Effect of acid-suppressive therapy on *Helicobacter pylori* production of interleukin-8 in the gastric mucosa

Masahiro Yoshinaga MD¹, Akira Ohtani MD¹, Satoru Tsuruta MD¹, Tetsuya Kato MD¹, Eishi Higashi MD¹, Yukio Yamada MD¹, Naohiko Harada MD², Hajime Nawata MD²

BACKGROUND: Recent studies have shown that acid-suppressive therapy increases the severity of *Helicobacter pylori*-associated gastritis in the corpus.

PURPOSE: To evaluate interleukin (IL)-8 production in the gastric corpus mucosa before and during acid-suppressive therapy in *H pylori*-infected patients.

PATIENTS AND METHODS: Ten patients with reflux esophagitis (five *H pylori*-positive and five *H pylori*-negative) were treated with omeprazole 20 mg. Serum gastrin concentrations, *H pylori* colonization density and mucosal IL-8 levels in the corpus were investigated at entry and two weeks after starting therapy. IL-8 levels were measured by ELISA. The organism density was determined, and scored according to area occupied by the bacterial colonies. The presence of *H pylori* was assessed by rapid urease testing and histological finding of gastric biopsy specimens.

RESULTS: In *H pylori*-positive patients, concentrations of IL-8 during therapy significantly exceeded those before therapy (36.2±6.8 versus 18.3±3.8 pg/mg protein; P<0.05) without altering *H pylori* density. In *H pylori*-negative patients, IL-8 levels were similar before and during therapy (6.1±2.7 versus 6.3±3.0 pg/mg protein). Concentrations of gastrin during therapy were significantly higher than those before therapy in all patients.

CONCLUSIONS: The results suggest that acid suppression increases mucosal IL-8 levels in *H pylori*-infected patients with reflux esophagitis.

Key Words: Helicobacter pylori; Interleukin-8; Proton pump inhibitor; Reflux esophagitis

Effet du traitement antiacide sur la production d’interleukine-8 dans la muqueuse gastrique dans les cas d’infection à *Helicobacter pylori*

CONTEXTE: Des études récentes montrent que le traitement antiacide accroît la gravité de la gastrite associée à *Helicobacter pylori* dans la muqueuse du corps de l’estomac.

voir page suivante

¹Department of Internal Medicine, Chikuhou Hospital; ²Third Department of Internal Medicine, Faculty of Medicine, Kyushu University, Fukuoka, Japan

Correspondence and reprints: Dr Masahiro Yoshinaga, Department of Internal Medicine, Fukuoka Teishin Hospital, 2-6-11 Yakuin, Fukuoka 810-8798, Japan. Telephone +81-92-741-3315, fax +81-92-781-2563, e-mail m-yoshi@winter.try-net.or.jp

Received for publication November 17, 1998. Accepted April 28, 1999
Helicobacter pylori infection elicits an infiltration of gastric mucosa with neutrophils, eosinophils, lymphocytes and plasma cells (1,2). This constellation of inflammatory responses constitutes chronic active gastritis and may remain clinically silent or progress to create the background for the development of diseases such as peptic ulcer, gastric adenocarcinoma or primary gastric B cell lymphoma (1,2). Interleukin (IL) -8 has been reported to be a factor causing neutrophil chemotaxis and activation (3). Previous studies have indicated that gene expression and the production of IL-8 are enhanced in H pylori-infected patients with chronic gastritis, duodenal ulcer or nonulcer dyspepsia (NUD) (4-8). Moreover, this IL-8 concentration is correlated with the degree of neutrophil infiltration and histological severity in H pylori-associated gastritis (7,9).

Recent studies have revealed that profound suppression of gastric acid is associated with increased severity of corpus gastritis caused by H pylori (10) and have suggested that patients with reflux esophagitis and H pylori infection treated with omeprazole are at increased risk of atrophic gastritis (11). However, these studies were evaluated in terms of the gastric histological features. The effect of acid suppression on H pylori-associated mucosal production of IL-8 in the corpus is not clear.

We, therefore, obtained the corpus mucosa from H pylori-infected patients with acid-related disorders before and during acid-suppressive therapy, and evaluated the effect of acid-suppressive therapy on the concentrations of H pylori-produced IL-8 in the corpus mucosa.

PATIENTS AND METHODS

Patients: Five H pylori-positive patients were chosen for this study with the following criteria: complaints of heartburn for longer than one month; diagnosed with reflux esophagitis by baseline endoscopy; H pylori assessed at diagnosis by rapid urease testing and histological finding of gastric biopsy specimens; and taking no form of acid-suppressive therapy, antibiotics, bismuth-containing medication or nonsteroidal anti-inflammatory drugs in the month before the entry. Patients ranged in age from 31 to 68 years (mean age 49.4 years). Three patients were male (60%), and all patients had positive findings in both a rapid urease testing and histological examination (Giemsa staining) for H pylori assessment.

BUT : Évaluer la production d’interleukine-8 (IL-8) dans la muqueuse du corps gastrique avant et durant le traitement antacidie chez les patients infectés à H. pylori.

MÉTHODE : Dix patients souffrant d’oesophagite peptique (cinq à H. pylori positif et cinq à H. pylori négatif) ont été traités à l’aide d’omeprazole, 20 mg. On a mesuré la concentration de gastrine dans le sang, la densité de colonisation par H. pylori et le taux d’IL-8 dans la muqueuse du corps au début du traitement et deux semaines plus tard. Le taux d’IL-8 a été déterminé à l’aide du test ELISA ; la densité du micro-organisme a été notée selon la région occupée par les colonies de bactéries et la présence d’H. pylori a été évaluée par une analyse rapide de l’uréase et des observations histologiques des échantillons de tissu gastrique prélevés à la biopsie.

RÉSULTATS : Chez les patients à H. pylori positif, le taux d’IL-8 durant le traitement dépassait largement le taux mesuré avant le traitement (36.2 pg/mg ± 6.8 c. 18.3 pg/mg ± 3.8; P<0.05), sans modification de la densité d’H. pylori. Chez les patients à H. pylori négatif, ce taux est resté stable avant et durant le traitement (6.1 pg/mg ± 2.7 c. 6.3 pg/mg ± 3.0). La concentration de gastrine s’est montrée passablement plus élevée durant le traitement qu’avant chez tous les patients.

CONCLUSION : Les résultats donnent à penser que la suppression d’acide augmente la concentration d’IL-8 dans la muqueuse gastrique chez les patients infectés à H. pylori souffrant d’oesophagite peptique.
density score was defined as zero if the H pylori colonization was negative. Scores of 1 to 3 were defined as follows: a score of 1 was given if the H pylori colonies comprised less than half of the whole culture; a score of 2 was given if the colonies occupied an area equal to or greater than half and less than three-quarters of the whole culture; and a score of 3 was given if the H pylori colonies occupied an area equal to or greater than three-quarters of the whole culture. In the preliminary evaluation, the number of H pylori (the colony forming unit/mL) was 10² in those given a score of 1, 10³ in those given a score of 2 and 10⁶ in those given a score of 3.

Measurement of mucosal IL-8: Three biopsy samples were immediately stored at −20°C and homogenized in 3 ml phosphate-buffered saline (PBS) (pH 7.4 for 1 min using an Ultra Turrax homogenizer (Janke & Kunkel Co, Staufen, Germany). Supernatants obtained by centrifugation (1500 × g for 30 mins) were frozen at −70°C in polypropylene tubes until assayed. Supernatants of homogenates were measured for IL-8 levels by ELISA according to the methods established by Ida et al (12). This assay uses the quantitative immunometric ‘sandwich’ enzyme immunoassay technique. Microtitre plates (Maxisorp 96-well, Nunc, Denmark) were coated with 0.5 µg/mL rabbit anti-human IL-8 polyclonal antibody (Toray Fuji Bionics, Tokyo, Japan) in 100 µL 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 100 µL of reaction buffer (0.1 M 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) labelled with horseradish peroxidase (Boehringer Mannheim, Mannheim, Germany) in 100 µL of PBS containing 0.25% BSA, 0.05% Tween 20 and 0.3 M sodium chloride was sequentially added and incubated at 25°C for 30 mins. After three washing steps, 100 µL of the substrate solution (0.1 M sodium acetate-citrate containing 0.006% hydrogen peroxide and 0.2 mg/mL 3,3′,5,5′-tetramethylbenzidine, pH 5.5) was added and incubated at 25°C for 30 mins. Finally, the colorimetric reaction was stopped by adding 100 µL of 1 N sulphuric acid, 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 are given as means ± SE. The two-tailed Mann-Whitney U test was used for unpaired comparisons between groups. For paired comparisons before and during acid-suppressive therapy, two-tailed Wilcoxon signed rank tests were used. A value of P<0.05 was accepted as statistically significant.

RESULTS

Table 1 shows the comparative levels of serum gastrin and mucosal IL-8 in the corpus between two groups of patients. The mucosal levels of IL-8 in H pylori-positive patients significantly exceeded those in H pylori-negative patients (18.3±3.8 versus 6.1±2.7 pg/mg protein; P<0.05). The levels of serum gastrin in H pylori-positive patients also significantly exceeded those in H pylori-negative patients (100.8±12.2 versus 39.5±1.5 pg/mL; P<0.05).

Figures 1 and 2 illustrate the comparative concentrations of mucosal IL-8 before acid-suppressive therapy (0 WK) with those during therapy (2 WKS) in Helicobacter pylori-positive patients. Concentrations of IL-8 during therapy significantly exceeded those before therapy (P<0.05).

Table 1: Comparison of mucosal interleukin (IL)-8 contents and serum gastrin levels in Helicobacter pylori-positive patients and in H pylori-negative patients.

<table>
<thead>
<tr>
<th></th>
<th>H pylori-positive</th>
<th>H pylori-negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8 (pg/mg protein)</td>
<td>18.3±3.8*</td>
<td>6.1±2.7</td>
</tr>
<tr>
<td>Gastrin (pg/mL)</td>
<td>100.8±12.2*</td>
<td>39.5±1.5</td>
</tr>
</tbody>
</table>

*P<0.05 H pylori-positive versus -negative
The frequency of \textit{H pylori} colonization in the corpus during acid-suppressive therapy was compared with that before therapy in Figure 3. The median \textit{H pylori} density score was 1.5 (range 1.0 to 2.0) before acid-suppressive therapy. During therapy, the \textit{H pylori} score was 1.0. This degree of remission of \textit{H pylori} density was not statistically significant.

Table 2 compares serum gastrin levels at entry with those two weeks after starting acid-suppressive therapy in \textit{H pylori}-negative and -positive patients.

Concentrations of gastrin during acid-suppressive therapy significantly exceeded those before therapy in two groups of patients (470.0±67.3 versus 100.8±12.2 pg/mL in \textit{H pylori}-positive patients [P<0.05]; 124.8±20.4 versus 39.5±1.5 pg/mL in \textit{H pylori}-negative patients [P<0.05]).

**DISCUSSION**

In the present study, levels of gastric mucosal IL-8 and serum gastrin in \textit{H pylori}-positive patients exceeded those in \textit{H pylori}-negative patients. This result agrees with that found in previous studies (4-7,9,13) and shows the accuracy of measurement of the mucosal IL-8 contents in the present study.

The main finding of the present study is that acid-suppressive therapy increases mucosal IL-8 levels in the corpus of \textit{H pylori}-infected patients with reflux esophagitis. To our knowledge, no studies have directly shown the increased production of gastric mucosal IL-8 in the corpus during acid-suppressive therapy in \textit{H pylori}-infected patients. We have not evaluated whether neutrophil numbers were increased according to the increased mucosal IL-8 content in this study. However, previous studies have already shown that acid-suppressive therapy, consisting of proton pump inhibitor administration, worsens the histological features of corpus or fundus lesions in \textit{H pylori}-infected patients with reflux esophagitis, peptic ulcer or gastric erosions (10,14-16). The present study indicates that the previous reported neutrophil chemotaxis is accelerated by increasing the mucosal IL-8 contents.

The \textit{H pylori} density in the corpus during acid-suppressive therapy or under conditions of acid hyposecretion remains to be evaluated. Previous studies indicate that the frequency of \textit{H pylori} in the corpus during acid-suppressive therapy or in acid hyposecretion exceeds that before therapy or that occurring with normal acid secretion (15,17,18). Conversely, recent studies have revealed that the density of \textit{H pylori} in the corpus is similar between these two conditions (10,19,20). The present study showed that the density of \textit{H pylori} during acid-suppressive therapy was similar to that at entry. However, more studies are needed because small numbers of subjects were examined in the present study. The present study proposes that local acid secretion may inhibit...
directly or through other factors (eg, supressing the formation of ammonia) the production of the mucosal IL-8. Solcia et al (14) suggested that the transformation from ammonium chloride to ammonia by acid suppression may be attributed to the inflammation in the gastric mucosa caused by H pylori. However, further examinations are needed to elucidate whether ammonia enhances the mucosal IL-8 contents in the H pylori-infected stomach.

Uemura et al (19) indicated that, in H pylori-infected duodenal ulcer patients, acid-suppressive therapy enhanced neutrophil infiltration, despite no significant change in the density of H pylori and the IL-8 contents in the corpus. On the contrary, their study showed that in the antrum, neutrophil chemotaxis was reduced after administration of famotidine, despite no significant change in the density of H pylori and the IL-8 contents (19). This discrepancy was not clarified in their study, and further examinations may need to be done.

H pylori-infected patients with peptic ulcer should undergo eradication of infection and not simply be prescribed a proton pump inhibitor (21-24). On the contrary, it is controversial whether H pylori-positive nonulcer patients require eradication of infection. Lee and O’Morain (25) summarized the national guidelines on H pylori eradication in eight European countries; most panels do not consider H pylori eradication in gastroesophageal reflux in their guidelines, but one panel considers it to be a relative indication for eradication (25). A few panels consider NUD to be an indication for eradication after investigation and in patients with severe or recurrent symptoms (26), whereas others do not consider it an indication. In the guidelines of the Canadian Helicobacter pylori consensus conference, gastroesophageal reflux is not recommended for eradication (27).

REFERENCES

Effect of acid-suppressive therapy on H pylori production of IL-8

NUD and gastritis are considered to be indications for eradication in patients with severe, long lasting or recurrent symptoms (27). Recent studies show that long term acid suppression in H pylori-positive patients with reflux esophagitis causes progression of gastric mucosal inflammation in the corpus and fundus (11,16). According to the Canadian guidelines, if a patient with gastroesophageal reflux on long term proton pump inhibitor therapy is already known to be H pylori-positive, treatment may be offered on a case-by-case basis (27). The present study implies that IL-8 contents in the corpus are increased with omeprazole administration in H pylori-infected patients with reflux esophagitis. Thus H pylori-infected patients with nonulcer disease may need attention when they are treated with proton pump inhibitors or H2 blockers.

Although intragastric pH was not examined directly, the serum gastrin levels were investigated at entry and during acid-suppression therapy in the present study. The present study showed that the serum gastrin levels during therapy significantly exceeded those at entry in both H pylori-positive and -negative patients. This finding suggests that this therapy can effectively suppress gastric acid secretion.

CONCLUSIONS
The present study indicates that mucosal IL-8 contents are increased by treatment with omeprazole 20 mg in the corpus of H pylori-infected patients with reflux esophagitis. This finding suggests that acid suppression may enhance the mucosal IL-8 of nonulcer patients with H pylori infection.

ACKNOWLEDGMENT: The authors thank Ms Yuriko Fukuda (SRL Inc, Tokyo, Japan) for technical assistance.

Can J Gastroenterol Vol 14 No 4 April 2000 281


