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Gastric Tumorigenicity of 1,2-Dimethylhydrazine on the Background of Gastric Intestinal Metaplasia Induced by X-Irradiation in CD (SD) Rats

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Five-week-old male CD (SD) rats were X-irradiated with a total of 20 Gy in 2 equal fractions with a 3-day interval. After the second irradiation, rats were fed normal diet supplemented with 1% sodium chloride, which is known to increase intestinal metaplasia. 1,2-Dimethylhydrazine (DMH) solution was injected i.m. into the back musculature at a dose of 20 mg/kg body weight weekly for 10 weeks, beginning 20 weeks after the final irradiation. Twelve months after the initial carcinogen treatment, gastric tumors in the glandular stomach were observed in 2 (3 lesions) of 30 animals in the X-irradiated and DMH-treated group fed diet supplemented with 1% sodium chloride. No gastric tumors were observed in the group which excluded X-irradiation from the experimental protocol.

Key words: Intestinal metaplasia — Gastric tumor — DMH — X-ray — Rat

Based on investigations in humans, intestinal metaplastic changes in the stomach have been considered as precancerous lesions or a basis for differentiated gastric carcinoma development.[1-7] We have reported an inverse relationship between the number of intestinal metaplasias with Paneth cells or without Paneth cells and the number of gastric tumors and we suggested that the presence of intestinal metaplasia does not exert a positive influence on induction of gastric neoplasia by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG)[8] or *N*-methylnitrosourea (MNU)[9] in rats. Nakagawa *et al.*[10] indicated that colorectal mucosa implanted into the glandular stomach, like the intrinsic large intestine, is sensitive to tumorigenesis caused by 1,2-dimethylhydrazine (DMH), whereas the normal gastric mucosa is not susceptible. The present study was designed to examine further whether intestinal metaplasia might be a sensitive site for DMH-induction of malignant tumors in the glandular stomach.

MATERIALS AND METHODS

Male 5-week-old Crj:CD (SD) rats were X-irradiated according to the method described previously,[11,12] with two X-ray doses of 10 Gy each at a three-day interval (total dose 20 Gy). After the second irradiation, rats were fed normal diet supplemented with 1% sodium chloride (NaCl), which is known to increase intestinal metaplasia,[12] and were provided with tap water *ad libitum*. DMH (Nacalai Tesque Inc., Kyoto) was injected i.m. into the back at a dose of 20 mg/kg body weight once weekly for 10 weeks beginning 20 weeks after the final irradiation of animals fed normal diet supplemented with 1% NaCl (group 1). In group 2, rats received DMH after sham irradiation and the 1% NaCl supplement diet. Animals were killed and autopsied when they became moribund and all remaining rats were killed 12 months after the initial DMH treatment. The stomach, and the small and large intestinal tracts were removed, opened and extended on cardboard for inspection. The location of individual tumors was recorded by measuring the distance from the pyloric ring in the small intestine and from the anus in the large intestine. The numbers and sizes of individual tumors were also noted. Whole tissues were fixed in 10% neutral formalin. Alkaline phosphatase (ALP)-positive foci in the gastric mucosa were detected by the naphthol-AS-MX-phosphate-fast blue RR staining method[13] and the numbers of ALP-positive foci in the whole gastric mucosa per rat were counted using a dissection microscope under a double-blind protocol. Sections of paraffin-embedded tissue were routinely stained with hematoxylin and eosin, and for clarification, when necessary, periodic acid Schiff-Alcian-blue (AB-PAS) staining of sialomucin (AB-positive) and sulfomucin [high-iron diamine (HID)-positive] was performed. In addition, tissue sections were subjected to paradoxical Con A, galactose oxidase-Schiff (GOS), anti-small intestinal alkaline phosphatase, and anti-pepsinogen antibody staining.[14,15] Intestinal meta-

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plasias were categorized using the following histological criteria[11, 12]: type A, gastric mucosa with goblet cells which were positive for AB-PAS and HID; type B, intestinal-type crypts without Paneth cells; type C, intestinal metaplasia with Paneth cells (alkaline phosphatase-positive foci). Using these criteria, the numbers of metaplastic crypts were counted separately for 2 sections through the lesser curvature (pylorus) and 4 through the greater curvature (fundus) in a double-blind fashion.

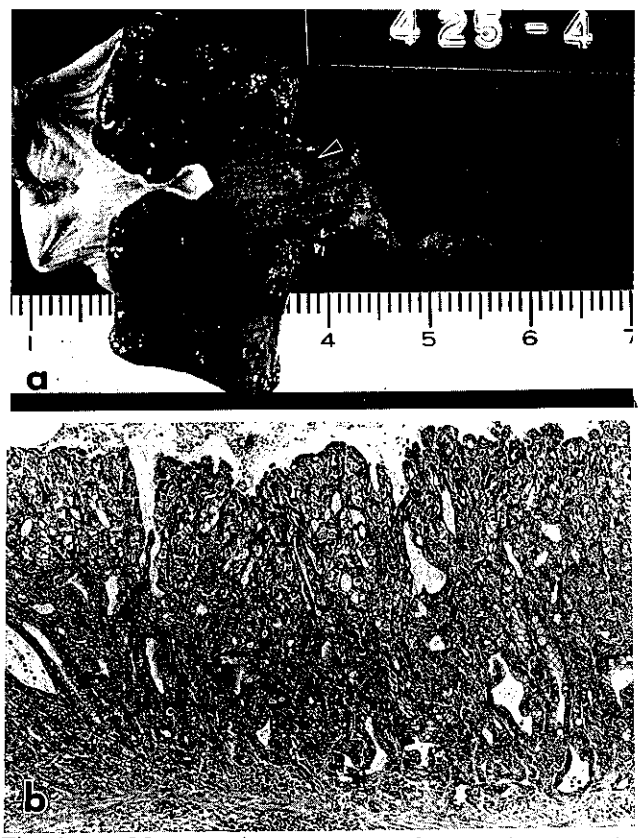

Fig. 1. a. Macroscopic appearance of a gastric tumor induced by DMH (◀). b. Microscopic details.

Tumors in the stomach, small intestine and large intestine were classified into well-differentiated and poorly differentiated types, the latter including both mucinous and signet ring cell forms.

The significance of differences in numerical data was evaluated by use of the chi-squared test and Student's *t* test.

RESULTS

Gastric tumors in the glandular stomach were observed in 2 of 30 (7%) animals in group 1 (one had 2 lesions), the rats being autopsied at 254 days and at 321 days after the beginning of DMH treatment. All tumors in the glandular stomach were well-differentiated, without goblet cells or mucin (Fig. 1, a and b). The tumors were located in the middle or upper portion and did not react with any anti-alkaline phosphatase of intestinal type or anti-pepsinogen I by immunohistochemistry, nor did they stain with paradoxical Con A or GOS, whereas positive staining was seen with paradoxical Con A or anti-pepsinogen in surrounding gastric mucosa and with anti-alkaline phosphatase in intestinal metaplasia or small intestine. No gastric tumors were observed in group 2 (Table I). The incidence of large intestinal tumors was 70% in both groups 1 and 2. The number of large intestinal tumors per rat was significantly higher in group 2 (2.32 ± 1.14) than in group 1 (1.48 ± 0.93) and mean tumor size was also larger in group 2. The tumors were multiple and of papillary and polypoid types, with a few adenomas. Signet ring cell carcinomas were more frequent (39%) in group 1 than in group 2 (19%) (Table II). Small intestinal tumors in group 1 (30%) were reduced as compared to group 2 (52%). ALP-positive-foci and type B metaplasia in the stomach were more frequently found in group 1 than in group 2 (Table III).

DISCUSSION

In the present experiment, induction of intestinal metaplastic mucosa in the glandular stomach was associated

Table I. Incidences of Tumors (%)

Group	Effective number of animals	Gastric tumors	Large intestinal tumors			Small intestinal tumors	Other tumors
			Incidence	No. of tumor/rats	Mean tumor size (mm)		
1	30	2 (7)[a]	21 (70)	1.48 ± 0.93**	4.99 ± 4.00	9 (30)	9 (30)[b], *
2	23	0	16 (70)	2.32 ± 1.14**	7.00 ± 6.35	12 (52)	1 (4)[c], *

a) One animal had 2 lesions.
b) Two sarcomas, 1 leukemia, 1 squamous cell carcinoma, 1 pancreatic tumor, 1 fibroadenoma, and 1 mammary tumor.
c) One seminoma.
Significantly different: * $P<0.05$, ** $P<0.01$.

Table II. Number of Different Large Intestinal Tumor Types

Group	Total number of tumors	Well-differentiated	Mucinous type	Signet ring cell
1	31	17 (55)[a]	6 (2)	12 (39)
2	37	27 (73)	3 (8)	7 (19)

a) Percentage of total in parentheses.

Table III. Frequency of Intestinal Metaplasia

Group	Effective number of animals	ALP-positive foci[a]	Intestinal metaplasia[b]		
			Type A	Type B	Type C
1	26	13.5±47.2	0.27±0.53	4.08±4.64	0.04±0.20
2	16	0.10±0.44	0	0	0

a) ALP-positive foci, intestinal metaplastic foci with alkaline phosphatase activity.
b) Type A, gastric mucosa with goblet cells; Type B, intestinal-type crypt without Paneth cells; Type C, intestinal-type crypt with Paneth cells.

with a susceptibility to tumorigenesis induced by DMH, in contrast to the non-susceptible normal gastric mucosa. Intestinal metaplasia in humans is considered as providing a region of incipient well differentiated gastric adenocarcinoma.[4-6] Early-stage small or large intestinal tumors are known to exhibit ductal structures lacking goblet cells or mucin, and large intestinal tumors tend to appear at the top of crypts near the lumen side.[16, 17] In the present experiment, gastric tumors also developed in the upper and middle parts of crypts, showing a similarity to intestinal tumorigenesis. Thus, gastric tumorigenicity on the background of gastric intestinal metaplasia induced by X-irradiation was demonstrated in the present experiment.

Azoxymethane and methylazoxymethanol, metabolites of DMH, are carcinogenic in both the large intestine and the small intestine, especially in the duodenum.[18, 19] Campbell *et al.* reported that these carcinogens could reach the target tissues by a route other than the fecal stream[20] and Zedeck *et al.* found that the proximate carcinogen form of the metabolized chemical reaches the mucosa mainly through the vascular system.[21] Matsubara *et al.* also indicated that carcinogens probably act on intestinal mucosa via the vascular system, as well as through biliary transport.[22] These findings indicate that the intestinal mucosal stem cell(s) is susceptible to DMH carcinogenesis independently of the luminal pathway, though the luminal pathway is still a possibility. We earlier reported that adenocarcinomas develop in successful implants of large intestinal mucosa in the glandular stomach of animals treated with DMH,[9] but not MNNG.[23] These findings further indicate that the intestinal mucosal target cell is the important determinant of interaction of DMH with the intestine, and not the intestinal environment itself. It is suggested that carcinogens can reach intestinal tissue within the glandular stomach via the vascular system. The results thus indicate that the target cells in intestinal metaplastic mucosa are sensitive to tumorigenesis by DMH, in contrast to their counterparts in normal gastric mucosa.

In conclusion, the presence of intestinal metaplasia may increase sensitivity to the induction of gastric tumors by carcinogens of the DMH type, but not the MNNG or MNU type. This route, however, is relatively minor compared to the main route of gastric carcinogenesis by carcinogens of the MNNG or MNU type acting on normal glandular mucosa in the stomach. Nevertheless, the protocol used in the present experiment may provide a new approach to distinguish between developmental events associated with intestinal metaplasia and gastric tumors. Further investigations are required to explain the number, size and type of intestinal tumors in the X-irradiated and DMH-treated group.

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