Reduced secretion of epidermal growth factor in duodenal ulcer patients with *Helicobacter pylori* infection

Xue-Qing Chen, Wan-Dai Zhang, Bo Jiang, Yu-Gang Song, Ri-Zi Reng, Dian-Yuan Zhou

**Abstract**

**AIM:** To investigate the concentration changes of epidermal growth factor (EGF) in duodenal ulcer patients with *Helicobacter pylori* (*H. pylori*) infection.

**METHODS:** Immunoreactive concentration of somatostatin, gastrin and epidermal growth factor of gastric and saliva juice in healthy volunteers, and chronic gastritis and duodenal ulcer patients with *H. pylori* infection were measured by radioimmunoassay.

**RESULTS:** Gastrin concentration of gastric juice in *H. pylori*-positive chronic gastritis (*P > 0.05*) and duodenal ulcer patients (*P < 0.01*) was higher than that of healthy volunteers (*P < 0.05*), whereas somatostatin concentration of gastric juice in chronic gastritis (*P < 0.05*) and duodenal ulcer patients (*P < 0.01*) was lower than that in healthy volunteers. Furthermore, EGF levels of gastric and saliva juice in duodenal ulcer patients with *H. pylori* infection (*n = 10*, 272.0 ng/L ± 96.3 ng/L and 8.3 ng/L ± 2.4 ng/L, respectively) were significantly lower than that in healthy volunteers (*n = 12*, 405.6 ng/L ± 35.6 ng/mL and 22.0 ng/L ± 17.0 ng/L, respectively) and in *H. pylori*-positive chronic gastritis patients (*n = 25*, 423.0 ng/L ± 104.0 ng/L and 22.0 ng/L ± 11.1 ng/L, respectively (*P < 0.05*).

**CONCLUSION:** A lower secretion of EGF may be a causative factor in the pathogenesis of *H. pylori*-positive duodenal ulcer.

**Key words:** Duodenal ulcer; *Helicobacter pylori*; Gastritis epidermal growth factor; Gastrins; Somatostatin

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.
active illnesses. Eligible patients gave informed written consent before enrolment. They were assigned to two groups based on the endoscopic results: Chronic gastritis group, consisting of 10 men and 17 women (mean age 38.5 years) and duodenal ulcer group, including 5 men and 7 women (mean age 32.8 years). Healthy group included 4 men and 10 women (mean age 37.4 years).

**Endoscopy and basal acid output measurement**
Gastroduodenoscopy was performed using an Olympus endoscope with standard biopsy forceps. Four fragments from the lesser curvature of the antrum at 1-2 cm from the pylorus and 2 fragments from the greater curvature of the corpus were obtained. The biopsy forceps were disinfected with 70% ethanol after each use. Before the biopsy, sampling of gastric juice as much as possible was obtained. The basal acid output was measured by titration of gastric juice in one hour with 10 mmol/L NaOH.

**H. pylori diagnosis**
The presence of *H. pylori* in the antrum and corpus was evaluated by microbiologic methods including culture[19,21], rapid urease test (commercial kits from Sanxing Biological Reagents Co, Fuzhou, China) and Warthin starry or Giemsa stains[25]. At least two of these tests should be positive in patients with *H. pylori* infection. *H. pylori* identified by Warthin Starry or Giemsa stains was graded as[21]: 0, null; 1+, a small number of bacteria (up to 20/gastric pit) presenting in a few of gastric pits; 2+, a large number of bacteria (more than 20/gastric pit) presenting in several gastric pits or a small number of bacteria in many gastric pits; 3+, a large number of bacteria presenting in nearly all gastric pits.

**Histology**
Biopsy fragments taken from the antral and oxyntic mucosa were fixed in 10% buffered formalin (pH 7.4), dehydrated and embedded in paraffin, and stained 5 μm thick with hematoxylin and eosin for histological evaluation. All sections were examined by one of the investigators (R.Z.R.), who was unaware of the previous histologic results, endoscopic findings, rapid urease tests and culture results.

**Extract and assay of somatostatin, gastrin and EGF from gastric and saliva juice**
Samples from gastric juice and saliva juice were boiled in water for 10 min, centrifuged, and pH adjusted to 7.0-7.5 by 10 mmol/L NaOH titration, then transferred to plastic tubes, and frozen with 500 KU/mL trasylol at 30 °C. Gastrin, somatostatin and EGF were measured using radioimmunassay kit from National Institute of Atomic Energy, Beijing, China, and Beijing Hai-Ke-Ri Biotech Centre, Beijing, China, respectively. Somatostatin, gastrin and EGF concentrations of gastric or saliva juice were expressed as ng/L.

**Statistical analysis**
Data of somatostatin, gastrin and EGF were expressed as mean ± SD and analyzed using one-way ANOVA (SNK test). The differences were considered significant when *P* < 0.05.

**RESULTS**

**H. pylori status, gastric histology, and basal acid output**
were investigated in 27 gastritis patients, 12 duodenal ulcer patients, and 14 volunteers. *H. pylori* were found in 2 of 14 healthy volunteers, 25 of 27 patients with chronic active gastritis, and 10 of 12 duodenal ulcer patients, and in none of those with normal gastric mucosa. (Table 1). Presence of *H. pylori* in chronic active gastritis patients was more common than in duodenal ulcer patients. In addition, active inflammatory infiltration tended to attack corpus mucosa in chronic gastritis patients (14/27), and those who had low basal acid output. Four of 12 duodenal ulcer patients had corpus inflammation and high basal acid output. Table 2 summaries the concentration of somatostatin, gastrin and EGF of gastric and saliva juice in *H. pylori*-positive patients and *H. pylori*-negative healthy volunteers. Gastrin concentration of gastric juice in duodenal ulcer was significantly higher than that in control group (*P* = 0.01). There were no significant differences in gastrin concentration between the chronic gastritis group and the control group. On the other hand, the somatostatin concentration of gastric juice in chronic gastritis and duodenal ulcer group was lower than that in the control group (*P* < 0.05 or 0.01). In *H. pylori*-positive chronic gastritis group, the levels of EGF in saliva juice and gastric juice were 423.0 ng/L ± 104.0 ng/L and 22.0 ng/L ± 11.1 ng/L, with no differences as compared with those in the control group (405.6 ng/L ± 35.6 ng/L and 22.0 ng/L ± 17.0 ng/L, respectively), (*P* > 0.05). However, the levels of EGF in saliva and gastric juice in chronic gastritis group and control group were both significantly higher than those in the duodenal ulcer group (272.0 ng/L ± 96.3 ng/L and 8.3 ng/L ± 2.4 ng/L, respectively), (*P* < 0.05).

**DISCUSSION**
This study showed for the first time that the levels of EGF in duodenal ulcer patients with *H. pylori* infection were much lower than those of the healthy volunteers and chronic gastritis patients with *H. pylori* infection, and also confirmed the previous findings that *H. pylori* infection can enhance gastrin secretion and lower somatostatin level, which can cause abnormal secretion of gastric acid[2-10].

A strong association between *H. pylori* and diseases of upper gastrointestinal tract has been reported[2-7,22]. The causal relationship between *H. pylori* and chronic superficial gastritis is well established, but that between *H. pylori* and peptic ulcer is rather difficult to establish on the basis of the available data[1]. The suggested mechanisms in antral organism cause a duodenal lesion including bacterial colonization of gastric meta-plasia in the duodenum[22], secondary changes in gastric acid or duodenal bicarbonate secretion[23,24] or the changes caused by the infected organism and/ or the inflammatory response to the host[25,26]. Recently, the changes in gastric acid caused by *H. pylori* infection have drawn more attention, for inhibition of gastric acid secretion promoted duodenal ulcer healing even in the presence of *H. pylori* and inflammation of gastric antrum[27,28].

The possible hypotheses in explaining the relationship between *H. pylori* infection and duodenal ulcer have been described as “gastrin link”[2,29,30] or “somatostatin link”[10,20] as duodenal ulcer patients with *H. pylori* infection often have hypergastrinemia, which may increase parietal cell mass and reduce somatostatin secretion known to promote the gastric secretion[6,27,30]. On the contrary, there was no consistent relationship between chronic *H. pylori* infection and acid secretion observed[21,23,24]. Kang et al[31] showed that patients with duodenal ulcer or combined gastric and duodenal ulcer had significantly higher gastric acid output compared with the presence or absence of *H. pylori*. However, gastric ulcer patients with *H. pylori* had higher basal and maximal acid output when compared to patients without *H. pylori*. McColl et al[32] have observed that after eradication of *H. pylori* in duodenal ulcer, daytime intragastric pH and nocturnal acid secretion were unchanged, even after 7 mo. Our results showed that hypochlorhydria in chronic gastritis patients and high acidity in duodenal ulcer patients with *H. pylori* infection, had both enhanced gastrin secretion and reduced somatostatin secretion. Low gastric acidity in chronic gastritis may be elicited by the action of *protein inhibitor of gastric acid*[29,31], but it would not be included that parietal cells may be damaged or inhibited by active inflammation of oxyntic mucosa because of host’s response to *H. pylori*. The above results suggested that “gastrin link” or “somatostatin link” could not elucidate the mechanism of *H. pylori* in the pathogenesis of duodenal ulcer[25,26], and other factor (s) should be taken into account.

The pathogenesis of peptic ulcer can be considered in terms of aggressive factors overwhelming mucosal defense. EGF should be one of such factors. In the previous studies it was shown that the EGF is localized in the submucular and Brunner’s glands of the rats and humans, and exerts protection of gastric mucosa and inhibition of gastric acid secretion[14,15]. Olsen et al[15] showed that the oral administration of human EGF/UGO may benefit the healing of chronic duodenal ulcers in rats. Gastric mucosal integrity in rats of removed submucular gland to reduce EGF levels in gastric juice was prone to be damaged[16]. Chen et al[33] compared patients with...
gastric ulcer and duodenal ulcer to healthy subjects and observed that EGF levels of plasma and saliva juice in the former were lower than that in the latter. Their results are similar to ours, but different from those of Hirasawa et al[34] who observed that salivary EGF output in patients with gastric, duodenal and gastroduodenal ulcers was higher than that in normal subjects, however, salivary EGF output in refractory peptic ulcer patients was much lower. Therefore, it is reasonable that any factor(s) which reduce or inhibit EGF secretion may be able to promote gastric mucosal damage. Based on the above evidences, we think that reduced EGF secretion may play an important role in the development of duodenal ulcer with *H. pylori* infection.

In conclusion, our study shows that gastric acidity is higher in *H. pylori*-positive duodenal ulcer patients than that in *H. pylori*-positive chronic gastritis and healthy subjects. Contents of gastrin of gastric juice in *H. pylori* positive chronic gastritis and duodenal ulcer patients were higher than in healthy subjects, and the somatostatin concentration was lower in healthy subjects. Levels of EGF gastric and salivary juice were also lower than those in the chronic gastritis patients and duodenal ulcer patients with *H. pylori* infection. Based on these results, we assume that EGF may play a causal role in the pathogenesis of *H. pylori*-positive duodenal ulcer.

REFERENCES

1 NIH Consensus Development Panel on Helicobacter pylori in Peptic Ulcer Disease. NIH Consensus Conference. Helicobacter pylori in peptic ulcer disease. *JAMA* 1994; 272: 65-69 [PMID: 8007082 DOI: 10.1001/jama.272.1.65]

2 Dunn BE. Pathogenic mechanisms of Helicobacter pylori. *Gastroenterol Clin North Am* 1993; 22: 43-57 [PMID: 8494957]

3 Tytgat GN, Nouh LA, Raouw EA. Helicobacter pylori infection and duodenal ulcer disease. *Gastroenterol Clin North Am* 1993; 22: 127-139 [PMID: 8495626]

4 Beardshall K, Moss S, Gill J, Levi S, Ghosh P, Playford RJ, Calam J. Suppression of Helicobacter pylori reduces gastrin releasing peptide stimulated gastrin release in duodenal ulcer patients. Gut 1992; 33: 601-603 [PMID: 1042474 DOI: 10.1136/gut.33.5.601]

5 McColl KE, Fullarton GM, el Nujumi AM, Macdonald AM, Brown IL, Hilditch TE. Lowered gastrin and gastric acidity after eradication of Campylobacter pylori in duodenal ulcer. *Lancet* 1989; 2: 499-500 [PMID: 25730202 DOI: 10.1016/S0140-6736(89)92105-3]

6 Graham DY, Opekun A, Lew GM, Evans DJ, Klein PD, Evans DG. Ablation of exaggerated meal-stimulated gastrin release in duodenal ulcer patients after clearance of Helicobacter (Campylobacter) pylori infection. *Am J Gastroenterol* 1990; 85: 394-398 [PMID: 2327380]

7 Kaneko H, Nakada K, Mitsuwa T, Uchida K, Furasawa A, Maeda Y, Morise K, Helicobacter pylori infection induces a decrease in immunoreactive-somatostatin concentrations of human stomach. *Dig Dis Sci* 1992; 37: 409-416 [PMID: 13465177 DOI: 10.1007/BF01037736]

8 Graham DY, Lew GM, Lechago J. Antcl G-cell and D-cell numbers in Helicobacter pylori infection: effect of *H. pylori* eradication. *Gastroenterology* 1993; 104: 1655-1660 [PMID: 8500723]

9 Queiruz DM, Mendes EN, Rocha GA, Moura SB, Resende LM, Barbosa AJ, Coelho LG, Passos MC, Castro LP, Oliveira CA. Effect of Helicobacter pylori eradication on antcl gastrin- and somatostatin-immunoreactive cell density and gastrin and somatostatin concentrations. *Scand J Gastroenterol* 1992; 28: 855-864 [PMID: 7903471 DOI: 10.3109/00365529309103125]

10 Moss SF, Legon S, Bishop AB, Polak JM, Calam J. Effect of Helicobacter pylori on gastric somatostatin in duodenal ulcer disease. *Lancet* 1992; 340: 930-932 [PMID: 15373474 DOI: 10.1016/0140-6736(92)92218-X]

11 Kang JW, Wee A. Helicobacter pylori and gastric acid output in peptic ulcer disease. *Dig Dis Sci* 1991; 36: 5-9 [PMID: 19880057 DOI: 10.1007/BF01039786]

12 Chittajallu RS, Howe CA, McColl KE. Effect of Helicobacter pylori on parietal cell sensitivity to pentagastrin in duodenal ulcer subjects. *Scand J Gastroenterol* 1992; 27: 857-862 [PMID: 1439539 DOI: 10.3109/00365529209080154]

13 Cohen S, Carpenter G. Human epidermal growth factor: isolation and chemical and biological properties. *Proc Natl Acad Sci USA* 1975; 72: 1317-1321 [PMID: 1055407 DOI: 10.1073/pnas.72.4.1317]

14 Dembinski A, Drezdowicz D, Gregory H, Konturej SJ, Warzecha Z. Inhibition of acid formation by epidermal growth factor in the isolated rabbit gastric glands. *J Physiol* 1986; 378: 347-357 [PMID: 3025433 DOI: 10.1113/jphysiol.1986.sp016223]

15 Olsen PS, Poulsen SS, Therkelsen K, Nexo E. Effect of sialodacryodenal and synthetic human urogastrone on healing of chronic gastric ulcers in rats. *Gut* 1986; 27: 1443-1449 [PMID: 3942412 DOI: 10.1136/gut.27.12.1443]

16 Skinner KA, Tepperman BL. Influence of desalivation on acid secretory output and gastric mucosal integrity in the rat. *Gastroenterology* 1981; 81: 335-339 [PMID: 7239410]

17 Amagase H, Murakami T, Miskaci M, Higashi Y, Hashimoto K, Funu T, Yata N. Possible mechanism of gastric mucosal protection by epidermal growth factor in rats. *Life Sci* 1990; 47: 1203-1211 [PMID: 2243536 DOI: 10.1016/0024-3205(90)90012-A]

18 Wright NA, Pike C, Elia G. Induction of a novel epidermal growth factor-secreting cyst cell lineage by mucosal ulceration in human gastrointestinal stem cells. *Nature* 1990; 343: 82-85 [PMID: 2296294 DOI: 10.1038/343082a0]

19 Queiruz DM, Mendes EN, Rocha GA, Indicator medium for isolation of Campylobacter pyloridis. *J Clin Microbiol* 1985; 23: 2378-2379 [PMID: 3429026]

20 Potters JV, Loffeld RJ, Snoebergerh E, van Spreewel JP, Arenz J. Rapid staining of Campylobacter pyloridis. *Histopathology* 1987; 11: 1223 [PMID: 2447004 DOI: 10.1111/j.1365-2559.1987.801683.x]

21 Satoh K, Kimura K, Yoshida Y, Kasuno T, Kihara K, Taniguchi Y. A topographical relationship between Helicobacter pylori and gastric: quantitative assessment of Helicobacter pylori in the gastric mucosa. *Am J Gastroenterol* 1991; 86: 285-291 [PMID: 1998309]

22 Wyatt JJ, Dixon ME. Chronic gastritis—a pathogenic approach. *J Pathol* 1988; 154: 127-134 [PMID: 3280764 DOI: 10.1002/path.1715140203]

23 Tarmaskey PR, Kovacs TO, Snytk B, Walsh JH. Asymptomatic *H. pylori* infection inhibits pH inhibition of gastrin and acid secretion during second hour of peptone meal stimulation. *Dig Dis Sci* 1993; 38: 1681-1687 [PMID: 8359081 DOI: 10.1007/BF01303178]

24 Kelly SM, Crompton JR, Hunter JO. Helicobacter pylori increases gastric antral juxtamucosal pH. *Dig Dis Sci* 1993; 38: 129-131 [PMID: 7974784 DOI: 10.1007/BF01296784]

25 Graham DY, Go MF, Lew GM, Genta RM, Rehfeld JF. Helicobacter pylori infection and exaggerated gastrin release. Effects of inflammation and progestin processing. *Scand J Gastroenterol* 1993; 28: 690-694 [PMID: 8210984 DOI: 10.3109/00365529309103201]

26 Murakami M, Saita H, Teramura S, Dekigki H, Asagoe K, Kusaka S, Kita T. Gastric ammonia has a potent ulcerogenic action on the rat stomach. *Gastroenterology* 1993;
27. Hu PL. (Comparison of acid and Helicobacter pylori in ulcerogenesis of duodenal ulcer disease. Zhonghua Yiye Za Zhi 1993; 73: 217-29, 253 [PMID: 8395315]
28. Levi S, Beardshall K, Swift I, Foulkes W, Playford R, Ghosh P, Calam J. Antral Helicobacter pylori, hypergastrinemia, and duodenal ulcers: effect of eradicating the organism. BMJ 1989; 299: 1504-1505. [PMID: 2514864 DOI: 10.1136/bmj.299.6714.1504]
29. Levi S, Beardshall K, Hadad G, Playford R, Ghosh P, Calam J. Campylobacter pylori and duodenal ulcers: the gastrin link. Lancet 1989; 1: 1167-1168 [PMID: 2566737 DOI: 10.1016/S0140-6736(89)9272-9]
30. McHenry L, Vuyyuru L., Schubert ML. Helicobacter pylori and duodenal ulcer disease: the somatostatin link? Gastroenterology 1993; 104: 1573-1575 [PMID: 8097735]
31. McCull KE, Fullarton GM, Chittajalu R, el Nujumi AM, MacDonald AM, Dhillon SW, Hilditch TE. Plasma gastrin, daytime intragastric pH, and nocturnal acid output before and at 1 and 7 mon after eradication of Helicobacter pylori in duodenal ulcer subjects. Scand J Gastroenterol 1991; 26: 339-346 [PMID: 1853158 DOI: 10.3109/00365529109025052]
32. Vargas M, Lee A, Fox JG, Cave DR. Inhibition of acid secretion from parietal cells by non-human-infecting Helicobacter species: a factor in colonization of gastric mucosa? Infect Immun 1991; 59: 3694-3699 [PMID: 1894369]
33. Chen SP, Lu GJ, Wen SH. Study on epidermal growth factor levels of saliva, gastric juice and serum in patients with peptic ulcer disease. Zhonghua Xiaohua Za Zhi 1994; 14: 15-17
34. Hirasawa Y, Asaki S, Hongo M, Shibuya D, Yamauchi N, Matsuda K, Toyota T. [Salivary epidermal growth factor in patients with peptic ulcer]. Nihon Shokakibyo Gakkai Zasshi 1991; 88: 1043-1050 [PMID: 1856997]
