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ORIGINAL ARTICLE

Delayed healing of gastric ulcer is associated with downregulation of connexin 32 in the gastric mucosa

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Summary *Background/Aims:* Most benign gastric ulcers are healed through suppression of gastric acid by a proton pump inhibitor (PPI). Despite prolonged use of a PPI, some gastric ulcers still do not heal. The primary goal of this study is to investigate the relationship between the expression of connexin 32 (Cx32), a major gap junction protein expressed in the gastric mucosa, and the healing response of gastric ulcers.
Methods: Patients with endoscopically verified gastric ulcer were treated with a standard dose of PPI for 12 weeks. Histological studies were performed to exclude malignancy. In total, 10 patients having endoscopically verified gastric ulcers with delayed healing at the end of the PPI course were included in this study. The control group consisted of 11 patients with gastric ulcers that healed normally. The expression of Cx32 in the gastric mucosa of the ulcer margin was analyzed by immunoblotting.
Results: Patients with gastric ulcer showing delayed healing had significantly reduced Cx32 expression in the gastric mucosa compared with the patients in whom the ulcers healed normally (i.e., controls). Age, sex, presence of duodenal ulcers, location and size of gastric ulcer, ulcer staging, *Helicobacter pylori* infection, use of nonsteroidal anti-inflammatory drugs (NSAIDs) and aspirin, smoking, and alcohol consumption were similar in both the control and delayed healing groups. *H. pylori* infection, use of NSAIDs, smoking, and alcohol consumption all had no significant impacts on the expression of Cx32. Age and expression of Cx32 were not correlated.

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Conclusion: Downregulation of Cx32 in the gastric mucosa of the ulcer margin may predict delayed healing in patients with gastric ulcer following acid-suppression therapy.

Introduction

The pathogenesis of gastric ulcer involves the interplay between injuries, defense, and repair of the gastric mucosa, as it is constantly exposed to a harmful environment. The chronic nature of gastric ulcers has been described to be associated with the size of the ulcer [1], smoking [2], persistence of *Helicobacter pylori* infection [3], continued use of nonsteroidal anti-inflammatory drugs (NSAIDs) [4], and an impaired response to antisecretory agents [5].

Suppressing acid secretion is the mainstay of treatment for a gastric ulcer [6]. During the period when histamine-2-receptor antagonists (H2RAs) were popular, gastric ulcers that did not healed well after treatment with a standard dose of H2RA for 12 weeks were termed "intractable" or "refractory ulcers" [7]. Proton pump inhibitors (PPIs) provide better healing effects and fewer relapses than H2RA in managing gastric ulcers [6,8]. However, even after aggressive and prolonged treatment with PPI, not all gastric ulcers heal completely [5,9]. Even after removing noxious environmental agents, poor healing of benign gastric ulcers can still be observed in certain circumstances [5,10]. The pathogenesis of this delayed healing response is not well understood. Transforming growth factor-β (TGF-β) and its receptors were found to be strongly expressed in well-healed ulcers but they were downregulated in half of the refractory gastric ulcers. In the other half of the refractory gastric ulcers, TGF-β was still normally expressed in the mucosa [11]. Factors other than growth factors may play a role in the healing process of a gastric ulcer.

Gap junction intercellular communication is crucial in diverse cellular processes such as homeostasis, morphogenesis, cell differentiation, and growth regulation. Communication spreads through gap junctional channels constructed by a family of connexin proteins [12]. Connexins are associated with a wide range of biological functions from providing restitution following cell damage to maintaining the integrity of the gastric mucosa [13]. Gap junction proteins in the gastric mucosa have been associated with several pathological states of the stomach, including intestinal metaplasia and gastric carcinoma [14,15]. A decrease in gap junctions has also been observed in the active stage of human gastric ulcers [16].

Connexin 32 (Cx32) is among the major gap junction proteins expressed in the gastric mucosa. Few studies have examined the role of Cx32 in the healing process of gastric ulcers. It is decreased or absent during the active stage of the ulcers, but its reappearance is reported to be related to the healing of gastric ulcers [17,18]. The role of gap junctions when gastric ulcers persist, however, remains unclear. The purpose of this study was to investigate the association of Cx32 expression between different healing responses of gastric ulcers. The expression level of Cx32 was compared between normally healed gastric ulcers and those with delayed healing.

Methods

Patients

From May 2007 to November 2008, patients with spontaneously developed gastric ulcers were recruited. The ulcers were documented by a standard procedure of upper gastrointestinal endoscopy. Patients were excluded if ulcers were related to malignancy, gastrinoma, Zollinger—Ellison syndrome, or opportunistic infection by microorganisms such as a virus or fungus, which was confirmed by standard histological procedures. Patients were treated with a standard-dose PPI (e.g., lansoprazole 30 mg daily, pantoprazole 40 mg daily, or rabeprazole 20 mg daily) for 12 weeks, and compliance was ensured. At the end of the treatment period, a repeat endoscopy was performed to evaluate the healing of the gastric ulcer. Patients with a gastric ulcer that did not heal after the 12-week treatment period were assigned to the delayed healing group, whereas patients with a gastric ulcer that healed completely were used as controls and assigned to the normal healing group. Patient histories were reviewed in detail for age, sex, concomitant use of NSAIDs including aspirin, and current smoking or alcohol consumption. The study protocol was approved by the Institutional Review Board of Mackay Memorial Hospital, Taipei, Taiwan. All patients provided written informed consent and agreed to be enrolled.

Endoscopic examination and histologic studies

An upper gastrointestinal endoscopic examination was performed, and the endoscopic findings were evaluated by experienced endoscopists. The location and size of gastric ulcers were recorded. The endoscopic staging of gastric ulcers was evaluated. Biopsies were taken from the mucosa of the ulcer margin using biopsy forceps at the first endoscopic examination in both study groups. The specimens subjected to histological analysis were fixed in 4% paraformaldehyde in 0.1M phosphate buffer. Tissues were then embedded in paraffin and sectioned. Histological sections were stained with hematoxylin and eosin. Detailed malignancy surveillance was performed carefully, and tissue sections were also subjected to modified Giemsa staining to detect the presence of *H. pylori*. Immunochemical analysis was then performed on paraffin-embedded sections.