Expression of Cyclooxygenases in Helicobacter pylori Gastritis and Residual Gastritis after Distal Gastrectomy

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Abstract. It has long been thought that duodenal reflux induces residual gastritis after distal gastrectomy. Helicobacter pylori infection appears to be another factor in residual gastritis; and H. pylori induced gastritis may exist preoperatively or may have been introduced postoperatively. Up until now, the surgical effect itself and H. pylori infection have not been well differentiated as causes of residual gastritis. Our aim in this study was to clarify the relationship between the surgical effect and H. pylori infection in residual gastritis. A residual gastritis model using the Mongolian gerbil has been established with microsurgical technique. Residual gastritis with and without H. pylori infection has been studied by histopathological examination and quantitated by Rausw’s score. The expression of cyclooxygenase (both COX-1 and COX-2) has also been examined immunohistologically. Elevation of pH in gastric juice after surgery was confirmed. H. pylori infection led to deterioration after surgery. The postoperative Rausw’s score with infection is higher than without infection. Levels of COX-1 were higher after surgery in both animals. COX-2 was not expressed in the animals without infection and only a little was expressed in the animals with infection. COX-2 was strongly expressed in the operated animals with infection, but the surgical effect was minute in the animals without infection. Residual gastritis consisted of both surgical gastritis and H. pylori gastritis. H. pylori gastritis is curable with eradication of the organism, but surgical gastritis is not. The COX inhibitor can be a good candidate in preventing residual gastritis after eradication of the H. pylori organism. The clinical implications of COX expression for patients with residual gastritis might deserve further study in the point of treatment of surgical and H. pylori gastritis.

The discovery of Helicobacter pylori and its clinical implications have been reported by leading gastroenterologists [1]. It is believed that H. pylori is one of the major causes of gastritis and peptic ulcer, although H. pylori is not the only causative factor in residual gastritis after gastric surgery [2]. The surgical effect itself and H. pylori infection have not been well distinguished as causes of residual gastritis. Indeed, residual gastritis appears to result from the surgical procedure, which produces duodenal reflux. H. pylori infection may then develop during the postoperative course. Further, some patients may have been infected with H. pylori before surgery.

Prostaglandin (PG) has an ability to protect the mucosal lining of the stomach against injury [3]. The PG endoperoxide synthase, cyclooxygenase (COX), is one of the rate limiting enzymes of PG synthesis derived from arachidonic acids. There are two isoforms of COX, a constitutively produced COX-1 and an inducible COX-2 [4–6]. COX-1 protein is seen in a variety of tissues, including the stomach. In contrast, COX-2 is induced in macrophages by lipopolysaccharide [7, 8], in fibroblasts by platelet-derived growth factor [9], and in epithelial cells by an epidermal growth factor (EGF) family peptide [10, 11]. Many studies in recent years have suggested that COX-1 is expressed in the intact stomach and contributes to protection of the gastric mucosa, whereas COX-2 contributes to the repair of lesions [12].

In the present study, using an animal model of distal gastrectomy, we investigate the association between H. pylori infection and the surgical procedure with the goal of identifying the extent of COX expression.

Materials and Methods

Animals and Surgical Procedures

The male Mongolian gerbils with and without H. pylori infection (8 week old, approximately 60 g of body weight) were obtained from Seyaku Yoshitomi Co. (Fukuoka, Japan) and kept under quarantine for one week before use in the experiments. Animals were kept in plastic cages, four per cage, and maintained in air-conditioned quarters with a room temperature of 20° ± 2°C, relative humidity of 50%; ± 10%, and an alternating 12:12-hour light-dark cycle. Under anesthesia, animals underwent laparotomy, and distal gastrectomy and a Billroth I reconstruction was performed under the microscope (Kona Medical Inc, Tokyo, Japan). Four weeks after operation, animals were sacrificed and bile reflux was confirmed with testing tape (Tokyo Roshi Co., Tokyo, Japan) to check the pH of the gastric juice. For the animals with H. pylori infection, the presence of H. pylori in the specimens was confirmed by rapid urease test and histologically using the Giemsa stain. Specimens of the stomach-joining anastomosis were rendered for analysis.

Histopathological Examination and Gastritis Scoring

The residual stomach was removed, opened longitudinally, and fixed in 10% buffered formalin. After the organ was embedded in...
paraffin, 4-mm serial sections were prepared and stained with he-
matoxylin and eosin. Gastritis was measured quantitatively by his-
tological examination of specimens taken from the gastric mucosa
using Rauws’ score.

Expression of COX

Expression of COX-1 and COX-2 was examined using an immuno-
histological staining kit (Histofine, Nichirei, Tokyo, Japan). Affin-
ity-purified goat anti-COX-1 and anti-COX-2 (Santa Cruz Bio-
technology, Inc, Santa Cruz, CA, USA) was used in dilution 1:200.
As controls, slides were incubated with the same concentration of
goat IgG (Santa Cruz Biotechnology, Inc, Santa Cruz, CA, USA) or
with phosphate-buffered saline alone. The color was developed us-
ing 3,3'-diaminobenzidine. Mayer’s hematoxylin was then added as
a counterstain.

Statistical Analysis

The data are expressed as arithmetic means (±SEM) unless other-
wise stated. Statistical comparisons between groups were per-
formed by Student’s t-test. The difference was considered to be sig-
nificant when p < 0.01.

Results

Elevation of pH in Gastric Juice after Distal Gastrectomy

To confirm the existence of duodenal reflux after distal gastrec-
tomy, the pH in the gastric juice was measured in animals with and
without H. pylori infection (Fig. 1). Although the pH before surgery
was 2.00 ± 0.16 in the animal without H. pylori infection and 2.08 ±
0.15 in the animal with infection, both animals showed significant
elevations of pH after distal gastrectomy, indicating that the opera-
tion was performed satisfactorily and there was no stenosis (4.13 ±
0.30 and 3.86 ± 0.37, respectively).

Scoring of Residual Gastritis

Residual gastritis with and without H. pylori infection was studied
by histopathological examination. Macroscopically, residual gastri-
tis was obvious in the animals 4 weeks after operation. In the case of
H. pylori infection, the gastritis was more severe after distal gastrec-
tomy. The degree of gastritis was quantitated using Rauws’ score
(Fig. 2). The score in animals without H. pylori infection was 0.20 ±
0.20 and increased after distal gastrectomy (2.25 ± 0.63). The score
was already high in the animal with H. pylori infection (5.33 ± 0.35)
and did not increase after the surgical procedure (5.43 ± 0.20). The
postoperative score with H. pylori infection was higher than that
without infection, indicating the additive effect of H. pylori infec-
tion for the gastritis.

Immunohistochemical Staining of COX-1 and COX-2

COX-1 was expressed both with and without H. pylori infection. The
expression of COX-1 was slightly augmented after distal gastrec-
tomy (Fig. 3). COX-2 was weakly expressed in the animals with
H. pylori infection and was augmented after operation (Fig. 3).
There was little expression of COX-2 in the animals without H.
pylori infection.

Discussion

After partial gastric resection, duodenal reflux is increased through
the anastomotic site because of the lack of a pyloric sphincter. In
patients with chronic atrophic gastritis, intestinal metaplasia (IM)
is observed after surgery and is considered a risk factor in gastric
cancer [13]. Meanwhile, H. pylori is commonly seen many years af-