group I and their incidence is sum
marized in Table I. After administration of
ENNG, carcinoma in the upper small intest
ine was found in a rat killed in week 26 and
gastric carcinoma was first found in a rat
killed in week 43. Gastric and intestinal
carcinomas or sarcomas were found in 6
(50%) and 9 (75%) of 12 rats killed after
week 26, respectively. Grossly, all carcinomas
were found in the pyloric gland area of the
glandular stomach. Microscopically, 7 lesions
of well-differentiated adenocarcinoma in the
stomach were found in 5 rats, and 3 of the 7
(43%) lesions had intestinal-type characteris
tics with goblet cells in the carcinoma tissue.

Experiment II  No differences in body weight
were detected among the 3 groups. The num
ber of rats which survived until the end of
the experiment (56 weeks) was counted as the
effective number, and the results are sum
marized in Table II.

IM was found in all rats in each group, but
the numbers of metaplastic glands per rat
differed among the groups. Group II showed a
lower number of metaplastic glands than the
other groups.

Gastric carcinomas were found in 2 of 6
(33%) rats and 7 of 14 (50%) rats in groups
I and III, respectively. Histologically, all
carcinomas, with the exception of one signet
ring cell carcinoma, were well-differentiated
adenocarcinomas infiltrating into the sub
mucosa to the serosa, but without meta
tasis. One of 7 (14%) well-differentiated
Fig. 1.  


b. Ulceration in the upper one-third of the pyloric gland of a rat in group II of Experiment I killed in week 1. H-E, ×50.
adenocarcinomas in group III showed an intestinal-type character with goblet cells. All gastric carcinomas and metaplastic glands were located in the pyloric gland area. A histologically well-differentiated type of upper small intestinal tumor was found in a rat of group I.

**DISCUSSION**

In this study, IM was induced effectively by a carcinogen but was not enhanced by regeneration. Several opinions exist as to the cause of IM. From recent epidemiological data, environmental factors have been considered to be implicated in the causation of IM and gastric cancers.3-5,15-18 Experimentally, administration of MNNG or PNNG has been shown to cause IM and gastric carcinoma.9-12,19 These results indicate that IM and gastric carcinoma are induced by similar environmental factors, possibly carcinogens in food. In contrast, from a pathological viewpoint, Moszkowicz13 has suggested that IM occurs through a regenerative process. Recently, Oohara et al.14 reported the induction of IM in the stomach of rats by simple alkaline treatment and emphasized that IM could be induced experimentally by a completely benign process alone. The results of the present study, however, showed that regeneration

Fig. 2. Cumulative incidence of IM which was found in 8 strips from each rat stomach in the 3 groups in Experiment I. ●, group I (ENNG administration); ○, group II (NaOH administration); ▲, group III (no treatment).

Fig. 3. Average number of metaplastic glands found in 8 strips from each rat stomach at each time of autopsy in Experiment I. ■■■, group I (ENNG administration); ●●●, group II (NaOH administration); □□□, group III (no treatment).
Fig. 4.  a. Focal metaplastic glands, bearing goblet cells, which are sparsely located in the pyloric gland area adjacent to a gastric carcinoma in a rat in group I of Experiment I killed in week 69. Alcian Blue-PAS, ×100. b. A few metaplastic glands in the pyloric gland area of a rat in group II of Experiment I killed in week 58. Alcian Blue-PAS, ×100.
following alkaline treatment alone did not enhance the occurrence of IM. The discrepancy between the results of this study and those of Oohara et al.\textsuperscript{14} may be attributable to the fact that we administered 0.1\textit{N} NaOH into the stomach of rats by intubation whereas Oohara introduced 0.5\textit{N} NaOH directly into the stomach via a catheter through a fistula in the gastric wall. Thus, the degree of ulceration in the gastric mucosa in our study was superficial and did not reach the generative cell zone except for some focal areas, whereas the entire gastric mucosa was exfoliated by Oohara’s method. In our experiment, 0.1\textit{N} NaOH was the maximum tolerable concentration that could be delivered to rats by gastric intubation. We felt that repeated regeneration is a more natural process, since in the human stomach, IM is almost always accompanied by “chronic” but not acute gastritis.\textsuperscript{20, 21} Thus, we chose repeated oral administration of alkali rather than a single administration.

The incidence of IM in the general population increases with age, and in stomachs obtained from Japanese patients at autopsy, IM is present even in persons in their twenties, and markedly increases in the forties until it reaches 60\% in the over-sixties.\textsuperscript{4} Thus, it has been suggested that IM occurs simply as a result of aging. However, in this study, along

Table I. Incidence of Neoplasms in the Stomach and Small Intestine in Group I of Experiment I: Sequential Study

<table>
<thead>
<tr>
<th>Time of autopsy (weeks)</th>
<th>No. of rats autopsied</th>
<th>Fore stomach Squamous cell carcinoma</th>
<th>Glandular stomach Well-differentiated adenocarcinoma</th>
<th>Signet ring cell carcinoma</th>
<th>Small intestine Well-differentiated adenocarcinoma</th>
<th>Sarcoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
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</tr>
<tr>
<td>34</td>
<td>2</td>
<td>0</td>
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<td>0</td>
<td>1</td>
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<tr>
<td>43</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>58</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>46</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>8</td>
<td>1</td>
</tr>
</tbody>
</table>

Table II. Incidence of IM in the Stomach and Neoplasms in the Stomach and Small Intestine in Experiment II: Two-stage Carcinogenesis

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Effective No.</th>
<th>No. of rats with IM</th>
<th>No. of IM$^a$</th>
<th>Stomach</th>
<th>Total (%)</th>
<th>Small intestine</th>
<th>Well-differentiated adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>ENNG</td>
<td>6</td>
<td>6</td>
<td>17.5</td>
<td>2</td>
<td>0</td>
<td>2 (33)</td>
<td>1</td>
</tr>
<tr>
<td>II</td>
<td>NaOH</td>
<td>5</td>
<td>5</td>
<td>6.8</td>
<td>0</td>
<td>0</td>
<td>0 (0)</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>ENNG → NaOH</td>
<td>14</td>
<td>14</td>
<td>20.6</td>
<td>7</td>
<td>1</td>
<td>8 (57)</td>
<td>0</td>
</tr>
</tbody>
</table>

$^a$ Average number of IM which was found in 8 strips from each rat stomach.
with previous studies, the appearance of IM occurred in control rats after 50 weeks, which is much later than in ENNG-treated rats. The incidence and number of metaplastic glands were significantly lower than in ENNG-treated rats. This observation corresponds to the epidemiological data. In areas where the population has a high risk of gastric cancer, such as Japan, South America, and Central and Eastern Europe, IM of the stomach occurs from a relatively early age. In contrast, in the United States, which has a low incidence of gastric cancer, IM only occurs from the forties and a mere 20% of subjects over sixty show metaplasia.

Histopathologically, IM has been thought to be one of the phenomena associated with chronic gastritis and the term “metaplastic gastritis” is often employed. In the present study, gastric mucosa of alkali-treated rats showed irregularity of pyloric glands, an increased amount of connective tissue in the stroma and small round cell infiltration. The combination of alkaline treatment after ENNG administration had no significant effect on the induction of IM. Thus, the regenerative process did not enhance or promote induction of IM by ENNG under these experimental conditions.

This experiment showed that a carcinogen is more effective for the induction of IM. However, this finding does not necessarily mean that IM is a precancerous condition, and the question of whether IM is a precancerous or paracancerous change is still unresolved. Watanabe et al. reported that X-ray irradiation of rats induced IM without induction of gastric cancer and emphasized that elevation of the pH value of gastric juice is one of the principal factors responsible for the development of IM. It has been suggested that metaplastic glands are rather stable or that IM is immunologically adapted to local antigen. Moreover, recent results on IM experimentally induced in rats by X-ray irradiation or MNNG have shown independent induction of IM and gastric cancer. These data favor the possibility that IM is a form of paracancerous change.

It thus seems clear that gastric carcinogens cause IM, but the significance of IM itself requires further investigation.

(Received Aug. 14, 1986/Accepted Jan. 5, 1987)

REFERENCES


