Demonstration of a Field Defect in Gastric Intestinal Metaplasia by Biological Marker Analysis

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Abstract

Gastric intestinal metaplasia (GIM) is a precursor lesion for gastric cancer. It most frequently involves the antrum and the angularis. At endoscopy, it is not possible to visually distinguish GIM from normal stomach. Furthermore, GIM frequently has a patchy distribution with areas of metaplasia coexisting with adjacent areas of other histologies, including normal stomach. In this study we sought to determine whether a "field defect" could be demonstrated in subjects with GIM, involving the entire region of the stomach. The biologic markers tested were ornithine decarboxylase (ODC) activity and bromodeoxyuridine labeling index (LI). Antral biopsies were obtained from 13 subjects with known GIM and 9 controls (no GIM based on multiple biopsies and absence of methylene blue staining). Three adjacent biopsies were obtained for ODC, LI, and histology. Group I consisted of a set of 3 biopsies from the 9 controls. In the 13 subjects with GIM, 2 sets of 3 biopsies were taken with methylene blue guidance in an attempt to obtain both GIM-free (group II) and GIM-containing (group III) tissue. ODC activities were markedly and statistically significantly ($P = 0.0001$) elevated in groups II and III *versus* group I; the mean ± SDs were 0.075 ± 0.117 for group I, 1.20 ± 0.83 for group II, and 1.14 ± 0.76 for group III. Group II *versus* Group III values were not different ($P = 0.979$). LI was less discriminatory with more overlap between the groups. The highest LI was in group II, which was significantly different from group I ($P = 0.014$) and group III ($P = 0.006$). LI values expressed as percentages were 3.9 ± 2.0 for group I, 9.5 ± 5.8 for group II, and 4.2 ± 2.8 for group III. Subjects with the gastric cancer precursor lesion GIM have a field defect demonstrated by increased ODC activity, whether or not the actual biopsy specimen assayed contains metaplasia. LI is highest in biopsies lacking metaplasia from subjects with GIM elsewhere but is not as "discriminatory" as ODC activity. Biological marker analysis, particularly reduction in ODC activity, may serve as a useful marker in gastric cancer prevention trials.

Introduction

Although rates of gastric cancer have been declining in Caucasian populations in recent decades, this disease is the most common neoplasm in the world (1). The therapeutic outcome for advanced disease is dismal, and advanced gastric cancer remains an incurable disease. Consequently, prevention, together with methods for detection of early disease, is the most promising approach for reducing morbidity and mortality from gastric cancer.

The most common subtype of gastric cancer is the intestinal type, which accounts for 70% of cases in some populations (2). The currently accepted hypothesis is that gastric carcinogenesis involves a series of histological stages from normal gastric epithelium to intestinal type gastric carcinoma, constituting sequential steps in the process of human gastric carcinogenesis (3). GIM[2] is a histologically identifiable precursor lesion in this pathway. GIM most commonly involves the antrum, angularis, and lesser curve of the stomach in a patchy distribution. At endoscopy it is visually difficult, if not impossible, to identify the patches of GIM among areas of more normal histology in order to target biopsies for diagnosing GIM. The approach often used has been to do multiple, random biopsies of high yield areas, such as the antrum and angularis. More recently, we have developed a technique using *N*-acetylcysteine and methylene blue to stain areas of GIM which could then be targeted for endoscopic biopsy in an effort to improve the yield of biopsies (4). Nevertheless, as is perhaps true for other carcinogen-affected "field cancerization" defects, there is need to develop biological markers that could be used to identify such "fields" in which the histological abnormalities can be patchy. Such markers would not only be useful diagnostically, but could be used to study the modulation of risk during intervention trials with chemopreventive agents.

ODC (EC 4.1.1.17) is the first, and often rate-limiting, enzyme in polyamine biosynthesis (5). The aliphatic polyamines play essential roles in cell proliferation and differentiation. They participate in various aspects of macromolecule synthesis (5, 6). Inhibitors of ODC block aspects of tumor promotion and induce cellular differentiation in numerous animal model systems (6). ODC activity has been shown to be increased in several premalignant conditions that are characterized by diffuse abnormalities in the affected mucosa. For example, elevated ODC levels have been documented in flat mucosa from familial colonic polyposis patients, an inherited condition with a high risk of developing colon cancer (7). We have previously demon-

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[2] The abbreviations used are: GIM, gastric intestinal metaplasia; ODC, ornithine decarboxylase; BrdUrd, bromodeoxyuridine; PCNA, proliferating cell nuclear antigen.

strated increased ODC activity in Barrett's esophagus, a condition with intestinal metaplasia of the esophagus that is associated with an increased risk of developing esophageal adenocarcinoma (8, 9).

Another putative biological marker that has been extensively studied in a variety of different tissues is measurement of the S-phase labeling index with tritiated thymidine or its equivalent, BrdUrd, incorporation. Increased and/or abnormal proliferation have been linked to carcinogenesis and have been reported in some of the precursor lesions for gastric cancer (10–13).

The objective of the present study was to prospectively evaluate the ability of these two biological markers to identify stomachs that harbor GIM in the antrum and angularis.

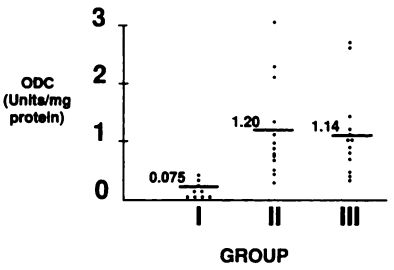

Fig. 1. ODC activity in endoscopic gastric biopsies. *Group I,* biopsies from 9 subjects without GIM; *Group II,* 13 GIM-free biopsies from subjects with GIM elsewhere in the antrum and angularis; *Group III,* 13 GIM-positive biopsies. Mean values for each group are shown (1 unit = 1 nmole $^{14}CO_2$ released/mg protein/h).

Materials and Methods

Subjects. The study population consisted of 22 subjects scheduled to undergo upper gastrointestinal endoscopy for evaluation of dyspepsia or as part of an ongoing natural history study of patients with GIM. There were 21 males and 1 female reflecting the demographics of a Veterans Affairs hospital. Mean age was 64 years with a range of 51–79 years. Nine subjects, all male, had no GIM, demonstrated by absence of the lesion in specimens obtained using a systematic sequence of biopsies from the entire stomach, which included a minimum of four biopsies from the antrum and angularis. Absence of GIM was also judged by absence of any methylene blue staining using methods described previously (4). Thirteen subjects (12 male, 1 female) had GIM in the antrum. Adjacent biopsies for BrdUrd labeling and ODC analysis were taken from the region of the angularis in the 9 GIM-free cases. In the 13 GIM-positive cases biopsies were also obtained from sites in the vicinity of the angularis. Both GIM-containing and GIM-free specimens were obtained using methylene blue staining for guidance. An extra biopsy was obtained from a site immediately adjacent to the sets for laboratory analysis in all cases in order to confirm the histology present at that location. GIM was histologically evaluated using standard criteria including alcian blue staining (14). The clinical data and identity of the specimens was unknown to the laboratory, thereby assuring blinded conduct of the assays. The study protocol was approved by the Institutional Human Subjects Committee and written informed consent was obtained from each participant.

BrdUrd Labeling Index. The labeling procedure used was based on standard, published methodology involving a 1-h incubation in BrdUrd medium in a 95% oxygen/5% carbon dioxide atmosphere (11, 15). Thin sections, 3 μm thick, were stained with anti-BrdUrd monoclonal antibody (Anti BrdU; Becton Dickinson, San Jose, CA), followed by immunoperoxidase development. Slides were counterstained with dilute Harris hematoxylin. The sections were counted at × 400 with epithelial cells lining pits being evaluated longitudinally from mucosal surface to glands and noted as labeled or unlabeled. The labeling index was expressed as a percentage of labeled over total cells counted.

ODC Assay. ODC activity was assayed using methods described in detail elsewhere (16). Briefly, specimens were placed in ice-cold buffer [25 mM Tris-HCl (pH 7.5), 2.5 mM dithiothreitol, 0.1 mM EDTA]. After centrifugation, the supernatant was removed, kept on ice, and assayed within 5 min. An aliquot of tissue supernatant (100 μl) was added to a radiolabeled ^{14}C-ornithine reaction mixture and lightly vortexed. The tubes were capped and the reaction allowed to proceed at 37°C for 30 min. The released labeled $^{14}CO_2$ was trapped on a Whatman filter paper disk and counted using a Beckman model LS 1801 liquid scintillation counter. The results were normalized to protein content. Each specimen was assayed in triplicate with the readings being within 10% of one another. All biopsies were stored immediately in a −70°C freezer within 10 min of collection and assayed within 8 weeks. One unit of ODC activity is defined as 1 nmol $^{14}CO_2$ released per 60 min with the values being normalized to protein content.

Statistical Analysis. The ODC and labeling index values in the different groups were analyzed by the Kruskal-Wallis test (17). The Wilcoxon rank sum test was used for comparison of pairs of groups with the *P* value adjusted for multiple comparisons using the Bonferroni procedure (18).

Results

Biopsy Specimens. There were 3 groups of biopsies obtained from the corresponding subjects and sites. Group 1 consisted of 9 sets obtained from the 9 GIM-free subjects. The histology was "normal" in 6 and "mild chronic gastritis" in 3. Group 2 consisted of the 13 GIM-free sets from subjects known to have GIM elsewhere in the antrum and angularis region. Histological diagnosis at these biopsy sites were "normal" in 4 cases and showed "chronic gastritis" in the remaining 9. Group 3 consisted of 13 GIM-containing sets from the subjects known to have GIM. Overall, there were no differences in either ODC activity or BrdUrd labeling index among the GIM-free biopsies that were considered normal or chronic gastritis. Consequently these are not separated further.

Ornithine Decarboxylase Activity. ODC activity in groups 2 and 3 were significantly higher than in group 1 (P = 0.0001; Fig. 1 and Table 1). The mean values of groups 2 and 3 were an order of magnitude greater than group 1. Overlap between group 1 and the rest of the biopsies was minimal (Fig. 1). There was no significant difference between groups 2 and 3 (P = 0.979), *i.e.,* biopsies obtained from a subject known to have GIM displayed increased ODC activity, whether the actual biopsy specimen assayed contained GIM (group 3) or was free of GIM (group 2).

BrdUrd Labeling Index. The BrdUrd labeling indices of the three groups are shown in Fig. 2 and Table 1. In two biopsies from group 1, an adequate BrdUrd study could not be performed because of improper specimen orientation

Table 1 ODC and BrdUrd labeling index in gastric intestinal metaplasia

ODC values of group 2 *vs.* 3 were not statistically significantly different, nor were the labeling index values of Group 1 *vs.* 3.

Group	Biopsy (No.)	ODC units[a] (range)	LI[b] % (range)
1	IM^- (9) (from IM-free subjects)	0.075 ± 0.117 (0–0.342)	3.9 ± 2.0 (2.2–8.0)
2	IM^- (13) (from subjects with IM)	1.20 ± 0.83[c] (0.303–3.11)	9.5 ± 5.8[d] (1.9–23.7)
3	IM^+ (13)	1.14 ± 0.76[c] (0.348–2.71)	4.2 ± 2.8 (1.2–9.4)

[a] 1 unit = nmol $^{14}CO_2$/mg protein/h; mean ± SD.
[b] LI, labeling index; IM^- (9), GIM-free biopsies from 9 subjects without any GIM; IM^- (13), GIM-free biopsies from 13 subjects with GIM elsewhere in the antrum; IM^+ (13), GIM-containing biopsies from 13 subjects with antral GIM.
[c] $P = 0.0001$ *vs.* group 1.
[d] $P = 0.014$ *vs.* group 1 and 0.006 *vs.* group 3.

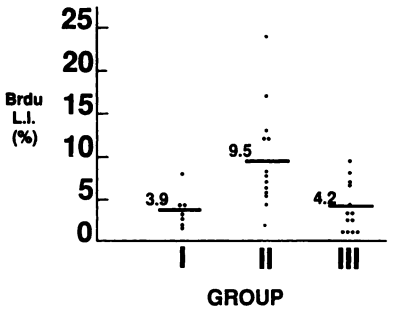

Fig. 2. BrdUrd-labeling indices in endoscopic gastric biopsies. *Group I,* biopsies from 9 subjects without GIM, however 2 were inadequate for labeling index measurement; *Group II,* 13 GIM-free biopsies from subjects with GIM elsewhere in the antrum and angularis; *Group III,* 13 GIM-positive biopsies. Mean values are shown.

and preparation. The highest BrdUrd-labeling indices were found in group 2, *i.e.,* in GIM-free biopsies obtained from a field harboring GIM ($P = 0.014$ *versus* group 1 and 0.006 *versus* group 3). Overall, however, BrdUrd-labeling index was less discriminatory with considerably more overlap than ODC activity.

Discussion

In this blinded study, ODC activity measured in endoscopic biopsies obtained from the antrum in the region of the angularis was found to be statistically significantly and markedly increased in subjects with GIM compared with GIM-free controls. Increased ODC was present whether the actual biopsy specimen assayed contained GIM or not, a finding of considerable clinical importance since it raises the possibility of determining the likelihood of presence of GIM in the gastric antrum and angularis based on random biopsies from a readily and reproducibly identifiable region, *i.e.,* the angularis. These data support the concept of a field defect affecting the entire antral and angularis area of the stomach when GIM is present, even though the histological changes can have a patchy distribution.

An increase in ODC activity in gastric atrophy and intestinal metaplasia biopsies has been reported recently (19, 20). Additionally, Nakanishi *et al.* (21) have described elevated ODC activity in the mucosa of gastric remnants following surgery. Our study results are in general agreement with these reports but extend the findings by including an analysis of GIM-free biopsies from subjects with GIM, thereby showing the existence of a "field defect."

Although ODC activity is often correlated with the degree of proliferative activity, we were unable to demonstrate a similar level of discrimination using BrdUrd-labeling index for proliferative activity assessment. One possible explanation for this might be that BrdUrd labeling only identifies cells in the S phase of the cell cycle and consequently will miss other proliferating cells that may not be in S phase. We are in the process of testing this by using procedures such as PCNA staining, a procedure that will identify most proliferating cells (22). Recent data suggests that PCNA labeling is increased in GIM, especially when Helicobacter pylori infection is also present (23). However, there are no studies of GIM-free tissues from a GIM-involved area or correlations with ODC activity.

The highest BrdUrd-labeling indices were found in GIM-free biopsies obtained from a region containing GIM (group 2). These biopsies also displayed the greatest amount of surface cell labeling, as well as increased labeling of compartments closer to the surface, than the normal biopsies from group 1 (GIM-free subjects) or the GIM biopsies from group 3 (data not shown). Although the overall BrdUrd-labeling index of group 2 was higher than that of group 3, their ODC activities were similar. Once again, comparisons using other measures of proliferation, such as PCNA, would be of interest.

The low level of ODC activity in normal stomach, or that showing only mild chronic gastritis, is consistent with our previous experience when comparing this tissue with another preneoplastic lesion, Barrett's specialized columnar epithelium (8, 9). In our experience thus far, normal upper gastrointestinal epithelium from squamous esophagus, stomach, or small bowel has consistently shown extremely low levels of ODC activity (8, 9). The values are often undetectable and are almost always less than 0.2–0.3 units/mg protein. Increased ODC activity, perhaps reflective of increased cell growth and turnover, may be a property of histological abnormalities associated with increased risk of neoplasia. Consequently, reduction in ODC activity in a group of subjects treated with chemopreventive approaches may be useful as an intermediate marker for decreasing cancer risk.

Recently, H. pylori infection has been associated with gastric carcinogenesis (24, 25). An increased incidence of H. pylori infection has also been found in patients with GIM (26, 27). We did not perform H. pylori stains in this study and it is very possible that diffuse H. pylori infection may be responsible for the increased ODC activity. Thus, since H. pylori is often present in areas adjacent to GIM, it would offer one explanation for our findings. Consequently, it would be of interest to determine whether H. pylori eradication would lower ODC activity, which may occur with or without altering the histological changes of GIM.

In summary, ODC activity was found to be markedly elevated in gastric antral biopsies from subjects with GIM, independent of whether the biopsies contained GIM or not. Therefore, ODC activity may prove to be a useful marker for identifying and following subjects with GIM of the gastric antrum and angularis, the region of the stomach most frequently affected by intestinal metaplasia. These data support the hypothesis that a field defect probably exists in the entire antral region of these stomachs, despite the fact that the histological change may have a patchy distribution.

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