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Influence of Gastric pH Modifiers on Development of Intestinal Metaplasia Induced by X-Irradiation in Rats

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The influence of gastric pH on intestinal metaplasia was examined in male Crj:CD(SD) rats. At the age of 5 weeks, animals were irradiated with two 10 Gy doses of X-rays to the gastric region at a 3-day interval (total 20 Gy), and 6 months after irradiation, received either secretin or histamine in silicon tubes for 2 months or had their bilateral submandibular salivary glands removed. The incidences of intestinal metaplasia in the fundus of animals after administration of secretin or histamine, or removal of the salivary glands were reduced, along with the pH values, as compared with values for rats given X-rays alone. In both the pyloric and the fundic gland mucosae, the numbers of alkaline phosphatase (ALP)-positive foci and type B metaplasias (intestinal crypts without Paneth cells) were also significantly decreased ($P<0.01$). In a second experiment, started six months after irradiation, rats were kept on 1% sodium chloride (NaCl) diet for 6 months. Subsequent removal of salivary glands along with histamine treatment brought about a marked drop in pH and in numbers of ALP-positive foci after three and five days. The present results thus indicated that development and maintenance of intestinal metaplasia can be influenced by a decrease of pH value.

Key words: Intestinal metaplasia — Rat — X-ray — Gastric pH

Intestinal-type mucosa, when it occurs in adult gastric mucosa, usually comprises highly differentiated epithelium resembling that of the small intestine.[1-6] It has been well investigated in man, and a number of authors consider such metaplastic changes as precancerous lesions or a base for differentiated gastric carcinoma development. However, the pathogenesis of intestinal metaplasia has not yet been clarified, although it can be experimentally induced by chemical carcinogens,[7-11] X-irradiation,[12-16] chemical carcinogens plus X-irradiation[17-19] or stomach antigens.[20] Previously we proposed that an elevation of gastric juice pH due to the disappearance of parietal cells in the fundic gland mucosa is one of the principal factors responsible for the development of intestinal metaplasia.[13, 15, 21] We suggested that elevation of pH in the gastric mucosa by pyloroplasty or pyloroplasty plus vagotomy plays a significant role in the subsequent development of intestinal metaplasia in the stomach.[21] In addition, the occurrence of intestinal metaplasia was found to be significantly increased by treatment with ranitidine,[15] which is a potent inhibitor of gastric acid production,[22-26] and decreased by cysteamine,[15] which increases gastric acid secretion.[27-29] In order to confirm our hypothesis, we therefore investigated the effects of various gastric pH modifiers on development and maintenance of intestinal metaplasias induced by X-irradiation.

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MATERIALS AND METHODS

Animals Male Crj:CD(SD) rats (Charles River Japan Inc., Hino), five weeks old at the commencement, were used in the present study. They were housed three or four per cage in polycarbonate cages and were kept under constant conditions of temperature (24 ± 2°C) and relative humidity (55 ± 10%) with a 12 h light/12 h dark cycle. Animals were maintained under the guidelines set forth in the "Guide for the Care and Use of Laboratory Animals" established by Hiroshima University.

X-Irradiation Rats were anesthetized with Nembutal and X-irradiated according to the method described previously.[15, 16] A 0.6-cm-thick lead cover, with a hole 1.8 cm in diameter, was positioned so that the hole lay over the gastric region of the rats. All animals were given two X-ray doses of 10 Gy each, with a three-day interval (total dose 20 Gy). Exposure factors were as follows: 200 kVp, filter 0.5 Cu + 1.0 Al, half-value layer 1.18 mm Cu, at a dose rate of 90 R/min as measured with a Radocon 555 dosimeter. The X-ray air dose (in R) was then converted to the absorbed dose (in cGy) using a factor of 0.95 cGy/R.

Experimental design

Experiment 1 One hundred and eleven rats divided into six groups. Rats of Groups 1 and 2 were respectively killed 6 and 8 months after the X-irradiation. Rats of Groups 3 and 4 received secretin (courtesy of Eisai Co., Tokyo) and histamine (Sigma), respectively, in silicon

capsules implanted into the back fat pads for 2 months, starting 6 months after the X-irradiation. The capsules were replaced after 1 month. In Group 5 the bilateral submandibular salivary glands were removed. Group 6 animals were kept as untreated controls maintained on a normal diet and tap water for 8 months. All animals had free access to food and water.

Experiment 2 Six months after irradiation the rats were given 1% sodium chloride (NaCl)-supplemented Oriental MF (Oriental Yeast Co. Ltd., Tokyo) diet for 6 months. At one year after the irradiation, the salivary glands were removed and histamine capsules were implanted. At this time and 3, 5 or 15 days thereafter groups of 4 animals were autopsied.

Examination of animals All animals were regularly observed and killed under ether anesthesia at the termination of the experiments. The stomachs were cut open along the greater curvature, stretched out and pinned on boards with the mucosal surface facing upward. After determination of the pH value of the gastric mucosa with a pH meter, each stomach was washed several times with physiological saline before gross examination, and 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.[30] The numbers of ALP-positive foci in the whole gastric mucosa per rat were counted by using a dissecting microscope employing a double-blind protocol.

Strips of the stomach were cut perpendicularly to the mucosal surface, two strips being taken through the lesser curvature and four through the greater curvature. The strips were embedded in paraffin and serially sectioned at 3 μm. Sections were routinely stained with hematoxylin and eosin, and for clarification when necessary, the periodic acid Schiff (PAS)-alcian blue and high-iron diamine (HID)-alcian blue (AB) staining procedures were introduced.

Intestinal metaplasia was categorized using the following histological criteria[15, 16]: type A, gastric mucosa with goblet cells which were positive for alcian-blue PAS and HID; type B, intestinal-type crypts without Paneth cells; type C, intestinal-type crypts with Paneth cells. Using these criteria, the numbers of metaplastic crypts were counted separately for both the 2 lesser curvatures (pylorus) and the 4 greater curvatures (fundus) in a double-blind fashion. Metaplastic crypts within 5 crypts from the pyloric ring were not scored. Gastric neoplastic lesions in the glandular stomach on the same slides were classified into atypical hyperplasia and adenocarcinoma categories.[19]

Statistical analysis The significance of differences in numerical data was evaluated by using the chi-squared test, or Student's t test and by fitting of the calibration line using a linear equation.

RESULTS

Experiment 1 Table I summarizes data on pH values of gastric mucosae along with incidences of X-irradiation-induced intestinal metaplasia and ALP-positive foci. The fundic pH value in Groups 3 to 5 tended to be lower than that in Group 2 (receiving X-rays alone), but there were no significant differences between individual groups. In Groups 4 and 5, erosion in the glandular stomach was noted in 9 of 15 animals, and 5 of 20, respectively.

ALP-positive foci were frequently found, especially in the pylorus of irradiated groups. In Groups 4 and 5, their incidences were lower than in Group 2 ($P<0.01$)

Table I. pH Values and Incidences of Intestinal Metaplasia and ALP-positive Foci Induced by X-Irradiation in CD Male Rats

Group	Treatment	Effective No. of animals	Pylorus		Fundus		Pylorus+Fundus (%)				
			pH (mean±SD)	Metaplasia (%)	pH (mean±SD)	Metaplasia (%)	ALP (%)[a]	A[b]	B[c]	C[d]	A+B+C
1	X-ray (6 mo)	40	5.5±1.5	27 (77)	3.6±0.7	8 (23)	(85)	14 (40)	35 (88)	12 (30)	35 (88)
2	X-ray (8 mo)	20	4.2±1.6	21 (81)	5.4±0.3	12 (46)	(83)	13 (50)	19 (95)	11 (55)	20 (100)
3	X-ray+ Secretin	16	4.9±1.0	15 (93)	4.8±1.2	1 (6)[e]	(82)	9 (56)	15 (94)	10 (38)	16 (100)
4	X-ray+ Histamine	15	3.9±1.4	14 (93)	3.1±0.9	5 (33)	(30)[e]	11 (73)	14 (93)	4 (27)	14 (93)
5	X-ray+ Removal of salivary glands	20	4.0±1.4	19 (95)	3.5±1.2	0[e]	(20)[e]	9 (45)	18 (90)	8 (40)	19 (95)
6	Untreated	21	3.5±0.9	1 (5)	4.0±1.2	0	(0)	0	1 (5)	0	1 (5)

a) ALP: Counted under a dissecting microscope.
b) A: Gastric mucosa with goblet cells.
c) B: Intestinal-type crypts without Paneth cells.
d) C: Intestinal-type crypts with Paneth cells.
e) Significantly different from group 2 ($P<0.05$).

Table II. Numbers of Metaplastic Foci Induced by X-Irradiation (mean±SE)

Group	Treatment	Pylorus			
		A	B	C	Total
1	X-ray (6 mo)	0.8±0.3	4.2±0.9	0.3±0.1	5.3±1.0
2	X-ray (8 mo)	0.5±0.2	6.4±1.3	0.4±0.1	7.0±1.3
3	X-ray+Secretine	1.1±0.3	3.9±0.5[a]	0.4±0.2	5.4±0.8
4	X-ray+Histamine	1.7±0.5	3.4±0.7[a]	0.5±0.3	5.7±0.9
5	X-ray+Removal of salivary glands	1.2±0.4	4.8±0.8	1.0±0.4	6.9±1.3
6	Untreated	0	0.05±0.04	0	0.05±0.04

Group	Fundus			
	A	B	C	Total
1	0.3±0.2	0.9±0.6	0.1±0.1	1.2±0.9
2	0.5±0.2	1.4±0.5	0.4±0.2	2.1±0.8
3	0	0.4±0.1[b]	0.2±0.2	0.6±0.6[b]
4	0.7±0.2	0.3±0.2[a]	0.1±0.1	0.6±0.3[a]
5	0	0[a]	0	0[a]
6	0	0	0	0

Group	Pylorus and fundus				
	ALP	A	B	C	Total
1	16.6±4.0	0.9±2.2	4.9±5.9	0.5±0.9	6.2±7.7
2	24.4±6.0	1.0±0.3	7.8±1.4	0.8±0.3	9.6±0.8
3	9.0±3.9[a]	1.1±0.3	4.4±0.7[a]	0.6±0.3	6.1±1.0[a]
4	0.8±0.04[a]	2.0±0.5	3.7±0.7[a]	0.6±0.3	6.3±1.0[a]
5	1.3±0.5[a]	1.2±0.4	4.8±0.8[a]	1.0±0.4	6.9±1.3[a]
6	0.1±0.01	0	0.05±0.04	0	0.05±0.04

a) Significantly different from group 2 ($P<0.01$).
b) Significantly different from group 2 ($P<0.05$).

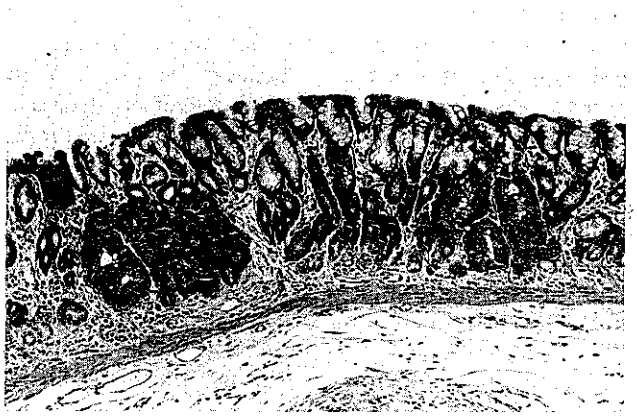

Fig. 1. Intestinal metaplasia induced by irradiation, ×200, HE staining.

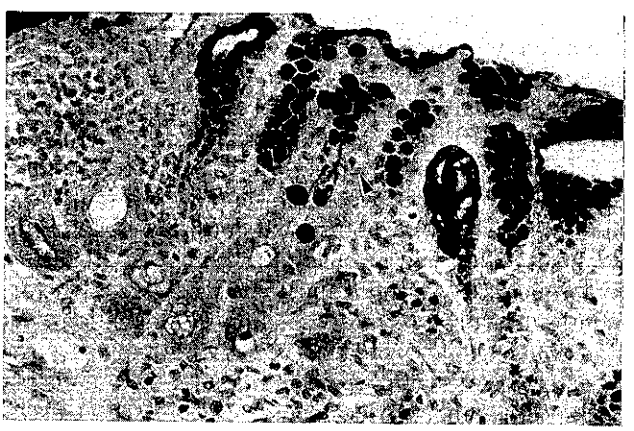

Fig. 2. A chimeric structure that consisted of both intestinal epithelium with goblet cells (Lower), mitosis (▲) and gastric mucosa (Upper), ×300, Alcian blue PAS staining.

(Table II). The numbers of ALP-positive foci in Groups 3 to 5 were also significantly decreased as compared to that in Group 2 ($P<0.01$).

Histologically, the incidences of intestinal metaplasia in all X-irradiated groups ranged from 77 to 95% in the pyloric gland, and there were no significant differences among the treated groups. In the fundus, the incidence of intestinal metaplasia was significantly decreased in Groups 3 and 5 as compared to Group 2 ($P<0.05$). In

Table III. Number of Intestinal Metaplasia after Salivary Gland Removal (mean±SE)

After operation (days)	ALP	A	B	C	Total
0	52.8±47.7	0.4±0.2	9.6±2.8	1.6±0.9	12.8±3.4
3	5.8±4.8	1.3±1.3	9.8±5.4	2.0±2.0	13.0±6.9
5	12.3±4.2	0	13.4±7.2	2.0±1.0	15.8±6.0
15	29.0±7.0	0	10.7±2.8	0.7±1.0	11.3±3.3

Table IV. pH Value after Salivary Gland Removal (mean± SD)

After operation (days)	Pylorus	Fundus
0	4.11±1.09	4.29±1.40
3	3.07±1.09	3.55±1.11
5	3.10±0.67	3.31±0.82
15	3.11±0.29	2.42±0.34

the group without irradiation (Group 6), one animal showed type B metaplasia in the pylorus.

The numbers of type B metaplastic foci developing in the fundus were also significantly decreased in Groups 3 to 5 as compared to the Group 2 value. Similarly, these lesions in the pylorus were significantly reduced by secretin or histamine and tended to decrease after removal of the submandibular glands (see Table II). No significant alteration in type A or type C metaplastic foci was observed.

An intestinal metaplasia induced by irradiation alone is shown in Fig. 1, many crypts normally being involved in Group 2 lesions. In contrast, in Groups 3 to 5, numbers of metaplastic crypts per lesion were few and some crypts showed a chimeric structure that consisted of both intestinal epithelium and normal gastric mucosa (Fig. 2).

Experiment 2 After administration of 1% NaCl for 6 months starting 6 months after X-irradiation, submandibular glands were removed and histamine exposure was started. As summarized in Table III, the numbers of ALP-positive metaplastic foci were decreased at Days 3 and 5 but recovered at Day 15 (not significantly different from Day 0).

Both fundic and pyloric pH value declined with the treatment but the differences among groups were not significant (Table IV). Histologically, the numbers of metaplastic foci remained unchanged, although degeneration within lesions (especially Paneth cells) was seen during the early period of the treatment. At Day 3, some crypts showed chimeric structures including both intestinal metaplastic and normal gastric mucosa features.

DISCUSSION

The present study, conducted to investigate the effects of pH value manipulation on the development of intestinal metaplasia caused by X-irradiation, revealed that treatment with histamine or removal of the submandibular glands resulted in a decline in the gastric pH value and a decrease in the number of APL-positive foci. It is well known that gastric acid secretion is increased by administration of histamine,[31] or removal of the submandibular glands,[32–34] with associated induction of duodenal ulcers. Secretin administration also generally decreases the gastric acid secretion,[35] but in this study, appeared to be without effect, possibly due to a rebound phenomenon after paroxysmal influence. Clarification of this point would require investigation of the serum concentrations of secretin or gastrin. Previously, we reported that the development of ALP-positive foci was accelerated by administration of ranitidine, an H_2 receptor antagonist, and decelerated by cysteamine, which increases gastric acid secretion.[15] The available data suggest that the numbers of ALP-positive foci are increased by a decrease of acid secretion and conversely decreased by increased acid and lower pH. Moreover, in the present study there was a close relationship between fundic pH and ALP-positive foci ($y=9.4x-30.5$, $r=0.92$, $P>0.05$) in animals receiving irradiation alone (8 months). When similarly irradiated animals were treated for 2 months with Tefrenon, an anti-gastric ulcer drug, which increases gastric mucin but does not influence the gastric pH value, the numbers of intestinal metaplasias did not change as compared to that 8 months after X-irradiation.[36]

The second experiment in the present study clearly showed that intestinal metaplasia is at least partially a reversible phenomenon associated with gastric alkalinity. The evidence of decreased numbers of ALP-positive foci as well as the chimeric structure of some crypts after 3 days and the increase thereafter suggest that intestinal metaplastic mucosa could redifferentiate into gastric mucosa and gastric mucosa could differentiate into intestinal mucosa according to the changes in the acidity of the stomach environment. Therefore it seems likely that

an elevation of gastric juice pH due to the disappearance of parietal cells in the fundic gland mucosa or a decrease in the gastric acidity caused by a dysfuncion of parietal cells is one of the principal factors responsible for the development of intestinal metaplasia. On the other hand, increased acid secretion is associated with partial disappearance of intestinal metaplasia, through a process of redifferentiation. Thus we found ALP-positive foci to decrease promptly in line with decrease in the gastric pH value. Histologically, degeneration of the metaplastic lesions was sometimes observed in the early period of the histamine treatment, so that ALP-positive foci, containing no neutral mucin, might be expected to disappear most promptly because of their low resistance to acidic conditions.

In human beings, well-differentiated adenocarcinomas are thought to be associated with or to develop from incomplete type intestinal metaplasias[37] rather than the complete type which is identical to the metaplasia with ALP-positive foci in our model. In our previous study, we found a reverse relationship between gastric tumors and ALP-positive foci when X-irradiated rats were treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.[19] Furthermore, in our recent experiment we found no increase in the incidence of gastric carcinoma in irradiated rats given 10% NaCl (considered a promoter of gastric carcinomas) although the height of the gastric mucosa was increased.[16] This result indicates that ALP-positive foci do not develop into gastric tumors under the influence of 10% NaCl.

In conclusion, the available data suggest that intestinal metaplasia is not a precancerous lesion and that the formation of ALP-positive foci is reversible.

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