Mucosal expression and luminal release of epidermal and transforming growth factors in patients with duodenal ulcer before and after eradication of *Helicobacter pylori*

P Ch Konturek, H Ernst, S J Konturek, A J Bobrzyński, G Faller, C Klingler, E G Hahn

Abstract

**Background**—Epidermal growth factor (EGF) and transforming growth factor-α (TGFα) are potent gastric acid inhibitors and stimuli of mucosal growth and protection but their involvement in *Helicobacter pylori*-associated duodenal ulcer has been little examined.

**Aim**—To assess gastric acid secretion, plasma gastrin concentrations, mucosal content of EGF and TGFα, and mucosal expression of these peptides and their receptor (EGFr) as well as salivary and gastric luminal release of EGF under basal conditions and after pentagastrin stimulation in 10 healthy subjects and in 25 *H pylori*-positive patients with duodenal ulcer before and after two weeks of triple anti-*H pylori* therapy and four weeks after the termination of this therapy.

**Results**—Pentagastrin stimulation caused a significant increase in salivary and gastric release of EGF both in healthy controls and patients with duodenal ulcers but in the patients, the eradication of *H pylori* resulted in several fold higher gastric luminal (but not salivary) EGF release than before the anti-*H pylori* therapy. Mucosal contents of immunoreactive EGF and TGFα and mucosal expression of EGF, TGFα, and EGFr in *H pylori*-positive patients with duodenal ulcer were significantly higher than those in healthy *H pylori*-negative controls and this increase persisted after eradication of *H pylori*. Basal plasma gastrin was significantly reduced after two weeks of triple therapy and four weeks after the *H pylori* eradication all ulcers were completely healed.

**Conclusions**—(1) *H pylori* infection in patients with duodenal ulcer was accompanied by enhanced plasma gastrin and increased mucosal content and expression of TGFα, EGF, and EGFr; (2) *H pylori* eradication resulted in ulcer healing, reduction in plasma gastrin, and enhancement of gastric (but not salivary) luminal release of EGF, particularly after pentagastrin stimulation; and (3) enhanced mucosal content and expression of TGFα, EGF, and EGFr and increased luminal release of EGF may contribute to ulcer healing after eradication of *H pylori*.

**Keywords:** *Helicobacter pylori*, EGF, TGFα, EGF receptors, gastrin, duodenal ulcer.

*Helicobacter pylori* infection is the cause of initially acute and then chronic active gastritis (type B) and plays a major part in the pathogenesis of duodenal ulceration. It is accompanied by an increment in plasma gastrin, possibly due to the impaired feedback control of the release of this hormone by luminal acid, probably due to the deficiency of its paracrine suppression by somatostatin. Epidermal growth factor (EGF) is secreted in humans mainly by salivary glands and is present in digestive secretions such as saliva, gastric, duodenal, and pancreatic juice, and in urine as urogastrone. Transforming growth factor-α (TGFα) is another growth factor produced predominantly in the gastric mucosa, especially when exposed to topical irritants. Similar to somatostatin, both EGF and TGFα are powerful inhibitors of gastric acid secretion and promoters of mucosal growth, repair, and ulcer healing. There is a pronounced increase in the EGF receptors and EGF producing cells around acetic acid induced gastric ulcers in rats and a novel cell lineage in human mucosal ulcerations secreting EGF adjacent to peptic ulcer suggesting that EGF plays an important part in ulcer healing. This notion has been supported by the findings that patients with peptic ulcer show reduced release of EGF into plasma, saliva, and gastric juice. Recent studies by Tunio and Hobsley showed, however, that there is an increased rather than decreased release of histamine stimulated EGF into the gastric juice in patients with duodenal ulcer possibly due to increased expression of an EGF producing new cell lineage in the ulcer area but the *H pylori* status in these patients was not determined.

Although *H pylori* infection is considered to be a major pathogenic factor in the formation of peptic ulceration and accompanying gastric secretory disorders, little information is available as to whether the presence of *H pylori* in gastric mucosa affects the expression and the release of EGF and TGFα under basal conditions and after gastric secretory stimulation in duodenal ulcer and whether the eradication of *H pylori* affects the mucosal expression and luminal release of these peptides in the stomach.

This study, carried out on healthy controls and *H pylori*-positive patients with duodenal
ulcer, was designed to determine the influence of *H pylori* eradication on plasma gastrin release and on luminal release (salivary and gastric) of EGF and mucosal expression of EGF, TGFα, and EGF receptor in gastric mucosa of these patients.

**Methods**

Twenty-five *H pylori* positive patients with duodenal ulcer and ten *H pylori* negative healthy controls were included in the study. All subjects gave informed consent and the project was reviewed and accepted by the ethical committee of the Jagiellonian University School of Medicine, Krakow, Poland.

Gastric contents were aspirated twice, firstly, during endoscopy using a special tube inserted through the endoscope, and secondly, in a separate secretory test, including continuous gastric juice collection during 30 minutes in basal conditions and 60 minutes of intravenous infusion of pentagastrin (2 μg/kg/h) (Peptavlon, ICI, Alderley Park, UK) using a standard technique of gastric aspiration. Both collections of gastric juice were performed after about 12 hours of overnight fasting with smoking and ethanol forbidden for this period. Also for at least 48 hours before the collection the subjects were asked to stop any medication affecting gastric acid secretion, including H2 receptor antagonists (ranitidine or cimetidine) or antibiotics. During basal and pentagastrin induced stimulation, the saliva was constantly expectorated and collected into 15 minute aliquots. Gastric juice was constantly aspirated and collected into separate 15 minute aliquots and their volumes were measured. The samples of saliva and gastric contents were immediately adjusted to pH 7.0 by adding 100 mM NaOH and stored at −70°C until radioimmunoaassay of EGF using commercially available reagents (Amersham, Buckinghamshire, UK) and following the protocol suggested by Amersham. The antisera, raised in rabbits against human EGF, was used at a final dilution of 1:20 000.

Plasma gastrin was also determined under basal conditions (just before endoscopy) using radioimmunoassay with antisera 4562 (kindly donated by Professor J E Rehfelt of Aarhus University, Aarhus, Denmark) at a final dilution of 1:140 000 as described.³

During endoscopy, at least six biopsy samples were obtained from the distal portion (antrum) of the stomach and fixed in buffered formalin and then used for the assessment of the severity of gastritis, the presence of *H pylori*, and immunocytochemical detection of EGF, TGFα, and EGF receptor. Two additional biopsy samples were taken from the fundic mucosa for determination of immunoreactive EGF and TGFα and immediately stored at −70°C until analysis.

The presence of *H pylori* was detected using haematoxylin and eosin staining and the quantity of *H pylori* was graded according to Marshall and Warren⁴ on a four point scale from absent (grade 0) to abundant (grade 3).

The severity of gastritis in antral mucosa was estimated on a five point scale² from normal (grade 0) to superficial gastritis (grade 1), and slight, moderate, and severe gastritis (grades 2, 3 and 4 respectively). The extent of intestinal metaplasia, the numbers of mononuclear inflammatory cells in the lamina propria, neutrophils, and eosinophils were estimated in grades showing a range of activity of gastritis from normal (grade 0) to slight (grade 1), moderate (grade 2), and heavy (grade 3) increase as described previously.²² These gradations closely resemble those of the Sydney system.²³

For immunocytochemistry, serial sections obtained from paraffin wax blocks were dewaxed, rehydrated, pretreated with citrate buffer (pH 6.0) in a microwave oven at 700 W for 10 minutes, and incubated with specific monoclonal antibody (12 hours, 4°C) for EGF (1/40; GF 0-1, Oncogene Science, New York, USA), TGFα (1/20, G10, Oncogene Science, New York, USA), or for EGF (1/40, Sigma Chemical Co, St Louis, MO, USA) followed by the avidin biotin complex (ABC) method (ABC kit, Oncogene Science, New York, USA). The cytoplasm staining reactions were graded in accordance with the intensity of staining by examining 100 consecutive cells in three regions of the gastric mucosa, the surface epithelium, the neck region (neck), and the basal portion of gastric glands (base) as described previously.²⁴ ²⁵ ²⁶ ²⁷ Coded specimens were independently assessed by two observers. The intensity of staining was graded as follows: 0=no staining, 1+=weakly staining, 2+=moderately positive staining (cytoplasm positive but other cytoplasm compounds also visible), or 3+=densely staining, equal to the simultaneously stained positive control. The number of cells positive for EGF receptor was evaluated for 100 consecutive cells in three different regions of the mucosa. Negative control sections were processed immunohistochemically after replacing the primary antibody with irrelevant monoclonal antibody or phosphate buffered saline (PBS). Positive control sections were obtained from pancreatic carcinoma (TGFα), oesophageal squamous carcinoma (EGF receptor), and mandibular gland (EGF) showing maximal labelling with the appropriate antibody. We validated our counts for EGF and TGFα expression and for EGF receptor labelling. The same person performed a second independent count on sections of mucosa immunostained for EGF, TGFα, and EGF receptor without knowledge of the results of the first counts. Results of one person were also compared with those obtained from the second person. There was less than 5% variation from the initial counting.

The stored mucosal samples for radioimmunoaassay of EGF and TGFα were weighed, thawed, and homogenised in 0.01 M PBS (pH 7.2; 5 ml/g wet tissue). The homogenates were centrifuged at 12 000 g for 20 minutes at 4°C until assay. The EGF radioimmunoaassay in tissue homogenates was performed as in the saliva and gastric juice samples described earlier.⁴ ⁹ ¹⁰ The TGFα
radioimmunoassay in these homogenates were performed with species specific human TGFα antiserum (<0.5% cross reactivity with rat or mouse TGFα or human EGF) and recombinant human TGFα (Berlex Bioscience Inc, Alameda, CA, USA) used both as a standard and as 125I-labelled tracer. Sheep anti-human TGFα was used at 1:40 000 in 1% normal sheep serum in radioimmunoassay buffer and incubated for 24 hours at 4°C. The assay was highly specific for human TGFα with no displacement of 125I-labelled tracer found with rat, mouse, or human EGF up to 0.1 μg/ml. The sensitivity of the assay was 5 pg TGFα per tube. The dose response curve of immunoreactive TGFα found in the gastric mucosa was parallel to standard (recombinant human TGFα) in the TGFα radioimmunoassay.

Gastric secretory studies, plasma gastrin determination, and endoscopy combined with biopsy and histology and immunocytochemistry were performed once in healthy H pylori negative subjects and repeated in patients with duodenal ulcer after two weeks of triple therapy (20 mg omeprazole twice daily, 500 mg amoxycillin four times a day, and 500 mg metronidazole twice daily) and four weeks after the termination of this therapy.

**STATISTICS**

Data are presented as means (SEM). The Wilcoxon signed rank test was used with paired data and the Mann-Whitney U test with unpaired data. A p value <0.05 was considered significant.

**Results**

Figures 1 and 2 show the values of basal plasma gastrin concentrations, gastric luminal concentrations of EGF, and gastric acid outputs under basal conditions and after pentagastrin stimulation in 10 healthy subjects and in 25 patients with duodenal ulcer before and after two weeks of triple therapy and four weeks after termination of this therapy. Values are means (SEM). *Significant increase above the value in healthy subjects. †Significant decrease below the value in patients with duodenal ulcer before the eradication of H pylori.

Basal plasma gastrin concentration in H pylori positive patients with duodenal ulcer before the start of therapy averaged 62 (8) pM/l and it was significantly higher than that recorded in healthy controls (Fig 1). After two weeks of triple therapy, when 15 out of 25 patients showed endoscopically healed ulcers, all patients showed a pronounced and significant reduction in plasma gastrin and this reduction was also seen four weeks after termination of the treatment in the patients with duodenal ulcer.

In patients with duodenal ulcer, basal gastric acid output (Fig 2) before and after the eradication of H pylori was not significantly different from that in H pylori negative healthy controls. The maximal acid response to pentagastrin in H pylori positive patients with duodenal ulcer before the anti-H pylori treatment and after two weeks of triple therapy was slightly but significantly higher than that in H pylori negative healthy controls but four weeks after the anti-H pylori therapy, this stimulated acid output was not significantly different from that in H pylori negative healthy controls.

Concentrations of EGF in gastric mucosa (collected at the time of endoscopy) in H pylori positive patients with duodenal ulcer averaged 100-8 (11-2) pM/l and this was significantly higher (by about 45%) than that in H pylori negative healthy controls (Fig 1). After two weeks of triple therapy and four weeks after stopping therapy the basal gastric EGF concentrations still remained significantly increased above the value recorded in healthy controls.

Figures 3 and 4 present the concentrations and outputs of EGF in the gastric juice aspirated continuously from the stomach (with
Healthy controls Hp (-)

Figure 3: Gastric EGF concentrations and outputs under basal conditions and during pentagastrin (P-gastrin) infusion in 10 healthy subjects. Values are means (SEM). *Significant increase above the basal values recorded during a 15 minute period just before the start of pentagastrin infusion.

saliva expectorated throughout the experiment) during the basal 30 minute period and after intravenous infusion of pentagastrin at a dose of 2 μg/kg/h in healthy subjects and patients with duodenal ulcer. There was no significant difference in basal gastric concentrations and outputs of EGF between healthy controls and H pylori positive patients

DU patients

Figure 4: Gastric EGF concentrations and outputs under basal conditions and during pentagastrin (P-gastrin) infusion in 25 patients with duodenal ulcer before triple therapy and four weeks after the termination of this therapy. Values are means (SEM). *Significant increase above the values obtained in corresponding periods in the same patients with duodenal ulcer before the eradication of H pylori.
with duodenal ulcer. In these patients, four weeks after the termination of triple therapy, when all ulcers were endoscopically healed and 

\[ H_pylori \] was detected histologically (Fig 5) in only two out of 25 patients (the rapid urease (CLO) test was negative in all 25 patients), the basal and pentagastrin stimulated EGF concentrations and outputs were considerably increased compared with the corresponding values recorded in these patients before the \( H_pylori \) eradication (Fig 4). For comparison, the basal salivary concentrations and outputs of EGF in normal \( H_pylori \) negative subjects were similar to those recorded in saliva of the patients with duodenal ulcer. After pentagastrin stimulation, the salivary EGF concentrations and outputs were significantly increased compared with basal values but they were lower than those recorded in gastric juice of these subjects (Table I).

**Table I** Basal and pentagastrin induced salivary EGF concentrations (pM/I) and outputs (nM/15 min) in \( H_pylori \) negative healthy controls (n=10) and in patients with duodenal ulcer (n=25) before and after eradication of \( H_pylori \)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>EGF</th>
<th>EGF expression (nM/15 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy ( H_pylori ) subjects</td>
<td>15</td>
<td>40 (7)</td>
</tr>
<tr>
<td>30</td>
<td>29 (9)</td>
<td>0.35 (0.08)</td>
</tr>
<tr>
<td>45*</td>
<td>38 (5)</td>
<td>0.42 (0.06)</td>
</tr>
<tr>
<td>60*</td>
<td>75 (8)*</td>
<td>0.66 (0.22)*</td>
</tr>
<tr>
<td>75*</td>
<td>70 (12)*</td>
<td>1.47 (0.22)*</td>
</tr>
<tr>
<td>90</td>
<td>68 (14)*</td>
<td>1.64 (0.34)*</td>
</tr>
<tr>
<td>( H_pylori ) positive patients with duodenal ulcer (before ( H_pylori ) eradication)</td>
<td>15</td>
<td>37 (6)</td>
</tr>
<tr>
<td>30</td>
<td>22 (5)</td>
<td>0.90 (0.05)</td>
</tr>
<tr>
<td>45*</td>
<td>26 (9)</td>
<td>0.54 (0.07)</td>
</tr>
<tr>
<td>60*</td>
<td>32 (12)</td>
<td>1.55 (0.08)*</td>
</tr>
<tr>
<td>75*</td>
<td>58 (6)*</td>
<td>3.62 (0.1)</td>
</tr>
<tr>
<td>90</td>
<td>64 (7)*</td>
<td>2.50 (0.2)</td>
</tr>
<tr>
<td>( H_pylori ) positive patients with duodenal ulcer (after ( H_pylori ) eradication)</td>
<td>15</td>
<td>52 (5)</td>
</tr>
<tr>
<td>30</td>
<td>28 (4)*</td>
<td>0.44 (0.10)*</td>
</tr>
<tr>
<td>45*</td>
<td>75 (9)*</td>
<td>2.25 (0.08)*</td>
</tr>
<tr>
<td>60*</td>
<td>49 (10)*</td>
<td>2.88 (0.30)*</td>
</tr>
<tr>
<td>75*</td>
<td>58 (9)*</td>
<td>2.94 (0.42)*</td>
</tr>
<tr>
<td>90</td>
<td>62 (7)*</td>
<td>2.17 (0.28)*</td>
</tr>
</tbody>
</table>

Values are means (SEM).
*Significant increase in basal values recorded before the start of pentagastrin infusion.
**Significant change in the values of EGF recorded in the gastric juice of these subjects.
*Time during pentagastrin infusion.

In the gastric mucosa of healthy subjects there was only a negligible EGF immunoreactivity, localised mainly in the lumen of gastric glands (Fig 6). TGFα immunoreactivity was more prominent and confined to surface epithelial cells and parietal cells. \( H_pylori \) positive patients with duodenal ulcer before the \( H_pylori \) eradication showed about 65% higher EGF immunoreactivity and only about 20% higher TGFα immunoreactivity than \( H_pylori \) negative healthy controls. Also the EGFs expressed mainly in the superficial epithelial cells and in the upper two thirds of the gland was about 45% higher in \( H_pylori \) positive patients with duodenal ulcer than in healthy controls (Fig 7). This increased EGF, TGFα, and EGFα immunostaining in patients with duodenal ulcer remained significantly raised after two weeks of triple therapy and four weeks after termination of therapy.

Mucosal contents of TGFα both in healthy controls and in \( H_pylori \) positive patients with duodenal ulcer were about eight to 10 times lower than in \( H_pylori \) negative controls before and after two weeks of therapy.
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and that the eradication of *H. pylori* in patients with duodenal ulcer results in a pronounced increase in this EGF release suggesting that this growth factor might contribute to ulcer healing in the course of anti-*H. pylori* therapy.

In the basal state during endoscopy, EGF concentrations in the gastric juice showed significantly higher values in *H. pylori* positive patients with duodenal ulcer when compared with those in *H. pylori* negative healthy controls. This agrees with enhanced mucosal content of immunoreactive EGF and TGFα and immunohistochemical expression of these peptides in the gastric mucosa of patients with duodenal ulcer. However, when the saliva was expectorated, the basal EGF concentrations in saliva and gastric juice of *H. pylori* positive patients with duodenal ulcer were not significantly different from those in healthy controls. This disagrees with previous studies, claiming lower basal concentrations of EGF in patients with peptic ulcer than in healthy normal subjects\(^\text{17-19}\) but supports a recent report of Tunio and Hobsley,\(^\text{20}\) who also found no significant difference in basal EGF release into the saliva and gastric juice between patients with duodenal ulcer and healthy controls.

In our study, when the saliva was constantly expectorated and the gastric content continuously aspirated, a fall in the gastric EGF content was found both in *H. pylori* negative healthy controls and *H. pylori* positive patients with duodenal ulcer, suggesting that basal EGF in gastric contents originates, at least in part, from the saliva. However, the salivary concentrations and outputs of EGF after pentagastrin infusion in healthy subjects and patients with duodenal ulcer were significantly lower than those measured in the gastric juice of these subjects. This indicates that during pentagastrin stimulation gastric luminal EGF originates from the gastric mucosa rather than from the swallowed saliva. As in previous reports with histamine stimulation,\(^\text{19}\) we also found a large increase in EGF release into gastric juice after the stimulation with pentagastrin both in normal *H. pylori* negative subjects and in patients with duodenal ulcer. This increase in EGF concentrations and outputs was accompanied by a large increase in gastric acid secretion caused by pentagastrin. This indicates that pentagastrin, similar to histamine, stimulates the production of EGF mainly in the stomach and this occurs in a manner that seems to be linked with gastric acid stimulation. It is unclear whether pentagastrin stimulates gastric EGF release via histamine release and what the cellular source of the gastric EGF is.

The major finding of this report is that after eradication of *H. pylori* and ulcer healing in patients with duodenal ulcer, there was a large increase in basal EGF release and a several-fold rise in gastric EGF outputs after pentagastrin stimulation. It is of interest that the mucosal content and expression of EGF and TGFα, which was significantly higher in *H. pylori* infected patients with duodenal ulcer than in healthy controls, remained increased also after

### Table II

<table>
<thead>
<tr>
<th>Treatment</th>
<th>EGF (pg/mg)</th>
<th>TGFα (pg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal controls</td>
<td>0.28 (0.09)</td>
<td>4.51 (0.42)</td>
</tr>
<tr>
<td>DU patients before treatment</td>
<td>0.79 (0.11)</td>
<td>6.82 (0.86)</td>
</tr>
<tr>
<td>After 2 weeks of treatment</td>
<td>0.67 (0.21)</td>
<td>5.04 (2.11)</td>
</tr>
<tr>
<td>After 4 weeks of treatment</td>
<td>0.53 (0.10)</td>
<td>4.72 (1.94)</td>
</tr>
</tbody>
</table>

Values are means (SEM). *p*<0.05 between healthy control and patients with duodenal ulcer. **p**<0.05 EGF and TGFα contents in healthy controls vs patients with duodenal ulcer before or after triple therapy.

Table II: Immunoreactive EGF and TGFα contents (pg/mg wet tissue) in biopsy samples of fundic mucosa of healthy subjects (n=10) and in patients with duodenal ulcer (DU) (n=25) before and after two weeks of triple therapy, and four weeks after termination of treatment.

Figure 8: Activity of gastritis in 25 patients with duodenal ulcer before and after two weeks of triple therapy and four weeks after termination of therapy. *Significant decrease below the value obtained in *H. pylori* (Hp) positive patients with duodenal ulcer before the triple therapy.

Figure 9: Severity of gastritis in 25 patients with duodenal ulcer before and after two weeks of triple therapy and four weeks after termination of therapy. *Significant decrease below the value obtained in *H. pylori* (Hp) positive patients with duodenal ulcer before the triple therapy.

Discussion

This study shows that pentagastrin stimulated EGF release in the human stomach originates, at least in part, directly from the gastric mucosa.
H pylori eradication. Thus, the increased EGF release coincided with greater expression of EGF and TGFα in the H pylori eradicated gastric mucosa. It is unclear, however, why during the actual H pylori infection, the content of EGF in the gastric juice was reduced despite the enhanced mucosal content of immunoreactive EGF and TGFα and mucosal expression of immunohistochemical content of these patients before the start of anti-H pylori therapy. It is possible that proteases produced by H pylori contribute to rapid degradation of the EGF released from the mucosal cells so that the measurable amounts of EGF in the gastric contents were much lower than those actually released from these cells. It is also possible that the adhesion of H pylori to the gastric mucosa somehow restrains the release of EGF from the mucosal cells. Further studies are needed to elucidate the interrelation between the expression and release of EGF and the infection of the mucosa by H pylori.

As EGF and TGFα are potent mitogens for epithelial cells both in vivo and in vitro,1-2 it is reasonable to assume that overexpression of these growth factors could contribute to the increase in the mucosal cell proliferation found in H pylori infected stomach.27-29 This increased epithelial cell proliferation during long term H pylori infection could serve as an indicator of risk for gastric cancer.30 Additional factors that could explain the mucosal cell proliferation in the H pylori infected stomach might be an increase in plasma gastrin possibly due to the reduced release of somatostatin.31 The overexpression of EGF receptors in the mucosa of patients with duodenal ulcer compared with healthy subjects could explain a possible link between growth factors and mucosal cell proliferation in these patients.27-30

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