HELCOBACTER PYLORI INFECTION

Vitamin C inhibits corpus gastritis in Helicobacter pylori-infected patients during acid-suppressive therapy

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Abstract

Background: Previous studies have shown that gastric acid suppression worsens corpus gastritis in Helicobacter pylori (H. pylori)-positive patients. We evaluated the effect of acid-suppressive therapy and vitamin C on H. pylori-associated gastritis.

Methods: Forty patients with reflux esophagitis were divided into three groups by the status of H. pylori and therapy: group A (n = 15), H. pylori (+) and omeprazole 20 mg; group B (n = 15), H. pylori (+) and omeprazole 20 mg + vitamin C 1200 mg; and group C (n = 10), H. pylori (−) and omeprazole 20 mg. In all three groups, the mucosal interleukin (IL)-8 contents, H. pylori colonization density, neutrophil infiltration in the corpus, and serum gastrin were evaluated at entry and 2 weeks after starting therapy; in group B, serum vitamin C levels were also measured.

Results: In group A, the IL-8 contents and the degree of neutrophil infiltration during therapy exceeded those at entry, whereas in groups B and C, these values did not change significantly with treatment. Helicobacter pylori colonization density during therapy was similar to that at entry in all three groups. The serum gastrin (in all groups) and vitamin C levels (in group B) during therapy exceeded those at entry.

Conclusions: Potent acid suppression worsens H. pylori-associated corpus gastritis, although such worsening gastritis may be inhibited by vitamin C.

Key words: gastritis, Helicobacter pylori, interleukin-8, omeprazole, vitamin C.

INTRODUCTION

In Helicobacter pylori (H. pylori)-associated gastritis, mucosal neutrophil infiltration has been shown to be enhanced.¹ Recent reports have shown that attachment of the organisms to the gastric epithelial cell surface activates the transcription factor, nuclear factor kappa B (NF-kappa B), eventually inducing mucosal interleukin (IL)-8 production, and that IL-8 production is correlated with the degree of neutrophil infiltration and histological severity in H. pylori-associated gastritis.²-⁴

Gastric acid-suppressive therapy has recently been reported to be associated with increased severity of corpus gastritis caused by H. pylori.⁵ Our preliminary study showed that mucosal IL-8 concentrations were enhanced during acid-suppressive therapy, although the mechanism of this enhancement remains unclear.⁶ Reactive oxygen intermediates (ROI) activate NF-kappa B to modulate IL-8 expression in the gastric mucosa, and vitamin C can act as a scavenger of ROI in patients with H. pylori infection.⁷-⁹

We therefore evaluated the effects of acid-suppressive therapy with or without a vitamin C supplement on the mucosal IL-8 levels, H. pylori colonization density, and on the degree of neutrophil infiltration.
METHODS

Patients
The 40 patients chosen for the present study all met the following criteria: they had experienced heartburn for at least 1 month, without any severe underlying diseases; they had been diagnosed with reflux esophagitis, which was grade B according to the Los Angeles classification by baseline endoscopy at entry; they had been assessed for \textit{H. pylori} positivity at diagnosis by means of both rapid urease testing and histological analysis of gastric biopsy specimens; and they had received no form of acid-suppressive therapy, antibiotics, bismuth-containing medication, or non-steroidal anti-inflammatory drugs in the month before entry. Two hours after performing baseline endoscopy, 30 patients showed positive findings in both the rapid urease testing and histological examination for \textit{H. pylori} assessment. These 30 patients were randomly divided into two gender- and age-matched groups (groups A, B). The remaining 10 patients (group C) showed negative results in both the examinations for \textit{H. pylori} assessment. All patients gave their informed consent for participation in the present study. Their clinical characteristics are summarized in Table 1.

Study design
At entry, biopsy specimens were obtained under baseline upper gastrointestinal endoscopy and fasting blood samples were obtained after the endoscopy from all 40 patients. Following these procedures, patients were divided into three groups (groups A, B, C) as described above. That is, patients in groups A and C were treated with omeprazole 20 mg per day, while patients in group B were treated with omeprazole 20 mg + vitamin C 1200 mg per day. Two weeks after starting the therapy, the examinations performed at entry were performed a second time. The mucosal IL-8 contents, \textit{H. pylori} colonization density, degree of neutrophil infiltration, and serum gastrin concentrations were evaluated in all three groups, and in group B the serum vitamin C levels were also examined. This protocol was approved by the ethical committee of the Fukuoka Teishin Hospital.

Endoscopic procedures
All endoscopies were performed under local anesthesia by using a videoscope (EVE-200; Fujinon, Tokyo, Japan). At entry, seven biopsy samples were obtained from the greater curvature of the upper site of the corpus (50 cm from the incisors). One sample was used for the rapid urease testing, three for the mucosal IL-8 assessments, two for histological examinations; and the remaining sample for the \textit{H. pylori} culture in groups A and B. On the second examination, five samples were obtained from the same location on the corpus. Three of these samples were used for the mucosal IL-8 assessments; one for the histological examinations; and one for the \textit{H. pylori} culture in groups A and B.

Serum gastrin and vitamin C level evaluation
By using fasting blood samples, serum gastrin levels were measured by using radioimmunoassay in all three groups. Serum vitamin C contents were also evaluated by using high performance liquid chromatography (HPLC) in group B.

Measurement of mucosal interleukin-8
Three biopsy samples were immediately stored at \(-20^\circ\text{C}\), and homogenized in 3 mL phosphate-buffered saline (pH 7.4) for 1 min using an ultra turrax homogenizer. Supernatants obtained by centrifugation (150 g for 30 min) were frozen at \(-70^\circ\text{C}\) in polypropylene tubes until assayed. Supernatants of homogenates were measured for IL-8 levels by using enzyme-linked immunosorbent assay (ELISA). This assay uses the quantitative immunometric ‘sandwich’ enzyme immunoassay technique. Microtiter plates (Maxisorp 96-well; Nunc, Roskilde, Denmark) were coated with 0.5 mg/mL rabbit antihuman IL-8 polyclonal antibody (Toray Fuji Bionics, Tokyo, Japan) in 100 µL phosphate-buffered saline (PBS) at 4°C overnight and blocked with 0.5% bovine serum albumin (BSA) in PBS at 25°C for 2 h. The plates were washed with 400 µL washing solution (twofold diluted PBS containing 0.025% Tween 20), followed by the addition of

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinical characteristics for three patient groups</th>
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</thead>
<tbody>
<tr>
<td>Characteristic</td>
<td>A ( (n=15) )</td>
</tr>
<tr>
<td>Age (years, mean ± SD)</td>
<td>49.7 ± 14.3</td>
</tr>
<tr>
<td>Gender (Male/Female)</td>
<td>10/9</td>
</tr>
<tr>
<td>Status of \textit{Helicobacter pylori}</td>
<td>Positive</td>
</tr>
<tr>
<td>Acid-suppressive drug</td>
<td>Omeprazole</td>
</tr>
<tr>
<td>Vitamin C supplement</td>
<td>Negative</td>
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</tbody>
</table>

Acid-suppressive therapy was done with omeprazole 20 mg/day.
100 μL of reaction buffer (0.1 mol/L Tris-HCl, pH 8.0, containing 0.25% BSA, 0.05% Tween 20, and 0.5% each of normal mouse and rabbit serum). Duplicate 50 μL aliquots of samples or standard IL-8 were added and incubated at 25°C for 1 h. The plate was shaken on a microplate mixer throughout the incubation time. After washing, 0.5 μg/mL mouse anti-IL-8 monoclonal antibody (Toray Fuji Bionics, Tokyo, Japan), which was labeled with horseradish peroxidase (Boehringer Mannheim, Mannheim, Germany), in 100 μL of PBS containing 0.25% BSA, 0.05% Tween 20 and 0.3 mol/L NaCl was sequentially added and incubated at 25°C for 30 min. After three washing steps, 100 μL of the substrate solution (0.1 mol/L sodium acetate-citrate containing 0.006% H2O2 and 0.2 mg/mL 3,3′,5,5′-tetramethylbenzidine, pH 5.5) was added and incubated at 25°C for 30 min. Finally, the colorimetric reaction was stopped by adding 100 μL of 1 N H2SO4, and the absorbance at 450 nm (reference at 595 nm) was measured. The IL-8 concentration of each sample was derived by comparing the optical density of the sample with the standard curve. This assay accurately measures natural, recombinant endothelial cell-derived, and monocyte-derived human IL-8, with no measurable cross-reactivity to other cytokines. The detection limit of this assay was 12.5 pg/mL, and the inter/intra-assay variability was less than 6%.

**Statistical analysis**

Values were given as mean ± SEM. The two-tailed Mann–Whitney U-test was used for unpaired comparisons between groups. For paired comparisons before and during the therapy, the two-tailed Wilcoxon signed rank tests were used. A value of P < 0.05 was accepted as being statistically significant.

**RESULTS**

**Effects of omeprazole with or without vitamin C supplementation**

Figure 1 shows that the mucosal IL-8 contents during therapy were compared with those at entry in groups A, B and C. In group A, the mucosal IL-8 contents during therapy significantly exceeded those at entry (45.1 ± 7.5

![Figure 1](image)

**Histological examinations**

On the examination at entry, two biopsy specimens were fixed in formalin and used for the histological examination of *H. pylori* (Giemsa staining) and the degree of neutrophil infiltration (hematoxylin-eosin staining). On the second examination, one specimen was used for evaluating the degree of neutrophil infiltration. Neutrophil infiltration (activity) was assessed on a scale of four grades: 0, 1, 2 and 3, corresponding to none, mild, moderate, and severe activity, according to the Sydney system. This evaluation was made without knowledge of the endoscopic diagnosis, *H. pylori* status or mucosal IL-8 contents.

**Helicobacter pylori colonization**

After homogenizing, one biopsy specimen was spread out with an applicator and placed in three successive cultures (No. 1–3). Each of the cultures consisted of Colombia blood agar mediums (pH 7.4; Oxoid, London, UK) containing 5% horse blood, vancomycin (10 μg/mL), amphotericin B (5 μg/mL), cefsulodin (5 μg/mL), and trimethoprim (5 μg/mL), and were incubated at 37°C under microaerophilic conditions (5% O2, 10% CO2 and 85% N2) for up to 7 days. The organisms were identified as *H. pylori* by Gram staining, colony morphology, positive oxidase, catalase, and urease reactions. The *H. pylori* colonization density was scored and graded from 0 to 4 as follows: 0 (no colonies); 1 (≤10 colonies in culture no. 1); 2 (colonizing throughout culture no. 1); 3 (colonizing throughout cultures no. 1 and 2); and 4 (colonizing in all cultures).


**Table 2** Comparison of neutrophil infiltration at entry and 2 weeks after starting acid-suppressive therapy in three patient groups

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Neutrophil infiltration</th>
<th>T=0 weeks</th>
<th>T=2 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.2 ± 0.9</td>
<td>1.9 ± 0.2*</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1.3 ± 0.2</td>
<td>1.4 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.2 ± 0.2</td>
<td>0.6 ± 0.4</td>
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*P<0.05; T=0 versus T=2 weeks.

**Table 3** Comparison of serum gastrin levels at entry and 2 weeks after starting acid-suppressive therapy in three patient groups

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Gastrin (pg/mL)</th>
<th>T=0 weeks</th>
<th>T=2 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>100.8 ± 12.2</td>
<td>267.5 ± 88.9*</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>109.7 ± 11.8</td>
<td>290.0 ± 121.1*</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>39.5 ± 1.5</td>
<td>124.8 ± 20.4*</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05; T=0 versus T=2 weeks.

Vitamin C and corpus gastritis

**Table 4** Comparison of serum vitamin C levels at entry and 2 weeks after starting acid-suppressive therapy in one patient group

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Vitamin C (µg/mL)</th>
<th>T=0 weeks</th>
<th>T=2 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>3.76 ± 0.88</td>
<td>7.64 ± 1.26*</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05; T=0 versus T=2 weeks.

**DISCUSSION**

It remains a subject of controversy whether acid-suppressive therapy worsens the histological features of corpus or fundus gastritis in *H. pylori*-infected patients with reflux esophagitis, peptic ulcer or gastric erosions. Most previous studies have discussed this issue solely from a histological viewpoint. In the present study, we evaluated the mucosal neutrophil-associated cytokine levels in addition to morphological findings. The present study clearly showed that omeprazole enhanced mucosal IL-8 contents and neutrophil infiltration in the corpus of *H. pylori*-positive patients.

One possible explanation of these results reported is that the suppression of acid production may have caused an increase in *H. pylori* colonization because acid-suppression has previously been shown to increase bacterial colonization in the corpus. However, *H. pylori* colonization during the acid-suppressive therapy was similar to that at entry in the present study. This finding indicates that the mechanism by which omeprazole exerts its effects on corpus gastritis may not be associated with the pattern of *H. pylori* colonization.

The present study showed that vitamin C inhibited the enhancement of the mucosal IL-8 contents and neutrophil infiltration during acid suppression in *H. pylori*-infected patients. Vitamin C has been shown to act as a scavenger of mucosal ROI in *H. pylori*-infected patients. The ROI activate NF-kappa B to modulate IL-8 expression in the gastric mucosa. Based on these results, the present study may indirectly suggest that the levels of gastric mucosal ROI are enhanced during acid suppression in *H. pylori*-infected patients. Previous studies have indicated that acid suppression induces the overproduction of ammonia (NH₃) in the gastric mucosa of *H. pylori*-positive patients, and that NH₃ has introduced neutrophils to produce ROI. Omeprazole might cause an overproduction of NH₃ in *H. pylori*-infected patients, and this surplus of NH₃ could pass through the gastric epithelium and stimulate neutrophils in the mucosa, ultimately leading to the enhanced production of ROI. Further examinations will be needed to prove this hypothesis.

We did not directly measure vitamin C concentrations in biopsy samples. To the best of our knowledge, three previous studies have evaluated the effect of high-dose vitamin C oral administration (≥1 g/day) on *H. pylori*-infected patients. These studies have shown that after a high dose of vitamin C administration, the gastric mucosal or gastric juice vitamin C levels were elevated parallel with the serum vitamin C levels. In our
preliminary study, mucosal vitamin C levels have been enhanced following vitamin C administration in *H. pylori*-infected patients. Moreover, we measured the mucosal IL-8, neutrophil infiltration, *H. pylori* colonization density, and serum gastrin levels at entry and 2 weeks after starting omeprazole therapy without administration of vitamin C in group B. In this trial, we found worsening gastritis during acid-suppression in group B patients (data not shown). This result was similar to that found in group A. These findings suggest that the high-dose vitamin C administration may enhance the gastric mucosal vitamin C levels and suppress the corpus gastritis during acid-suppression in *H. pylori*-infected patients. Moreover, in our preliminary evaluation, the serum vitamin C levels in groups A and C during acid-suppressive therapy were similar to those at entry (data not shown).

In conclusion, the present study showed that omeprazole enhanced the mucosal IL-8 contents and neutrophil infiltration in *H. pylori*-infected patients, and that vitamin C inhibited these enhancements. These findings suggest that potent gastric acid suppression may worsen corpus gastritis in *H. pylori*-infected patients, and that vitamin C administration may prevent this effect.

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REFERENCES