Role of nuclear factor-κB in gastric ulcer healing in rats

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Takahashi, Satoru, Takuya Fujita, and Akira Yamamoto. Role of nuclear factor-κB in gastric ulcer healing in rats. Am J Physiol Gastrointest Liver Physiol 280: G1296–G1304, 2001.—We investigated the role of nuclear factor-κB (NF-κB) in gastric ulcer healing in rats. NF-κB was activated in ulcerated tissue but not in normal mucosa, and the level of the activation was decreased with ulcer healing. NF-κB activation was observed in fibroblasts, monocytes/macrophages, and neutrophils. Treatment of gastric fibroblasts, isolated from the ulcer base, with interleukin-1β activated NF-κB and the subsequently induced cyclooxygenase-2 and cytokine-induced neutrophil chemoattractant-1 (CINC-1) mRNA expression. Inhibition of activated NF-κB action resulted in suppression of both their mRNA expression and increases in PGE2 and CINC-1 levels induced by interleukin-1β. Persistent prevention of NF-κB activation caused an impairment of ulcer healing in rats. Gene expression of interleukin-1β, CINC-1, cyclooxygenase-2, and inducible nitric oxide synthase in ulcerated tissue had been inhibited before the delay in ulcer healing became manifest. The increased levels of cyclooxygenase-2 protein and PGE2 production were also reduced. These results demonstrate that NF-κB, activated in ulcerated tissue, might upregulate the expression of healing-promoting factors responsible for gastric ulcer healing in rats.

interleukin-1β; cytokine-induced neutrophil chemoattractant-1; cyclooxygenase-2; inducible nitric oxide synthase

GASTRIC ULCER HEALING PROCEEDS through multiple steps, such as formation of granulation tissue, angiogenesis, and epithelial regeneration. The healing response is largely coordinated by a variety of inflammatory mediators, cytokines, and growth factors that are produced locally in the ulcerated portion. There has been accumulating evidence that prostaglandin/cyclooxygenase (COX)-2, nitric oxide (NO)/inducible NO synthase (iNOS), cytokine-induced neutrophil chemoattractants (CINC), interleukin (IL)-1, and hepatocyte growth factor are induced or increased by gastric ulceration and might contribute to ulcer healing (9, 10, 13, 21, 24, 25, 33). The genes of these factors have nuclear factor-κB (NF-κB) responsive elements in their promoter regions, and their expression is regulated by NF-κB in many cell types (2, 11, 22).

NF-κB exists in the majority of cell types and consists of homo- or heterodimers of structurally related proteins including p65 (Rel A), c-Rel, and p50 (NF-κB1) (2, 11, 22). In resting cells, NF-κB is coupled with inhibitor-κB (I-κB) and resides in the cytosol as an inactive form. In response to inflammatory stimuli and mitogens, I-κB is phosphorylated and dissociates from NF-κB. Subsequently, NF-κB translocates into the nucleus to function as an active transcription factor. We speculated that NF-κB is activated in ulcerated gastric tissue because gastric ulcers are associated with inflammation. In the present study, we found that NF-κB is activated only in ulcerated tissue and that its activation is sustained during the healing of gastric ulcers in rats. Then we examined whether NF-κB upregulates the expression of healing-promoting factors such as COX-2, iNOS, CINC-1, and IL-1β in vitro (isolated gastric fibroblasts) and in vivo (ulcerated gastric tissue in rats). In addition, we examined the effect of pyrroliidine dithiocarbamate (PDTC, an inhibitor of NF-κB activation) on ulcer healing in rats. Here we describe the crucial role of NF-κB in gastric ulcer healing in rats.

METHODS

Evaluation of gastric ulcer healing in rats. Male Wistar ST rats (270–360 g; Nihon SLC, Hamamatsu, Japan) were anesthetized with pentobarbital (50 mg/kg ip), and the stomachs were exposed through an abdominal incision. Acetic acid (100%) was applied to the anterior serosal wall surface at the border between the antrum and the fundus through a plastic tube (15). Since deep and well-defined ulcers had been generated 3 days later, we defined the third day as day 0 (the onset of ulcer healing). The ulcerated area (mm²) was determined under a dissecting microscope (Olympus, Tokyo, Japan).

Histological examination of gastric ulcer healing was according to our previously reported method (21, 23). After histological sections were prepared from ulcerated tissues on day 14 and then stained with hematoxylin and eosin, the length of the regenerated mucosa on the ulcer base (mm), the thickness of the base (mm), and the density of neutrophils infiltrated in the base were determined under a light microscope (Olympus). Alternatively, the sections were immunostained with the antibody against von Willebrand factor (factor VIII-related endothelial antigen; DAKO, Glostrop, Denmark). The numbers of neutrophils and microvessels in the base were measured in three randomly chosen fields (0.0625 mm²), and their densities were expressed as the...
number of neutrophils and microvessels per square millimeter, respectively. The person evaluating ulcer healing was unaware of the treatment given the animals.

**Isolation of gastric fibroblasts.** Gastric fibroblasts were isolated from ulcerated tissue on day 7. Under ether anesthesia, rats were perfused via the left cardiac ventricle with medium A ([in mM]: 25 HEPES-NaOH (pH 7.4), 132.4 NaCl, 5.4 KCl, 5 Na2HPO4, 1 NaH2PO4, 1.2 MgSO4, and 1 CaCl2 with 2 mg/ml glucose and 1 mg/ml BSA]. After ulcerated tissues were finely minced in medium A, they were incubated in medium A containing 0.05% collagenase (Wako Pure Chemicals, Osaka, Japan) and 1 mM EDTA for 60 min at 37°C under 95% O2-5% CO2 and then filtered through a mesh (pore size 0.1 mm). The culture of the isolated cells was maintained at 37°C under 5% CO2 in air in DMEM supplemented with 10% FBS (Life Technologies, Gaithersburg, MD), 100 U/ml penicillin, 100 U/ml streptomycin, and 0.25 μg/ml amphotericin B for >4 wk, after which contaminated immune and epithelial cells died and cultures were >95% fibroblasts. The remaining cells were spindle-shaped and reacted to anti-vimentin (an antibody to K0) but not to anti-smooth muscle α-actin antibody ( Nichirei, Tokyo, Japan). Their growth was significantly stimulated by basic fibroblast growth factor. For experiments, gastric fibroblasts were allowed to grow to confluence in 60-mm dishes.

**Effect of NF-κB decoy oligonucleotide in gastric fibroblasts.** Double-stranded NF-κB consensus motifs (decoys) were synthesized as single-stranded oligonucleotides (NF-κB decoy, 5′-AGTTGAGGGCAATTCCAGGC-3′ and 5′-GCTTGGGAAAGTCCGCTCAACT-3′; mutant decoy, 5′-AGTTGAGGGCAGTTCCAGGC-3′ and 5′-GCTTGGGAAATCCGCTCAACT-3′) (Life Technologies) and annealed (17). The decoys (5 μg) were introduced to gastric fibroblasts with LipofectAMINE PLUS Reagent (Life Technologies), according to the instruction manual. Sixteen hours later, medium was replaced with DMEM supplemented with 5% FBS, and then gastric fibroblasts were stimulated with 10 ng/ml IL-1β. At the indicated times, nuclear extracts and total RNAs were prepared, and then gastric fibroblasts were stimulated with 10 ng/ml IL-1β and subjected to measurement of PGE2 and CINC-1 by enzyme immunoassay (PGE2 EIA kit, Cayman, Ann Arbor, MI; GRO/CINC-1 EIA kit, Immuno Biological Laboratories, Fujisawa, Japan). NS-398 at 10 μM was added 5 min before IL-1β stimulation. PGE2 and CINC-1 production were expressed as picograms per milliliter per 16 h.

**Preparation of nuclear extracts.** Gastric tissues taken from normal and ulcerated stomachs were homogenized in buffer A ([in mM]: 10 HEPES-NaOH (pH 7.8), 10 KCl, 0.1 EDTA, 1 dithiothreitol (DTT), and 0.5 phenylmethylsulfonyl fluoride (PMSF) with 2 μg/ml aprotinin, 2 μg/ml leupeptin, and 1% Triton X-100] and then left on ice for 15 min. Gastric fibroblasts were scraped in PBS, followed by centrifugation at 800 g for 5 min. The cell pellets were suspended in buffer A, vortexed for 1 min, and then left on ice for 15 min. After the homogenates and cell lysates were centrifuged at 5,000 g for 5 min, the precipitates were suspended in 0.5 ml and 0.1 ml, respectively, of buffer B ([in mM]: 50 HEPES-NaOH (pH 7.8), 420 KCl, 0.1 EDTA, 5 MgCl2, 1 DTT, and 0.5 PMSF with 20% glycerol, 2 μg/ml aprotinin, and 2 μg/ml leupeptin] and then left on ice for 30 min with frequent agitation. Nuclear extracts were prepared by centrifugation at 15,000 g for 10 min. Protein concentration was determined using a protein assay kit (Bio-Rad, Hercules, CA) with BSA as a standard.

**Gel shift assay.** Nuclear extracts (5–10 μg) were incubated with 32P-labeled NF-κB oligonucleotide probe (2 pmol, 50,000 cpm) in 20 μl of binding buffer ([in mM]: 10 HEPES-NaOH (pH 7.8), 50 KCl, 1 EDTA, 5 MgCl2, and 5 DTT with 100 μg/ml poly[dI-dC]] at 30°C for 30 min. NF-κB consensus oligonucleotide probe (5′-AGTTGAGGGCAATTCCAGGC-3′) (Promega, Madison, WI) was end labeled with [γ-32P]ATP (Amersham Pharmacia Biotech, Little Chalfont, UK) and MEGALABEL (TaKaRa, Kyoto, Japan). The specificity of the oligonucleotide probe-protein binding was determined by competition reaction in which 200-fold excess of unlabeled NF-κB probe or SP-1 probe (Promega) was added to the binding buffer. Supershift assay was performed by adding anti-p65 subunit of NF-κB antibody (Roche Diagnostics, Indianapolis, IN) or anti-activating transcription factor (ATF)-2 antibody (New England Biolabs, Beverly, MA). The DNA-protein complexes were subjected to nondenaturing 4% polyacrylamide gel electrophoresis. The gel was dried, and detection of labeled complexes was carried out with an imaging analyzer (BAS2000; Fuji Film, Tokyo, Japan).

**Western blot analysis.** Gastric tissues taken from normal and ulcerated stomachs were homogenized in 25 mM Tris-HCl (pH 8.0) buffer containing 250 mM sucrose, 1 mM EDTA, 0.5% Triton X-100, 0.1% SDS, 0.1% Na deoxycholate, and 2 μg/ml leupeptin, and then COX proteins were partially purified as we previously described (26). Nuclear extracts and COX proteins were subjected to SDS-PAGE (10%), and then the separated proteins were electrophoretically transferred to Hybond P membranes (Amersham Pharmacia Biotech) (28). The membranes were incubated with anti-p65 subunit of NF-κB antibody, anti-COX-1 antibody (Cayman), or anti-COX-2 antibody (Cayman) after nonspecific binding sites had been blocked. Immunoreactive proteins were visualized on X-ray films (Phototope-HRP Western Blot Detection kit; New England Biolabs).

**Immunohistochemical analysis.** Histological sections of gastric tissues were incubated with anti-p65 subunit of NF-κB antibody after deactivation of endogenous peroxidase with 0.3% H2O2 and blocking of nonspecific binding sites. This antibody only reacts to the activated NF-κB because it binds to a p65 epitope that is exposed only after I-κB dissociation (8). Activated NF-κB was visualized by the avidinin-peroxidase complex method (Vectastain ABC kit; Vector Laboratories, Burlingame, CA), and then the sections were successively stained with hematoxylin.

**Determination of gastric acid secretion in rats.** Gastric acid secretion on day 14 was determined by the pylorus ligation method. Two hours after the pylorus was ligated the gastric contents were collected, and then acidity was determined by titration of the contents against 100 mM NaOH to pH 7.0. Total acid output was calculated as volume × acidity and expressed as milliequivalents per hour.

**Determination of PGE2 and CINC-1 production by gastric tissues of rats.** PGE2 and CINC-1 production in the animals treated with PDTC for 7 days was determined. Normal and ulcerated gastric tissues were incubated at 37°C in 1 ml of DMEM supplemented with 5% FBS and the antibiotics for 1 h. The medium was subjected to enzyme immunoassay of PGE2. The levels of CINC-1 protein in the remaining tissues were also determined as we previously reported (33). PGE2 and CINC-1 production was expressed as picograms per milligram per hour.

**RT-PCR.** Total RNAs were extracted from gastric tissues with TRIzol Reagent (Life Technologies) (6). First-strand cDNAs were prepared from 1–3 μg of total RNAs with 150 units Moloney murine leukemia virus reverse transcriptase (Life Technologies) and 0.3 μg random hexamer oligonucleotides (TaKaRa). The DNAs were subjected to PCR with 0.625 units Taq DNA polymerase (Life Technologies). The primers were synthesized as we described previously (25), except for...
glyceraldehyde-3-phosphate dehydrogenase primers (5). After denaturation at 94°C for 5 min, PCR was performed for 22–25 cycles consisting of denaturation at 94°C for 45 s, annealing at 55–60°C for 45 s, and extension at 72°C for 30 s. The amplification was terminated by a 10-min final extension step at 72°C. The PCR products were subjected to 2% agarose gel electrophoresis and then visualized by ethidium bromide staining. The sequences of the PCR products were confirmed to be identical to known ones (with reference to the databases of GenBank and EMBL).

**Materials.** Recombinant IL-1β (Otsuka Pharmaceutical, Tokushima, Japan) was dissolved in DMEM. NS-398 (Calbiochem, La Jolla, CA) was dissolved in dimethyl sulfoxide, followed by dilution with culture medium. The final concentration of dimethyl sulfoxide was 0.5%, at which concentration cell viability and PGE2 production were not affected. PDTC (Nacalai Tesque, Kyoto, Japan) was dissolved in saline and administered twice daily starting from day 0 for the indicated times. The drug was administered orally in a volume of 5 ml/kg. Control animals received saline alone. All other chemicals used here were of reagent grade.

**Statistical analysis.** The data are presented as means ± SE. Statistical differences in the dose-response studies were evaluated by Dunnett’s multiple comparison test. Student’s t-test was applied to comparisons between two groups. P values of <0.05 were considered significant.

**RESULTS**

**NF-κB activation during gastric ulcer healing in rats.** We examined whether NF-κB is activated in gastric tissues of rats. There were round and deep ulcers in all animals on day 0, and thereafter the ulcers healed spontaneously (Fig. 1A). Gel shift assay with radiolabeled NF-κB-specific oligonucleotide probe revealed that NF-κB activation was induced in ulcerated tissue (Fig. 1B). NF-κB activation was maintained during healing of gastric ulcers, although the activation was reduced in a time-dependent manner. However, activated NF-κB was not detected in normal tissue. When the antibody against the p65 subunit of NF-κB was added to the nuclear extract from ulcerated tissue on day 7, the NF-κB-radiolabeled oligonucleotide probe complex was supershifted to the upper band. On the other hand, anti-ATF-2 antibody (an NF-κB-unrelated antibody) did not affect mobility of the NF-κB-oligonucleotide probe complex (data not shown). In addition, binding of the radiolabeled probe to NF-κB was abolished in the presence of 200-fold molar excess of unlabeled probe. In contrast, the complex formation was not affected by the same amount of an unrelated oligonucleotide, SP-1 probe (data not shown). Activation of NF-κB was also assessed by Western blotting (Fig. 1C). Similar to the results with gel shift assay, activated NF-κB was detected only in the nuclear extracts from ulcerated tissues, and the amount of activated NF-κB decreased with ulcer healing.

We determined the localization of activated NF-κB by immunohistochemical staining (Fig. 2). In ulcerated tissue on day 7, NF-κB immunoreactivity was abundant in spindle-shaped cells, mononuclear cells, and polymorphonuclear cells. Considering the existence in granulation tissue, these cell types were morphologically identified to be fibroblasts, macrophages/mono-

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**Fig. 1.** Nuclear factor (NF)-κB activation during the healing of acetic acid-induced gastric ulcers in rats. A: healing process of gastric ulcers. The 3rd day after acid application was defined as day 0, when deep and round ulcers had been firmly formed. Ulcerated area was determined on days 0, 7, 14, and 21. Data are presented as means ± SE for 6 animals/group. B: gel shift analysis of NF-κB activation in gastric tissues. Nuclear extracts were prepared from ulcerated (lane 1, day 3; lane 2, day 7; lane 3, day 14) and normal (lane 4) tissues and then subjected to gel shift assay with 32P-labeled NF-κB oligonucleotide probe. In addition, the nuclear extract, prepared from ulcerated tissue on day 7, was incubated with radiolabeled NF-κB probe in the absence (lane 5) and presence (lane 6) of anti-NF-κB antibody or 200-fold molar excess of unlabeled NF-κB probe (lane 7). C: Western blot analysis of activated NF-κB in gastric tissues. Nuclear extracts were prepared from ulcerated (lane 1, day 3; lane 2, day 7; lane 3, day 14) and normal (lane 4) tissues and then subjected to Western blot analysis with the antibody recognizing only activated NF-κB.
cytes, and neutrophils. NF-κB-immunoreactive mononuclear cells and polymorphonuclear cells were also positive to macrophage- and neutrophil-specific esterases, respectively. Activated NF-κB was evident both in the nucleus and cytosol in the immunoreactive cells. However, there were no immunoreactive cells in normal gastric mucosa.

NF-κB-mediated expression of CINC-1 and COX-2 in response to IL-1β in rat gastric fibroblasts. We already found that endogenous IL-1 plays an important role in gastric ulcer healing by upregulating the gene expression of healing-promoting factors in rats (25). Therefore, we isolated fibroblasts from ulcerated tissue and investigated the role of NF-κB in IL-1β-stimulated fibroblasts.

As shown in Fig. 3, exposure of gastric fibroblasts to 10 ng/ml IL-1β caused activation of NF-κB in a time-dependent manner. NF-κB activation was evident 15 min after the addition of IL-1β and reached a maximal level at 1 h. Neither CINC-1 (IL-8-like neutrophil chemokine in rats) nor COX-2 mRNAs were expressed in control cells, but their mRNA expression was also time-dependently increased by IL-1β. IL-1β-induced expression of CINC-1 and COX-2 mRNAs followed NF-κB activation in gastric fibroblasts. In contrast, COX-1 mRNA was expressed in control cells and remained unchanged on stimulation with IL-1β.

NF-κB decoy oligonucleotide specifically binds to activated NF-κB, preventing the action of NF-κB to its responsive element (14, 17). When gastric fibroblasts introduced with NF-κB decoy were incubated with 10 ng/ml IL-1β for 1 h, the formation of NF-κB-radio-labeled probe complex was completely abolished (Fig. 4A). In contrast, the complex formation was not affected by mutant decoy, which has no binding activity to activated NF-κB. NF-κB decoy also markedly inhibited the induction of CINC-1 and COX-2 mRNA expression in response to IL-1β, but COX-1 mRNA expression was similarly observed even on treatment with NF-κB decoy (Fig. 4B). Mutant decoy had no effect on the IL-1β-induced mRNA expression. Furthermore, IL-1β at 10 ng/ml promoted the production of CINC-1 protein and PGE2 in gastric fibroblasts (Fig. 4C). NF-κB decoy significantly inhibited both the IL-1β-induced inflammasome of gastric fibroblasts.
creases in CINC-1 protein and PGE$_2$. The inhibition rates by the decoy were ~70%. NS-398, a COX-2-selective inhibitor, also potently reduced the increased PGE$_2$ production. In contrast, mutant decoy did not affect the effect of IL-1$\beta$.

**Prevention of gastric ulcer healing by persistent inhibition of NF-$\kappa$B activation in rats.** We next examined the effect of PDTC on gastric ulcer healing in rats. PDTC inhibits I-$\kappa$B degradation, and the resultant NF-$\kappa$B activation, and has been widely used as an NF-$\kappa$B inhibitor both in vitro and in vivo (12, 19). PDTC (100 mg/kg) or vehicle was repeatedly administered, and nuclear extracts from the ulcer bases were subjected to gel shift assay (Fig. 5A). The activation of NF-$\kappa$B was potently inhibited by PDTC on day 7. The effect of PDTC on ulcer healing is shown in Fig. 5B. There was no difference of ulcerated area between control and PDTC-treated groups on day 7. However, ulcer healing was prevented by PDTC from day 10. On day 14, significant delay of ulcer healing caused by PDTC was observed in a dose-dependent manner. In contrast, administration of 100 mg/kg PDTC for 14 days did not cause any gastric lesions or ulcers in normal rats.

In addition, histological study revealed prevention of ulcer healing by PDTC (Table 1). Regeneration of the gastric mucosa, reduction in the ulcer base size, and angiogenesis were inhibited, although no significant differences were observed in length of the regenerated mucosa. Infiltration of neutrophils into ulcerated tissue was also inhibited by PDTC. Acid secretion was not affected by PDTC, i.e., acid output was $146.3 \pm 25.4$ $\mu$eq/h in the control group, $206.3 \pm 34.0$ $\mu$eq/h in the 50 mg/kg PDTC group, and $182.7 \pm 24.4$ $\mu$eq/h in the 100 mg/kg PDTC group.

**Effect of NF-$\kappa$B inhibition on the mRNA expression of CINC-1, COX-2, and iNOS in ulcer healing.** We examined the effect of PDTC on the mRNA expression of CINC-1, COX-1, COX-2, and iNOS in gastric ulcers by means of RT-PCR (Fig. 6A). Neither CINC-1, COX-2, nor iNOS mRNAs were expressed in normal gastric mucosa, but their mRNA expression was induced in
ulcerated tissue on day 7. Treatment with 100 mg/kg PDTC suppressed the induction of CINC-1, COX-2, and iNOS mRNAs. COX-1 mRNA expression was observed in both normal and ulcerated tissues, and the level was not affected by PDTC. In addition, expression of COX-2 protein as well as its mRNA was also induced in ulcerated tissue on day 7, whereas the expression level of COX-1 protein in ulcerated tissue was similar to that in normal mucosa (Fig. 6B). PGE2 production in ulcerated tissue markedly increased by 3.4-fold compared with that in normal mucosa (Fig. 6C). PDTC at 100 mg/kg inhibited the expression of COX-2 protein without affecting COX-1 protein level and significantly reduced the increased PGE2 production. CINC-1 protein level was also significantly reduced by PDTC treatment (15.3 ± 3.4 pg·mg⁻¹·h⁻¹ in the control group and 6.7 ± 3.1 pg·mg⁻¹·h⁻¹ in the 100 mg/kg PDTC group).

DISCUSSION

In the present study, NF-κB is found to be activated in ulcerated gastric tissue during a healing phase in rats. We had confirmed that ulcer size reaches maximum and that granulation tissue (ulcer base) is firmly formed until day 0 in the acetic acid-induced gastric ulcer model. Thereafter, ulcer size decreases with time. It is evident that NF-κB activation was evaluated during an ulcer healing process (from day 0) but not during an ulcer developing process (until day 0). Normal inflammatory response to gastric ulceration is essential for mucosal repair and is reduced with ulcer healing. There has been accumulating evidence that inflammatory proteins, such as IL-1β, COX-2, iNOS, and CINC-2, play important roles in gastric ulcer healing (10, 13, 21, 25, 33). It is reasonable that NF-κB activation is maintained until gastric ulcers are remarkably reduced.

The activation of NF-κB was detected in fibroblasts, monocytes/macrophages, and neutrophils in the ulcer base. Immunoreactive NF-κB was observed both in the nucleus and the cytosol. This is consistent with IκB dissociation and NF-κB activation in the cytosol and the subsequent nuclear translocation of activated NF-κB (2, 11, 22). Gastric fibroblasts are a major component of the ulcer base and produce PGE2, cytokines, and growth factors, contributing to ulcer healing (3, 21, 23–27). The activation of NF-κB was confirmed in IL-1β-stimulated cultured fibroblasts, because the activation occurred in the fibroblasts in the ulcer base. Furthermore, our results clearly indicate that, in response to IL-1β, NF-κB actually functions as a positive regulator of CINC-1 and COX-2 gene expression in gastric fibroblasts.

We found that NF-κB might play a crucial role in gastric ulcer healing in rats. Schmassmann et al. (18) and we (21) reported that epithelial regeneration, maturation of the ulcer base (reduction of the ulcer base size), angiogenesis, and neutrophil infiltration are relevant events for efficient healing of gastric ulcers. It is evident that NF-κB is implicated in all of the above

Table 1. Histological evaluation of gastric ulcer healing after repeated administration of PDTC

<table>
<thead>
<tr>
<th>PDTC, mg/kg</th>
<th>50</th>
<th>100</th>
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<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
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<tr>
<td>Length of regenerated mucosa, mm</td>
<td>1.67 ± 0.15</td>
<td>1.22 ± 0.08</td>
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<tr>
<td>Thickness of ulcer base, mm</td>
<td>1.22 ± 0.07</td>
<td>1.83 ± 0.08*</td>
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<tr>
<td>Density of microvessels, counts/mm²</td>
<td>89.8 ± 14.9</td>
<td>44.8 ± 11.0*</td>
</tr>
<tr>
<td>Density of infiltrated neutrophils, counts/mm²</td>
<td>918.2 ± 139.6</td>
<td>653.9 ± 120.4</td>
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Values are means ± SE (n = 5–6). Pyrrolidine dithiocarbamate (PDTC) or vehicle (control) was administered twice daily for 14 days, and then the length of the regenerated mucosa, thickness of the ulcer base, and densities of the microvessels and neutrophils in the base were histologically determined. *P < 0.05 vs. control.
events. The mechanism of the NF-κB action in gastric ulcer healing is discussed below. The present study revealed that NF-κB might be involved in the gene expression of healing-promoting factors such as COX-2, iNOS, and CINC-1 in gastric ulcers in rats as well as in cultured gastric fibroblasts. Our group (21, 26) reported that COX-2 mRNA and protein are induced by gastric ulceration and that the increased prostaglandins, derived from COX-2, have stimulatory effects on epithelial regeneration, maturation of the base, and angiogenesis. Konturek et al. (10) reported that NO might maintain an increased blood flow at the ulcer margin and stimulate angiogenesis in the ulcer base, contributing to gastric ulcer healing. Most of the NOS activity in the ulcer base is due to iNOS activity (25). These results strongly suggest that iNOS is also an important factor for ulcer healing. CINC-1 exhibits potent neutrophil chemotactic activity in vitro (20) and acts as a functional chemoattractant for neutrophils in vivo (30, 31). Since CINC belongs to IL-8 chemokine family, and a rat counterpart of human IL-8 has not been discovered to date, CINC's are considered to play a predominant role in neutrophil infiltration in rats (30, 31). The activation and infiltration of neutrophils are essential for the elimination of wounded cells, cell debris, and extracellular matrix proteins in tissue regeneration. It was reported that CINC induces neovascularization in a rat corneal pocket model and that NF-κB activation results in CINC production and participates in the induction of retinal neovascularization in rats (34). As we described previously (25, 33) and here, CINC-1 mRNA and protein are induced in ulcerated gastric tissue. CINC-1 not only elicits neutrophil infiltration but also may enhance angiogenesis in the ulcer base. In addition, we recently reported that IL-1 upregulates the gene expression of COX-2, iNOS, and CINC-1 in the ulcer base, and the inhibition of IL-1 action causes an impairment of ulcer healing in rats (25). This, together with in vitro data, indicates that NF-κB might partly mediate IL-1 signaling to induce these factors.

Administration of PDTC for more than 10 days prevented gastric ulcer healing, although the drug did not affect the healing on day 7 when NF-κB activation was inhibited. These findings are similar to COX inhibitor-delayed healing (21). Neither indomethacin nor NS-398 affect ulcer healing in early phase (by day 7), but PGE2 production in ulcerated tissue has been markedly inhibited since day 0. It is considered that there is a lag period in which the delay of ulcer healing has not
yet become manifest, because the persistent inhibition of tissue repair responses of cells results in visible healing impairment. In addition, NF-κB inhibition suppresses new production of the healing-promoting factors but has no influence on the levels of the preexisting ones. The lag period might be attributable partly to the remaining factors.

NF-κB decoy oligonucleotide and PDTC potently inhibited NF-κB activation in IL-1β-stimulated gastric fibroblasts and ulcerated tissue, respectively, but their inhibitory effects on the expression of healing-promoting factors were weaker than those on NF-κB activation. Since it is known that several transcription factors coordinately upregulate them, it is suggested that other pathways as well as the NF-κB pathway might be important in their expression in gastric fibroblasts and ulcers. Actually, we have recently found that a mitogen-activated protein kinase pathway is also involved in the induction of these factors in gastric ulcers (Takahashi et al., unpublished data).

In contrast to the present results, it has been accepted that NF-κB plays a pivotal role in the pathogenesis of gastric mucosal inflammation and injury caused by *Helicobacter pylori* infection. NF-κB mediates *Helicobacter pylori*-induced IL-8 expression in gastric epithelial cells (8). Thus NF-κB has both favorable and harmful actions in the gastric mucosa. The bilateral actions are similarly observed in the case of IL-1. IL-1 provides protection of the gastric mucosa against various noxious stimuli (16, 29) and might contribute to gastric ulcer healing (25). On the other hand, IL-1 is responsible for inflammation and injury in *Helicobacter pylori*-infected mucosa (4, 7) and causes recurrence of healed gastric ulcers (32). Factors controlling the roles of NF-κB and IL-1 remain unclear, but it is likely that NF-κB and IL-1 exert injurious effects on the gastric mucosa when severe inflammation is sustained. In the case of ulcer healing delayed by indomethacin, severe inflammation is persistently observed (1, 33). Sustained severe inflammation disrupts epithelial functions and therefore is deleterious to the gastric mucosa (1, 4, 33). In conclusion, NF-κB, activated in fibroblasts, monocytes/macrophages, and neutrophils in the ulcer base, might upregulate the expression of healing-promoting factors, contributing to gastric ulcer healing in rats.

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