Endothelin-1, an ulcer inducer, promotes gastric ulcer healing via mobilizing gastric myofibroblasts and stimulates production of stroma-derived factors


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Am J Physiol Gastrointest Liver Physiol 290: G1041–G1050, 2006. First published December 29, 2005; doi:10.1152/ajpgi.00462.2005.—Endothelin-1 (ET-1) is a potent inducer of peptic ulcers. The roles of ET-1 in ulcer healing, however, have remained unclear, and these were investigated in mice. Gastric ulcers were induced in mice by serosal application of acetic acid. Three days later, mice were given a neutralizing ET-1 antibody or nonimmunized serum. The ulcer size, amount of fibrosis and myofibroblasts, and localization of ET-1 and ET-1 receptors were analyzed. To elucidate the mechanisms underlying the effects of ET-1, we examined the proliferation, migration, and release of growth and angiogenic factors in gastric myofibroblasts with or without ET-1. The expression of prepro-ET-1 (an ET-1 precursor) and ET-converting enzyme-1 was examined in gastric myofibroblasts using RT-PCR. Immunoneutralization of ET-1 delayed gastric ulcer healing. The areas of fibrosis and myofibroblasts were smaller in the anti-ET-1 antibody group than in the control. ET-1 was expressed in the gastric epithelium, myofibroblasts, and other cell types. ETA receptors, but not ETB receptors, were present in myofibroblasts. ET-1 increased proliferation and migration of gastric myofibroblasts. ET-1 stimulated the release of hepatocyte growth factor, VEGF, PGE2, and IL-6 from gastric myofibroblasts. mRNA for prepro-ET-1 and ET-converting enzyme-1 was also expressed. ET-1 promotes the accumulation of gastric myofibroblasts and collagen fibrils at gastric ulcers. ET-1 also stimulates migration and proliferation of gastric myofibroblasts and enhances the release of growth factors, angiogenic factors, and PGE2. Thus ET-1 has important roles not only in ulcer formation but also in ulcer healing via mobilizing myofibroblasts and inducing production of stroma-derived factors.

endothelin-A receptors; α-smooth muscle actin; human growth factor; prostaglandin E2; vascular endothelial growth factor

ENDOTHELIN (ET)-1 is a potent vasoconstrictive peptide (43) present in the gastrointestinal tract (35). The effect of ET-1 is exerted predominately by paracrine and autocrine mechanisms through the stimulation of specific receptors, namely, ETA and ETB. ET-1 has a major role in the development of gastric mucosal injury (12, 17, 18, 20, 42). We have previously reported that ET-1 has a pivotal role in the development of gastric ulcers in rats after hemorrhagic shock (20) and ethanol administration (17, 18). A neutralizing antibody against ET-1 (17, 18) and an ETA-specific antagonist (20) suppress the development of gastric injury, indicating important roles for endogenous ET-1 and its receptors in ulcer development. In critically ill patients, plasma and mucosal ET-1 concentrations are significantly higher on admission compared with those of control subjects, and ET-1 levels are significantly higher in injured mucosa than in normal adjacent mucosa, suggesting that ET-1 is closely related to the development of stress ulcers in humans (21). Plasma and mucosal ET-1 levels are also elevated in patients with gastric ulcers, suggesting that the stomach is an important source of circulating ET-1 (16). Moreover, mucosal ET-1 levels are higher in the margins of healing ulcers than in those of active stage ulcers (16). Akimoto et al. (2, 3) also confirmed that ET-1 levels are higher in healing ulcers than in active stage ulcers. They reported that in addition to ET-1, inducible nitric oxide synthase, VEGF, and certain chemokines are increased during gastric ulcer healing. All of these findings suggest that ET-1 influences peptic ulcer healing. Although Akimoto et al. (3) argued that there was a possible interaction among ET, nitric oxide, and VEGF during gastric ulcer healing, there has been no evidence demonstrating that endogenous ET-1 promotes or delays ulcer healing.

Myofibroblasts transiently appear at the site of tissue injury and are believed to have a pivotal role in wound healing (7). Because they secrete extracellular matrix proteins and promote contraction of granulation tissue through the expression of contractile α-smooth muscle actin (α-SMA), these cells are particularly essential for wound closure and scarring (7, 28). In the heart, myofibroblasts also serve as a local source for ET-1 and their receptors (11, 34).

The roles of ET-1 in the stomach in ulcer healing are not known. We hypothesized that endogenous ET-1 promotes or delays ulcer healing. In particular, we investigated 1) the expression of ET-1 and its receptors in mice using immunohistochemistry; 2) whether a neutralizing antibody against ET-1 in mice with ulcers delays or accelerates ulcer healing in vivo; and 3) the role of ET-1 in migration, proliferation, and ET-1 induced expression of growth factors and cytokines in gastric myofibroblasts in vitro.

MATERIALS AND METHODS

Animal experiments. Acetic acid ulcers were produced in male BALB/c mice aged 6 wk (n = 80) using the method of Okabe et al.

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(25) with minor modifications. In brief, mice were laparotomized under anesthesia with sevoflurane (Maruishi Pharmaceutical; Osaka, Japan). The anterior wall of gastric angle was touched with 100% acetic acid in a cotton-plugged plastic tube, 2 mm in diameter, for 30 s. If the procedure was carefully performed, 3 days later (designated as day 0), penetrating gastric ulcers developed and began to heal with 100% survival.

On day 0, mice were randomly divided into two groups. The anti-ET-1 antibody group (n = 40) was intraperitoneally injected with a neutralizing rabbit antibody against ET-1 (0.5 μl/g body wt, Peptide Institute; Osaka, Japan) daily from day 0 to the day of death. This antibody is an unrefined rabbit polyclonal antiseraum that neutralizes the vasoconstrictive action and ulcer-inducing action of ET-1 (17, 18).

A 1:20,000 dilution of the serum binds to 50% of ET-1 at a concentration of 1 pmol/ml (17). Pretreatment with 0.02 μl/g body wt of the same serum successfully blocks microcirculatory disturbances and gastric mucosal injury caused by intragastric ethanol in rodents (18). The nonimmunized serum (NIS) group (n = 40) received the same volume of nonimmunized normal rabbit serum (Peptide Institute).

Animals were humanely killed on days 0, 3, 6, or 9 under anesthesia. The stomach was harvested and opened along the greater curvature. The longest diameter of the ulcer was measured under a microscope, and the gastric wall was sectioned along the longest diameter.

Half of the tissue was fixed in 4% paraformaldehyde-PBS for 24 h and embedded in paraffin, and the other half of the tissue was mounted in Tissue-Tek OCT compound (Sakura Finechemical; Tokyo, Japan) and embedded in paraffin, and the other half of the tissue was mounted on chamber slides and immunohistochemically stained for α-SMA using the ABC method. Cells were used as gastric myofibroblasts on chamber slides and immunohistochemically stained for ET-1 receptor antagonist, cells were preincubated with BQ-123 (CN Biosciences; Darmstadt, Germany) at concentrations of 0.1 nM for 1 h before the addition of ET-1. Viable cell numbers were estimated using water-soluble tetrazolium dye (WST-8, Nacalai Tesque; Kyoto, Japan) after 24 h of culture, according to the manufacturer's protocol. The percent increase in viable cells was calculated and used as an index of cell proliferation.

**Elaborate:**

**Evaluation of cell proliferation.** Myofibroblasts were seeded onto 96-well culture plates at a density of 1.0 × 10^4 cells/well in DMEM-0.5% FCS and preincubated for 24 h. At the start of the experiment, the medium was replaced with DMEM-0.5% FCS supplemented with or without ET-1 (Peptide Institute) at concentrations between 0.1 and 1.000 nM. In experiments using a selective ET_A receptor antagonist, cells were preincubated with BQ-123 (CN Biosciences; Darmstadt, Germany) at concentrations of 0.1 μM for 1 h before the addition of ET-1. Viable cell numbers were estimated using water-soluble tetrazolium dye (WST-8, Nacalai Tesque; Kyoto, Japan) after 24 h of culture, according to the manufacturer’s protocol. The percent increase in viable cells was calculated and used as an index of cell proliferation.

**Cell migration assay.** Cell migration was assessed using cell culture inserts with an uncoated, translucent membrane with 8-μm pores (Falcon 3097, Becton Dickinson; Franklin Lakes, NJ), which was placed in 24-well plates (Falcon 3047) (1, 13). Myofibroblasts (7.85 × 10^4 cells) suspended in 350 μl DMEM-0.1% FCS were seeded in the upper part of the insert. DMEM-0.1% FCS supplemented with ET-1 (1–1,000 nM) was placed in the lower chamber. After 6 h of incubation, cells adhering to the membrane were fixed with methanol and stained with Mayer’s hematoxylin solution. Cells in the upper part of the insert were removed, and cells that migrated to the lower surface were counted at ×400 magnification in 15 fields for each filter. The average number of cells per field was used as the index of cell migration. All experiments were performed at least three times in duplicate.

**RT-PCR.** To determine whether gastric myofibroblasts express prepro-ET-1 and ET-converting enzyme (ECE)-1 de novo, we performed a RT-PCR assay using GeneAmp PCR System 9600 (PerkinElmer Applied Biosystems; Roissy, France) and Ready-To-Go PCR Beads (Amersham Pharmacia Biotech; Piscataway, NJ). To monitor cDNA synthesis efficiency, β-actin was used as an internal control.

PCR primers for mouse prepro-ET-1 (370 bp), ECE -1 (128 bp), and β-actin (540 bp) cDNA were selected from the mouse sequence as previously reported (24, 27, 41). The sequences of the primers were as follows: prepro-ET-1 (mouse) forward 5'-TTC CGG TGA TCT TCT CTC TGT 3' and reverse 5'-TCT TCT GGG CAT CAA AAA TTC CA-3', ECE (mouse) forward 5'-ATG ACG CCG CCC ATG GTG AAC-3' and reverse 5'-TGG TGT GGC TAA GAC CAT AC-3', and β-actin (mouse) forward 5'-GTG GGC CGC TCT AGG CAA CAA-3' and reverse 5'-TCT TTT GAT GTC ACG CAC GAT TTC-3'. The amplification conditions were 30 cycles for prepro-ET-1, 35 cycles for ECE-1, and 25 cycles for β-actin. PCR products were electrophoresed in 2.0% agarose gels containing ethidium bromide in 1× Tris-acetate-EDTA buffer, and photographs were taken.

**ELISA and Western blot analysis.** Cells were seeded at a density of 4.0 × 10^4 cells/6-cm dish in DMEM-0.5% FCS and preincubated for 24 h. The medium was replaced with DMEM-0.5% FCS supplemented with or without ET-1 (0.1–1,000 nM). After 24 h of incubation, the supernatant was used for the measurement of hepatocyte growth factor (HGF), VEGF, PGE_2, IL-1β, IL-6, and TNF-α by ELISA using the appropriate kits (Institute of Immunology, Tokyo, Japan; and R&D Systems, Abingdon, UK). Data were corrected for the amount of total protein of the cells.

To examine the expression of α-SMA, cells were incubated for 24 h with and without 100 nM ET-1 and in the presence and absence of BQ123. Cells were lysed in RIPA buffer containing 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin, 100 μg/ml phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate, and 50 mM sodium fluoride in PBS (pH 7.4). Lysates (10 μg protein/sample) were electrophoresed and transferred to a membrane, which was incubated with anti-α-SMA antibody (Progen Biotechnik; Heidelberg, Germany) and with the appropriate horseradish peroxidase-conjugated biotinylated secondary antibody (Pierce; Rockford, IL). The signal was detected using the
enhanced chemiluminescence method, as described by the manufacturer.

Statistical analysis. Data are shown as means ± SD and were analyzed using either the unpaired Student’s t-test, rank sum test, ANOVA followed by Bonferroni t-test, or rank-based ANOVA followed by Dunn’s multiple-comparison test. A P value of <0.05 was considered to be statistically significant.

Ethical issues. Experiments were performed in accordance with American Physiological Society guiding principles for the care and use of animals and were preapproved by the Ethicas Committee on Experimental Animals at Osaka University.

RESULTS

Immunohistochemical staining for ET-1 in the stomach in sham-operated mice and mice with ulcers. There was ET-1 immunoreactivity in endothelial cells, vascular smooth muscle cells, and gastric epithelial cells in the stomach. In acetic acid-induced gastric ulcers, there was strong ET-1 immunoreactivity not only in vascular endothelial cells but also in gastric epithelial cells (Fig. 1, B and C), whereas there was only weak immunoreactivity in gastric epithelial cells in sham-operated gastric mucosa (Fig. 1, A). Preincubation of the primary antisera antibody for ET-1 produced no immunoreactivity, indicating that the immunoreactivity was specific for ET-1 (Fig. 1, D).

Immunohistochemical staining of ETA and ETB receptors in the gastric ulcer bed. Figure 2 shows immunohistochemical micrographs of ETA receptors (A and B) and ETB receptors (C and D) in gastric mucosa with gastric ulcers. There was ETA receptor immunoreactivity in vascular smooth muscle cells (Fig. 2, A) and myofibroblasts (Fig. 2, B). ETB receptor immunoreactivity was mainly observed in vascular smooth muscle cells (Fig. 2, C) and was absent in myofibroblasts in the ulcer bed (Fig. 2, D).

Fig. 1. Immunohistochemical analysis of endothelin (ET)-1 in the gastric mucosa of sham-operated mice (A), mice with gastric ulcers (B and C), and a negative control specimen (D). A: only weak ET-1 immunoreactivity was observed in gastric epithelial cells in the sham-operated gastric mucosa (original magnification: ×20). B: strong ET-1 immunoreactivity was observed in the gastric mucosa surrounding acetic acid-induced gastric ulcers (original magnification: ×20). C: at higher magnification, ET-1 immunoreactivity was observed in vascular endothelial cells and in epithelial cells (original magnification: ×50). D: preincubation of the primary antisera antibody for ET-1 produced no immunoreactivity, indicating that the immunoreactivity was specific for ET-1 (original magnification: ×50).

Fig. 2. Immunohistochemical analysis of ETA (A and B) and ETB (C and D) receptors in the gastric mucosa of mice with gastric ulcers. A: ETA receptor immunoreactivity was observed in vascular smooth muscle cells (original magnification: ×60). B: ETA receptor immunoreactivity was also observed in myofibroblasts in the ulcer bed (original magnification: ×60). C: ETB receptor immunoreactivity was mainly observed in vascular smooth muscle cells (original magnification: ×60). D: ETB receptor immunoreactivity was absent in myofibroblasts in the ulcer bed (original magnification: ×60).
reactivity was mainly in vascular smooth muscle cells (Fig. 2C) but was absent in myofibroblasts beneath the ulcer bed (Fig. 2D).

**Effect of neutralizing antibody against ET-1 on the gastric ulcer healing.** Figure 3 shows changes in the diameter of acetic acid-induced ulcers in mice treated with either neutralizing antibody against ET-1 (anti-ET-1) or NIS. Ulcers of the NIS group healed almost completely by day 9. The ulcer diameter in the anti-ET-1 group was consistently and significantly larger than that of the NIS group on days 3, 6, and 9, indicating that the neutralizing antibody against ET-1 significantly delayed ulcer healing.

Figure 4, A and B, shows the histological appearance of acetic acid-induced ulcers on day 6 in mice treated with NIS and anti-ET-1 antibody, respectively. Serosal application of acetic acid produced gastric ulcers that penetrated from the lumen to the serosa, which was gradually replaced by granulation tissue during ulcer healing. In the NIS group, the gastric epithelium regenerated and developed toward the ulcerated area (Fig. 4A), which was not obvious in the anti-ET-1 group (Fig. 4B). The granulation tissue, which was weakly stained with eosin, was smaller in the NIS group than in the anti-ET-1 group. The granulation tissue contained collagen fibrils, which were demonstrated with Masson’s trichrome staining (Fig. 4, C and D) and α-SMA-positive myofibroblasts (Fig. 4, E and F). The magnified view shows that myofibroblasts colocalized with collagen fibrils. Immunoneutralization of ET-1 suppressed the expression of collagen fibrils (Fig. 4D) and α-SMA in the ulcer bed (Fig. 4F) compared with NIS (Fig. 4, C and E).

Histometric image analyses revealed that the area of fibrosis in the ulcer bed was significantly smaller in the anti-ET-1 group than in the NIS group on both days 6 and 9 (Fig. 5). As with the fibrosis area in the ulcer bed, there was a significant reduction in the myofibroblast area in the anti-ET-1 group compared with the NIS group on both days 6 and 9 (Fig. 6).

**Expression of ETA/ß receptors on murine myofibroblasts in culture.** Immunohistochemical analysis of α-SMA (Fig. 7A), ETA receptors (Fig. 7B), and ETß receptors (Fig. 7C) in cultured myofibroblasts revealed that all of the cells had a spindle-like shape with several extensions and were positive for α-SMA. Immunohistochemical analysis of ETA and ETß receptors revealed ETA, but not ETß receptor immunoreactivity in myofibroblasts.

**Effect of ET-1 and BQ123 on murine gastric myofibroblast proliferation.** Figure 8 shows the cell proliferation of myofibroblasts after 24-h incubation in DMEM-0.5% FCS without or with ET-1 (0.1–1,000 nM). ET-1 significantly increased cell proliferation at concentrations of 10 and 100 nM compared with control cells incubated with DMEM + 0.5% FCS (P < 0.001 and P < 0.0001, respectively, by Bonferroni t-test; Fig. 8).

To determine whether the effects of ET-1 on myofibroblast proliferation were via ETA receptors, myofibroblasts were incubated with or without ET-1 (10 and 100 nM) for 24 h in the absence or presence of BQ123 (0.1 μM), a selective ETA receptor antagonist. ET-1-stimulated cell proliferation was completely blocked by BQ123 at concentrations of 0.1 μM, and values were similar to the control level (Fig. 8).

**Migration of murine gastric myofibroblasts.** Figure 9A shows cells that migrated through the pores of the uncoated, translucent membrane in response to DMEM-0.1% FCS alone and with ET-1 (1–100 nM). Figure 9B shows quantitative cell migration data. ET-1 stimulated migration of mouse gastric fibroblasts in a dose-dependent manner. The maximum effect of ET-1 occurred at a concentration of 10 nM, and the maximum migration of ET-1 was 13.5 ± 0.9 cells/high-powered field (×400). ET-1 treatment induced an additional 2.8-fold increase (P < 0.0001) in migration in controls.

**RT-PCR analyses of murine gastric myofibroblasts.** Expression of the prepro-ET-1 gene (the only known precursor gene that encodes big ET-1) and ECE-1 were examined in myofibroblasts by RT-PCR. RT-PCR experiments resulted in PCR products of the predicted size for prepro-ET-1 (370 bp) and ECE-1 (128 bp) (Fig. 10). These results demonstrated that myofibroblasts express prepro-ET-1 and ECE-1, the key enzyme that converts big ET-1 to the active peptide ET-1. The PCR products for ET-1 and ECE-1 were of the predicted sizes, and their identities were confirmed by cycle sequencing.

**Production of growth factors and angiogenic factors in gastric myofibroblasts.** We examined the effect of ET-1 stimulation on HGF, PGE2, VEGF, IL-1ß, IL-6, and TNF-α secretion in murine gastric myofibroblasts. ET-1 significantly increased the concentrations of HGF, PGE2, VEGF, and IL-6 released from myofibroblasts into the culture medium (Fig. 11). IL-1ß and TNF-α levels were undetected in culture media of gastric myofibroblasts incubated with and without ET-1.
ET-1 upregulates expression of α-SMA in gastric myofibroblasts. Gastric myofibroblasts expressed α-SMA protein without exogenous ET-1. After ET-1 stimulation, the expression of α-SMA protein was upregulated in myofibroblasts (Fig. 12). The elevated expression of α-SMA was markedly reduced by BQ123 compared with control cells incubated without ET-1 and cells treated with ET-1 alone.

DISCUSSION

In this study, immunoneutralization of ET-1 significantly delayed gastric ulcer healing and scarring, demonstrating important roles of this peptide in ulcer healing. Because gastric mucosal epithelial cells do not have ET\(_{A/B}\) receptors nor proliferate in response to ET-1 (unpublished data), we focused our attention on gastric myofibroblasts. Myofibroblasts reside beneath the epithelium, which is an ideal location for paracrine action on epithelial cells. When the stomach is ulcerated, myofibroblasts migrate toward the ulcer bed to form granulation tissue. Later, they produce collagen fibrils and other extracellular matrixes and form ulcer scar. Furthermore, we demonstrated that myofibroblasts are a potential source of growth factors, angiogenic factors, and PGE\(_2\).

To investigate the targets of ET-1, we visualized ET\(_A\) and ET\(_B\) receptors in stomach ulcers. ET\(_A\) receptor immunoreactivity was localized mainly at spindle-like cells in the ulcer bed as well as in vascular smooth muscle cells, whereas ET\(_B\) receptor immunoreactivity was observed mainly in vascular smooth muscle cells. ET\(_A\) receptor-positive, spindle-like cells were identified as myofibroblasts because they were positive for α-SMA. Murine myofibroblasts isolated from the gastric wall also expressed ET\(_A\) receptors but not ET\(_B\) receptors in vitro. These results indicated that myofibroblasts have ET\(_A\) receptors and therefore are one of the targets of ET-1.
Immunoneutralization of endogenous ET-1 delayed ulcer healing as well as influenced the accumulation of α-SMA-positive myofibroblasts and the development of fibrosis in penetrating gastric ulcers. Immunohistochemistry indicated that gastric epithelial cells and gastric myofibroblasts are possible sources of gastric ET-1. Gastric myofibroblasts expressed both ET-1 and ETA receptors, suggesting that exogenous ET-1 as well as endogenous ET-1, produced by gastric myofibroblasts per se, contribute to express actin stress fibers in gastric myofibroblasts. Consequently, we also investigated phenotypic changes of gastric myofibroblasts in response to ET-1 in vitro. ET-1 stimulates (30) or inhibits (15) proliferation of myofibroblasts/fibroblasts derived from other organs. In the present study, ET-1 significantly induced the proliferation and chemotaxis of gastric myofibroblasts in vitro. Western blot analysis demonstrated that ET-1 also enhances α-SMA expression in gastric myofibroblasts. Cotreatment with BQ123, the ET_A receptor antagonist, suppressed α-SMA expression in myofibroblasts. These results indicate that ET-1 has a pivotal role in the accumulation and phenotypic activation of murine gastric myofibroblasts in vitro, confirming our results of the histological analysis of gastric ulceration in mice in vivo.
Because ET-1 attracted and activated myofibroblasts in gastric ulcers, the effects of ET-1 on myofibroblast-derived factors are an important issue. In the present study, however, we were unable to analyze gastric mucosal levels of growth factors, PGE2, and cytokines in the mouse stomach, which is too small to measure them quantitatively using methods such as ELISA. Although we confirmed VEGF expression in myofibroblasts (data not shown), immunohistochemistry was not suitable for quantitative analyses of VEGF and other factors in the mouse stomach in vivo. Instead, we analyzed their expression in gastric myofibroblasts with and without ET-1 in vitro.

HGF is one of the most potent stimulants of gastric epithelial cell proliferation (37) and provides regular gastric mucosal maintenance. Gastric epithelial cells express a HGF receptor gene, c-met (39), and proliferate (37), migrate, and form gland structures in response to HGF (38). In the majority of tissues, HGF gene expression is restricted to myofibroblasts/fibroblasts or other mesenchymal cells, whereas expression of c-met is confined to nonmesenchymal cells that do not secrete HGF (33). In the present study, we confirmed that cultured myofibroblasts did not express the c-met gene (data not shown). On the other hand, gastric myofibroblasts secreted HGF in response to ET-1 stimulation. The results retained that a paracrine mechanism of the ligand-receptor interaction is required for wound repair (38). Such epithelial cell replication, migration, and maturation are essential for ulcer healing. In addition, HGF increases angiogenesis, and the HGF receptor has been detected on cultured endothelial cells (19). The development of a vascular supply is also essential for wound healing.

VEGF is a potent angiogenic factor that has a fundamental role in the growth and differentiation of vascular cells (5) including invasion of the endothelium into the extracellular matrix (6). VEGF is expressed in a wide variety of cells, including epithelial cells, neoplastic cells, and stromal cells. Our preliminary immunohistochemistry indicated that VEGF is expressed in not only the gastric epithelium but also in myofibroblasts in the ulcer margin (unpublished data). In the present study, ET-1 stimulated VEGF expression in gastric myofibroblasts in vitro. Consequently, ET-1-induced modulation of myofibroblasts might support not only gastric reepithelialization but also angiogenesis in the granulation tissue of the gastric wall in a paracrine manner. On the other hand, ET-1 is also a potent vasoconstrictor and possibly reduces gastric...
mucosal blood flow, which also has a pivotal role in gastric ulcer healing. It might be that ET-1, upregulated in the ulcerated mucosa, negatively influences gastric mucosal blood flow surrounding ulcers. Indeed, ET-1 is a potent ulcerogen that acts by reducing gastric mucosal blood flow in ethanol-induced gastric damage (17, 18) as well as in hemorrhagic shock-induced gastric injury (20). ET-1 stimulates gastric myofibroblasts to produce VEGF, HGF, and other angiogenic factors, however, that promote neovascularization in gastric ulcer. Newly formed capillaries frequently lack vascular smooth muscles and sphincters that reduce blood flow in response to

ET-1. In addition, ET-1 also stimulates the production of PGE₂, a potent vasodilator, in gastric myofibroblasts. Thus ET-1 might enhance tissue blood flow by promoting neovascularization without exerting its vasoconstricting activity.

We examined the effect of ET-1 stimulation on inflammatory cytokine (IL-1β, IL-6, and TNF-α) secretion in cultured myofibroblasts. We also measured the production of another inflammatory mediator, PGE₂, in isolated gastric myofibroblasts. ET-1 stimulation significantly increased IL-6 production in a dose-dependent manner; however, IL-1β and TNF-α levels were below the detection limits. IL-6 is a multifunctional cytokine and causes proliferation and migration of systemic endothelial cells in culture (31). Myofibroblast-derived IL-6 might support angiogenesis in coordination with VEGF and HGF. Moreover, PGE₂ production is increased in gastric myofibroblasts in response to ET-1. PGE₂ is not only an inflammatory mediator and a cytoprotective factor but also a proangiogenic factor. We demonstrated that cyclooxygenase (COX)-2 and the subsequent increase in PGE₂ promote angiogenesis via upregulating several angiogenic factors, including VEGF (40). In rodents, COX-2 and the subsequent PGE₂ production accelerate ulcer healing (29). Other investigators have reported that PGE₂ derived from COX-2 and microsomal PGE synthesis upregulates VEGF expression in gastric stromal cells (22). Interestingly, IL-6 possibly enhances COX-2 expression via NF-IL-6 (14). Furthermore, several growth stimuli are reported to upregulate COX-2 expression in cultured cells. Although it is possible that ET-1 enhances the production of growth factors such as VEGF, IL-6, and HGF by upregulating COX-2 and subsequent PGE₂ production, it is not clear whether ET-1 directly or indirectly increased the expression of these factors. The mechanisms for ET-1-induced expression of COX-2, growth factors, and angiogenic factors in gastric myofibroblasts remain to be investigated.

Furthermore, the source of ET-1 was investigated in gastric cells in vivo and in vitro in the present study. Immunohistochemistry clearly demonstrated ET-1 immunoreactivity in gastric epithelial cells as well as in myofibroblasts at ulcer margin. Because myofibroblasts at the ulcer margin possessed ETA receptors, it was unclear whether myofibroblasts were able to produce ET-1. To examine this hypothesis, we analyzed the expression of prepro-ET-1 and ECE in gastric myofibroblasts using RT-PCR. mRNA of these genes was detected in gastric myofibroblasts. The results show that ET-1 stimulates myofibroblasts in both a paracrine and autocrine manner.

In conclusion, ET-1, a well-known ulcerogen, has pivotal roles in gastric ulcer healing. ET-1 promotes chemotaxis,
proliferation, expression of α-SMA, and subsequent accumulation of ETα receptor-positive myofibroblasts. ET-1 also enhances the production of growth factors and angiogenic factors, i.e., HGF, VEGF, PGE2, and IL-6, in gastric myofibroblasts. ET-1, released from the ulcer margin and ulcer bed, mediates the activation of myofibroblasts in both a paracrine and autocrine manner and supports ulcer healing through ETα receptors. These data strongly suggest an important role for ET-1 in the interactions between the mesenchyme and epithelium during wound healing, which occurs predominantly during the remodeling phase of healing.

REFERENCES


