GASTRIC MUCOSAL EXPRESSION AND LUMINAL RELEASE OF GROWTH FACTORS IN GASTRIC CARCINOMA AND DUODENAL ULCER PATIENTS BEFORE AND AFTER ERADICATION OF *HELICOBACTER PYLORI*

Department of Medicine, University Erlangen, Germany and Institute of Physiology, Jagiellonian University School of Medicine, Cracow, Poland

Epidemiological studies have consistently shown an association between infection of *Helicobacter pylori* (Hp) and duodenal ulcer (DU) and gastric cancer. The mechanism of the ulcerogenic effect of Hp has been related to excessive gastrin release, gastric acid hypersecretion and gastric metaplasia in duodenum. The implication of Hp in gastric carcinogenesis has not been explained. In this study, mucosal expression of EGF and TGFα and luminal release of EGF as well as basal and pentagastrin-stimulated acid secretion and plasma gastrin levels have been determined in healthy subjects, gastric carcinoma and DU patients. It was found that Hp positive DU patients show excessive gastrin release and gastric acid secretion combined with increased expression and luminal release of EGF and TGFα. These changes returned to normal values two years after the eradication of Hp. Gastric cancer patients also showed increased expression of EGF and TGFα and highly increased plasma gastrin but their gastric acid secretion was markedly reduced possibly due to atrophy of oxyntic mucosa. This study indicates that overexpression of growth factors in gastric mucosa may be implicated in the pathogenesis of both duodenal ulcer and gastric cancer and that Hp positive hypochlorhydric and hypergastrinemic patients may be predisposed to development of gastric cancer.

**Key words:** duodenal ulcer, gastric secretion, gastric carcinoma, gastrin, EGF, TGFα.

**INTRODUCTION**

*Helicobacter pylori* (Hp) infection is the major risk of peptic ulceration (1—3) and gastric carcinoma (3) but the involvement of Hp in the ulcerogenesis and carcinogenesis has not been explained.

Growth factors such as epidermal growth factor (EGF) and transforming growth factor alpha (TGFα) are implicated in the maintenance of mucosal integrity and repair of already established mucosal damage within the
gastrointestinal tract (4—7). EGF originates mainly from the salivary secretion and is present in gastric and duodenal juices and in urine as urogastrone (8, 9). TGFα is produced predominantly in the gastric mucosa, especially when exposed to topical irritants (5, 6) and present in intestinal type of gastric carcinoma (10) and gastric carcinoid tumours (11). There is a pronounced increase in the EGF receptors and EGF producing cells around chronic gastric ulcers in rats (12) and in the area adjacent to peptic ulcerations in humans (13). This suggests that EGF plays an important role in ulcer healing and mucosal repair. This is supported by recent finding that there is an increase of gastric luminal EGF release following histamine stimulation in patients with DU (14) though earlier studies suggested a decreased release of EGF into saliva and gastric juice in peptic ulcer patients (15). The expression of growth factors in the gastric cancer has been little studied (10, 11) and no information is available regarding the expression and role of growth factor in gastric carcinogenesis.

This study was undertaken to compare the changes in EGF and TGFα expression in gastric mucosa and in luminal release of these growth factors in normal Hp negative subjects and those with Hp positive gastric carcinoma and duodenal ulcer (DU) patients before and after the eradication of Hp.

METHODS

Ten Hp-negative healthy controls (mean age 45 years, range 18—55 years), ten Hp-positive gastric carcinoma (mean age 48 range 35—67 years) and twenty Hp positive DU patients (mean age 42, range 30—55 years) were included into the study. The Hp status was assessed by CLO-test (Campylobacter-like Organism test, Delta West Pty Ltd, Bentley, Western Australia Ltd). CLO test result was assigned as Hp-positive when a change of color from yellow to red was immediate or up to 3 h after adding the biopsy sample to the slide containing urea and phenol red at 30—40°C. All patients with gastric cancer or duodenal ulcer were diagnosed by clinical history and actual gastroduodenal endoscopy, gastric biopsy and histopathology. The study was approved by Institutional Research Review Committee at the University School of Medicine, Cracow, Poland and informed consent was obtained from each subject.

For luminal release of EGF, gastric content was aspirated after an overnight fast during 30 min basal conditions and 60 min of pentagastrin (2 μg/kg-h) infusion. The saliva was constantly expectorated and the gastric juice was collected separately, its volume was measured and the samples adjusted to pH 7.0 by adding 100 mM NaOH were stored at −70°C until the radioimmunoassay of EGF using commercially available reagents (Amersham, Buckinghamshire, UK) as described previously (9). The antiserum raised in rabbits against human EGF was used at a final dilution of 1:20000.

Additional samples of gastric juice were collected during basal state and following pentagastrin infusion for measurement of HCl concentration and output as described previously (9). Blood samples were also obtained for determination of basal concentration of gastrin using radioimmunoassay (9).

During endoscopy, at least two samples were obtained from the mucosa of antral portion of the stomach or carcinoma tissue for determination of immunoreactive EGF and TGFα and immediately stored at −70°C until analysis. The stored samples were weighed, thawed and
homogenized in 0.01 M phosphate buffered saline (PBS). The homogenates were centrifuged at 12000 g for 20 min at 4°C until assay of EGF and TGFα was performed as in gastric juice samples described earlier (9). For immunocytochemistry, two additional biopsy samples were obtained from gastric antrum or gastric carcinoma. Formalin-fixed paraffin-embedded examples of normal gastric mucosa (normal subjects), antral gastritis (DU patients) and gastric carcinoma were selected for the evaluation of immunostaining. 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 min and incubated with specific monoclonal antibody directed against EGF (1/40; GF 0.1, Oncogene Science, New York, USA, and TGFα (1/20, G10 Oncogene Science, New York, 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 examination of 100 consecutive cells in three regions of the gastric mucosa, the surface epithelium, the neck region and the basal portion of gastric glands as described previously (12). 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 components also visible), or 3+ = densely staining equal to simultaneously positive control. Positive control sections were obtained from pancreatic carcinoma (TGFα) and submandibular gland (EGF), showing maximal labeling with the appropriate antibody.

Gastric secretory studies and endoscopy combined with biopsy, histology and immunocytochemistry were performed once in healthy Hp-negative subjects and in gastric carcinoma patients. In Hp-positive DU patients the studies were repeated 4 weeks and 2 years after the eradication of Hp using 2 week triple therapy including omeprazole 20 mg twice daily, amoxicillin 500 mg four times daily and metronidazole 500 mg twice daily).

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 and P value <0.05 was considered significant.

RESULTS

Fig. 1 shows the values of basal plasma gastrin concentrations and gastric acid outputs under basal conditions and after pentagastrin stimulation in healthy Hp-negative subjects, gastric carcinoma patients and DU patients before and 4 weeks and 2 years after the eradication of Hp. Basal and pentagastrin-induced acid outputs were significantly reduced in gastric carcinoma patients as compared to healthy controls. In DU patients, pentagastrin-stimulated but not basal acid output was significantly higher before the Hp eradication but then declined to the level similar to that in healthy controls after four weeks and two years after the eradication therapy.

Plasma gastrin concentrations in gastric carcinoma and in Hp infected DU patients were several fold higher than those in healthy controls. Following the eradication of Hp in DU, the plasma gastrin fell within 4 weeks to the levels observed in healthy controls. This reduced plasma gastrin level remained decreased two years after the eradication of Hp.
Basal concentrations of EGF in gastric juice were similar in healthy controls and gastric carcinoma or DU patients without or with eradicated Hp infection but following the stimulation with pentagastrin there was a marked rise in luminal concentration of EGF (Fig. 2). The highest increment in luminal EGF was observed in DU patients four weeks after the eradication of Hp. In gastric carcinoma patients the rise in luminal concentration of EGF after pentagastrin stimulation was higher than that in Hp infected DU patients and did not differ significantly from that recorded in healthy controls.

The contents of immunoreactive EGF and TGFα in biopsy samples of antral mucosa of healthy controls, gastric carcinoma and DU patients before and 4 weeks and 2 years after eradication of Hp are presented on Table 1.
Table 1. Immunoreactive EGF and TGFα contents (pM/g wet tissue) in biopsy samples of antral mucosa of healthy subjects (N = 10), gastric carcinoma (N = 10) and patients with duodenal ulcer (DU) (N = 20) before and four weeks and 2 years after triple anti-Hp therapy.

<table>
<thead>
<tr>
<th></th>
<th>EGF (pM/g)</th>
<th>TGFα (pM/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal controls</td>
<td>0.15 ± 0.06</td>
<td>5.31 ± 0.62</td>
</tr>
<tr>
<td>Gastric carcinoma</td>
<td>2.5 ± 0.21*</td>
<td>9.4 ± 0.63*</td>
</tr>
<tr>
<td>Duodenal ulcer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>before therapy</td>
<td>1.1 ± 0.25*</td>
<td>7.4 ± 0.35*</td>
</tr>
<tr>
<td>4 wks after therapy</td>
<td>1.7 ± 0.15*</td>
<td>6.9 ± 0.28*</td>
</tr>
<tr>
<td>2 yrs after therapy</td>
<td>0.18 ± 0.09</td>
<td>4.85 ± 0.57</td>
</tr>
</tbody>
</table>

Asterisk indicates significant (P < 0.05) increase above the values in healthy controls.

In healthy subjects there was only a negligible content of immunoreactive EGF and it was localized mainly in the lumen of gastric glands. Mucosal contents of TGFα in healthy controls were about 6 times higher than that of EGF. Both these peptides were present in significantly higher amounts in Hp-positive gastric carcinoma and in antral mucosa of Hp-positive DU patients. After the eradication of Hp in the latter patients the tissue contents of EGF and TGFα remained elevated after four weeks upon the Hp eradication but two years later the contents of growth factors fell to the values not significantly different from those in healthy controls.

![Fig. 3. EGF and TGFα immunocytochemical expression in antral mucosa of healthy controls, Hp-positive gastric cancer and duodenal ulcer patients before and four weeks and two years after the eradication of Hp. Asterisk indicates significant increase above the value recorded in healthy subjects.](image)

As shown on Fig. 3, the EGF immunostaining of antral mucosa in healthy controls was relatively weak as compared to that of TGFα. In gastric carcinoma, which was histologically classified as intestinal-type, the staining of both EGF and particularly of TGFα was several times stronger than that in intact antral mucosa of healthy subjects. The intensity of TGFα cytoplasmic
staining in carcinoma was invariably stronger than that of surrounding morphologically normal gastric epithelium. In Hp-positive DU patients before the therapy and 4 weeks after the eradication of Hp, both EGF and TGFα immunostaining was significantly higher than that in healthy controls but after 2 years upon the eradication of Hp the immunostaining for both EGF and TGFα returned to the values observed in normal subjects.

**DISCUSSION**

This study demonstrates that there is an overexpression of TGFα, and to lesser extent, EGF in Hp infected gastric mucosa of DU and in gastric carcinoma. The immunoreactivity of TGFα and EGF was confined to the differentiated compartment of antral mucosa and greatly augmented in the mucosa of Hp-positive DU patients Hp and in intestinal type of gastric carcinoma.

The role of increased expression of EGF and TGFα in Hp infected patients is not clear. It is well established that gastric epithelial cells proliferate more rapidly in patients with Hp infection as compared to noninfected healthy controls (16, 17). This hyperproliferation may have tumor promoting effect possibly due to an overexpression of growth factors, particularly of TGFα in the Hp infected mucosa.

Another factor responsible for the enhanced mitogenesis could be an increased plasma level of gastrin which is a potent mucosal growth stimulating agent. Plasma gastrin was greatly increased in Hp infection both in DU and in gastric carcinoma patients. In DU patients, the hypergastrinemia seems to be directly associated with the gastric infection by Hp because the eradication of p in these patients was followed by almost immediate normalization of plasma gastrin and the fall in gastric acid secretion.

This study does not explain how the Hp infection could lead to carcinogenesis. It is likely that Hp infection is indirectly mutagenic because it increases the chances of converting DNA damage caused by endogenous breakdown of DNA or by ingested carcinogens into stable mutations (17). The concept of „chronic mitogenesis“ facilitating mutagenesis has been proposed by Correa et al. (18) before the Hp area. The increased expression of growth factors in gastric carcinoma observed in our study could contribute to the uncontrolled mitogenesis. At present, the role of Hp infection in gastritis and peptic ulcer is well recognized but epidemiological data suggest that Hp infection may also be a risk in cancer causation (17). It is noteworthy that a Working Group of the WHO International, Agency for Research on Cancer concluded in 1994 that Hp is a group 1 carcinogen in humans (19).

Our gastric carcinoma patients were Hp-positive but unlike Hp-positive DU patients they showed markedly reduced gastric acid secretion possibly due
to atrophic gastritis affecting the oxyntic gland area. The increased plasma gastrin in these patients probably reflected the increased luminal pH as low acid state is known to be associated with hypergastrinemia that in turn could increase the synthesis and release of growth factors (11).

Low acid secretion in atrophic gastritis may allow the colonization of the stomach by a mixed bacterial flora capable of producing nitrosoamines that could damage epithelial cell DNA (17). This might result in the development of atrophy, intestinal metaplasia, dysplasia and finally cancer. Early diagnosis of acid hyposecretion in Hp infected subjects and the reversal of this hyposecretion by the eradication of Hp may have important therapeutic implications (20). The identification of Hp infected patients with hyposecretion and eradication therapy could prevent the progression of gastric atrophy to cancer. Such eradication could also decrease the expression and the release of growth factors, especially TGFα which (together with gastrin) may be responsible for the hyperproliferation with the tumor promoting effect (17). This is supported by the finding that enhanced tumor growth as measured by bromodeoxyuridine (BrdU) labeling index was positively correlated with the tissue expression of EGF, TGFα and their common receptor (21).

REFERENCES


Received: April 25, 1997
Accepted: May 2, 1997

Authors address: P.Ch. Konturek, Department of Medicine I, University of Erlangen-Nuremberg, Krankenhausstrasse 12, 91054 Erlangen, Germany.