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PROTECTIVE EFFECT OF MUCIN ON EXPERIMENTAL GASTRIC CANCER INDUCED BY N-METHYL-N'-NITRO-N-NITROSOGUANIDINE PLUS SODIUM CHLORIDE IN RATS*1

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Studies were made on the effect of mucin on the induction of gastric carcinomas by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), with or without sodium chloride, in male Wistar rats. Seven groups of rats were treated as follows: Group 1 was given continuously 50 mg MNNG/liter solution to drink and 1 ml of saturated sodium chloride once a week and fed on stock diet supplemented with 4% mucin. Group 2 was given 50 mg MNNG/liter solution and fed on stock diet supplemented with 4% mucin. Group 3 received 1 ml of saturated sodium chloride once a week and 50 mg MNNG/liter solution to drink. Group 4 was treated with MNNG only. Group 5 was fed on stock diet supplemented with 4% mucin. Group 6 was given sodium chloride only. Group 7 was untreated. The incidence of gastric cancer in Group 3 was significantly higher than that in Group 4 ($P<0.05$) or in Group 1 ($P<0.05$). The difference in the incidence of gastric cancer in Groups 2 and 4, and of intestinal tumors in Groups 1 to 4 were not statistically significant. No malignant tumors were seen in Groups 5, 6, and 7. Thus mucin reduced the high incidence of gastric cancer induced by MNNG and sodium chloride to the level induced by MNNG alone, but it had no effect on the incidence of intestinal tumors. The effect of mucin in preventing destruction of the gastric mucosal barrier by sodium chloride and so reducing the induction of gastric cancer is discussed.

The mucosal barrier of the glandular stomach has been suggested to be important in preventing the induction of stomach carcinomas.[3,10,12] The gastric mucosal barrier is destroyed by detergents.[5] Hypertonic sodium chloride solution has been found to enhance the uptake of 7,12-dimethylbenz[*a*]-anthracene by the glandular stomach.[4] Adenocarcinomas in the glandular stomach of rodents were induced by various kinds of carcinogens.[2,15,16,18~21] Moreover, some detergents[7,21~23] and sodium chloride[24] enhanced the gastric carcinogenic activity of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and 4-nitroquinoline 1-oxide in rats by decreasing the gastric mucosal barrier. Gastric mucus is one of the most important components of this barrier[9] and it contains much mucin. Therefore, it seemed possible that administration of mucin might stregthen the gastric mucous barrier against carcinogen.

In this work studies were made on the induction of carcinomas by MNNG in both normal rat stomach and stomach injured by sodium chloride.

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Materials and Methods

The gastric carcinogen used was N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) from the Aldrich Chemical Co. (Milwaukee, Wis.). Mucin from porcine stomach was supplied by Sigma Chemical Co. (St. Louis, Mo.). NaCl was a commercial product from Nakarai Chemical, Ltd. (Kyoto).

A total of 140 male Wistar rats, initially weighing about 200 g, were treated as follows:

Group 1: Twenty-five rats were given 50 mg MNNG/liter in their drinking water and were fed freely on stock diet (Oriental MF, Oriental Yeast Co., Tokyo) supplemented with 4% mucin. They were also given 1 ml of saturated NaCl solution (29.0%) by gastric intubation once a week.

Group 2: Twenty-five rats were given 50 mg MNNG/liter to drink and fed on stock diet supplemented with 4% mucin.

Group 3: Thirty rats were given a solution of 50 mg MNNG/liter to drink, fed on stock diet, and given 1 ml of saturated NaCl solution by gastric intubation once a week.

Group 4: Thirty rats were given a solution of 50 mg MNNG/liter to drink and fed on stock diet.

Group 5: Ten rats were fed on stock diet supplemented with 4% mucin.

Group 6: Ten rats were given 1 ml of saturated NaCl solution (29.0%) by gastric intubation once a week.

Group 7: Ten rats were given stock diet and tap water.

Rats were housed 5 to a wire cage under natural lighting in an air-conditioned room at 24° and were weighed weekly. The MNNG solution was kept in light-proof bottles and renewed every day from a stock solution (1 g/liter) prepared weekly. The rats were treated with MNNG, mucin, and/or NaCl for 20 weeks and then maintained on stock diet and tap water.

All surviving rats were killed and autopsied after 50 weeks and animals which became moribund after the 40th week were also included in the effective number. The stomach and other organs were examined carefully and fixed in 10% formaldehyde solution for histological examination. Tissues were embedded in paraffin and stained with Hematoxylin and Eosin. Selected tissues were stained with Alcian Blue, periodic acid-Schiff, Mayer's Mucicarmine, or Van Gieson's stain. The results were analyzed by the χ^2 test.

Results

The average body weight of the rats in each group is shown in Table I. There was no significant difference in the intake of drinking water in each group. The total intake of MNNG by animals in Groups 1, 2, 3, and 4 was about the same, but the exact intake is unknown, because the MNNG solution was supplied freely.

Gastrointestinal Tumors The incidence of gastrointestinal tumors in each group is summarized in Tables II and III. In Groups 1, 2, 3, and 4 treated with MNNG, tumors were mainly found in the glandular stomach with a few intestinal tumors.

Glandular Stomach: Most tumors of the glandular stomach were in the lesser curvature of the pyloric region. They were sessile polypoid lesions or ulcerated tumors with a pale, irregularly elevated border, and appear-

Table I. Experimental Groups of Rats Treated with MNNG, Mucin, and/or NaCl, and Changes in Their Body Weight

Group No.	Experimental group			Initial No. of rats	Body weight (g)	
	Drinking water	Gastric intubation	Diet		Initial	After 50 weeks
1	MNNG	Satd. NaCl	4% Mucin	25	195.7	493.3
2	MNNG	—	4% Mucin	25	185.1	491.3
3	MNNG	Satd. NaCl	Stock diet	30	190.8	465.5
4	MNNG	—	Stock diet	30	186.3	486.8
5	Tap water	—	4% Mucin	10	195.2	489.8
6	Tap water	Satd. NaCl	Stock diet	10	190.5	464.7
7	Tap water	—	Stock diet	10	198.0	470.5

MNNG=50 mg MNNG/liter solution. Satd. NaCl=1 ml of saturated NaCl, once a week.
4% Mucin=stock diet supplemented with 4% mucin.

Table II. Incidence of Gastric Tumors in Rats Treated with MNNG, Mucin, and/or NaCl

Group No.	Effective No. of rats	Glandular stomach: Well-differentiated adenocarcinoma	Glandular stomach: Poorly differentiated adenocarcinoma	Sarcoma[a)]	Total malignant tumor (%)
1	23	12	0	0	12(52.2)*,**
2	21	9	1	(1)	10(47.6)*,**
3	25	18	2	(1)	20(80.0)
4	28	14	1	0	15(53.6)*
5	10	0	0	0	0(—)
6	9	0	0	0	0(—)
7	10	0	0	0	0(—)

a) Numbers in parentheses indicate the number of rats with a sarcoma plus an adenocarcinoma.
* Significantly different from incidence in Group 3 ($P<0.05$).
** Not significantly different from incidence in Group 4.

Table III. Incidence of Instetinal Tumors in Rats Treated with MNNG, Mucin, and/or NaCl

Group No.	Effective No. of rats	Small intestine: Carcinoma	Small intestine: Sarcoma	Large intestine: Carcinoma	Large intestine: Sarcoma	Total malignant tumors (%)
1	23	1	6	0	1[1]	8(34.8)
2	21	2	2[1]	0	0	4(19.0)
3	25	0	4	0	0	4(16.0)
4	28	2	6[1] (1)	0	0	8(28.6)
5	10	0	0	0	0	0 —
6	10	0	0	0	0	0 —
7	10	0	0	0	0	0 —

Numbers in brackets indicate the number of rats with metastatic changes, and numbers in parentheses indicate the number of rats with a sarcoma plus an adenocarcinoma.

ed as smooth or irregular surfaced globules extending into the serosa. Some adhered to the omentum and the liver.

The adenocarcinomas of the glandular stomach were of two histological types; well-differentiated and poorly differentiated adenocarcinomas like those described in previous papers.[8,24)] The well-differentiated tumors had a tubular, papillary, or cystic glandular structure (Photo 4). Cellular atypism was usually slight and tumor cells contained much or little mucin. Poorly differentiated tumors were characterized by loss of the glandular arrangement. Anaplastic tumor cells showed pleomorphism of the cytoplasm and nucleus (Photo 3). Mucinous adenocarcinomas, variants of poorly differentiated adenocarcinomas, often producing much extracellular mucus and containing only a few tumor cells, were found in Groups 3 and 4 (Photos 1 and 2). No significant difference was found in the histological appearance of the adenocarcinomas in the different groups.

Adenocarcinomas of the glandular stomach were found in 12 of 23 effective animals in Group 1, in 10 of 21 effective animals in Group 2, in 20 of 25 effective animals in Group 3, and in 15 of 28 effective animals in Group 4. Among these adenocarcinomas, all 12 in Group 1, 9 of the 10 in Group 2, 18 of the 20 in Group 3, and 14 of the 15 in Group 4 were well-differentiated. No metastases were found. The differences in the incidence of adenocarcinomas between Groups 1 and

3, 2 and 3, and 3 and 4 were statistically significant ($P<0.05$), but those between Groups 1 and 4 and between 2 and 4 were not. All the rats with sarcoma of the glandular stomach also had an adenocarcinoma of the glandular stomach.

Intestine: The tumors in the intestine grew into the serosa and had a rough surface, and they almost completely obstructed the alimentary canal. Some of them were hemorrhagic and invaded adjacent tissue such as the omentum, liver, and small and large intestine forming conglomerates. Almost all the adenocarcinomas of the small intestine were composed of anaplastic cancer cells and had an irregular tubular structure, and they showed invasive growth into the serosa.

The sarcomas were classified as angiosarcomas, fibrosacomas, leiomyosarcomas, and unclassified sarcomas. One adenocarcinoma, 3 angiosarcomas, and 3 fibrosarcomas of the small intestine were found in Group 1. One unclassified sarcoma of the large intestine was also found in Group 1 and this metastasized to the liver, small intestine, and omentum. Two adenocarcinomas and 2 fibrosarcomas of the small intestine were found in Group 2 and one of the fibrosarcomas metastasized to the liver and omentum. Three angiosarcomas and one leiomyosarcoma of the small intestine were found in Group 3. Two adenocarcinomas, 3 angiosarcomas, 2 fibrosarcomas, and one leiomyosarcoma of the small intestine were found in Group 4. One rat with an adenocarcinoma also had a fibrosarcoma. One fibrosarcoma metastasized to the intestinal lymph nodes. No intestinal tumors were found in Groups 5, 6, and 7. The differences in the incidence of malignant tumors of the intestine between Groups 1 to 4 were not statistically significant.

Nontumor Areas of the Gastrointestinal Tract Marked hyperplastic and atrophic lesions of the pyloric mucosa were detected in groups treated with MNNG. Some pyloric gland metaplasia in the fundic mucosa was also seen in these groups and in a few rats in Group 6 given saturated NaCl. Slight erosion or ulceration of the gastrointestinal tract was found in a few rats in the groups treated with MNNG and in the group given saturated NaCl only.

Discussion

In the present work a significant difference ($P<0.05$) was found in the incidence of gastric cancer in rats treated with MNNG with and without saturated NaCl (Group 3 vs. Group 4), but the difference in the incidence of intestinal tumors in these groups was not significant. These results show that NaCl enhanced the carcinogenic effect of MNNG on the stomach but not on the intestine of rats, as reported in our previous paper.[24] As we suggested previously,[24] NaCl may enhance the carcinogenic activity of MNNG by removing the mucous layer and so destroying the gastric mucosal barrier[4] and by decreasing the viscosity of the gastric mucus.[24]

The incidence (47.6%) of gastric cancer in the group treated with MNNG and mucin (Group 2) was lower than that (53.6%) in the group treated with MNNG alone (Group 4), but the difference was not statistically significant. Thus, mucin did not prevent induction of gastric cancer by MNNG, but the difference in the incidence of gastric cancer in the groups treated with MNNG and saturated NaCl solution, with and without mucin (Group 1 vs. Group 3), was statistically significant ($P<0.05$). Mucin suppressed the induction of gastric cancer by MNNG and saturated NaCl solution in Group 1 to the level in the group treated with MNNG alone (Group 4). Erosion and ulceration of the glandular stomach were seen after administration of 1 ml of saturated NaCl by a stomach tube both to rats on stock diet and to those on stock diet supplemented with 4% mucin (unpublished data). Thus, mucin did not inhibit erosion or ulceration caused by NaCl. Moreover, mucin did not change the histological pattern of the adenocarcinomas induced in the stomach.

The exact locus and nature of the gastric mucosal barrier are unknown. However, one component must be the lipoprotein layer which forms the plasma membrane of the mucosal cells, and another component may be the mucous layer at the distal end of the epithelial cells.[9] Mucin is a chief component of the mucous layer. Administered mucin coated the ulcers and protected them against the proteolytic action of gastric secretion and it also readily combined with free acid.[6] Mucin from porcine stomach contains chondroitinsulfate[13] which inhibits the action of pepsin[14] both *in vitro* and *in vivo*.[11] However, Anderson[1] reported that several sulfated polysaccharides protect the gastric mucosa from pepsin by combining with protein of the ground substance rather than by inhibiting the action of pepsin. These reports and the present results suggest that mucin reinforces the gastric mucosal barrier after its injury by NaCl, both by enhancing the protective activity of the gastric mucosa and by inhibiting the actions of toxic factors, such as pepsin and free acid in the gastric juice.

MNNG may have a toxic action on the gastric mucosa, but very few histopathological changes, such as erosion and ulceration, were observed in the pyloric mucosa from 2 weeks to 5 months after the beginning of its administration (50 mg/liter in the drinking water) to rats.[17] Thus, destruction of the gastric mucosal barrier by MNNG must be slight. Although mucin had no protective effect against the slight destruction of the gastric mucosal barrier by MNNG, mucin strengthens the gastric mucosal barrier after its injury by NaCl so that it becomes as effective against MNNG as the normal gastric mucosal barrier.

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Explanation of Plate

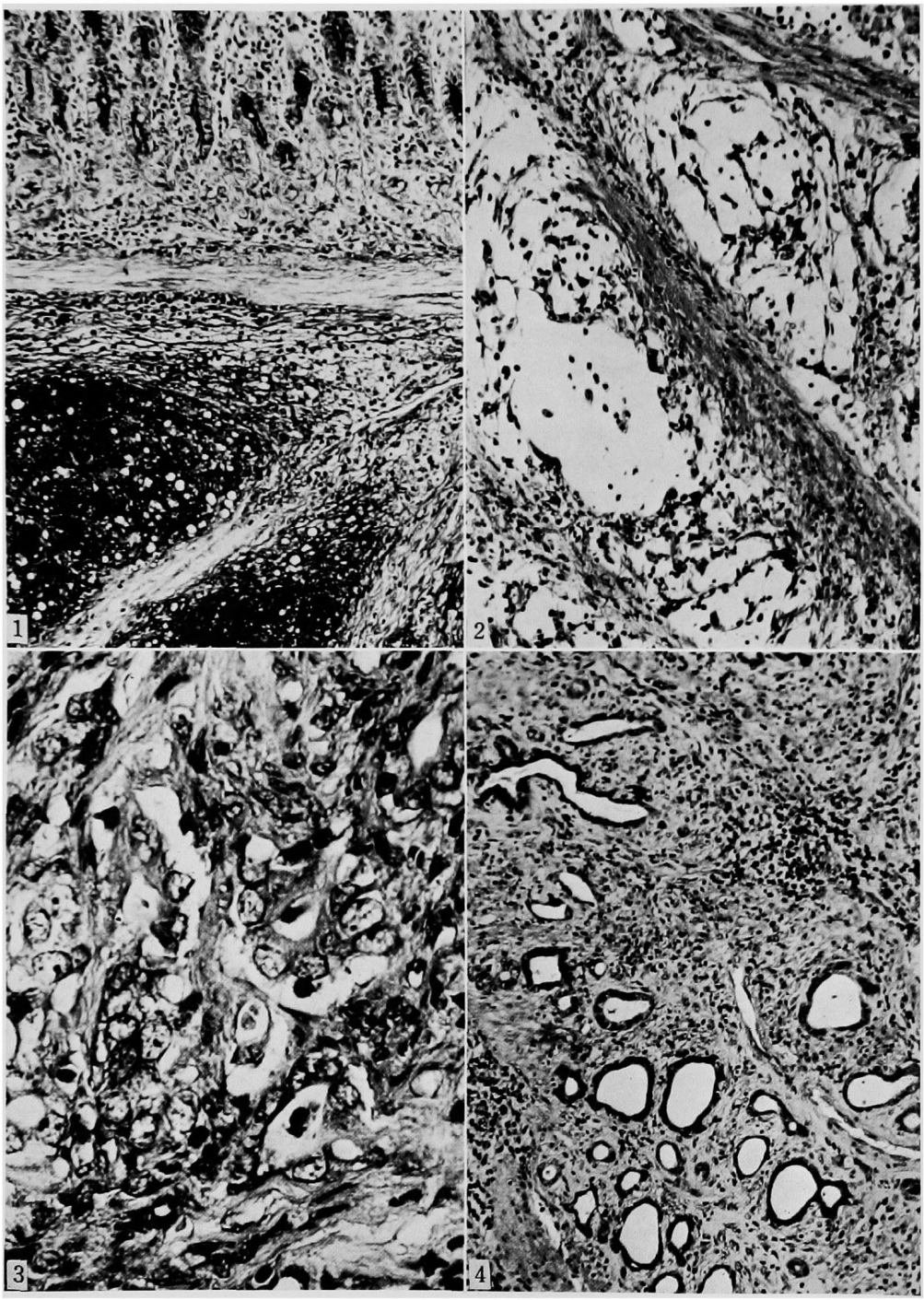
Photo 1. Invasive growth of poorly differentiated adenocarcinoma (mucinous adenocarcinoma) consisting of mucin-secreting tumor cells into the muscle layer of the glandular stomach (Group 3). Alcian Blue and periodic acid-Schiff. ×100.

Photo 2. Higher magnification of the tumor in the muscle layer of the glandular stomach shown in Photo 1 (Group 3). H-E. ×200.

Photo 3. Poorly differentiated adenocarcinoma consisting of anaplastic tumor cells (Group 2). H-E. ×400.

Photo 4. Invasive growth of a well-differentiated adenocarcinoma into the muscle layer of the glandular stomach (Group 4). H-E. ×200.

H-E=Hematoxylin and Eosin stain.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