Ornithine Decarboxylase Activity in the Mucosa of Gastric Remnant following Gastric Surgery

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Abstract
Ornithine decarboxylase (ODC) activity and polyamine levels in mucosal specimens obtained by endoscopic biopsy from the stomal portion and greater curvature of the gastric remnant mucosa taken from 181 patients were determined and compared with the histopathological findings. The results obtained can be summarized as follows. (a) The ODC activity was significantly higher in the stomal portion [455 ± 340 (mean ± SD)] of the gastric remnant than in the greater curvature (148 ± 107). The ODC activity of the stomal portion was significantly higher following a Billroth's II method (599 ± 417) than following any other operative method that consists of a Billroth's I method (327 ± 172) and a Roux-en-Y (341 ± 191). (b) The levels of total polyamine, putrescine, spermidine, and spermine were also significantly higher in the stomal portion of the gastric remnant than in the greater curvature. There were no significant differences in the stomal tissue level of any of these substances among operative procedures used. (c) Histopathological changes consisting of glandular dilatation and an irregular glandular structure were detected more frequently in the stomal mucosal specimens, especially following a Billroth's II method.

In summary, the present findings suggest that the measurement of ODC activity may thus be considered as one method of estimating the risk of carcinogenesis.

Introduction
Many studies have been reported on the likely facilitatory effects of the duodenogastric reflux of bile acids (which is a tumor promoter) on stomach carcinogenesis. The activity of ornithine decarboxylase, which is the rate-limiting enzyme of polyamine biosynthesis, is generally accepted as the index of cell proliferation and as a useful index of tumor promotion in many experimental studies. We measured the levels of ODC activity and those of putrescine, spermidine, and spermine in tissue samples obtained by endoscopic biopsy from the stomal portion and the greater curvature of the gastric remnant, and we examined the relation of those levels to the histopathological findings in order to clarify whether any tumor-promoting stimuli continuously existed in the anastomosed region and if the ODC activity and polyamine levels in the stomal mucosa might provide useful information on the risk of carcinogenesis of the gastric remnant.

Materials and Methods
Tissues. Mucosal specimens were obtained via endoscopic biopsy from the stomal portions and greater curvatures at least 5 cm from the stomal portions of the gastric remnants of 181 patients who were followed up at the First Department of Surgery, Osaka City University Medical School. Almost all of them underwent curative operation for gastric cancer. The patients comprised 64 females and 117 males, ranging in age from 27 to 87 years (average, 60.7 years). The time after gastrectomy ranged from 1 to 37 years (average, 4.3 years). The types of operations performed were B-I for 87 patients, B-II for 85 patients, and Roux-en-Y for 9 patients. There were no significant differences between patients treated by B-I and those treated by B-II with regard to the range in age, sex, and the period after operation. The specimens were immediately frozen in liquid nitrogen, preserved at -85°C, and analyzed within 2 weeks.

Enzyme Assay. The ODC activity was measured as described previously (1). Briefly, the specimens were minced and homogenized in 500 μl of 50 mM Tris (pH 7.5) containing 0.25 M of sucrose, 2.5 mM of dithiothreitol, 0.1 mM of EDTA, and 0.2 mM of pyridoxal phosphate with an ultrasonic homogenizer. After centrifugation at 100,000 × g for 60 min at 4°C, 200 μl of the supernatant and 42 nmol of L-[1-14C]-ornithine (0.125 μCi; 10 μl) were put into a test tube. The test tube was tightly stoppered with a rubber stopper equipped with the syringe needle and a filter paper disk pierced with the needle and impregnated with 70 μl of Soluene-350 (Pakard Co, Ltd.). The mixture was incubated at 37°C for 60 min. The reaction was stopped by the addition of 1 ml of 2 M citrate using a syringe through the rubber stopper and the syringe needle. After another incubation at 37°C for 60 min, the paper disk was transferred to a vial containing 5 ml of toluene scintillation fluid (Omnifluor, Daiichi Chemicals, Tokyo, Japan), and the radioactivity was measured in a Beckmann liquid scintillation counter.

Statistics. Tests of statistical significance were performed using nonparametric methods. The generalized Wilcoxon T test was used to evaluate the significance of the differences in the mean ODC activity and polyamine levels in the mucosal specimen from the stomal portion versus those from the greater curvature, and the generalized Kruskal-Wallis' H test was used to compare the different operative methods.

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1 To whom requests for reprints should be addressed.
2 The abbreviations used are: ODC, ornithine decarboxylase; B-I, Billroth's I method; B-II, Billroth's II method.
Results

Fig. 1 shows ODC activity in the mucosa at different biopsy sites. The ODC activity in the mucosa of the stomal portion was $455 \pm 340$ pmol CO$_2$/h/mg protein (mean $\pm$ SD) and was significantly higher than that of the greater curvature ($148 \pm 107$ pmol CO$_2$/h/mg protein). Fig. 2 shows the ODC activity in the mucosa for the different operative methods. The ODC activity was significantly elevated at the stomal portion compared with that at the greater curvature, irrespective of operative methods. As for the stomal portion, the ODC activity following a B-II was $599 \pm 417$ pmol CO$_2$/h/mg protein, and that following a B-I was $327 \pm 172$ pmol CO$_2$/h/mg protein, and that following a Roux en Y method was $341 \pm 191$ pmol CO$_2$/h/mg protein. The ODC activity following a B-II was more significantly elevated than it was with any other operative method.

Table 1 shows the polyamine levels of the stomal mucosa and of the greater curvature after gastric resection by the various operative methods. As for each operative method and to summarize them, the putrescine, spermidine, spermine, and the total polyamine levels were significantly higher in the mucosa of stomal portion than in the greater curvature. No significant differences were recognized among the different operative methods. Table 2 shows the histopathological findings consisting of intestinal metaplasia, glandular dilatation, irregular glandular structure, and round cell infiltration. The glandular dilatation and the irregular glandular structure were significantly more frequent in the stomal mucosa than in the greater curvature (65.8

versus 5.8%, and 50.0 versus 2.3%); and were significantly more frequent in the stomal mucosa following a B-II than following a B-I (91.9 versus 13.5% and 78.4 versus 2.7%). They were seldom recognized at the greater curvature in at least one-half of the whole specimens studied.

Discussion

The frequency of primary cancer developed in the gastric remnant surgically treated for peptic ulcer varies from one reported study to another. Totgaad (2) showed the fact that the risk of carcinogenesis of the gastric remnant more than 20 years after gastrectomy is severalfold higher than was expected. Sowa et al. (3), Tokudome et al. (4), and Kondo (5) demonstrated that stomal cancer develops more frequently following a B-II than following a B-I (91.9 versus 13.5% and 78.4 versus 2.7%).
for long periods. The higher levels of polyamines in the stomal mucosa, especially in the stomal portion, was stimulated to proliferate continuously and rapidly because of the B-II promoting stimulus. Saito et al. (7) suggested that the stomal mucosa, ODC activity was significantly higher following a B-II than following a B-I. This result also supports the concept of ODC activity as a biochemical marker of cell proliferation and tumor promotion. Saito et al. (7) also suggested that the degree of increase in ODC activity is greater than that of the increase in the polyamine levels. On the other hand, polyamine levels may change slowly, and it is difficult to detect the changes because of the fact that we cannot distinguish polyamines produced de novo from those stored in cells during the measurement of polyamine levels of tissues. Therefore, the existence of and any changes in the stimuli causing cell proliferation may reflect rapidly upon the ODC activity in tissues, rather than on polyamine levels, suggesting that ODC activity is useful for considering the risk of carcinogenesis clinically rather than polyamine levels in tissues.

In Japan, there are many patients who survive for a long period after curative operations for early gastric cancer. However, the prognosis of remnant stomach cancer in advanced stage must be said to be poor in general, and, therefore, few patients having stomal cancer have been diagnosed in the early stage. The significance of strict postoperative follow-up is reconfirmed. B-II reconstruction should be avoided if possible, because it carries a high risk of stomal cancer. Therefore, few patients having stomal cancer have been diagnosed in the early stage. The significance of strict postoperative follow-up is reconfirmed. B-II reconstruction should be avoided if possible, because it carries a high risk of stomal cancer. Therefore, few patients having stomal cancer have been diagnosed in the early stage. The significance of strict postoperative follow-up is reconfirmed. B-II reconstruction should be avoided if possible, because it carries a high risk of stomal cancer.

B-II were significantly higher than those following a B-I. Reflux of duodenal juice containing bile acids following a B-II causes continuous activation of cell proliferation at the stomal portion for long periods, resulting in these histological changes and eventually in cancer.

Polyamine biosynthesis was closely associated with cellular growth and proliferation (6) because polyamine levels were elevated in proliferating cells and neoplastic tissues. The ODC activity is regarded as the general index of cell proliferation. Recently there has been increasing interest in the concept of ODC activity as a biochemical marker of tumor promotion. Saito et al. (7) suggest that the degree of increase in ODC activity is related to the power of the tumor promotion. They clearly showed here that ODC activity was significantly higher in the stomal portion of the gastric remnant than in the greater curvature, and that regarding the stomal mucosa, ODC activity was significantly higher following a B-II than following any other operative method. These results suggest that the stomal mucosa, especially reconstructed by B-II, is stimulated to proliferate continuously for long periods. The higher levels of polyamines in the stomal mucosa also support this conclusion. Any tumor-promoting stimulus elevates ODC activity promptly, and as the stimulus disappears, rapid reduction of the ODC activity occurred due to its short half-life. Also, the extent of the increase in ODC activity is greater than that of the increase in the polyamine levels. On the other hand, polyamine levels may change slowly, and it is difficult to detect the changes because of the fact that we cannot distinguish polyamines produced de novo from those stored in cells during the measurement of polyamine levels of tissues. Therefore, the existence of and any changes in the stimuli causing cell proliferation may reflect rapidly upon the ODC activity in tissues, rather than on polyamine levels, suggesting that ODC activity is useful for considering the risk of carcinogenesis clinically rather than polyamine levels in tissues.

Table 1. Changes in mucosal ornithine decarboxylase activity and polyamine levels of 57 cases at different biopsy sites following varying operative procedures

<table>
<thead>
<tr>
<th>Sample/assay</th>
<th>ODC (pmol Co2+/mg protein)</th>
<th>Polyamine (nmol/mg)</th>
<th>Putrescine (nmol/mg)</th>
<th>Spermidine (nmol/mg)</th>
<th>Spermine (nmol/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-I (n = 25)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Stomal portion</td>
<td>422 ± 231± b,c</td>
<td>3.42 ± 1.98c</td>
<td>0.273 ± 0.153c</td>
<td>1.03 ± 0.63c</td>
<td>2.11 ± 1.38c</td>
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<tr>
<td>Greater curvature</td>
<td>172 ± 150</td>
<td>2.81 ± 1.51</td>
<td>0.230 ± 0.102</td>
<td>0.901 ± 0.53</td>
<td>1.68 ± 0.99</td>
</tr>
<tr>
<td>B-II (n = 28)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Stomal portion</td>
<td>737 ± 534± b,c</td>
<td>3.78 ± 2.40c</td>
<td>0.278 ± 0.112c</td>
<td>1.41 ± 1.08c</td>
<td>2.10 ± 1.32c</td>
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<tr>
<td>Greater curvature</td>
<td>201 ± 154</td>
<td>2.94 ± 1.95</td>
<td>0.208 ± 0.078</td>
<td>1.02 ± 0.74</td>
<td>1.71 ± 1.22</td>
</tr>
<tr>
<td>B-II/Roux en Y (n = 4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomal portion</td>
<td>267 ± 59± b,c</td>
<td>2.93 ± 0.61c</td>
<td>0.297 ± 0.051c</td>
<td>1.04 ± 0.30c</td>
<td>1.60 ± 0.32c</td>
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<tr>
<td>Greater curvature</td>
<td>93 ± 61</td>
<td>2.04 ± 0.37</td>
<td>0.254 ± 0.066</td>
<td>0.621 ± 0.02</td>
<td>1.16 ± 0.20</td>
</tr>
<tr>
<td>Sum (n = 57)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomal portion</td>
<td>566 ± 439</td>
<td>3.56 ± 2.15c</td>
<td>0.276 ± 0.129c</td>
<td>1.22 ± 0.88c</td>
<td>2.07 ± 1.31c</td>
</tr>
<tr>
<td>Greater curvature</td>
<td>181 ± 150</td>
<td>2.82 ± 1.71</td>
<td>0.221 ± 0.089</td>
<td>0.939 ± 0.64</td>
<td>1.68 ± 1.09</td>
</tr>
</tbody>
</table>

a P < 0.01 (significant difference between stomal portion and greater curvature).
b P < 0.05 (significant difference among different operative methods).
c P < 0.05.

Table 2. Histopathological changes in the gastric remnant mucosa at different biopsy sites following varying operative procedures

<table>
<thead>
<tr>
<th>Operative method</th>
<th>Biopsy site</th>
<th>% of Intestinal metaplasia</th>
<th>% of Glandular dilatation</th>
<th>% of Irregular glandular structure</th>
<th>% of Round cell infiltration</th>
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</thead>
<tbody>
<tr>
<td>B-I (n = 45)</td>
<td>GC</td>
<td>8.9</td>
<td>0</td>
<td>2.2</td>
<td>8.9</td>
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<tr>
<td></td>
<td>SP</td>
<td>26.7</td>
<td>(4/45)</td>
<td>(0/45)</td>
<td>(1/45)</td>
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<tr>
<td>B-II (n = 37)</td>
<td>GC</td>
<td>10.8</td>
<td>(4/37)</td>
<td>(5/37)</td>
<td>(5/37)</td>
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<tr>
<td></td>
<td>SP</td>
<td>32.4</td>
<td>(12/45)</td>
<td>(22/45)</td>
<td>(14/45)</td>
</tr>
<tr>
<td>B-II/R-Y (n = 4)</td>
<td>GC</td>
<td>0</td>
<td>(0/4)</td>
<td>0</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>SP</td>
<td>0</td>
<td>(0/4)</td>
<td>0.0</td>
<td>50.0</td>
</tr>
</tbody>
</table>

a GC, greater curvature; SP, stomal portion.

References
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