Effects of Atrophic Changes in Mucosa and Endogenous Gastrin (Hypergastrinemia) on Development of Experimental Gastric Cancer and its Growth in Rats: A Pilot Study[*]

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ABSTRACT

In the present experiment the influence of atrophic mucosa and resulting endogenous hypergastrinemia on the induction of gastric cancer by N–methyl–N'–nitro–N–nitroso-guanidine (MNNG) was examined in male Donryu rats. All rats were fed MNNG in tap water for 6 months and were killed after more 5 months. The incidence of gastric cancer was 55% in rats with atrophic gastritis and hypergastrinemia, which is significantly higher than the incidence of 10% observed in rats treated with MNNG alone. Gastric cancer was not found in any rats with atrophic gastritis without associating with hypergastrinemia. These data suggest that atrophic mucosa and resulting hypergastrinemia are important in development and growth of gastric cancer in rats.

INTRODUCTION

The incidence of chronic atrophic gastritis and hypochlorhydria as well as gastric achlo-rhydria is elevated in patients with gastric cancer[3,10,16,24] and gastric cancer is rare in duodenal ulcer patients with hyperchlorhydria[9,10], but its incidence increases following ulcer surgery[8]. These observations suggest that atrophic mucosa of the stomach in a hypoacidic environment may be responsible for the development of gastric cancer.

It is well known that gastrin stimulates gastric acid secretion[5] and exerts a trophic action on the gastric mucosa[12]. Gastrin, therefore, may accelerate the induction and growth rate of gastric cancer by its trophic action.

In the present study attempts were made to determine whether atrophic gastrtis and endogenous hypergastrinemia induced experimentally have any effect on the development of the gastric cancer by MNNG in Donryu rats.

MATERIALS AND METHODS

Thirty five male Donryu rats weighing 180 g were divided into the following four experimental groups: Group (A):MNNG alone. Ten rats were administered MNNG without any treatment. Group (B):Atrophic gastritis. Ten rats underwent immunizatation of homologous gastric mucosal homogenate as antigen and were administered MNNG. Group (C): Atrophic gastritis. Ten rats were administered taurocholate orally daily together with MNNG. Group (D):Five rats which were given Tween 60 without any treatment were used as control.

Each ten of three groups of rats were given MNNG at a concentration of 50mg/liter in

drinking water containing 0. 004% Tween 60 for 6 months and were observed for an additional 5 months before being sacrificed. The drinking bottles were shielded with foil to prevent deterioration of MNNG by light exposure and the water was changed daily.

Experimental atrophic gastritis was induced by the following two methods: (1) Immunization[20]. Rats were given two injections of 0.5 ml of dialysed mitochondrial fraction of homologous stomach (protein 6 mg) emulsified incomplete Freund adjuvant in multiple intradermal injection sites at one-month interval (Experimental group B). (2) Taurocholate[15]. Rats received 2 mM taurocholic acid through a tube into the stomach daily during the entire experimental period (Experimental group C).

Five months after suspension of MNNG, the animals were sacrificed by decapitation after 24 hours. Blood was drawn for the determination of gastrin levels by radioimmunoassay. The abdomen was opened and the abdominal contents were examined for gross evidence of tumors. The site and size of grossly detectable tumors were recorded.

After gross examination, the entire stomach was pinned on cork mats to prevent shrinkage of the mucosal folds and oriented. They were then fixed in Bouin's solution containing 15 ml of saturated picric acid, 5 ml of neutral formaldehyde and 1 ml of glacial acetic acid for 3 to 6 hours, oriented, embedded in paraffin, and sectioned 3 microns in thickness. A couple of different sections were taken from each tumor, body and antral mucosa of each rat and stained with hematoxylin and eosin stain for histopathological study. Each section taken from the body and antral mucosa was also stained with Azan for fibrosis and PAS–alcian blue at pH 2. 5 for intestinal metaplasia. The number of parietal cells per unit are $(1 cm^2 \times 400$ magnification) was counted for evaluation of atrophic changes in the gastric mucosa. Atrophic gastritis was defined in the present study to have a reduction of parietal cells in the mucosa of the body and fundus of the stomach.

RESULTS

(1) Incidence of gastric cancer (Table 1, Fig. 1)

The incidence of gastric cancer was higher

Table 1. Animals with gastric cancer

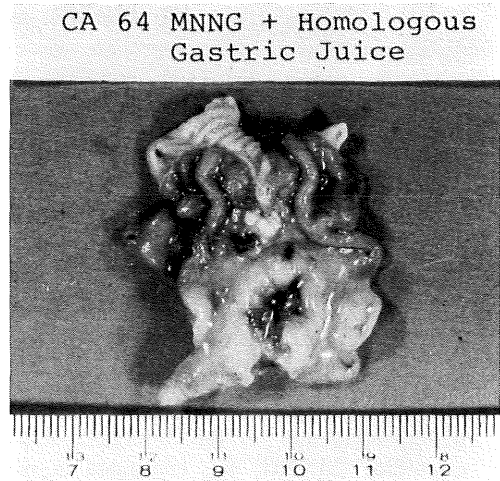| Experimental group	Survived No	Gastric cancer Animal No	Percent Incidence
(A)	10	1	10
(B)	10	6	60
(C)	10	5	50
(D)	5	0	0

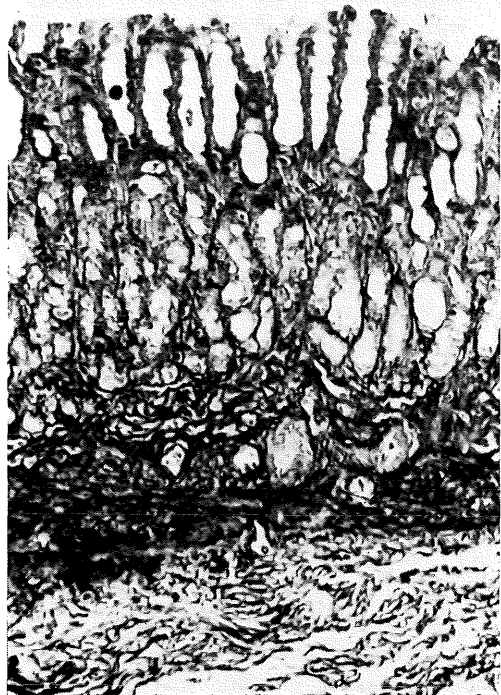**Fig. 1.** A gastric cancer invaded into muscle layer in the rat with experimental group B.

Fig. 2. Markedly fibrous tissue was found in the body mucosa of experimental group B (Azan stain, ×200).

in rats with atrophic gastritis (60% in experimental group B and 50% in C) than in rats given MNNG alone (10% in experimental group A).

(2) Atrophic gastritis

Histopathologically, the mucosae of both the antral and body areas were abnormal in all specimens of experimental groups A, B and C. Various grades of cellular changes were observed characterized by disruption on the normal linear arrangement of the cells composing the branched glands. Decreased in thickness of the mucosa with fibrosis (Fig. 2) and intestinalization (Fig. 3) were observed in animals belonging to experimental groups B and C. The number of parietal cells was significantly smaller in animals of three experimental groups than in control group D (Table 2 and Fig. 4).

Fig. 3. Intestinal metaplasia was observed in the antral mucosa of experimental group C (PAS-alcian blue at pH 2.5, ×400).
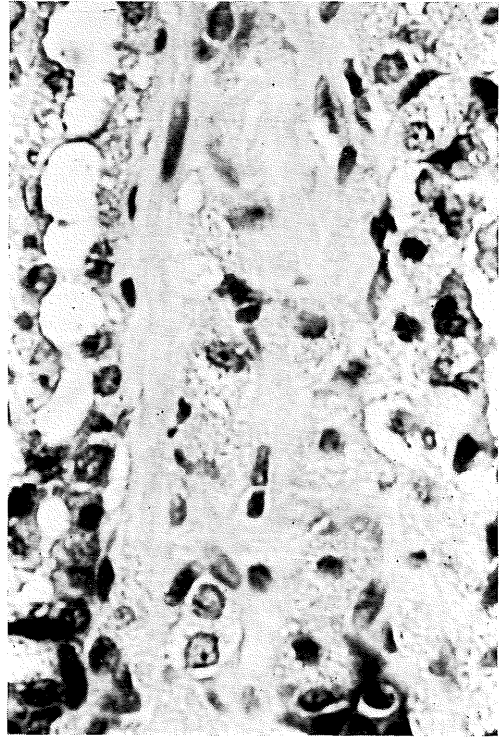

Fig. 4. There was a marked reduction of parietal cells in the body mucosa of experimental group B (Hematoxylin and eosin stain, ×400).

Table 2. Animal weight, mucosal pH, number of parietal cells, fasting levels of immunoreactive gastrin

Experimental group		Survived No	Weight (g)	Mucosal pH	Parietal cells (cm²)	Cancer (%)	Serum gastrin (pg/ml)	SDH activities	Fibrosis
(A)	With gastric cancer	1	470	5	57.5	10	148	(2+)	(1+)
	Without gastric cancer	9	483.3±68.9	4-5	61.1±5.1		63.4±28.9	(2+)	(1+)
(B)	With gastric cancer	6	370.2±26.8	8	38.0±9.8	60	168.5±25.6	(+)	(3+)
	Without gastric cancer	4	380.4±24.3	6	44.8±11.0		64.5±20.0	(1+)	(2+)
(C)	With gastric cancer	5	412.5±67.7	5-6	54.5±25.1	50	13.0±30.3	(1+)	(2+)
	Without gastric cancer	5	411.5±23.2	5-6	58.1±13.4		68.8±20.8	(1+)	(2+)
(D)	Control	5	473.3±20.8	2-3	96.0±15.3		61.0±14.6	(2+)	(−)

$*p < 0.05$ $**p < 0.01$

No difference was observed in the number of parietal cells between experimental animals with gastric cancer and without gastric cancer (Table 2). Mucosal pH examined by test papers ranged from 5 to 8 in animals of the experimental groups which is significantly higher than pH 2 to 3 observed in animals of the control group. These data are summarized in Table 2.

Fibrotic changes were generally prominent both in the antral mucosa and in the body mucosa of animals of groups B and C. These atrophic changes were most prominent in animals of group B.

(3) Immunoreactive gastrin (Table 2)

Immunoreactive gastrin values in the blood were significantly elevated in animals with gastric cancer of all experimental gpours (group A: 148, group B: 168.5±25.6, group C: 131.0±30.3, group D: 61.0±14.6 pg/ml). These values were not different among animals without gastric cancer of three groups (group A: 63.3±28.9, group B: 64.5±20.0, group C: 68.8±20.8 pg/ml).

DISCUSSION

It is well known that atrophic gastritis in man is associated with gastric cancer[2,3,10,24]. There is little doubt as to its predisposing role in gastric cancer. In order to as certain this hypothesis in this study, therefore, atrophic gastritis as well as gastric cancer were developed simultaneously in Donryu rats in which it is rather difficult to induce gastric tumors[22].

In the present study, a significant reduction in the number of parietal cells was noted in rats with group B (immunization) and C (taurocholate) when compared to the number of parietal cells in rats with group D. The changes in the gastric mucosae observed after immunization or oral administration of taurocholate were defined as atbophic gastritis (group B and C) since atrophic gastritis is essentially a result of reduction in the number of parietal and chief cells in the body stomach[21]. The incidence of gastric cancer was only 10% in rats with group A (MNNG alone) while the incidence was significantly higher in group B and C (atrophic gastritis with MNNG). This suggests that atrophic mucosa can enhance experimental carcinogenesis of MNNG, but the exact mechanism of this enhancement is unknown. One of possible mechanisms is that

the atrophic changes in the mucosa can be the possible precursors of malignant changes since cell turnover is more rapid[11] and rapid dividing cells are immatured and these may be prone to cancer induction in such mucosa[7].

A significant reduction in the number of parietal cells observed in group B and C implies decreased acid secretion since the parietal cell mass is congruous with gastric acid output[6]. Therefore, increased pH in gastric surroundings was found in this study. Mucosal pH of gastric surroundings is likely important for carcinogenic effect of MNNG, since in acid medium MNNG is converted to carcinogenic derivatives N-methyl-N'-nitrosoguanidine and nitrous acid[17]. Conversely, in alkaline solution MNNG is degraded to azoxymethane, a strong methylating agent, which is considered the carcinogenesis of MNNG, because azoxymethane methylates many nucleic acids[18]. However, the elevated pH in the gastric surroundings observed in this sudy was equally observed in all experimental animals regardless of induction of gastric cancer after 44 weeks of the experiment, suggesting that acid surroundings in the stomach is not necessarily essential for induction of gastric cancer with the exception of cases with hyperchloric stomach, in which gastric cancer is extremely rare. In fact, it seems likely that increased acid secretion may protect animals from the carcinogenesis of MNNG[4,7,27].

Gastrin exerts a trophic effect on the gastric mucosa as well as on gastric acid secretion[5,13,19,27]. Thus, it is of interest to observe whether gastrin enhances experimental carcinogenesis and growth of gastric tumors. Marked hypergastrinemia in the study was exclusively observed in animals bearing gastric cancer among the three experimental groups, but the gastrin values in animals without gastric cancer were not higher than those in animals of control group D. This suggests that endogenous hypergastrinemia has some effect on incidence or growth of experimental gastric cancer. Although this may explain the trophic action of gastrin on gastric cancer[25,26], the trophic action on gastric cancer has been questioned[1,4,7,27]. The contradictory data may in part on different experimental conditions including different animals used. In this study, endogenous hypergarinemia was caused by increased pH in the

antrum due to experimentally induced atrophic gastritis. Endogenous hyprgastrinemia is correlated with increased DNA synthsis and mitotic activities of cells[28] and further endogenous gastrin (G34 and G17) is several times more potent in stimulating DNA synthsis than exogenous gastrin (pentagastrin) on a molar basis[12]. It may be suspected that endogenously released gatrin in this study has a greater effect on induction or growth of experimental gastric cancer than exogenously administered gastrin which has been used in some experiments reported[4,27]. Thus, experimentally induced atrophic mucosa and its resulting endogenous hypergastrinemia observed in this study enhance experimental carcinogenesis of MNNG in Donryu rats.

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