BIOCHEMICAL STUDY OF PYLORIC METAPLASIA IN THE MUCOSA REGENERATING OVER IODOACETAMIDE-INDUCED FUNDIC ULCERS IN RAT STOMACH*1

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The molecular species and content of pepsinogen in regenerated mucosa over fundic ulcers induced in a rat stomach by iodoacetamide were found to be the same as those in normal pyloric mucosa, but different from those in normal fundic mucosa.

Addition of iodoacetamide to the drinking water of rats induces chronic ulcers in the fundic region of the glandular stomach adjacent to the limiting ridge. Recently, Shirai et al. reported sequential morphological studies on the changes in this region during ulceration induced by iodoacetamide and during the regeneration process. The healing process began soon after iodoacetamide administration was stopped, the ulcers were almost completely covered with regenerated mucosa within 6 weeks, and appearance of the regenerated mucosa did not change for at least 56 weeks. Histologically, the regenerated mucosa appeared similar to normal pyloric mucosa. When the mucosa over ulcers induced by iodoacetamide had regenerated, administration of N-methyl-N'-nitro-N-nitrosoguanidine induced adenocarcinoma of the fundus adjacent to the limiting ridge in a high incidence.

Pepsinogens, zymogens of pepsins, are produced in the glandular mucosa of the stomach. In the fundic mucosa pepsinogens are produced by chief cells and mucous neck cells, whereas they are produced by pyloric gland cells in the pyloric mucosa. Multiple forms of pepsinogens have been found in various animal species. Furihata et al., using polyacrylamide gel electrophoresis, separated the pepsinogen of the fundus of normal rats into 4 isozymes (Pg 1, 2, 3, and 4) and that of the pylorus into 3 isozymes (Pg 1, 3, and 4). They also found that the fundus contained more than 5 times as much pepsinogen as the pylorus. These findings suggested that examination of the pepsinogen isozyme pattern and pepsinogen content of regenerated mucosa should show whether this regenerated mucosa resembles normal pyloric mucosa biochemically. The present paper describes studies on this subject.

MATERIALS AND METHODS

Ten male Wistar strain rats, weighing about 130 g, were used. They were fed on commercial stock diet Oriental MF (Oriental Yeast Co., Tokyo) and given iodoacetamide (Tokyo Kasei Industry Co., Tokyo) in their drinking water for 12 weeks. The concentration of iodoacetamide in the drinking water was increased gradually from 0.01 to 0.02, 0.04, and 0.08% in the first 4 weeks, and then it was maintained at 0.1% for the remaining 8 weeks.

Two weeks after the end of iodoacetamide administration, animals were anesthetized with ether and the stomach was rapidly removed, opened along the greater curvature, and stretched out on a cork plate. Then the areas of regenerated mucosa were carefully cut out from the ulcer under a binocular microscope, taking care not to include

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fundic mucosa. The area surrounding the ulcers was also examined histologically to confirm the presence of mucosa, and normal regions of the fundus and pylorus were also examined as a control. In 8 of the 10 rats the regenerated mucosa was large enough for examination.

For the assay of pepsin, the tissue was homogenized with 9 volumes of 0.05M Tris-acetate buffer (pH 8.2) in a Potter-Elvehjem glass homogenizer fitted with a Teflon pestle. Then the homogenate was centrifuged at 105,000g for 60 min and the potential peptic activity of pepsinogen in the resulting supernatant was determined with hemoglobin (type I, Sigma Chemical Co., St. Louis, U.S.A.) as a substrate, as described previously. Protein was measured by the method of Lowry et al. One unit of enzyme activity was defined as the amount releasing a peptide equivalent to 1 unit of absorption at 280 nm in 10 min.

Electrophoresis of pepsinogen in the supernatant was performed on polyacrylamide gel as described previously. For routine histological examination, pieces of the stomach were fixed in 10% Formalin for 24 hr and then dehydrated and embedded in paraffin. Sections were stained routinely with Hematoxylin and Eosin. For electron microscopy, small pieces of the tissue from the area of the ulcer were fixed for 1 hr in ice-cold 3% glutaraldehyde buffered at pH 7.4 with cacodylate and postfixed in 1% OsO4 in the same buffer at 4°C for 1 hr. The tissues were dehydrated by passage through a graded concentrations of ethanol, and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead acetate, and examined with a Hitachi Hu-12 electron microscope.

RESULTS AND DISCUSSION

Pepsinogen Content of the Mucosa As shown in Table I, the pepsinogen content of four specimens (Nos. 1, 2, 7, and 8) of regenerated mucosa over iodoacetamide-induced ulcers was the same as that of normal pyloric mucosa, whereas that of the other four (Nos. 3, 4, 5, and 6) was slightly higher but still much less than that of normal fundic mucosa.

Electrophoresis of Pepsinogen In the glandular mucosa of normal stomach, the pepsinogen of the fundus can be separated into 4 components (Pg 1, 2, 3, and 4) and that of the pylorus into 3 components (Pg 1, 3, and 4) by electrophoresis, as shown in

<table>
<thead>
<tr>
<th>Mucosa</th>
<th>Proteolytic activity at pH 2.0 (units/mg protein)</th>
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<tbody>
<tr>
<td>Regenerated fundic</td>
<td>10.5</td>
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<tr>
<td>2</td>
<td>8.35</td>
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<tr>
<td>3</td>
<td>17.7</td>
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<td>4</td>
<td>18.6</td>
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<td>5</td>
<td>18.3</td>
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<td>6</td>
<td>14.2</td>
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<td>7</td>
<td>7.22</td>
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<tr>
<td>8</td>
<td>10.3</td>
</tr>
<tr>
<td>Normal pyloric</td>
<td>10.1±2.0</td>
</tr>
<tr>
<td>Normal fundic</td>
<td>75.0±20.0</td>
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Table I. Potential Peptic Activity of Pepsinogen in the Mucosal Supernatant

Fig. 1. The content of Pg 1 was greatest in the fundus, but the content of the 3 pepsinogens was similar in the pylorus. On electrophoresis of pepsinogen from regenerated mucosa, only three pepsinogens (Pg 1, 3, and 4) were found, as in normal pyloric mucosa (Fig. 1).

Morphological Findings Appearance of the regenerated mucosa was described previously; it was very similar to normal pyloric mucosa (Photo 1) but contained no parietal or chief cells. Electron microscopic examination showed that the apical parts of the cell in regenerated glands contained many secretory granules. These granules were round or oval with a slightly irregular outline and fused with each other. The rough-surfaced endoplasmic reticulum was sparse. Golgi bodies, consisting of several small sacculi, and some vesicles and the nucleus were often flattened against the base of the cell (Photo 2).

The present results confirmed both morphologically and biochemically that the mucosa regenerating over ulcers of the fundic region of a rat stomach induced by iodoacetamide treatment showed pyloric gland metaplasia. Namely, it seems that the mucosa regenerating over ulcers induced by iodoacetamide is biologically similar to normal pyloric mucosa.

Almost all gastric adenocarcinomas in rats induced by N-methyl-N’-nitro-N-nitrosog-
BIOCHEMICAL STUDY OF MUCOSA IN IODOACETAMIDE-INDUCED ULCER

Fig. 1. Electropherograms of pepsinogens on polyacrylamide gel

Zymogen extracts were run on 7.5% polyacrylamide gel in 0.05M Tris-acetate buffer (pH 8.2). After electrophoresis, the gel was immersed in a solution of 0.65% hemoglobin in 0.06N HCl, incubated in a humid chamber at 37°C, and stained with 1% Amido Black 10B in 7% acetic acid. Gels were destained in 7% acetic acid.

Fig. 2. Diagrammatic representation of Fig. 1.

The isozyme pattern of pepsinogen in well-differentiated adenocarcinomas in the fundus was found to be the same as that of well-differentiated adenocarcinoma in the

guanidine (MNNG) in the drinking water arise from the pyloric mucosa. Thus the pyloric mucosa seems to be susceptible to this carcinogen while the fundic mucosa is resistant. When MNNG was given in the drinking water to rats with regenerated mucosa over iodoacetamide-induced ulcers, adenocarcinomas developed in the fundic region where ulcers had been induced. The present finding that the regenerated mucosa in the fundic region resembles pyloric mucosa explains why MNNG induced carcinomas in the fundus rather than the pylorus in these animals.

The isozyme pattern of pepsinogen in well-differentiated adenocarcinomas in the fundus was found to be the same as that of well-differentiated adenocarcinoma in the
pylorus; namely, Pg 2 and Pg 3 were present but Pg 1 was absent or present in only a small amount (Furihata et al., unpublished data). This suggests that well-differentiated adenocarcinomas in the pyloric and fundic areas both arise from the same lesion.

During MNNG carcinogenesis without iodoacetamide, pyloric gland metaplasia was occasionally found in the fundus, and it was suggested that this metaplasia was related to carcinogenesis in the fundic region. Histopathological observations and examination of pepsinogen isozymes have confirmed this. Thus it is concluded that pyloric gland metaplasia in the fundus is an intermediary step in the induction of adenocarcinomas in this region.

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REFERENCES
10) Samloff, I. M., Gastroenterology, 61, 185~188 (1971).

EXPLANATION OF PLATE
Photo 1. Regenerated mucosa showing pyloric gland metaplasia over an ulcer induced by iodoacetamide. Hemotoxylin and Eosin stain. × 100.

Photo 2. Electron microscopic appearance of the mucosa showing pyloric gland metaplasia. There are many round or oval secretory granules in the apical parts of the cells. The rough-surfaced endoplasmic reticulum is sparse. ×12,000.