Helicobacter pylori infection. Several studies on pathology and clinicopathology

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CHAPTER 6

Effect of *Helicobacter pylori* infection
on cyclooxygenase-2 express
in gastric antral mucosa

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Abstract

Background/ Aim: Helicobacter pylori (H. pylori) infection is a major etiological cause of chronic gastritis. The inducible cyclooxygenase (COX-2) is an important regulator of mucosal inflammation. Recent studies indicate that expression of COX-2 may contribute to gastrointestinal carcinogenesis. Our aim is to investigate the effects of H. pylori infection on COX-2 expression in gastric antral mucosa before and after antibiotics therapy using immunohistochemistry.

Methods: Antral biopsies were taken from 46 patients with chronic gastritis and H. pylori infection both before and after anti- H. pylori treatment. COX-2 protein was stained by immunohischemical method and expressed as a percentage of the total number of epithelial cells. Gastritis and H. pylori infection status were graded according to the Sydney system.

Results: Cytoplasmic staining of COX-2 protein could be detected in epithelial cell both before and after eradication of H. pylori. The mean percentage of cells staining with COX-2 was significantly higher in H. pylori-infected mucosa, compared with mucosa after successful H. pylori eradication (20.1%±13.1% vs 13.8%±5.9%; p<0.05) and normal controls (18.0%±14.1% vs 12.3%±4.6%, p<0.05). COX-2 immunostaining was correlated with the degree of chronic inflammation (r=0.78, p<0.05).

Conclusions: Our results showed that H. pylori infection leads to gastric mucosal overexpression of COX-2 protein, suggesting that the enzyme is involved in H. pylori-related gastric pathology in humans.

Key words  Cycooxygenase-2; Helicobacter pylori; Chronic gastritis
Introduction

Cyclooxygenase (COX) is the rate-limiting enzyme required for the conversion of arachidonic acid to prostaglandins (PGs). Two cyclooxygenase isoforms have been identified and referred to COX-1 and COX-2. Under many circumstances, the COX-1 enzyme is produced constitutively in site of normal tissues while COX-2 is inducible in macrophages, fibroblasts and other cell types in inflammation. COX-2 expression is induced by phorbol esters and proinflammatory cytokines, including interleukin (IL)-1 β and tumor necrosis factor (TNF)-α. Recent studies indicate that it is inducible COX-2 that plays an important role in gastrointestinal carcinogenesis. *H. pylori* infection is a major cause of chronic active gastritis and can be associated with gastric adenocarcinoma and MALToma. This study was designed to investigate the effects of *H. pylori* infection on COX-2 expression in gastric antral mucosa.

Materials and Methods

Patient subjects

All patients underwent upper gastrointestinal endoscopy at the center of endoscopy of Renji Hospital affiliated to Shanghai Second Medical University during 1997 and 1998 before and 4~6 weeks after treatment, including 46 (32 men and 14 women; age range, 16~64yr). According to the mapping principle of our study, during each endoscopic examination, three biopsy specimens each were taken from the antrum and the corpus, respectively. Among these, one specimen each were used for rapid urease test, the others were fixed in 10% formalin and
stained with H&E for histologic examination and Giemsa stain for H. pylori assessment. H. pylori assessment was performed by the rapid urease test and histopathology (Giemsa stain). All patients were positive for H. pylori. Twenty-seven patients with superficial chronic gastritis and 19 with mild atrophic gastritis proven by endoscopy and histopathology were selected in the study.

All patients with H. pylori positive chronic gastritis were given a 7-day course of bismuth triple therapy: De-Nol (CBS) 240mg Bid with two kinds of antibiotics. The patients again underwent endoscopy and biopsy at 4~6 weeks after treatment. Eradication of H. pylori infection was defined as absence of microorganisms in both rapid urease test and tissue sections 4-6 weeks after cessation of therapy.

Ten H. pylori negative patients (5 men and 5 women; age range, 16~38yr) with endoscopically and histologically confirmed normal gastric mucosa were selected as control group.

None of patients had been taking NSAIDs or glucocorticoid regularly before the endoscope in our study.

**Histological assessment**

All biopsy specimens for histologic examination were fixed in 10% formalin, and embedded in paraffin on oriented edge, cut in sequential 5 μm sections. All tissue sections were stained with haematoxylin and eosin for histologic examination and with a Giemsa stain for H. pylori assessment. Only gastric biopsies from the antrum were performed immunostain in our study.
The severity of chronic gastritis and activity, and atrophy and intestinal metaplasia, as well as *H. pylori* density were evaluated and graded on a four point scale (absent or normal, mild, moderate and severe abnormality), according to Sydney classification for the histological grading of gastritis.

**Immunohistochemistry**

COX-2 polyclonal antiserums and ABC kit were brought from Santa Cruz Corporation USA. Tissue sections were dewaxed in xylene, rehydrated through graded alcohol to distilled water. Antigen retrieval was performed by microwave treatment in sodium citrate buffer (10mmol/L, pH 6.0), and then cooled to room temperature. Subsequently, they were immersed in 1%H$_2$O$_2$ in methanol for 15 min and then immersed in normal monkey serum for 15 min to block unspecific binding sites. After extra normal serum removal, slides were incubated with goat polyclonal IgG specific for human COX-1/COX-2 in a dilution of 1:100 overnight at 4°C. Washing in PBS for 5 min for three times, the sections were thereafter treated with biotinylated secondary antibodies at 37°C for 60 min. Washing in PBS for 5 min for three times, they were then stained with the standard avidin-biotin-peroxide complex (ABC) methods and visualised using 3,3'-diaminobenzidine(DAB). The sections were counterstained with hematoxylin.

**Evaluation of immunostaining**

Immunohistochemical staining for COX-2 was assessed by counting the number
of positive and negative staining cells on the surface epithelial cells, fovealar and inherent glands of gastric mucosa of each biopsy. The number of positive staining cells was expressed as a percentage of the total number of cells. The pathologist was blinded to the *H. pylori* status of the patient. Positive cells showed cytoplasmic and perinuclear brownish staining. Only the blue nuclear staining was recognised as negative. From the ten fields counted, the percentage of average positive cells was graded semiquantitatively.

**Statistical Analysis**

Statistical significance was calculated with the Student t test and Spearman signed rank test, and $p<0.05$ was selected as the statistically significant value.

**Results**

**COX-2 protein expression in gastric antral mucosa**

Cytoplasmic staining of COX-2 protein could be detected in the surface epithelial cells and inherent glands in the antral mucosa of normal group and *H. pylori* positive group both before and after eradication of *H. pylori*. Positive cells were mainly located in the neck of foveolae and superficial propria. Almost all intestinal-type epithelium displayed COX-2 protein positive staining. Interstitial cells, including monocyte, endothelium and smooth muscle fiber also sporadically displayed COX-2 protein positive staining.
Correlation between COX-2 protein expression and *H. pylori* infection

The mean percentage of cells staining with COX-2 was significantly higher in *H. pylori*-infected mucosa, compared with normal controls (18.0% ± 14.1% vs 12.3% ± 4.6%, p<0.05). Successful eradication of *H. pylori* has obtained in 32 out of 46 (70%) patients after a triple antibiotic therapy. The mean percentage of cells staining with COX-2 before treatment was 20.1% ± 13.1% and decreased to 13.8% ± 5.9% after successful *H. pylori* eradication (p<0.05). There was no significant difference between normal controls and patients after successful *H. pylori* eradication (13.8% ± 5.9% vs 12.3% ± 4.6%; p>0.05). The mean percentage of cells staining with COX-2 was 17.8% ± 10.1% in 14 patients in whom *H. pylori* infection remained 4–6 weeks after treatment, there was also no significant difference compared with those before treatment (p>0.05).

Correlation between COX-2 immunostaining and chronic inflammation

COX-2 immunostaining was correlated with the severity of chronic inflammation (r=0.78, p<0.05). However, no correlation was found between COX-2 protein expression and the density of *H. pylori* colonization and the activity of gastritis (p>0.05).

Discussion

Several studies have demonstrated that COX-2 protein expression was significantly increased in gastric and colonic adenocarcinoma. The studies of animal model of human familial adenomatous polyposis (FAP) has shown that elevated
levels COX-2 mRNA and protein expression were also observed in epithelial cells of colonic adenomas [4,5]. NSAIDs and specific inhibition of COX-2 could reduce the size and number of adenoma, and may play a prophylactic role in the adenoma-carcinoma sequence, suggesting that COX-2 may play an important role in the early onset of gastrointestinal carcinogenesis. This effect has also been demonstrated in humans [6].

*H. pylori* infection is the major cause of gastroduodenal diseases, including chronic active gastritis, peptic ulcers and gastric malignancy (both adenocarcinoma and lymphoma), and leads to gastric inflammation. The International Agency for Research on Cancer has classified *H. pylori* as a class I carcinogen, a definite cause of gastric cancer in human, but its mechanism on carcinogenesis deserves further study.

We have shown that elevated COX-2 protein expression was observed in epithelial cells, monocytes and intestinal-type epithelial cells of the normal controls and *H. pylori* positive group both before and after eradication of *H. pylori* in our study. COX-2 protein expression was significantly increased in epithelial cells of *H. pylori*-infected mucosa and was closely associated with the severity of chronic gastritis. Recently, the study by Zarrilli et al have demonstrated that COX-2 mRNA expression was significantly increased in *H. pylori*-infected mucosa compared with *H. pylori* negative mucosa. It's still unknown how *H. pylori* infection induces expression of COX-2 protein in the gastric epithelial cells. The study by Romano found that cyclooxygenase-2 mRNA and prostaglandin E₂ levels increased by 5-fold.
and 3-fold at 24 h after incubation of MKN 28 cells with broth culture filtrates or bacterial suspensions from wild-type H. pylori strain. These have suggested that COX-2 expression was induced by H. pylori and its bacteria toxins or inflammatory cytokines or by a paracrine-signaling pathway between macrophages and the neighboring epithelial cells.[14].

It remains unclear how COX-2 protein overexpression contributed to the carcinogenesis. Studies of animal model have reported that high level of COX-2 protein could increase PGE₂ biosynthesis which could induce cell proliferation and further stimulate over expression of bcl-2 protein and therefore resistant to apoptosis. Loss of balance on proliferation and apoptosis may promote the progression of tumor. H.pylori infection can induce proliferation in gastric epithelial cells. Several studies have demonstrated that there is high level proliferation in the gastric epithelial cells, but no corresponding change in apoptosis. COX-2 protein may play an important role in the multistep process of "H.pylori related gastritis→precancerous lesion→gastric cancer". This support the specific COX-2 inhibitors as chemopreventive agents in prevention of gastrointestinal carcinogenesis.

Reference


