Increased Mucosal Ornithine Decarboxylase Activity in Human Gastric Cancer

Junichi Okuzumi, Tetsuro Yamane, Yoshitaka Kitao, Kazuaki Tokiwa, Toshiharu Yamaguchi, Yoshihiro Fujita, Hoyoku Nishino, Akio Iwashima, and Toshio Takahashi

Introduction

Ornithine decarboxylase catalyzes the conversion of ornithine into putrescine, which is the rate-limiting step in polyamine biosynthesis (1). The activity of this enzyme in vivo can fluctuate rapidly in response to various growth-promoting stimuli (2). It has been suggested that the induction of ODC activity plays an important role in the tumor-promoting step of the two-stage carcinogenesis model, initiation and promotion. Initially, an excellent correlation between ODC activity and tumor-promoting ability was shown in mouse skin carcinogenesis (3). In the large bowel, not only in the rect colon but also in surgically resected human colon bearing colon cancer, ODC activity plays a significant role (4-6). Increased levels of ODC were induced in the normal-appearing mucosa of human carcinoma-bearing large bowel. ODC activity may be a good biological marker to detect individuals at high risk for large bowel cancer (5). The stomach carcinoma MNNG as well as bile acids have been proven to induce transitional ODC activity in the rat stomach (7, 8). But few reports are available concerning ODC activity in the human stomach (9).

In this study, ODC activity was compared in human gastric cancer tissue and in the normal-appearing mucosa of cancer-bearing stomach surgically resected for gastric cancer. Gastric mucosa without gastric cancer was also studied. Furthermore, in gastric carcinogenesis the property of ODC was investigated.

Materials and Methods

Chemicals. Reagent grade chemicals were obtained from standard sources. N-(1-14C)Orotate monohydrochloride and Protosol were purchased from New England Nuclear, Boston, MA. Bio-Rad protein assay kit was purchased from Bio-Rad Laboratories, Richmond, CA. MNNG and GTP were obtained from Sigma Chemical Co., St. Louis, MO.

Human Specimens. Tissue samples of grossly normal-appearing mucosa and carcinoma were obtained from patients who received gastrectomy. Of these, 31 were gastric cancer cases, composed of 22 men and 9 women, ranging from 26 to 90 years old with a mean age of 59.3. One case, a 46-year-old man had no gastric cancer, but required gastrectomy for a benign gastric ulcer. Immediately after gastrectomy, the stomach was opened along the greater curvature and cleansed, small pieces of the normal-appearing mucosa were dissected from the fundic and pyloric gland areas, respectively. A small piece of cancer tissue was also obtained. Mucosa without gastric cancer was obtained by gastrofiberscopic biopsy from 6 persons (3 men and 3 women) ranging from 30 to 75 years old. Of these 6 patients, 3 were proven to have a gastrroduodenal ulcer and others had no obvious lesions. The biopsy site was selected so as to avoid grossly evident lesions. Tissue samples were frozen immediately in liquid nitrogen, stored at −80°C, and were analyzed for ODC activity within 2 weeks.

Animal Experiment. Male Wistar rats at 8 weeks of age were purchased from Shimizu Experimental Materials Co. Ltd., Kyoto, Japan. To measure transient ODC activity induction in rat glandular stomach by MNNG (gastric intubation), rats were given 0.5 ml of a solution of MNNG (200 mg/kg body weight) in dimethyl sulfoxide by gastric intubation. Twenty-four h after intubation, rats were sacrificed by cervical dislocation, the stomachs were removed, opened along the greater curvature, and rinsed with saline. The glandular stomach mucosa was scraped off with a razor blade and homogenized. To measure the constitutively expressed ODC activity of cancer-bearing stomach, rats were given drinking water containing 100 mg/liter MNNG for 28 weeks. Animals were sacrificed at the end of week 44. The stomachs were removed, opened along the greater curvature, and rinsed with saline. Mucosa was scraped off with a razor blade and homogenized. Gross examination of the stomach revealed elevated lesions which were defined histologically as adenocarcinoma. Homogenates were processed in the same way as human specimens for ODC assay.

Preparation of Tissue Extract. Tissue samples of 100 mg were homogenized in 1.0 ml of 50 mM sodium phosphate buffer (pH 7.2), containing 0.1 mM pyridoxal phosphate and 0.1 mM EDTA. Insoluble material was removed by centrifugation at 30,000 × g for 20 min, and the protein content of the supernate was determined by the Bio-Rad protein assay kit, using bovine serum albumin as standard.

ODC Activity Determination. ODC activity was determined by measuring the release of CO2 as described by Fujiki et al. (10) (modification of the method of Russel and Snyder). The incubation mixture (final volume, 2 ml) in Warburg flasks consisted of 0.4 μmol pyridoxal phosphate, 1.0 μmol dihydrobiopterin, 100 μmol sodium phosphate (pH 7.2), and 0.1 to 0.4 ml tissue extracts containing approximately 1.0 mg of protein. After preincubation at 37°C for 10 min, 0.2 ml of solution containing 18.5 kBq DL-[1-14C] orotate monohydrochloride (1.954 GBq/mmol) and 0.2 μmol L-ornithine were added. Incubations were carried out for 60 min at 37°C. The reaction was stopped by adding 0.8 ml of 2 M citric acid, and released CO2 was trapped in 0.2 ml Protosol (New England Nuclear). The assay was done in duplicate. One enzyme unit is defined as 1 pmol of CO2/h/mg protein.

Sufficient human tissue specimens were available to assay ODC...
activity and polyamines. Putrescine, spermidine, and spermine levels in tissue specimens were determined by high performance liquid chromatography as described elsewhere (11). Student’s t test was used to analyze the statistical significance of the results.

RESULTS

Mean ODC activity ± SE in the gastric cancer tissue was 157.8 ± 68.2 pmol CO₂/h/mg protein (n = 12). Mean ODC activity in the normal-appearing mucosa with gastric cancer was 45.7 ± 6.8 pmol CO₂/h/mg protein (n = 30; Fig. 1; Table 1). The cancer tissue had significantly higher ODC activity than the normal-appearing mucosa with gastric cancer (P < 0.05). Among the normal-appearing mucosa with gastric cancer, the mean mucosal ODC activity of the fundic gland mucosa and of the pyloric gland mucosa were 21.6 ± 4.4 and 42.8 ± 7.2 pmol CO₂/h/mg protein, respectively (Table 2). The activity of ODC was significantly higher in the mucosa of the pyloric gland than in that of the fundic gland (P < 0.05) in gastric cancer cases. Mean ODC activity in mucosa without gastric cancer was 11.8 ± 3.5 (n = 7; Fig. 1; Table 1). The mucosa with gastric cancer had higher ODC activity compared with the mucosa without gastric cancer and the difference was significant (P < 0.05). Among the mucosa without gastric cancer, there was no difference in enzyme activity levels between the mucosa of the fundic gland and that of the pyloric gland.

Polyamine levels in gastric cancer tissue and mucosa in gastric cancer cases (pyloric and fundic gland mucosa) were statistically higher than those in fundic gland mucosa. Putrescine, spermidine, and spermine levels in pyloric gland mucosa were higher than those in fundic gland mucosa but the difference was not significant.

It has been reported that ODC of some murine and human tumors is activated by GTP and GTP-activatable ODC is considered to be structurally and functionally different from the usual form of ODC (12, 13). We investigated whether or not ODC of the stomach is activated by GTP. Three human gastric cancer tissues were activated by GTP. In rat glandular stomach, transiently induced ODC by a single gastric intubation of MNNG was not activated. Without GTP, ODC was not detectable in 2 mucosae of MNNG-induced cancer-bearing stomach, but in the presence of 0.1 mM GTP, ODC activity was 6.7 and 9.7, and was activated by GTP in one mucosa.

It is known that gastric cancer is often accompanied by atrophic gastritis (especially in intestinal type gastric cancer). Of 30 cancer cases, 29 exhibited more or less atrophic gastritis in the pyloric gland area. The degree of atrophic gastritis was classified into none, slight, moderate, and severe. The degree of atrophic gastritis and mucosal ODC activity in gastric cancer cases is summarized in Table 5. There was no correlation between ODC activity and the degree of atrophic gastritis.

In gastric cancer cases, tumor size ranged 1.1 to 16.0 cm with a mean value of 4.9 cm. Mean mucosal ODC activity in tumors larger than 5.0 cm was 57.0, whereas in tumors smaller than 4.9 cm, it was 36.4. Of 30 gastric cancer cases 15 were early gastric cancer (tumor limited to the mucosa or submucosa) and 15 were advanced gastric cancer (tumor with invasion of more than muscularis propria). Mean mucosal ODC activity in early gastric cancer cases was 37.9 while that in advanced gastric cancer was 49.8. More advanced or larger tumor cases tended toward high ODC activity but the difference was not statistically significant. Mean mucosal ODC activity in diffuse type cancer (n = 14) and intestinal type cancer (n = 16) was 45.2 and 44.7, respectively. Type of the tumor did not affect mucosal ODC activity. In gastric cancer cases, the mean mucosal ODC activity

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**Table 1 ODC activity of gastric cancer tissue and mucosa of gastric cancer cases and non-gastric cancer cases**

<table>
<thead>
<tr>
<th>Source</th>
<th>No. of cases</th>
<th>ODC activity (pmol CO₂/h/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric cancer tissue</td>
<td>12</td>
<td>157.8 ± 68.2°</td>
</tr>
<tr>
<td>Mucosa of gastric cancer cases</td>
<td>30°</td>
<td>45.7 ± 6.8°</td>
</tr>
<tr>
<td>Mucosa of non-gastric cancer cases</td>
<td>7</td>
<td>11.8 ± 3.5°</td>
</tr>
</tbody>
</table>

* Mean ODC activity ± SE.
* Significantly different from gastric cancer tissue (P < 0.05).
* Significantly different from mucosa of gastric cancer cases (P < 0.05).

**Table 2 ODC activity in mucosa of fundic gland and pyloric gland in gastric cancer cases and non-gastric cancer cases**

<table>
<thead>
<tr>
<th>Case</th>
<th>Fundic gland mucosa</th>
<th>Pyloric gland mucosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric cancer (n = 29)</td>
<td>21.6 ± 4.4°</td>
<td>42.8 ± 7.2°</td>
</tr>
<tr>
<td>Non-gastric cancer (n = 7)</td>
<td>6.0 ± 3.4</td>
<td>9.4 ± 4.0</td>
</tr>
</tbody>
</table>

* Mean ODC activity ± SE.
* Significantly different from the fundic gland mucosa of gastric cancer.
* Four cases with gastroduodenal ulcer and 3 cases with no obvious lesion.

**Table 3 Polyamine levels**

<table>
<thead>
<tr>
<th>Polyamines (nmol/mg protein)</th>
<th>n</th>
<th>Putrescine</th>
<th>Spermidine</th>
<th>Spermine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer tissue</td>
<td>7</td>
<td>1.40 ± 0.21°</td>
<td>0.73 ± 0.15°</td>
<td>1.02 ± 0.27</td>
</tr>
<tr>
<td>Fundic gland mucosa</td>
<td>11</td>
<td>0.98 ± 0.11°</td>
<td>0.34 ± 0.12°</td>
<td>0.50 ± 0.13</td>
</tr>
<tr>
<td>Pyloric gland mucosa</td>
<td>11</td>
<td>1.63 ± 0.44°</td>
<td>0.48 ± 0.10°</td>
<td>0.77 ± 0.22</td>
</tr>
</tbody>
</table>

* Mean ± SE.
* Statistically significant difference (P < 0.05).
early and essential event in tumor promotion. Induction of this enzyme activity is considered to be an
important role. MNNG, a gastric carcinogen, and bile acids, gastric tumor promoters, increased ODC activity in the mucosa of the rat
glandular stomach after administration by gastric intubation (7,
8).

in patients over 60 years old was 53.0, whereas that in patients
less than 59 years old was 38.4. The difference was not
statistically significant (Table 6).

DISCUSSION

ODC activity is induced by respective tumor promoters in various animal organs (3, 4, 14). Therefore, in animal carcino-
genesis, induction of this enzyme activity is considered to be an
early and essential event in tumor promotion.

Recently, it has been reported that ODC activity is elevated
in human premalignant lesions. Garewal et al. (15) reported
that ODC activity in Barrett's mucosa, a premalignant lesion
associated with an increased risk of development of esophageal
adenocarcinoma, was higher than in squamous esophageal or
gastric mucosa. ODC activity in the large bowel has been
reported (5, 6, 13, 16) and is elevated not only in colon cancer
but also in adenoma. Moreover, ODC activity is higher in
mucosa with large bowel cancer than in that without large
bowel cancer. The mucosal ODC activity was higher in cases
of multiple tumors (adenocarcinoma plus adenoma) than in
those of solitary tumors (one adenocarcinoma alone) (5).

In gastric carcinogenesis, ODC activity plays an important
role. MNNG, a gastric carcinogen, and bile acids, gastric tumor
promoters, increased ODC activity in the mucosa of the rat
glandular stomach after administration by gastric intubation (7,
8).

Table 5 Correlation between ODC activity of pyloric gland mucosa of gastric
cancer cases and degree of atrophic gastritis

<table>
<thead>
<tr>
<th>Degree of gastric atrophy</th>
<th>Mean ODC activity ± SE (pmol CO₂/h/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (n = 1)</td>
<td>84.5</td>
</tr>
<tr>
<td>Slight (n = 11)</td>
<td>36.7 ± 7.0</td>
</tr>
<tr>
<td>Moderate (n = 15)</td>
<td>47.6 ± 13.3</td>
</tr>
<tr>
<td>Severe (n = 3)</td>
<td>28.0 ± 7.7</td>
</tr>
</tbody>
</table>

There are few reports concerning ODC activity in human
gastric cancer. Lundell and Rosengren (9) reported that there
was ODC activity in gastric cancer tissue, but the enzyme
activity of the normal-appearing mucosa was not mentioned.
In this study of human stomach specimens we found that (a)
gastric cancer tissue had higher ODC activity than the normal-
appearing mucosa with gastric cancer; (b) normal-appearing
mucosa with gastric cancer had higher ODC activity than that
without gastric cancer; (c) in gastric cancer, the mucosa of the
pyloric gland had higher enzyme activity compared with that
of the fundic gland; (d) in some, but not all cancer tissues, and
in gastric cancer mucosae, ODC was activated by GTP.

O'Brien et al. (12) reported that ODC of mouse epidermal
tumor induced by a two-stage carcinogenesis protocol is struc-
turally and functionally different from that transiently induced
by tumor promoter, and they have demonstrated that tumor
ODC is activated by GTP while transiently induced ODC is
not. Hietala et al. (13) reported that in some human colon
cancers ODC is activated by GTP not only in cancer tissue but
also in adjacent normal-appearing mucosa. They attributed this
to a qualitatively different form and suggested that a phenotypic
change occurred in tumor progression. In order to clarify the
properties of stomach ODC, we investigated the effects of GTP
on ODC. In 3 of 3 gastric cancer tissues ODC was activated
to 347.8, 115.9, and 220.2% of the control in the presence of 0.1
mm GTP. In mucosa of the gastric cancer case, ODC in the
fundic gland mucosa was activated by GTP, whereas it was not
in the pyloric gland mucosa. Furthermore, using rat glandular
stomach, the properties of transiently induced ODC and con-
stitutively induced ODC were analyzed. A single gastric intu-
bation of MNNG induced ODC activity in the rat glandular
stomach which reached maximum at 24 h and returned to zero
at 48 h: i.e., transiently induced. Mucosal ODC induced by
continuous administration of MNNG is considered to be con-
stitutively induced. Transiently induced ODC was not affected
by GTP but constitutively expressed ODC was activated (as
was the same in mouse skin carcinogenesis, as described by
O'Brien et al.). Since eukaryotic ODC is not usually activated
by GTP, the ODC in gastric cancer and cancer-bearing stomach
mucosa may be different from usual ODC.

The mechanism of ODC activity elevation in human gastric
cancer is not well understood. Our present rat study demon-
strated that gastric carcinogens induce ODC in rat glandular
stomach mucosa by a single administration but the induction
is transitional and the ODC was not activated by GTP. How-
ever, continuous administration of a gastric carcinogen for 28
weeks, followed by a 16-week carcinogen-free period, induced

Table 6 Correlation between ODC activity in mucosa of gastric cancer and tumor
size, invasiveness, histology, and patients' age

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ODC activity ± SE (pmol CO₂/h/mg protein)</th>
</tr>
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<tbody>
<tr>
<td>Tumor size</td>
<td></td>
</tr>
<tr>
<td>4.9 cm (n = 17)</td>
<td>36.4 ± 4.6*</td>
</tr>
<tr>
<td>≧5.0 cm (n = 13)</td>
<td>57.0 ± 15.2*</td>
</tr>
<tr>
<td>Invasiveness</td>
<td></td>
</tr>
<tr>
<td>Early gastric cancer* (n = 15)</td>
<td>37.9 ± 4.9*</td>
</tr>
<tr>
<td>Advanced gastric cancer* (n = 15)</td>
<td>49.8 ± 13.0*</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
</tr>
<tr>
<td>Diffuse type (n = 14)</td>
<td>45.2 ± 11.4*</td>
</tr>
<tr>
<td>Intestinal type (n = 16)</td>
<td>44.7 ± 8.2*</td>
</tr>
<tr>
<td>Patients' age</td>
<td></td>
</tr>
<tr>
<td>60&lt; (n = 15)</td>
<td>38.4 ± 5.3*</td>
</tr>
<tr>
<td>≧60 (n = 15)</td>
<td>53.0 ± 12.2*</td>
</tr>
</tbody>
</table>

ad: n.s., difference not significant.
*: tumor limited to the mucosa or submucosa.
/: tumor with invasion of more than muscularis propria.
ODC in the mucosa (carcinogen-induced cancer-bearing stomach), which was activated by GTP. An epidemiological study revealed that dietary habits or foods affect gastric carcinogenesis. It may be speculated that repeated administration of carcinogens or tumor-promoting stimuli such as nitroso compounds or salt are concerned in gastric carcinogenesis and ODC is induced in the process and qualitative changes occur as in the rat. In the process of such stimuli ODC gene may be altered. We are now investigating the nature of the change in the ODC gene. More detailed investigation is recommended.

As shown in Fig. 1, about one-third of gastric cancer tissue and gastric cancer mucosa had as low ODC activity as that of non-gastric cancer cases. The reason can be attributed to heterogeneity of the tumor, for in cancer tissues not all cells grow simultaneously, and in fact various values can be found in fragments from the same cancer tissue (data not shown).

There are two distinct clinopathological types of gastric carcinoma, intestinal type and diffuse type (17). The gastric mucosa, the background of gastric carcinogenesis, is composed of the fundic and pyloric glands which are gradually replaced with age by atrophic gastritis (18, 19). Diffuse type gastric cancer arises from the proper gastric gland (fundic and pyloric gland) and the gastric cancer arising from the fundic gland mucosa is the diffuse type. However, intestinal type gastric cancer arises from atrophic gastritis (more or less accompanied by intestinal metaplasia) (19–21). In our study, ODC activity of the pyloric gland mucosa was significantly higher than that of the fundic gland mucosa in gastric cancer cases. Interestingly, ODC activity of fundic gland mucosa was activated by GTP while that of pyloric gland mucosa was not activated in gastric cancer cases. Most of the pyloric gland mucosa used in this study exhibited some atrophic gastritis. Our study failed to reveal a relationship between the severity of atrophic gastritis and the level of ODC activity. In non-gastric cancer cases the enzyme activity was low in mucosae of both the fundic and pyloric glands. It is suggested that the pyloric gland mucosa has higher susceptibility to tumor-promoting stimuli than fundic gland mucosa, or that a different type of tumor promoter is concerned with fundic gland mucosa. This fact is interesting when considering the different histogeneses of intestinal and diffuse type gastric cancers. Further investigation is necessary to clarify this aspect.

In conclusion, ODC activity may be important in human gastric carcinogenesis and measuring the enzyme activity levels of the mucosa may provide an indication of the risk for malignancy.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the technical assistance of Dr. Hirota Fujiki and Dr. Masami Suganuma, the National Cancer Center Research Institute, Tokyo, Japan.

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