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. The measurement of ODC activity may thus be considered as one method of estimating the risk of carcinogenesis.

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 85 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 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 mM 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.
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 and that duodenogastriic reflux might play a role in the carcinogenesis. The endoscopic finding of a remnant stomach is redness, while that of the stomal mucosa is rubor, edema, or erosion. The positive rates of these findings increased according to postoperative duration and were especially high in patients who had undergone the B-II method. Biopsy of sections revealed a high frequency of glandular dilatation and irregular glandular structure. These changes were much more frequently recognized at the stomal portion in cases having a long postoperative period after B-II operation. In this study, the frequencies of these changes at the stomal portion following a
These results suggest that the stomal mucosa, especially re-
stomal mucosa, ODC activity was significantly higher fol-
tumor promotion. Saito the concept of ODC activity as a biochemical marker of
The ODC activity is regarded as the general index of cell
els were elevated in proliferating cells and neoplastic tissues.
flux of duodenal juice containing bile acids following a B-II
mal portion for long periods, resulting in these histological
B-II were significantly higher than those following a B-I. Re-
Change 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 activity (pmol CO2/h/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>
</tr>
<tr>
<td>Stomal portion</td>
<td>422 ± 231ab</td>
<td>3.42 ± 1.98c</td>
<td>0.273 ± 0.153c</td>
<td>1.01 ± 0.63c</td>
<td>2.11 ± 1.38c</td>
</tr>
<tr>
<td>Greater curvature</td>
<td>172 ± 150b</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>
</tr>
<tr>
<td>Stomal portion</td>
<td>737 ± 534ab</td>
<td>3.78 ± 2.40e</td>
<td>0.278 ± 0.112c</td>
<td>1.41 ± 1.08c</td>
<td>2.10 ± 1.32c</td>
</tr>
<tr>
<td>Greater curvature</td>
<td>201 ± 154a</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 ± 59ac</td>
<td>2.93 ± 0.61e</td>
<td>0.297 ± 0.051c</td>
<td>1.04 ± 0.30c</td>
<td>1.60 ± 0.32c</td>
</tr>
<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 ± 0.88e</td>
<td>2.07 ± 1.31r</td>
</tr>
<tr>
<td>Greater curvature</td>
<td>181 ± 150a</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>

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

B-II were significantly higher than those following a B-I. Re-
flux 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 lev-
els were elevated in proliferating cells and neoplastic tissues.
The ODC activity is regarded as the general index of 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 carcino-
geneis 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 ad-
vanced stage must be said to be poor in general, and, there-
fore, 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
carcinogenesis. Patients who are found to have glandular
dilatation, irregular glandular structure histologically, and
high ODC activity in the stomal mucosa must be carefully
followed up so that stomal cancer, if it develops at all, can
be detected as early as possible.

References
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Ikejiri, T., Oka, N., and Tsurumaru, H. A prospective study on primary gastric
mal mucosa also support this conclusion. Any tumor-
promoting stimulus elevates ODC activity promptly, and as
the stimulus disappeared, rapid reduction of the ODC ac-
tivity 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 meas-
urement of polyamine levels of tissues. Therefore, the exis-
tence of and any changes in the stimuli causing cell pro-
liferation 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 carcino-
geneis clinically rather than polyamine levels in tissues.

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>(12/45)</td>
<td>48.9</td>
<td>(1/45)</td>
</tr>
<tr>
<td>B-II (n = 37)</td>
<td>GC</td>
<td>10.8</td>
<td>1.3</td>
<td>2.7</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td>SP</td>
<td>32.4</td>
<td>(12/45)</td>
<td>91.9</td>
<td>(1/45)</td>
</tr>
<tr>
<td>B-II/Roux en Y</td>
<td>GC</td>
<td>0.4</td>
<td>0</td>
<td>0</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>SP</td>
<td>0.4</td>
<td>(0/4)</td>
<td>0.4</td>
<td>(0/4)</td>
</tr>
</tbody>
</table>

* GC, greater curvature; SP, stomal portion.


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