Enhancement of Gastric Carcinogenesis in Dogs Given N-Methyl-N'-nitro-N-nitrosoguanidine following Vagotomy

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ABSTRACT

In an attempt to improve the method of induction of gastric cancer in dogs, selective vagotomy without drainage procedure was performed before administration of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). Twenty dogs were divided into two groups of 10 dogs (five beagles and five mongrels). The control group received MNNG alone. Dogs of the experimental group were vagotomized 2 months before administration of MNNG. MNNG solution (50 μg/ml) was administered in the drinking water for 12 months. In the vagotomized group, gastric adenocarcinomas were induced in all of eight effective cases, whereas in the control group they were seen in six of eight cases. Gastric cancer was found to be multiple in many dogs, but the majority of cancer lesions remained in the early stage. The total numbers of cancer lesions induced in the vagotomy and control group were 42 and 16, respectively. Vagotomy resulted in the production of more advanced cancers. One lesion in the control group and four lesions in the vagotomized dogs. Gastric acid secretion of the vagotomized dogs measured after termination of MNNG was reduced, on the average, to one-fourth of that of the control. The possible role of vagotomy in the enhancement of gastric carcinogenesis in dogs by MNNG is discussed in terms of the inactivation of MNNG in gastric juice and ulcerogenic action of the carcinogen to the gastric mucosa.

INTRODUCTION

The experimental induction of gastric cancer in dogs permits one to investigate the developmental process of gastric cancer by serial radiographic and endoscopic examination without sacrificing the animal.

A high incidence of adenocarcinomas in the stomach has been demonstrated in rats (15) and dogs (2, 4, 17) following p.o. administration of MNNG.3 However, dogs given MNNG in their drinking water developed leiomyosarcomas in the small intestine (9) while the gastric cancers remained in the early stage without metastasis at the time when the animals were sacrificed because of the intestinal sarcomas (4, 14, 17). In view of this difficulty, attempts have been made to improve the methods for inducing more advanced carcinomas with metastases (3, 10), such as the use of vagotomy to enhance gastric carcinogenesis in rats (8, 11).

The present study is designed to evaluate whether selective vagotomy performed before p.o. administration of MNNG will enhance the production of gastric cancers in dogs.

MATERIALS AND METHODS

Animals and Operation. Twenty dogs weighing 6 to 11 kg were used in this experiment. Ten male beagle dogs and 10 mongrel dogs (7 males and 3 females), 10 months old, were divided into 2 groups, each consisting of 5 beagles and 5 mongrels. The animals were housed in individual cages and given about 300 g of dog chow (No. 1: CLEA, Japan) every morning except Sunday. In one group, selective vagotomy was performed under i.v. anesthesia with sodium pentobarbiturate. After an upper median incision, the vagus nerves in the lesser omentum from the esophagus to the antrum were cut. The nerves around the esophagus and to the extragastric organs were preserved (13). No gastric drainage operation was carried out. No operative procedure was done in the other group, which served as controls.

Carcinogen. At 12 months of age, all the dogs were given an aqueous solution of MNNG (50 μg/ml; Aldrich Chemical Co., Milwaukee, Wis.) ad libitum for 12 months and then returned to tap water. Stock solution of MNNG dissolved in deionized water at a concentration of 1 mg/ml was diluted with tap water just before use. The amount of MNNG solution consumed was determined twice a week.

Morphological Observations. Routine endoscopic examination was performed under i.v. anesthesia every 3 months. The time the first tumor was observed in each dog was recorded.

All animals were autopsied, and entire organs were grossly examined when the animals died or were killed when moribund. The vagus nerves in the experimental group were examined to ascertain that vagotomy was done properly. The stomach was opened along the greater curvature and was step sectioned into 5-mm widths for the recognition of geographic relationship of each lesion (Fig. 1). Other organs which showed grossly abnormal findings were also sectioned. Sections were routinely stained with hematoxylin and eosin. The Alcian blue-periodic acid-Schiff method was also used to stain selected sections.

Assay of Gastric Acid Secretion. The estimation of gastric acid secretion was performed 3 to 6 months after the termination of MNNG administration. After the dogs had been fasted for 36 hr, stomach tubes were inserted into their stomachs under i.v. anesthesia with sodium pentobarbiturate. Gastric juice in 15-min aliquots was collected for

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2 To whom requests for reprints should be addressed.
3 The abbreviations used are: MNNG, N-methyl-N'-nitro-N-nitrosoguanidine; MAO, maximal acid output.

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30 min before and 90 min after s.c. injection of tetragastrin at 2 µg/kg body weight. Each aliquot was titrated to pH 7.0 with 0.1 N NaOH. The peak acid concentration and MAO 1 hr after injection were then calculated.

RESULTS

Gastric Acid Secretion. The peak acid concentration was 76 ± 17 mEq/liter (mean ± S.E.) in the vagotomized dogs while it was 128 ± 6 mEq/liter in the control dogs (p < 0.025). The volume of gastric juice in the vagotomized dogs was reduced to about one-half of that in the control dogs (19 ± 3 ml versus 42 ± 6 ml, p < 0.01). MAO in the vagotomized dogs was 1.1 ± 0.2 mEq/hr versus 4.3 ± 0.7 mEq/hr in the control dogs (p < 0.01), which represents a reduction of gastric acid secretion by approximately 75% in the vagotomized dogs, as compared to that in the control dogs.

MNNG Intake and Tumor Incidence. Dogs that survived 12 months or more were judged as effective cases because the first case of gastric cancer was noted in a dog that died accidentally from anesthesia for endoscopic examination 12 months after the beginning of MNNG administration. Comparison of body weight, MNNG intake, latent period, survival, stomach cancer incidence, and metastasis in the control and vagotomy groups is presented in Table 1. The average body weight gain during the experimental period in the 2 groups of dogs was similar. Cumulative MNNG intake was not different. Of the 8 control dogs at risk, 6 developed gastric cancer, whereas all the dogs in the vagotomy group had cancer. Endoscopic observation also indicated that stomach cancer appeared earlier (latent period) in the vagotomy group than in the controls.

Gastric Cancer. Multiple epithelial lesions which showed various degrees of atypia were found in almost all of the stomachs examined. In our previous report (4), these lesions were classified histologically into 5 grades ranging from A (minimal atypia) to E (severe atypia) according to the degree of departure from the normal structure. The same criteria were used in the present study, especially Grade E and D lesions, which were considered to be carcinomatous. Grade E lesion refers to typical adenocarcinoma, which consists of neoplastic glands showing marked cellular and structural atypism with clear evidence.
of invasion regardless of the depth of invasion. The lesion which is composed of the same nature of neoplastic glands as Grade E lesion, but which is confined to the mucosa without definitive evidence of invasion, has been listed as Grade D.

Multiple gastric cancers (Grade D + E), ranging from 2 to 10 lesions, were found in all of the dogs of the vagotomy group except one. On the other hand, gastric cancer was seen in 6 of 8 dogs of the control group. Four of the 6 dogs had multiple (2 to 5) cancers. The vagotomy group had 20 Grade E invasive cancers, whereas the control group had 8. The total number of grade D cancers which were preinvasive reached 22 in the vagotomy group, but there were only 8 found in the control group. The average number of cancer lesions in the vagotomy group and the control group was 5.3 ± 1.2 and 2.0 ± 0.7, respectively.

Gross Features. In both groups, the majority of the gastric cancers were found in areas where atrophy of the gastric mucosa was frequently seen (the anterior wall of the upper body of the stomach and the gastric angle along the lesser curvature). These gastric cancers in dogs showed a variety of sizes, gross appearances, and histological types. As shown in Table 2, the gastric cancers ranged in size from microscopic foci under 2 mm to large lesions over 60 mm in diameter. In the control group, all of the 16 lesions found were within 20 mm in diameter, and only 3 lesions were between 11 and 20 mm. On the contrary, 9 of 42 lesions in the vagotomy group were over 11 mm, and 4 of them were more than 21 mm in diameter.

Macroscopic features of gastric cancers obtained in this experiment showed that only 4 of the cancers found were applicable to Borrmann classification for human advanced cancer, but the rest of the cancers had macroscopic features fairly similar to those of human early gastric cancers. Therefore, these cancers were classified by the system established for the classification of early gastric cancer at the Japan Gastroenterological Endoscopic Society (11). Table 3 shows the frequency of appearance of the different macroscopic classes of gastric cancer in the 2 groups. Although the superficial type II was commonly prevalent in both groups, the relative incidence of the various subtypes showed some differences. More type IIa elevated cancers were observed in the control group, whereas incidence of type IIb depressed cancers was higher in the vagotomy group. The Borrmann type of advanced cancers was found exclusively in 4 lesions of the vagotomy group.

Histological Types. The distribution of the cancers by histological types in both groups, together with the degree of penetration, is given in Table 4. The cancers were classified by the predominant histological type. Every type of gastric cancer except mucoid carcinoma was found in both groups. The majority of lesions in both groups were highly or moderately differentiated adenocarcinomas with a papillary or tubular pattern. Signet ring cell cancers in which nuclei completely lost their polarity and possessed an abundant amount of mucus were found in one of the controls and 2 of the vagotomy group. In both groups, poorly or moderately differentiated adenocarcinomas showed a more conspicuous tendency to penetrate the stomach wall than did well-differentiated adenocarcinoma or signet-ring cell cancer. One lesion of advanced gastric cancer with moderate differentiation in which the invasion of cancer cell nests reached the serosa was found in the control group, whereas 4 lesions of this type were seen in the vagotomy group, 2 of them moderately differentiated adenocarcinomas, one well differentiated, and one poorly differentiated. Carcinomas with vessel invasions (Fig. 2) were revealed in 7 lesions, 1 in the control group and 6 in the vagotomy group.

The cancer lesions were without exception surrounded by mucosa which showed marked atrophy or loss of chief cell and parietal cell. Intestinal metaplasia was not observed. Vagotomy in dogs had not been shown to affect significantly the histological structure of normal gastric mucosa.

Metastases. Furthermore, distant metastases of advanced gastric cancers were revealed in 2 of the vagotomized dogs. One of these cases was a male beagle dog (Dog 59) that was killed on Day 967 because of heavy weight loss and weakness. In the stomach, a Borrmann type II poorly differentiated adenocarcinoma (2.5 x 2.5 cm) was present on the anterior wall of the upper body, in addition to multiple lesions of superficial type gastric cancers. Metastases of the advanced cancer to the liver and lungs were found in addition to the perigastric lymph nodes metastases. The details were described in our recent report (3). It was considered to be the first report of hematogenic metastases of chemically induced gastric cancer.

The other advanced cancer with metastases was seen in a female mongrel dog (Dog 42) that was killed when moribund on Day 1163. At autopsy, 3 Borrmann type III tumors with deep craters and a Borrmann type I tumor were seen on the fundic gland area of the stomach (Fig. 1). There

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**Table 2**

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<thead>
<tr>
<th>Distribution of dog gastric cancers according to diameter</th>
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<tbody>
<tr>
<td>No. of lesions with diameters of</td>
</tr>
<tr>
<td>≤ 2 mm</td>
</tr>
<tr>
<td>Control (16)</td>
</tr>
<tr>
<td>Vagotomy (42)</td>
</tr>
</tbody>
</table>

**Note:**
- Even the lesions with the largest diameters were measured in millimeters.
- Number in parentheses, total number of lesions.

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**Table 3**

<table>
<thead>
<tr>
<th>Macroscopic appearances of dog gastric cancers</th>
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<tr>
<td>Classification systems for early gastric cancer established by the Japanese Gastroenterological Endoscopic Society (12) and for advanced cancer by Borrmann were used.</td>
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</table>

<table>
<thead>
<tr>
<th>Advanced Borrmann (types II and III)</th>
<th>Early cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (polypoid)</td>
<td>IIa (elevated)</td>
</tr>
<tr>
<td>Control (16)</td>
<td>0</td>
</tr>
<tr>
<td>Vagotomy (42)</td>
<td>3</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Type</th>
<th>Number in parentheses, total number of lesions.</th>
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<tbody>
<tr>
<td>IIa + IIb</td>
<td>17</td>
</tr>
<tr>
<td>IIa + IIb + III</td>
<td>14</td>
</tr>
</tbody>
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**Note:**
- Combination types of IIa + IIb and IIb + III are included in type IIa.
- Number in parentheses, total number of lesions.
also were a polypoid lesion and 3 tiny lesions with central depression resembling superficial II, depressed-type early gastric cancer in humans. Grossly multiple metastatic foci were seen in the liver and perigastric lymph nodes. The most advanced carcinoma, which was composed of moderately differentiated tubular glands and situated on the posterior wall near the cardia, revealed invasion to the pancreas tissue and metastases to perigastric lymph nodes. Microscopic findings confirmed that the liver metastases consisted of tubular structures which were similar to those of the advanced cancer (Figs. 3 and 4).

**Sarcoma.** Sarcomas of the small intestine were induced in 4 dogs in each group. In the vagotomy group, sarcomas of the stomach were also seen. Histologically, these lesions were leiomyosarcomas composed of interlacing bundles of spindle cells. The intestinal sarcomas frequently caused death from obstruction and bloody diarrhea.

**DISCUSSION**

Selective vagotomy performed in dogs before the p.o. administration of MNNG solution resulted in the production of more advanced gastric cancers with distant metastases, as well as an increase in the number of gastric cancers compared to that of the controls. The results in the control showed sufficient similarity to our previous study (4) in which the early stage of the gastric cancer was found in 2 of 6 dogs given MNNG (60 μg/ml). Shimosato et al. (14) also reported that 4 dogs given MNNG developed adenocarcinomas with no metastasis. In these experiments and another report (9), sarcomas, in addition to gastric adenocarcinomas, were frequently found in the gastrointestinal tract. These sarcomas induced simultaneously by MNNG seemed to interrupt further development of the gastric cancer.

In the present study, selective vagotomy without drainage procedure was performed mainly with 2 aims: (a) to reduce the gastric acid secretion with resulting retardation of MNNG inactivation in the stomach to increase the gastric cancer production; and (b) to reduce the transportation of active MNNG into the duodenum to result possibly in an increase of tumor incidence in the stomach and a decrease of the intestinal tumor production. Eventually, gastric acid secretion expressed by MAO after tetragastrin injection was reduced by approximately 75% in the vagotomized dogs. It is known that the inactivation of MNNG occurs rapidly under the strong acidity of the normal stomach (16). The significant reduction of the gastric acid secretion induced by selective vagotomy is conceivably the main cause of the enhancement of the gastric cancer production by MNNG. While this type of vagotomy was suggested to be associated with delay of gastric emptying, the procedure did not reduce the induction of the intestinal sarcoma in dogs.

There have been some reports which attempted to evaluate the relationship between vagotomy and experimental gastric cancer in rats. Kowalewsky (8) indicated that vagotomy contributed to the development of gastric adenocarcinoma in rats fed 2,7-diacetylaminofluorene. Morgenstern (11) reported that gastrojejunostomy combined with vagotomy enhanced the methylcholanthrene-induced gastric cancer in rats. These investigators have speculated that the effect of vagotomy on carcinogenesis may be correlated chiefly with artificially induced hypochlorhydia.

In the present study, atrophic changes were observed in the mucosa surrounding the gastric cancers in both the control and the vagotomy group. It has been demonstrated that MNNG acts not only as a strong carcinogen but also as an ulcerogenic agent to the gastric mucosa. Our recent study (18) revealed that the atrophic area where gastric cancers were frequently induced was closely related to the particular region of the stomach where diffuse erosive changes or shallow ulcers had been observed by endoscopy during MNNG administration. The ulcerogenic action of MNNG will influence the gastric carcinogenesis in 2 different manners. It is possible that ulceration may provide more cells which will be susceptible to the carcinogen, resulting in an increase of the number of transformed cells in the regenerating epithelium. On the contrary, it may decrease the cancer formation by sloughing off the mucosal cells previously transformed by MNNG. The strong acid in the gastric juice may also act as a promoting factor influencing the ulcerogenic activity of MNNG. It was found in the present study that, in addition to the formation of more advanced gastric cancers, a significant increase in the number of minute cancer foci was seen in the vagotomized dogs in which gastric acid secretion was considerably suppressed. The present results suggest that the decrease of gastric acidity in the vagotomized dogs has resulted in certain reduction of sloughing off of the mucosal cells transformed during administration of MNNG. This factor seems to be another reason why vagotomy has enhanced gastric carcinogenesis in dogs given MNNG.

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**Table 4**

<table>
<thead>
<tr>
<th>Highly differentiated adenocarcinoma</th>
<th>Moderately differentiated adenocarcinoma</th>
<th>Poorly differentiated adenocarcinoma</th>
<th>Signet ring cell cancer</th>
</tr>
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<tbody>
<tr>
<td>Control (16)</td>
<td>5 m</td>
<td>1 sm</td>
<td>0 s</td>
</tr>
<tr>
<td>Vagotomy (42)</td>
<td>18 m</td>
<td>5 sm</td>
<td>1 s</td>
</tr>
</tbody>
</table>

Note: The abbreviations used are: m, mucosa; sm, submucosa; s, serosa.

* Number in parentheses, total number of lesions.
Recently, long-term intake of carcinogen or procarci-
gen present in food has been considered to be responsible
for gastric cancer in man. It is also well-known that chronic
atrophic gastritis accompanying achlorhydria is associated
with gastric cancer (6), but the etiological correlation be-
tween these conditions remains unclear. On the other hand,
it is recognized that gastric cancer is seldom found in the
duodenal ulcer patients with hyperacidity. The results of
the present study indicate that strong acidity in the stomach
could be one of the factors which reduces the incidence of
gastric cancer. Some clinical reports (1, 5) imply that a
causal relationship exists between vagotomy for benign
diseases and gastric cancer. The present study suggests
that patients undergoing vagotomy without gastric resec-
tion should be carefully followed up to evaluate their risk
for gastric cancer.

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Fig. 1. Mucosal surface of the stomach of Dog 42, which had multiple tumors. Huge Borrmann type III tumors with deep ulcer can be seen in the posterior wall and the anterior wall near cardia, as well as a Borrmann type I tumor and several small lesions with slight elevation or depression. The whole stomach was step sectioned into sections about 5 mm wide along the lesser curvature. Metastatic foci were found in perigastric lymph nodes and the liver.

Fig. 2. Section of poorly differentiated adenocarcinoma with vessel invasion (Dog 57). Mucosal lymphatics contain clusters of anaplastic cells. H & E, × 115.

Fig. 3. Section of metastatic carcinoma in the liver of the dog in Fig. 1. Arrows, metastatic foci. H & E, × 7.

Fig. 4. Higher magnification of metastatic carcinoma shown in Fig. 3 (moderately differentiated adenocarcinoma). H & E, × 100.
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