Effects of antisecretory agents on angiogenesis during healing of gastric ulcers

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Background. We studied the effects of a proton pump inhibitor (PPI) and an H2-receptor antagonist (H2-blocker) on angiogenesis during gastric ulcer healing, by examining stromal cell-derived factor (SDF-1) and CXC chemokine receptor 4 (CXCR4) expression in the gastric mucosa. Methods. Patients with gastric ulcers were allocated to an untreated control group, consisting of patients with active ulcers (GA), healing ulcers (GH), and ulcer scars (GS) or a PPI group (P; given rabeprazole at 20mg/day), or an H2-blocker group (H; given nizatidine at 800mg/day). Frozen sections of biopsy specimens were examined by reverse transcription-polymerase chain reaction (RT-PCR) to analyze SDF-1 and CXCR4 mRNA. Results. CXCR4 mRNA levels were elevated in the control (GH and GS patients) group and the H2-blocker group. CXCR4 was significantly elevated in the P-GA subgroup of the PPI group (P < 0.01), but its level decreased with time. Conclusions. In the PPI group, CXCR4 levels were increased in the early phase of ulcer healing and returned to a level similar to that in the control group during the scar phase. These results suggest that PPIs increase the expression of CXCR4 mRNA and thus promote vessel regeneration and maturation, facilitating ulcer healing.

Key words: gastric ulcer healing, angiogenesis, CXC chemokine receptor 4 (CXCR4), stromal cell-derived factors-1 (SDF-1), proton pump inhibitor (PPI)

Introduction

The initial stage of blood vessel formation in the fetus involves angiogenesis, which includes the differentiation of mesenchymal cells, derived from the mesoderm, into endothelial cells and the formation of a vessel lumen. Angiogenesis also involves the extension and union of the lumens to form an initial capillary network, followed by tissue- and organ-specific remodeling that leads to the formation of mature blood vessels. During early fetal vasculogenesis, vascular endothelial growth factor (VEGF) and its receptors, kinase insert domain-containing receptor (KDR) and fms-like tyrosine kinase-1 (Flt-1), are required for the development of the systemic vasculature. During late fetal angiogenesis, stromal cell-derived factor (SDF-1) and its receptor, CXC chemokine receptor 4 (CXCR4), play an organ-specific role in the formation of large blood vessels supplying the stomach and intestines.1 Similar receptor-ligand systems play a role in angiogenesis occurring in mature individuals. SDF-1 has been reported to promote angiogenesis by microvascular endothelial cells.2 CXCR4 is expressed by normal human colonic epithelial cells and may play a role in the maintenance and renewal of the colonic epithelium.3

We previously reported that SDF-1 and CXCR4 participated in the development and maturation of blood vessels during gastric ulcer healing in humans and in rats.4,6 Proton pump inhibitors (PPIs) are more potent than H2-receptor antagonists (H2-blockers) in terms of inhibiting gastric acid secretion7,8 and promoting ulcer healing. Unlike PPIs, H2-blockers suppress angiogenesis and granulation tissue formation during ulcer healing.9-11 To further clarify the differences between these two classes of drugs, we measured SDF-1 and CXCR4 mRNA levels in human gastric ulcers and evaluated the effects of PPI and H2-blocker therapy on angiogenesis during ulcer healing. Rabeprazole was chosen as the PPI because it has been reported to mainly undergo nonenzymatic metabolism, so the response to this drug is therefore not affected by genotype, and individual differences in the inhibition of gastric acid secretion are minimal.12,13
Subjects and methods

Subjects

The subjects comprised 54 patients with ulcers located at the gastric angle, 7 patients with previously untreated Helicobacter pylori-positive gastritis (gast+), and 7 with H. pylori-negative gastritis (gast–). All diagnoses were confirmed endoscopically. The mean age of the subjects was 51.5 ± 12.4 years. Thirty-three patients were men and 35 were women. Informed consent was obtained from all of the subjects. The patients with gastric ulcers were all positive for H. pylori and were allocated to either an untreated control group, a PPI group, or an H2-blocker group. The untreated control group comprised 7 subjects with active ulcers (GA), 7 with healing ulcers (GH), and 7 with ulcer scars (GS). The PPI group (P) was administered rabeprazole at 20mg/day; the P-GA subgroup consisted of 4 subjects treated for 2 to 3 days, the P-GH subgroup consisted of 6 subjects treated for 7 to 10 days, and the P-GS subgroup consisted of 6 subjects treated for 8 weeks. Subjects in the H2-blocker group (H) received 300mg/day of nizatidine; the H-GA subgroup comprised 4 subjects treated for 5 to 7 days, the H-GH subgroup comprised 7 subjects treated for 4 to 8 weeks, and the H-GS subgroup comprised 6 subjects treated for 2 to 5 months. The subjects in the P-GA, P-GH, and H-GA subgroups presented with upper abdominal pain and received treatment before endoscopic examination.

Methods

Histological examination

Two biopsy specimens were taken from both the gastric angle and the central indentation of the ulcer scar in the GS group, and were immediately frozen in hexane/acetone and dry ice.

Formalin-fixed sections were stained with hematoxylin-eosin (H&E) and Giemsa stains. When specimens were negative for H. pylori on both culture and histological examination, and serum anti-H. pylori IgG was also negative, the subject was considered to have no H. pylori infection.

Immunostaining of SDF-1 and CXCR4 in the gastric mucosa

Immunostaining was performed, using the immunoperoxidase method, to detect SDF-1 and CXCR4, using frozen sections. The localization of SDF-1 and CXCR4 in the gastric wall was determined using anti-SDF-1 antibody (R&D Systems, Minneapolis, MN, USA) and anti-CXCR4 antibody (Santa Cruz Biotechnology, Palo Alto, CA, USA).

Analysis of SDF-1 and CXCR4 mRNA expression in the gastric mucosa

Frozen tissue samples were stored at −80°C and were analyzed by the reverse transcription polymerase chain reaction (RT-PCR). The primers used were 5'‐TGG TCG TGC TGG TCC TG-3' and 5'‐AAG GGT TCC TCA GGC GTG TC-3' for SDF-1, 5'‐GTT ACC ATG GAG GGG ATC AG-3' and 5'‐TCC TTG GCC TCT GAC TGT TG-3' for CXCR4, and 5'‐CAA GAG ATG GCC ACG GCT GCT-3' and 5'‐TCC TTC TGC ATC CTG TCG GCA-3' for β-actin.

Total cellular RNA was isolated from gastric biopsy samples, using Trizol (Invitrogen, Carlsbad, CA, USA), and 1 μg of total RNA was reverse transcribed, using a PCR Thermal Cycler (MP model; Takara Bio, Shiga, Japan). Amplification required 40 cycles for SDF-1 and 35 cycles for CXCR4, with each cycle consisting of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and polymerization at 72°C for 30 s (60 s for CXCR4).

PCR products (5 μl) were electrophoretically separated on 3% NuSieve 3 : 1 agarose gel (CBM Intellectual Properties, Rockland, ME, USA), after which the gel was stained with ethidium bromide. The integrated optical density (IOD) of the amplified bands was measured with a fluorescent image analyzer (FMBIO II Multiview, Takara Bio, Shiga, Japan). Then the IOD of each target band was divided by the IOD of the β-actin band for normalization. The level of expression in each sample is shown relative to the baseline value of one gast– subject.

Statistical analysis

Values are expressed as means ± SDs. Significance was determined by Bonferroni’s method, and P < 0.05 was considered significant.

Results

SDF-1 mRNA expression in the gastric mucosa

SDF-1 mRNA expression in the gastric mucosa is shown in Fig. 1a. In the control group, SDF-1 expression was significantly lower in the GH (0.46 ± 0.08) and GS (0.52 ± 0.11) subgroups than in the GA subgroup (1.43 ± 0.26) and the gast+ and gast– groups (P < 0.05). In the H2-blocker group, SDF-1 expression was similar to that in the control group. In the PPI group, SDF-1 decreased in the order of: P-GA subgroup (1.27 ± 0.09) > P-GH subgroup (0.87 ± 0.25) > P-GS subgroup (0.77 ± 0.28),