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Early Changes in the Gastric Mucosa with MNNG Exposure in the Rat

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ABSTRACT

The morphology of the rat gastric mucosa was examined by light microscopy after short term-administration of a carcinogenic agent, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG, at a dose of 50 mg/l in 0.04% of Tween 60), which was given to rats ad libitum from light-sealed bottles for 3 months. Tween 60 (0.04%) was given to rats as control for 3 months. Rats thus treated were sacrificed by decapitation at the end of 3 months after the beginning of the experiment. Tissue samples were taken from the area of the gastric mucosa designated before the experiment. Tissue sections three microns in thickness were stained with hematoxylin and eosin for histopathological evaluation and stained with azan for evaluation of proliferation of collagenous fiber. Slight atrophic changes, such as reduction in the number of parietal cells per unit area (78.8 ± 15.4 in the MNNG group and 110.1 ± 20.8 in the control group), shortened mucosal thickness (5.36 ± 0.69 in the MNNG group and 6.72 ± 0.53 in the control group), slight infiltration (grade 1) of inflammatory cell infiltration, and also slight proliferation (grade 1) of collagenous fibers were present in the fundic mucosa of rats treated with MNNG, while mucosal changes of hyperplastic gastritis, such as hyperplastic glands, increased mucosal thickness (2.77 ± 0.15 in the MNNG group; 2.64 ± 0.21 in the control group), and mild infiltration (grade 2) of inflammatory cells, and mild proliferation (grade 2) of collagenous fibers were present in the pyloric mucosa of rats treated with MNNG. Furthermore, in the pyloric mucosa, focal and diffuse lesions composed of atypical cells with erosions considered to be precancerous stage were present. Diffuse mucosal surface injuries (erosions) existed in both the fundic and pyloric mucosa. Development of gastric cancer does not seem to be involved in the background such as mucosa with atrophic gastritis but with hyperplastic gastritis in short term-administration of MNNG.

Sugimura and his colleagues[13] were the first in successfully producing adenocarcinoma similar to human gastric carcinoma in the glandular stomach of the rat by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in 1967. Thereafter a vast number of studies dealing with experimental cancer of the stomach by MNNG have been published[2-4,11,14]. However, not many studies have been made on alterations such as gastritis, especially atrophic gastritis, in the gastric mucosa as a background in the process of gastric carcinoma development by MNNG in the rat[8,11,12]. Therefore, the authors attempted to ascertain the mucosal changes induced by MNNG from the standpoints described in the materials and methods. The ultimate goal of this study was to clearly whether or not the mucosa with atrophic gastritis which may lead to gas-

tric carcinoma can be developed at such an early stage as 12 weeks after the administration of MNNG.

MATERIALS AND METHODS

(1) Animals

Male Wistar rats, weighing 180 g at the beginning of the experiment, were used in this study. Their average body weight was 350 g at the end of the experiment.

(2) Administration of MNNG and control

Solution containing 50 mg of MNNG (Sigma) dissolved in one liter of 0.04% Tween 60 (Seikagaku Kogyo Co.) was administered to rats ad libitum in light-sealed bottles for three months (experimental group, N=5). The solution was prepared and adjusted every day. Solution containing 0.04% of Tween 60 alone was given to the control rats for three months (control group, N=5).

(3) Preparation of tissue samples

The rats of both groups were sacrificed three months after the beginning of the experiment. The abdomen was opened and the stomach was resected and opened along the greater curvature. After microscopic examination for possible tumorous lesions as well as mucosal lesions in the stomach and small intestine of both the experimental and control groups, Swiss rolled tissue specimens for observation from the cardiac to the pyloric region along the line of each section of the numbered mucosa under the microscope were each taken from five sites of the stomach of rats of both groups (Fig. 1). These were immediately fixed in Bouin's solution for 6 hr at 4°C. After fixation, the rolled tissue specimens were embedded in paraffin wax and sectioned 3 microns in thickness.

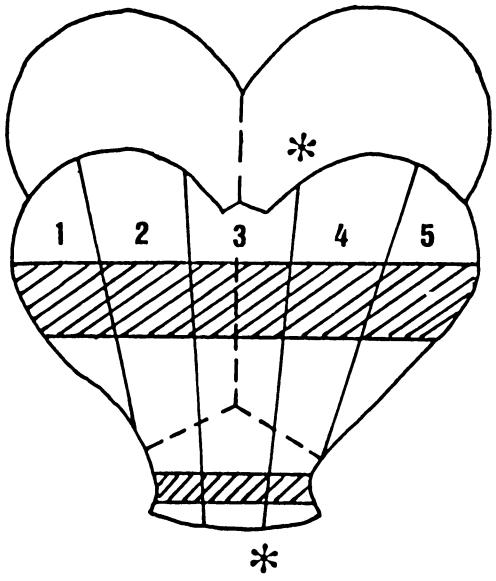

Fig. 1. Gastric mucosa of the rat. The figure shows Swiss rolled tissue samples. The number of parietal cells and the mucosal thickness are counted and measured in the shaded area, respectively.

(4) Conventional histopathology

Sections were stained with hematoxylin and eosin for histopathological evaluation of each tissue. Azan stain was made for evaluation of the fibrotic changes in the gastric wall.

Using ocular eyegrid equipped in the eyepiece of the light microscope, the length of mucosal surface lesions (erosions) was measured on the mucosal line of each numbered mucosa as shown in Fig. 1 and expressed as the total length (cm) per stomach. The length of the body and the antral mucosa was measured in the shaded area of the mucosa as shown in Fig. 1 by the ocular eyegrid under × 100 magnification described above and express as mm. The number of parietal cells was counted in the shaded area of the mucosa as shown in Fig. 1 by the same ocular eyegrid as in the case of the length of erosions under × 400 magnification and expressed as the number per unit area (0.25 cm^2). The reason why the shaded area was arranged before the study was because the length of the mucosa and the distribution of parietal cells varied from the cardiac to the pyloric region of the mucosa. The exact horizontal sections were used for this purpose. The length of the mucosa was measured and the number of parietal cells was counted in three to five visual fields and expressed as the mean per visual field or unit area, respectively. The degree of inflammatory cell infiltration and fibrotic proliferation was evaluated slight (grade 1), moderate (grade 2), and severe (grade 3) according to the number of inflammatory cells in the lamina propria and the extent of staining with azan, respectively.

(5) pH in the gastric juice

pH in the gastric juice was checked by dipping of pH paper.

(6) Markers of atrophic gastritis

In the present study, the markers of atrophic gastritis were defined as 1) reduction in the number of parietal cells, 2) shortening of the mucosal length in the body and antrum, 3) proliferation of interstitial fibrosis, and 4) inflammatory cell infiltration in the lamina propria mucosae and the base of the mucosa. These are also referred to in the evaluation of atrophic gastritis.

7) Statistical analysis

Data on the length of erosions, mucosal length and number of parietal cells are expressed as mean ± standard error of the mean. Student-t test was used as the statistical test. Wilcoxon rank-sum test was utilized for statistical evaluation of the degree of fibrotic proliferation and inflammatory cell infiltration. Difference with p value of less than 0.05 was considered statistically significant.

RESULTS

(1) Gross pathology

There were neither tumourous lesions both in the fundic and pyloric regions nor ulcerative lesions with the exception of small, hemorrhagic spots and localized hyperemia on the mucosal surface.

(2) Microscopic pathology

Mucosal surface injuries (erosions) were observed on the whole mucosa of the fundus (Fig. 2). Ulcerative lesions, however, which reached the deep region of the mucosa and muscularis mucosae were not present in the fundus. The glandular structure was relatively regular, but the glandular tubules were slightly tortuous and irregular as well as cystic in shape (Fig. 3). Inflammatory cell infiltration was not marked in the fundic mucosa with the exception of the regions close to the antrum and of the cardia, where inflammatory cell infiltration was marked. Focal reduction of parietal cells was present in the fundus. Fibrotic proliferation was not marked in the fundic region. Pseudopyloric or intestinal metaplasia and tumourous lesions as well as malignant lesions were not observed in the region.

In the pylorus, erosive lesions were present bu they were shallow, superficial and focal, accompanied with occasional inflammatory cell infiltration at the margins (Fig. 4). The lesions were sometimes covered with exudate and necrotic

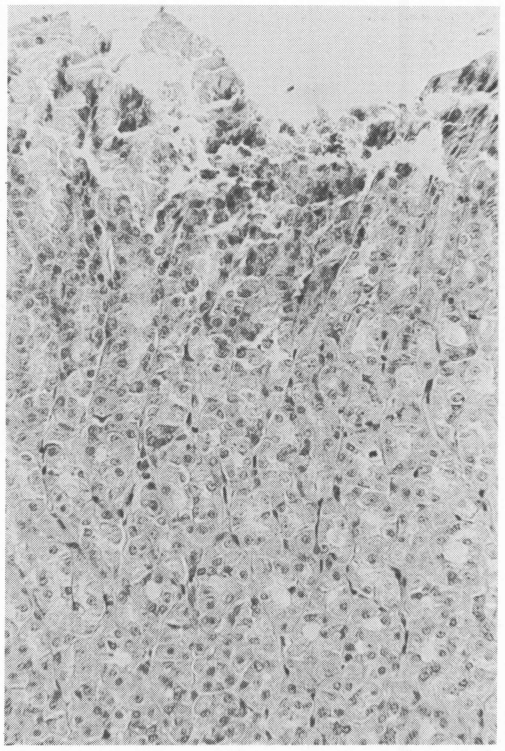

Fig. 2. Mucosal surface injuries (erosions) on the mucosa of the rat treated with MNNG (HE stain, × 160).

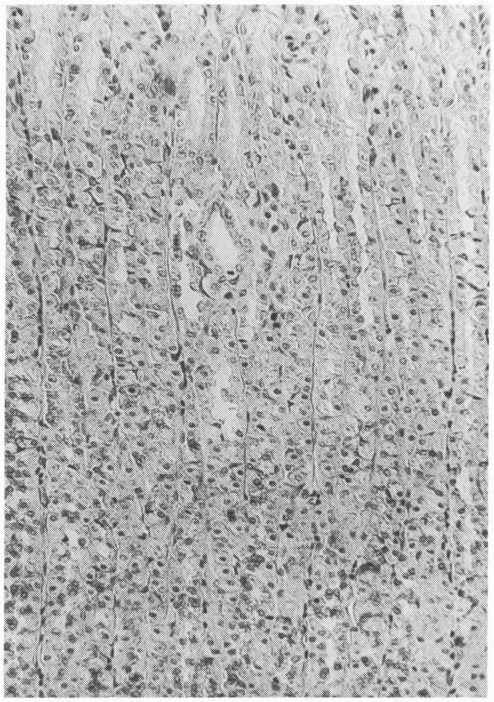

Fig. 3. Glandular tubules were dilated cystically in the fundic mucosa of the rat treated with MNNG (HE stain, × 160).

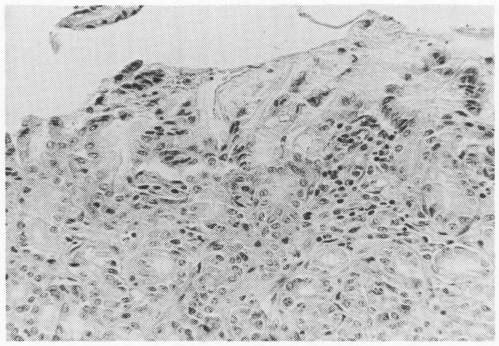

Fig. 4. Inflammatory cells occasionally surrounded the erosion in the pyloric mucosa of the rat treated with MNNG (HE stain, × 160).

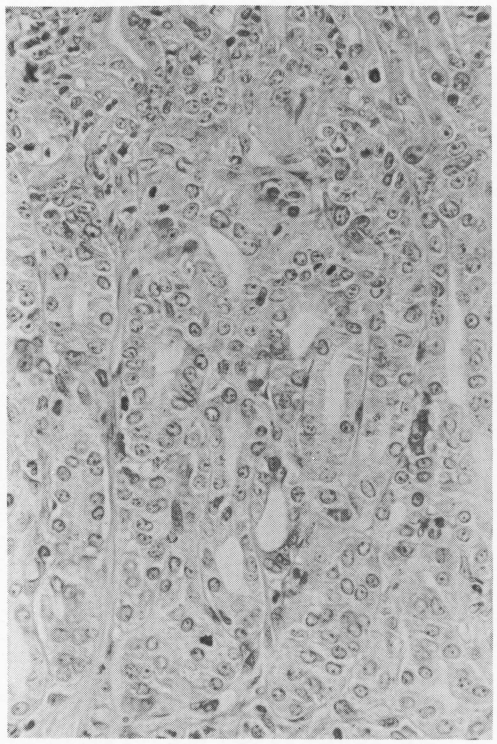

Fig. 5. A diffuse lesion composed of atypical cells and irregular glandular structure. In the lesion, precancerous stage of focal cancer is observed in the pyloric mucosa exposed to MNNG (HE stain, × 160).

mass, which reacted weakly to PAS stain. Atypical glands which were composed of cells with cytoplasmic and nuclear atypism and clearly different from usual pyloric gland cells were found focally but in a large area in the pylorus in No.3638 rat and also in the region around the

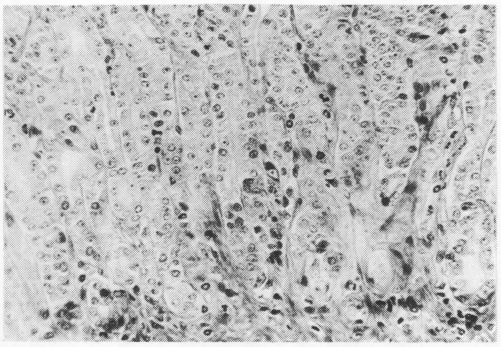

Fig. 6. Proliferation of collagenous fiber is seen in the base upward and some pyloric glands are replaced by fibrosis (Azan stain, × 160)

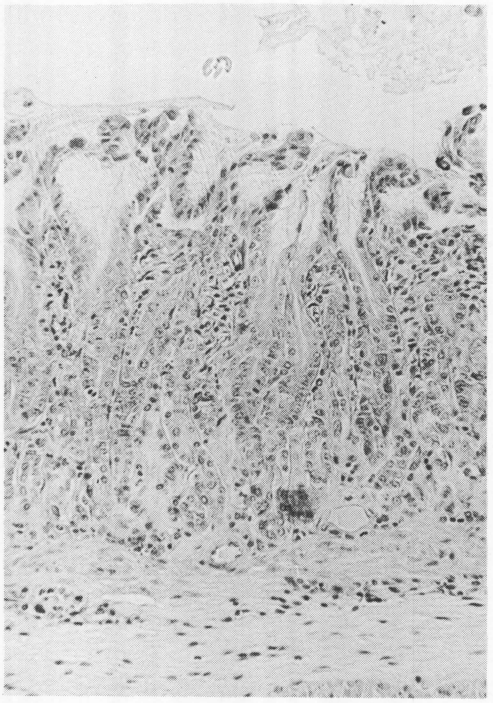

Fig. 7. Edema and inflammatory cell infiltration were found in the pyloric mucosa after exposure to MNNG (HE stain, × 160).

border of the fundus and pylorus in No.3637 rat (Fig. 5). The typical glands were tubular and surrounded with fibrosis and inflammatory cells. The collagenous fiber proliferated from the base to the surface of the mucosa and seemed to replace the glands, resulting in reduction and / or disappearance of pyloric glands (Fig. 6).

The arrangement of pyloric glands with the exception of atypical lesions was relatively regu-

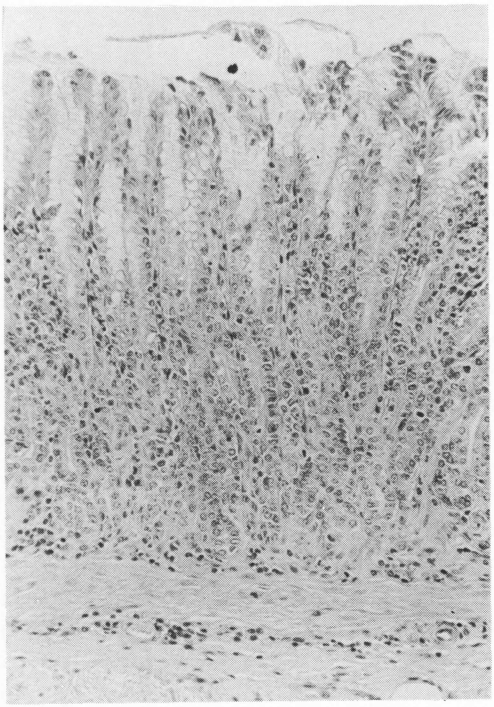

Fig. 8. Glandular hyperplasia is present in the pylorus treated with MNNG (HE stain, × 160).

lar, but there were edema and cell infiltration composed mainly of lymphocytes and monocytes and rarely of plasma cells in the lamina propria (Fig. 7). Glandular hyperplasia was also observed chiefly in the pylorus, which caused elongation of crypts to raise the mucosal height and proliferation of the pyloric glands (Fig. 8). In addition, hyperplastic proliferation of epithelial cells was also noted in the diffuse area of the pylorus. Polyporous or flat lesions as well as malignant lesions were not present in the pylorus and intestinal metaplasia could not be seen at this stage.

(3) Mucosal surface lesions (erosions)

The length of erosions was 16.82 ± 2.23 cm in the experimental group, while it was 0.90 ± 0.11 cm in the control group (Table 1). A statistically significant difference existed in the length between the two groups.

(4) Mucosal length (thickness) (Table 1)

The length of the fundic mucosa was 5.36 ± 0.69 mm in the experimental group and 6.72 ± 0.53 mm in the control group. A significant difference between the two could be demonstrated. The length of the antral mucosa was 2.77 ± 0.15 mm in the experimental group and 2.64 ± 0.21 mm in the control group, showing a significant difference between the two.

(5) Parietal cells (Table 1)

The number of parietal cells per unit area was 78.8 ± 15.4 cells in the experimental group, while it was 110.1 ± 20.8 cells in the control group. A statistically significant difference was observed between the two groups.

(6) Interstitial fibrosis

Proliferation of interstitial fibrosis was significantly marked in the stomach wall of the experimental group in which the degree of fibrosis

Table 1. Summary of histoquantitative data in parietal cells and mucosal thickness

Exptl. Group	Erosion (cm)	P cells	Mucosal Thickness (mm)	
			Fundus	Antrum
MNNG	16.82 ± 2.23	78.8 ± 15.4	5.36 ± 0.69	2.77 ± 0.15
Control	0.90 ± 0.11	110.1 ± 20.8	6.72 ± 0.53	2.64 ± 0.21
	*	*	*	*

** $p<0.01$
*** $p<0.001$

Table 2. Summary of inflammatory cell infiltration and fibrotic proliferation

Extl. Group	Cell infiltration		Fibrosis	
	Fundus	Pylorus*	Fundus	Pylorus*
MNNG	0.9 ± 0.1	2.0	1.1 ± 0.1	2.0
Control	0.8 ± 0.1	1.0	0.9 ± 0.1	1.0
	NS		NS	

* As the number of the pylorus samples was small, statistical analysis was not made.

NS: Not statistically significant

was grade 2 in the pylorus but grade 1 (1.1 ± 0.1) in the fundus of rats when compared to that (grade 1 in the pylorus and, 0.9 ± 0.1 in the fundus) in the control group as shown in Table 2.

(7) Inflammatory cell infiltration

Inflammatory cells consisting mainly of lymphocytes and occasionally of plasma cells infiltrated into the lamina propria and the base of the mucosa, which was grade 2 in the pylorus and grade 1 (0.9 ± 0.1) in the fundus. Cell infiltration tended to be marked in the experimental group when compared to that (grade 1 in the pylorus and 0.8 ± 0.1 in the fundus) in the control, but the difference was not statistically significant (Table 2).

(8) pH in the gastric juice

pH in the gastric juice was around 2.0 in rats of both groups.

DISCUSSION

Early changes in the mucosa of the stomach after exposure of MNNG, a carcinogenic agent, were examined in the present study. For this purpose, a carcinogenic dose of MNNG was administered to rats for 12 weeks. The gastric mucosa stained with hematoxylin or azan was examined histopathologically and histoquantitatively mainly for atrophic changes in the mucosa as a background lesion for development of gastric carcinoma. The present study showed that a short term administration of MNNG caused serious and variable changes in the gastric mucosa. In the fundic mucosa, diffuse mucosal surface injuries (erosions), inflammatory cell infiltration in the lamina propria mucosae though not marked around the erosions, and proliferation of collagenous fibers from the base upward in the mucosa were observed, suggesting that these were mucosal changes of erosive gastritis. In addition to these, there were reduction in the number of parietal cells per unit square and shortened mucosal thickness, indicating that the atrophic change was progressing gradually but evidently in the mucosa of the rats treated by MNNG, since the authors observed that marked atrophic gastritis occurred in the mucosa of the rats after a long term exposure of MNNG[5,6]. The authors assume that repetition of repair and excerbation of erosions may be one of causative factors of atrophic changes in the mucosa after exposure of MNNG. These findings and hypothesis support some of the studies reported to date[11,12].

On the contrary, the changes in the pyloric mucosa were peculiar after a short term exposure of MNNG. The mucosal thickness increased, hyperplastic pyloric glands existed in the pyloric mucosa, inflammatory cells infiltrated more markedly, and collagenous fibrosis proliferated more intensely in the lamina propria and mucosal base when compared to those in the fundic mucosa. Again diffuse erosions were observed. These findings suggest that the changes were erosive and hyperplastic gastritis which was progressing in the pyloric mucosa during exposure of MNNG. There were a few atypical lesions in the pyloric mucosa with such gastritis in all rats treated with MNNG. The lesions had irregular arrangement and structure of glands was composed of atypical cells, some of which were in the precancerous stage or focal cancer. The downgrowth of the lesions to the submucosa was not observed in all rats treated with MNNG. Atrophic changes were not present around the lesions. These suggest that in such a short duration of exposure of MNNG the significance was low of atrophic gastritis serving as a background lesion for the development of gastric carcinoma, from which carcinomas might grow after a long term exposure of MNNG. Pyloric hyperplastic gastritis observed in the present study may be also important as a background lesion for the development of gastric carcinomas as suggested by some investigators[7,9,12,15].

Finally, intestinal metaplasia, which appeared in a high frequency after 4 or 5 months after the exposure of MNNG[10], was not observed in both the fundic and pyloric mucosa. This also suggests that such a short term exposure of the agent as in the present study may be not sufficient for developing atrophic gastritis with intestinal metaplasia.

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