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ROLE OF INTERLEUKIN-4 IN DOWN-REGULATION OF ENDOTHELIN-1 DURING GASTRIC ULCER HEALING: EFFECT OF SUCRALFATE

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Background: The course of events associaed with healing gastric mucosal injury involves an orderly interplay between the array of signaling molecules that exert their influence on the processes leading to the restoration of the mucosal integrity. In this study, we investigated the effect of antiulcer agent, sucralfate, on the mucosal apoptotic processes during gastric ulcer healing by analyzing the expression of interleukin-4 (IL:4), endothelin-1 (ET-1), tumor necrosis factor-α (TNF-α), and the mucosal activity of capase-3, and constitutive (cNOS) and inducible nitric oxide synthase (NOS-2). *Methods:* Rats with experimentally induced chronic gastric ulcers were administered twice daily for 14 days either sucralfate at 100 mg/kg or vehicle, and at different stages of treatment their stomachs were used for macroscopic and biochemical assessments. *Results:* The ulcer onset was characterized by a massive epithelial apoptosis associated with a 33-fold increase im caspase-3 activity, 5.7-fold increase in TNF-α, 17.5-fold increase in NOS-2 and a 3.9-fold increase in ET-1, while the mucosal expression of cNOS activity showed a 7.6-fold drop and IL-4 expression fell by 37.2%. The healing was reflected in a rapid recovery in IL-4, and a decrease in apoptosis, caspase-3, TNF-α, ET-1 and NOS-2, and a slow recovery in cNOS activity, and the process was accelerated in the sucralfate-treated group. While in the absence of sucralfate the expression of IL-4 returned to that of the normal mucosa by the 7th day of healing and that of ET-1 and TNF-α by the 14th day, an accelerated ulcer healing with sucralfate treatment was associated with IL-4 recovery by the 4th day and that of ET-1 and TNF-α by the 10th day when the ulcer heated, while recovery in cNOS activity required 14 days. Yet, in both groups of animals the apoptotic DNA fragmentation rate, caspase-3 and the expression of NOS-2 activity remained significantly elevated even after the ulcer healed. *Conclusions:* The results suggest that a decrease in the mucosal expression of the regulatory cytokine IL-4 at the ulcer onset may well be a key factor causing dysregulation of ET-1 production, induction of TNF-α, and triggering the apoptotic events that affect the efficiency of mucosal repair process. We also show that accelerated ulcer healing by sucralfate may be the result of a rapid mucosal IL-4 generation that leads to the suppression of the mucosal apoptotic events.

Key words: *gastric mucosa, chronic ulcer healing, IL-4, ET-1, TNF-α, cNOS, NOS-2, caspase-3, sucralfate.*

INTRODUCTION

The course of events associated with healing gastric mucosal injury involves a diverse array of signaling cues exerting their influence on the processes that propel the mucosal cells to proliferation, differentiation and migration to the site of injury, or to signal the programmed cell death (1—5). An orderly and synchronized interplay between the signaling molecules and timely processing of signaling cues assures not only the restoration of the mucosal integrity, but also the quality of ulcer healing (4—8). Indeed, studies indicate that the mucosal injury and the ulcer onset are accompanied by a dynamic variation in the mucosal expression of growth factors, induction of proinflammatory cytokine production, and the distribances in endothelium-dependent vasoactive substances, nitric oxide and endothelins (5, 8—12).

Nitric oxide, a pluripotent molecule, is an important biological messenger that plays a role in phyiological and patjophysiological conditions such as regulating blood pressure, neurotransmission, inflammation, and septic shock. One of its major intracellular targets is the heme group o soluble guanylate cyclase with NO markedly stimulating enzymatic activity and thereby increasing the intracellular cGMP concentration. Most recent data also indicate that NO regulates gene expression via cGMP activation of G-kinase (cGMP-dependent protein kinase) (13, 14).

The endothelins (ET) are a family of cysteine-rich peptides consisting of 21 amino acids and containing two intramolecular disulfide bridges (15, 16). At the present, the existence of three active isoforms of endothelin, ET-1, ET-2, and ET-3, and two distinct receptors, ET_A and ET_B, is well documented (16, 17). The ET_A receptor mediates vasoconstriction and displays high affinity for ET-1, while the ET_B receptor exhibits an equal affinity for ET-1 and ET-3, and its activation results in vasoconstriction as well as vasodilatation (16, 18—20). Aside of vascular tissues, the ET receptors are also found in many nonvascular tissues, including gastric and intestinal mucosa where ET-1 plays a major role in the pathogenesis of gastric mucosal injury (9, 21, 22). ET-1, the most prevalent member of endothelin family, is a cleavage product of preproET-1 by as yet not well defined furin-like enzyme to yield a peptide of 39 amino acids, termed big ET-1, which is subsequently cleaved at the Trp^{21} residue by a specific membrane-bound endothelin-coverting enzyme-1 (ECE-1) comprising of ECE-1α and ECE-1β isoforms. The two isoforms share the same C-terminal domain and appear to be the alternatively spliced product of the same gene (20).

The enhanced ET-1 levels accompany local and systemic inflammations, and affect the formation of several proinflammatory cytokines, including TNF-α (19, 23, 24). The expression of this mediator of inflammatory process is controlled at the translational level by the regulatory pleiotropic cytokine, IL-4, that remains under the influence of nitric oxide (25, 26). Indeed, IL-4,

through its specific bipartite IL-4 receptor activation, triggers a rapid onset of tyrosine protein transphosphorylation and activation of JAK-STAT pathway (28, 29), thus leading to down-regulation a wide variety of TNF-induced effects, including suppression of TNF-induced NFκB and AP-1 activation, inhibition of apoptotic caspase-3 activity and abrogation of TNF-induced c-Jun N-terminal kinase activation (26—29). Furthermore, IL-4 through the involvement of a specific metalloprotease is capable of inducing TNF receptor sheadding (26).

In this study, we examined the course of events associated with gastric mucosal repair by analyzing the relationship between the apoptotic processes and the expression of ET-1, TNF-α, IL-4, and cNOS and NOS-2 during chronic ulcer healing, and assessed the result of treatment with antiulcer agent, sucralfate.

MATERIALS AND METHODS

Animals

This study was conducted with 180 to 200 g Sprague-Dawley rats cared for by the professional personnel of the Research Animal Facility of UMDNJ. The animals were deprived of food 24 h before the experiment and water was withheld 2 h before the procedure. Under ether anesthesia, the rats were subjected to laparotomy, and the serosal surface of the stomach was exposed for 20 sec to contact with glacial acetic acid, with the use of a plastic tube of 6 mm in diameter (4). This produced an immediate mucosal necrosis within affected area followed 2 days latter by the development of chronic ulcer with a well-defined crater, which normally healed within 14 days (4, 5). After recovery from the anesthesia, the animals were divided into groups and given twice daily for 14 days, by intragastric route, either a 100 mg/kg of surface or vehicle consisting of 5% gun Arabic in saline (4). Animals were killed at different time intervals of ulcer healing, their stomachs dissected, and the gastric mucosa from the ulcer area together with its margin excised and subjected to the quantification of TNF-α, ET-1, and IL-4, assays of epithelial cell apoptosis, and the measurements of caspase-3, cNOS and NOS-2 activities. The protein content of samples was measured with the BCA kit, and the rate of ulcer healing was assessed by planimetry (4).

Apoptosis assay

Quantitative measurements of apoptosis were conducted with epithelial cells prepared from gastric mucosal scrapings (5, 10). The cells were incubated in the lysis buffer in accordance with the manufacturer's (Boehringer Mannheim) instruction, centrifuged, and the diluted sepematant containing the cytoplasmic histone-associated DNA fragments was reacted with in the microtitrator wells with immobilized antihistone antibody. The retained complex was reacted with anti-DNA peroxidase, and the bound peroxidase probed with ABTS reagent for spectrophotometric detectin (10).

TNF-α expression assay

TNF-α was quantitated with an enzyme-linked immunosorbent assay according to the menufacturer's (Genzyme) instructions. The microtitrator wells were precoated with monoclonal anti-TNF-α to capture TNF-α from the gastric mucosal homogenates, and, after washing, the

retained complex was probed with horseradish peroxidase-conjugated anti-TNF-α. The complex was then incubated with TMB reagent for TNF-α quantitization (10).

Caspse-3 activity assay

Caspase-3 activity measurements were carried out with gastric epithelial cells using a Quanti Zyme assay system (Biomol Res. Lab., Inc.) Following lysis and centrifugation, the aliquots of the resulting cytosolic fraction were incubated in the microtitrator wells with DEVD-pNA substrate and the caspase-3 activity measured spectrophotometrically (10).

ET-1 expression assay

ET-1 assays were carried out on the individual specimens of gastric mucosal tissue following lyophilization and homogenization with 4 volumes of 1 M acetic acid containing 10 μg/ml of pepstain (12, 30). The homogenates were heated for 5 min at 100°C, centrifuged, and the resulting suoernatants applied to a Sep-Pack C-18 reverse phase cartridges. After initivl washing with 0.1% trifluoroacetic acid, the adsorbed ET-1 was eluted with methanol-water-trifluoroacetic acid (90:10:0.1, v/v/v). The eluates were dried under vacuum, reconstituted in the assay buffer, and subjected to ET-1 quantitization using double-antibody sandwich technique according to the manufacture's (Alexis Corporation) instruction. The sample aliquots were applied to the microtitrator wells coated with ET-1 capture antibody and incubated at 4°C for 16 h. After washing, the wells were probed with Ellman's reagent, incubated at room temperature for 2 h, and ET-1 quantified spectrophotometrically (30).

IL-4 expression assay

IL-4 measurements were conducted using a solid-phase enzyme-linked immunosorbent system (Bio-Source International). The individual specimens of gastric mucosal tissue were homogenized with 5 volumes of the sample buffer, centrifuged, and the resulting supernatant diluents were pipetted to the microtitrator wells precoated with antibody specific for rat IL- (30). Following incubation, the complex was probed with biotinylated second antibody, reacted with streptavidin-peroxidase, and processed with TMB reagent for IL-4 quantification (12).

NOS activity assay

Gastric mucosal activity of cNOS and NOS-2 was measured by monitoring the conversion of L-[^{3}H]arginine to L-[^{3}H]citrulline using NOS-detect kit (Stratagene). The specimens of gastric mucosa were homogenized in a sample buffer containing either 10 mM EDTA (NOS-2) or 6 mM $CaCl_2$ (cNOS), and centrifuged at 800 × g for 10 min (5). The aliquots of the resulting supernatants were incubated for 30 min at 25°C in the presence of L-[2,3,4,5-^{3}H]arginine (50 μCi/μl), 10 mM NAPDH, 5 μM tetrahydrobiopterin, and 50 mM Tris-HCl buffer, pH 7.4, in a final volume of 250 μl (5, 30). Following addition of stop buffer and Dowex-50 W (Na^+) resin, the mixtures were transferred to spin cups, centrifuged and the formed L-[^{3}H]citrulline contained in the flow through was quantitated by scintillation counting.

Antiulcer drug

The antiulcer drug sucralfate was kindly provided by Chugai Pharmaceutical Co. Ltd., Tokyo, Japan. The agent was stored at 4°C in the dark and was suspended in saline shortly before experimentation. The drug or vehicle was given orally in a volume of 1 ml through dull metal tubing attached to a 2-ml syrine.

Data analysis

All experiments were carried out in duplicate, and the results are expressed as the means ± SD. Analysis of variance (ANOVA) was used to determine significance, and the significance, and the significance level was set at $p < 0.05$.

RESULTS

Chronic gastric mucosal ulcers were developed in the rat by exposing the serosal surface of the stomach to a contact with acetic acid (4, 5). The mean ulcer area at the onset of the experiments (day, 0) averaged 27.6 mm^2, which decreased to 21.3 mm^2 by the fourth day and to 4.8 mm^2 by the tenth day, and essentially disappeared by the fourteenth day. The animals treated with sucralfate showed a 50% decrease in ulcer area by the 4th day and an 86% decrease by the 7th day, and the ulcers were healed by the 10th day.

The data on gastric mucosal expression of TNF-α with ulcer healing in the absence and the presence of sucralfate administration are summarized in *Fig. 1*.

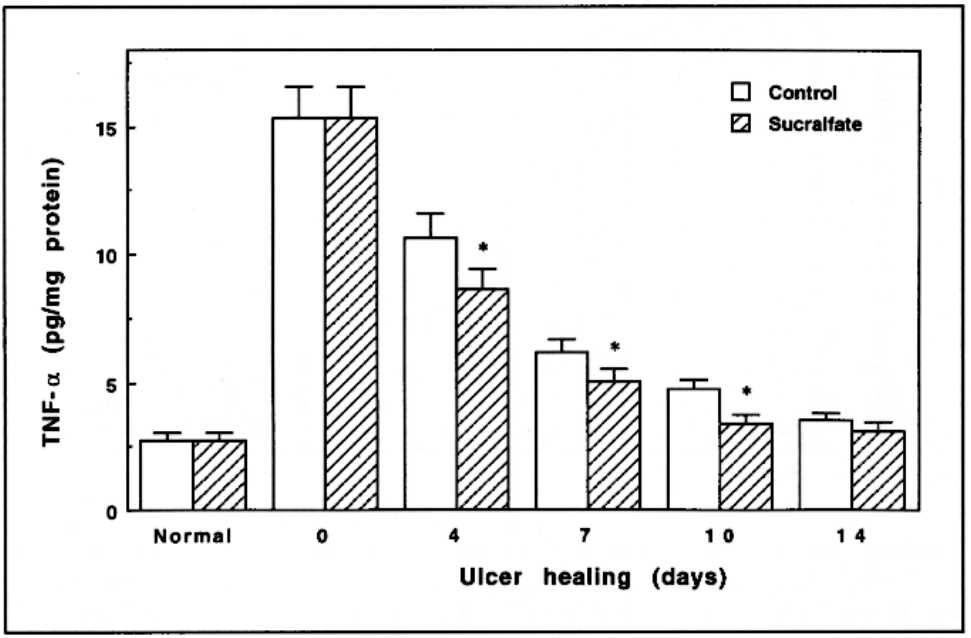

Fig. 1. Effect of sucralfate administration, 100 mg/kg twice daily, on the expression of TNF-α in gastric mucosa during chronic gastric ulcer healing. Values represent the means ± SD of duplicate analyses performed with 10 animals in each group. * $P < 0.05$ compared with that of the control.

The results of assays revealed that comparing to the normal mucosa the ulcer onset was characterized by a massive (5.7-fold) elevation in TNF-α expression which, although declining steadily, remained significantly elevated during the

first 10 days of healing and reached a level comparable to that of nornal mucosa by the fourteenth day when the ulcer was healed. Accelerated ulcer healing with sucralfate was reflected in a more pronounced drop in TNF-α, which reached the level comparable to that of normal mucosa by the 10th day of healing.

The results of apoptotic assays conducted with epithelial cells isolated from gastric mucosa of the animals at different stages of ulcer healing with and without of sucralfate treatment revealed that, compared to normal mucosa (1.4 unit/mg protein), the ulcer onset (day, 0) was characterized by a marked increase (24.8-fold) in DNA fragmentation (*Fig. 2*). This was followed by a rapid decline in apoptosis, giving in the absence of sucralfate by the 7th day of healing a value of 12.8 unit/mg protein and an 8.2 unit/mg protein in the preaence of sucralfate treatment, while the respective values of 7.9 and 5.3 unit/mg protein were obtained by the 10th day of healing. In both groups of animals, however, the apoptotic DNA fragmentation rate remained significantly elevated over that of normal mucosa levels beyond the 14 days period.

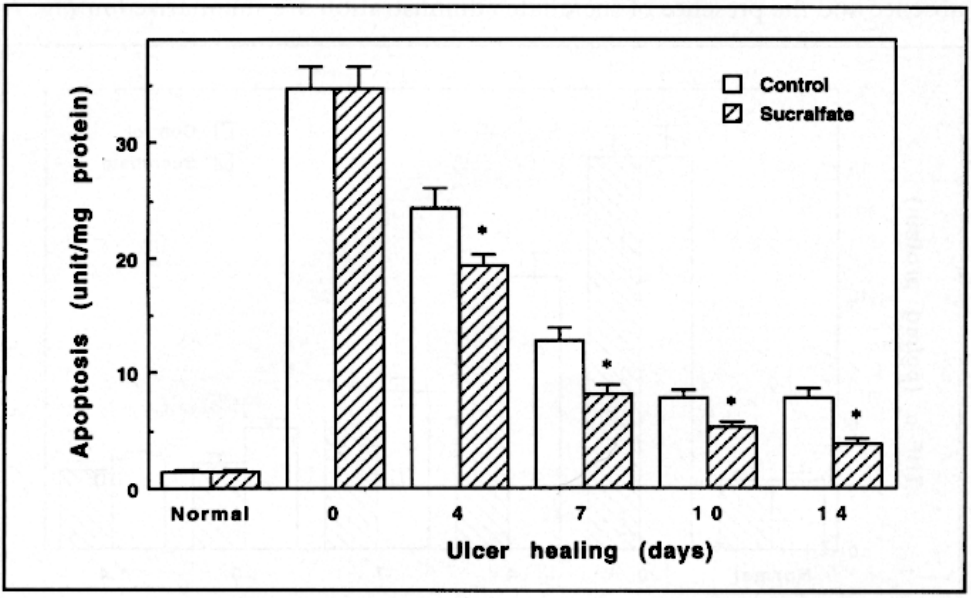

Fig. 2. Effect of sucralfate administration on gastric epithelial cell apoptosis during chronic gastric ulcer healing. Values represent the means ± SD of duplicate analyses performed with 10 animals in each group. * $P < 0.05$ compared with that of the control.

Figure 3 shows the expression of gastric mucosal caspase-3 activity with chronic ulcer healing in the presence of sucralfate treatment. The ulcer onset, associated with a 33-fold increase in caspase-3 activity, was followed in both groups by a rapid decline in the activity with healing. However, compared to the controls, the values attained for caspase-3 in the presence of sucralfate

administration showed a 41% greater decline by the 10th day of healing and were a 72% lower at the end of 14 day period. Nevertheless, the caspase-3 activity still remained about 2 times greater than that of the normal mucosa.

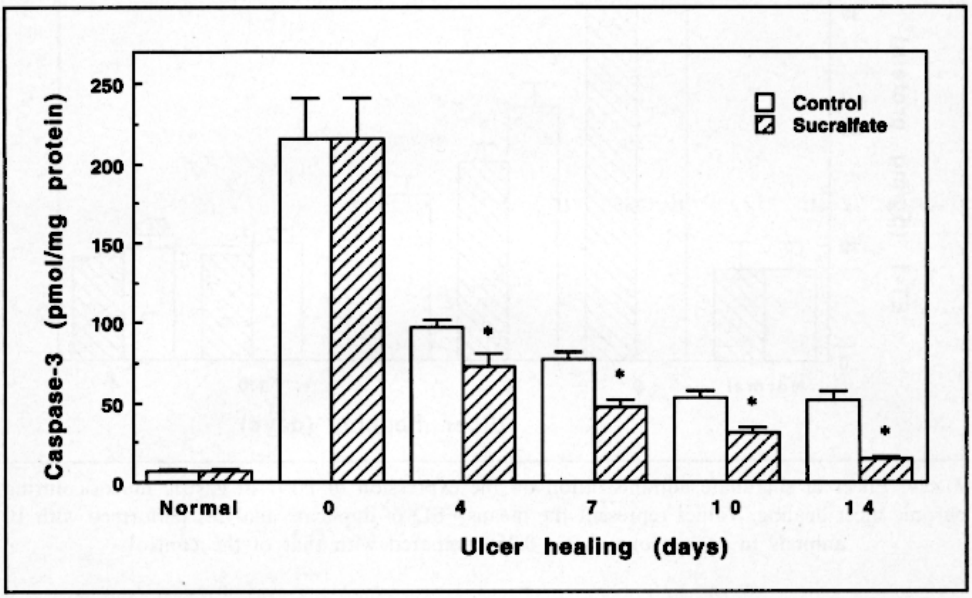

Fig. 3. Effect of sucralfate administration on the expression of caspase-3 activity in gastric mucosa during chronic gastric ulcer healing. Values represent the means ± SD of duplicate analyses performed with 10 animals in each group. * $P < 0.05$ compared with that of the control.

The expression of gastric mucosal ET-1 during chronic ulcer healing is presented in *Fig. 4*. The ulcer onset (day, 0) was associated with a 3.9-fold increase in the ET-1 level over that of the normal mucosa, followed by a gradual decline to 10 pg/mg protein by the 10th day od healing and reaching ET-1 values comparable with that of normal mucosa by the 14th day. Comparing to the controls, the accelerated ulcer healing with sucralfate treatment was manifested by a significant decline in the mucosal ET-1 level up to the 7th day of healing.

The data on the gastric mucosal expression of IL-4 during ulcer healing in the absence and the presence of treatment with sucralfate are summarized in *Fig. 5*. Compared with the values for normal mucosa, the ulcer onset (day, 0) was characterized by a 36.2% reduction in the IL-4 expression. This was followed by a rapid increaae in IL-4 level with healing. In the absence of sucralfate, the IL-4 values reached that of the normal mucosa by the 7th day of healing and exceeded (13%) by the 14th day. The values attained for IL-4 in the presence of sucralfate treatment reached those of normal mucosa by the 4th day of healing and remained consistently, although not significantly, elevated over that of control throughout the entire 14 day period of healing.

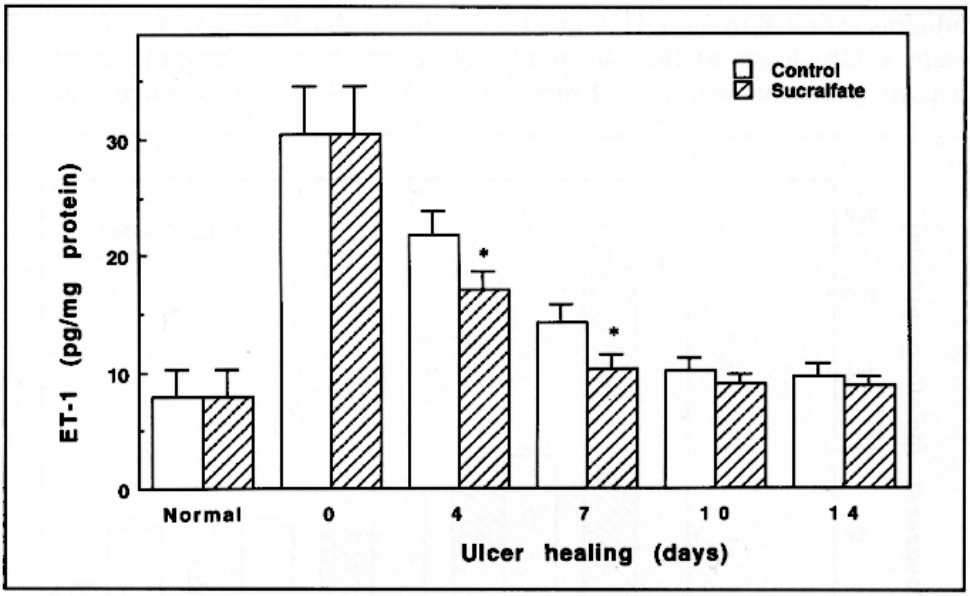

Fig. 4. Effect of sucralfate administration on the expression of ET-1 in gastric mucosa during chronic ulcer healing. Values represent the means ± SD of duplicate analyses performed with 10 animals in each group. * $P < 0.05$ compared with that of the control.

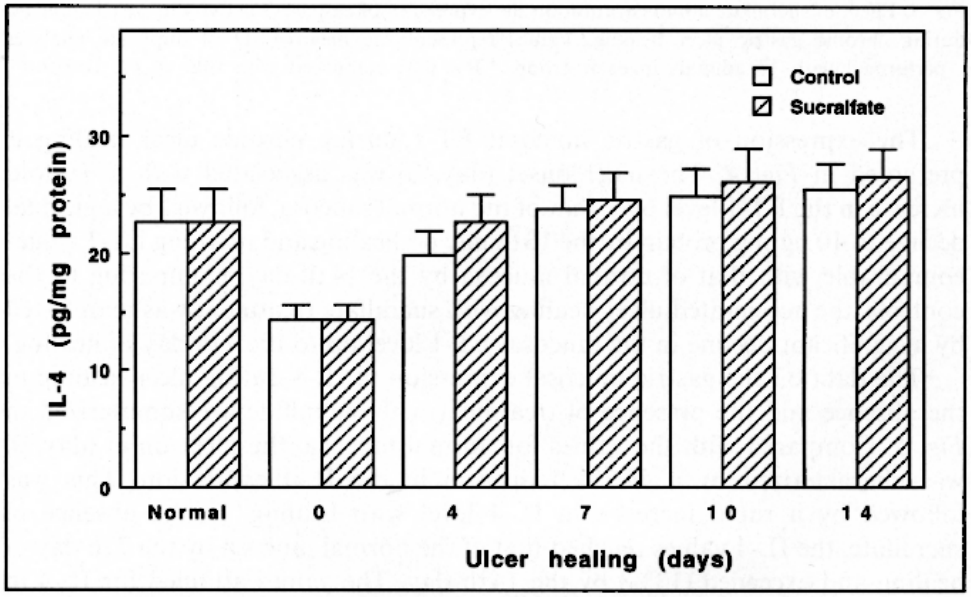

Fig. 5. Effect of sucralfate administration on the expression of IL-4 in gastric mucosa during chronic gastric ulcer healing. Values repesent the means ± SD of duplicate analyses performed with 10 animals in each group. * $P < 0.05$ compared with that of the control.

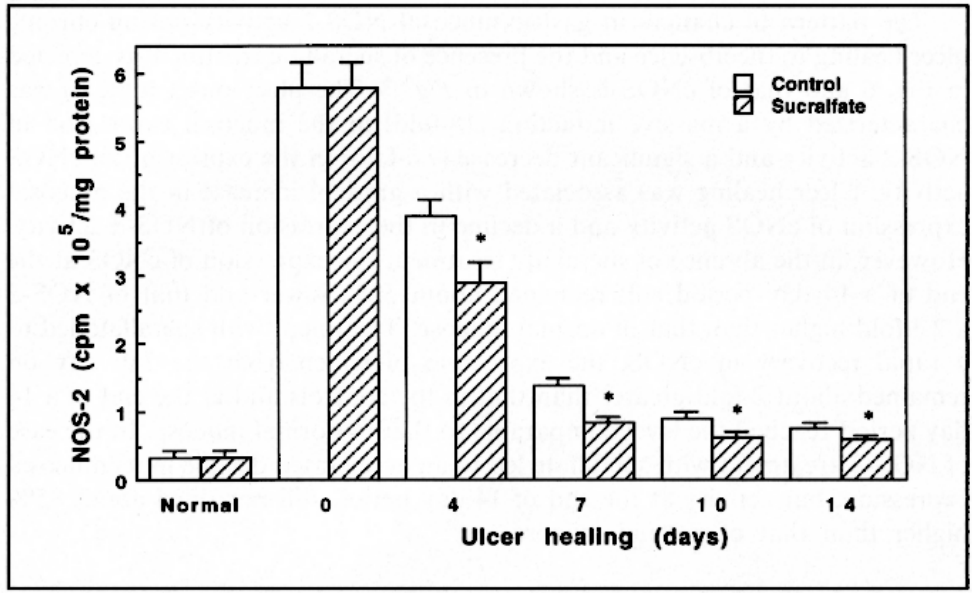

Fig. 6. Effect of sucralfate administration on the expression of NOS-2 activity in gastric mucosa during chronic gastric ulcer healing. Values represent the means ± SD of duplicate analyses performed with 10 animals in each group. * $P < 0.05$ compared with that of the control.

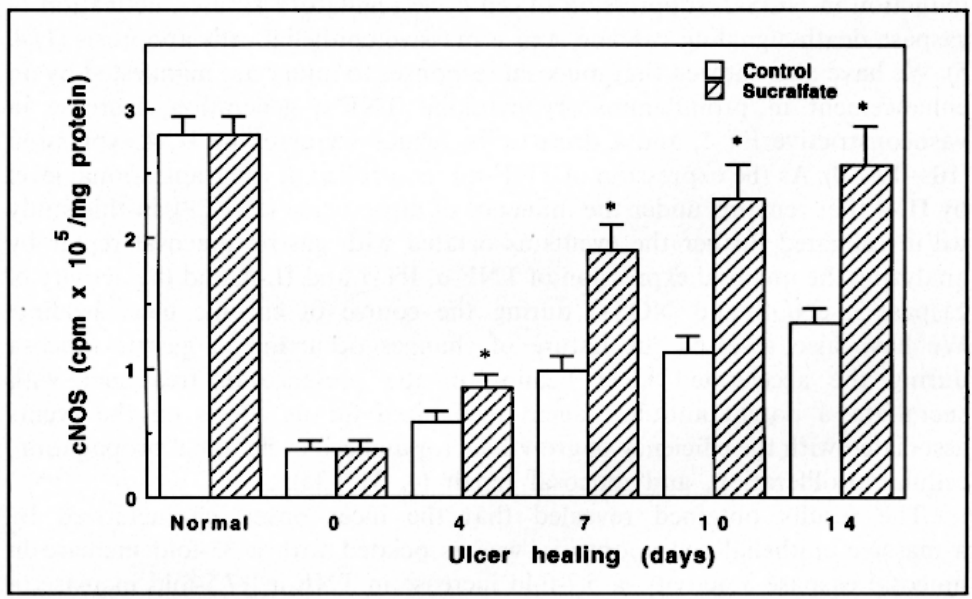

Fig. 7. Effect of sucralfate administration on the expression of cNOS activity in gastric mucosa during chronic ulcer healing. Values represent the means ± SD of duplicate analyses performed with 10 animals in each group. * $P < 0.05$ compared with that of the control.

The pattern of changes in gastric mucosal NOS-2 activity during chronic ulcer healing in the absence and the presence of sucralfate treatment is depicted in Fig. 6 and that of cNOS is shown in *Fig.* 7. The ulcer onset (day, 0) was characterized by a massive induction (18-fold) in the mucosal expression of NOS-2 activity and a significant decrease (7.6-fold) in the expression of cNOS activity. Ulcer healing was associated with a gradual increase in the mucosal expression of cNOS activity and a decline in the expression of NOS-2 activity. However, in the absence of sucralfate treatment the expression of cNOS at the end of a 14 day period still remained about 52% lower and that of NOS-2 a 2.3-fold higher than that of normal mucosa. Treatment with sucralfate led to a rapid recovery in cNOS, the expression of which from the 7th day on remained about 2-fold greater than that in the controls and at the end of a 14 day period reached the level comparable to that of normal mucosa. In the case of NOS-2, treatment with sucralfate led to an accelerated decline in its mucosal expression, but activity at the end of 14 day period still remained about 85% higher than that of normal mucosa.

DISCUSSION

Our previous studies with animal model of acetic acid-induced chronic gastric ulcer demonstrated that the ulcer onset is characterized by a marked induction in NOS-2, suppression of cell cycle regulatory kinases, activation of caspase death signaling cascade, and a massive epithelial cells apoptosis (1, 4, 5). We have also showed that mucosal responses to injury are manifested by an enhancement in proinflammatory cytokine TNF-α generation, increase in vasoconstrictive ET-1, and a decrease in regulatory cytokine IL-4 expression (10—12, 30). As the expression of TNF-α is controlled at the translational level by IL-4 that remains under the influence of nitric oxide (25—29), in this study we investigated further the events associated with gastric mucosal repair by analyzing the mucosal expression of TNF-α, ET-1 and IL-4, and the activity of caspase-3, cNOS and NOS-2 during the course of chronic ulcer healing. We have also assessed the nature of changes occurring in gastric mucosa during the accelerated ulcer healing in the presence of treatment with sucralfate, a potent antiulcer agent recognized for its effects on the events associated with the efficiency of growth factor and cell cycle signal propagation, cellular proliferation, and mucosal repair (4, 31—35).

The results obtained revealed that the ulcer onset, characterized by a massive epithelial cell apoptosis, was associated with a 33-fold increase in mucosal caspase-3 activity, a 5.7-fold increase in TNF-α, 17.5-fold increase in NOS-2 and a 3.9-fold increase in ET-1, while the mucosal expression of cNOS activity showed a 7.6-fold drop and IL-4 expression fell by 37.2%. The ulcer healing was reflected in a rapid recovery in IL-4 and a decrease in apoptosis,

caspase-3, TNF-α, ET-1 and NOS-2, and a slow recovery in gastric mucosal cNOS activity. While in the absence of sucralfate the expression of IL-4 returned to that of the normal mucosa by the 7th day of healing and that of ET-1 and TNF-α by the 14th day, an accelerated ulcer healing with sucralfate treatment was reflected in IL-4 recovery by the 4th day and that of ET-1 and TNF-α by the 10th day when the ulcer healed. However, even in the presence of sucralfate treatment the rate of apoptosis and the expression of caspase-3 and NOS-2 activity remained significantly elevated over that of normal mucosa beyond the 14 day period. The finding that the ulcer onset was reflected in a marked induction in NOS-2 expression and a drop in cNOS, while the healing process in the presence of sucralfate was characterized by a pronounced decrease in NOS-2 and a rapid recovery in cNOS provides a strong indication as to the importance of cNOS in the process of gastric mucosal repair and attests to a value of sucralfate in ulcer treatment. In this connection it is pertinent to note that the activity of cNOS is also compromised during gastric mucosal injury induced by ischemia-reperfusion or caused by NSAIDs (22, 31, 36, 37).

The fact that the enhanced NOS-2 expression with ulcer onset coincided with a massive epithelial cell apoptosis and a 33-fold increase in mucosal expression of caspase-3 activity points towards participation of NOS-2 in the events involved in apoptotic cell death. Indeed, the sustained induction in NOS-2 expression is known to lead to the activation of apoptotic caspase cascade and results in the formation of NO-related species that exert a direct inhibitory effect on NFkB causing transcriptional disturćances that lead to apoptosis (30—40). On the other hand, the constitutively expressed and largely cytosolic cNOS plays an active role in the inhibition of apoptogenic signals generated by caspase activation (38, 41—43). This inhibitory effect of cNOS occurs through S-nitrosylation of the apoptotic caspase-3 which leads to the suppression of Bcl-2 cleavage, thus preventing the mitochondrial release of cytochrome c, and resulting in the inhibition of apoptosis (38, 41, 44). The cNOS is also involved in the inhibition of caspases activity through a cGMP-dependent mechanism that functions in the catosol at the level of caspase zymogens activation that requires cleavage adjacent to aspartates (41, 44).

Our findings on the enhanced mucosal expression of ET-1 and TNF-α at the ulcer onset followed by a slow decline with healing, strongly imply a key involvement for ET-1 in triggering the prolonged mucosal inflammatory responses that interfere with cessation of apoptotic events required for the efficient repair process. Indeed, an accelerated ulcer healing (10 days) with sucralfate treatment coincided with the return of ET-1 and TNF-α levels to that of normal mucosa, and the literature indicates that ET-1 affects formation of such proinflammatory catokines as IL-2 and IL-6, and stimulates

biosynthesis of TNF-α (19, 23). The later process apparently involves the activation by ET-1 of ET_A receptor which, in turn, leads to the activation of tyrosine kinase intracellular protein cascade and ultimately culminates in translation of the TNF-α gene (19, 44). Apparently the interaction of ET-1 with its receptor is subject to interference by nitric oxide, which is capable of both displacing the bound ET-1 from the ET_A receptor and interfering with postreceptor pathways related to calcium mobilization through the channel protein S-nitrosylation (22, 46, 47). The vasoactive form of ET-1 is a 21 amino acid peptide derived from the initial gene product, a 39 amino acids containing inactive big ET-1, by a series of proteolytic steps culminating in the hydrolysis of $Tryp^{21}$-Val^{22} bond by a specific cell surface metallopeptidase, known as endothelin-converting enzyme, ECE (16, 48). The findings indicate that the expression of ECE and other metalloproteinase genes are controlled at the translational level by IL-4 (25—29). Interestingly, the results of our study revealed that the ulcer onset is associated with a significant drop in gastric mucosal IL-4 level followed by a more rapid recovery (4 days) with healing in the presence of sucralfate treatment. Therefore, the activity of ECE is not only of paramount importance to ET-1 generation and the production of TNF-α, but also to the apoptotic signal propagation as well as its cessation.

The interaction of IL-4 cytokine with the α-chain and γc-chain of the specific IL-4 receptor on target cells leads to receptor subunit dimerization and the activation of Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway resulting in the transcriptional activation of specific genes (26, 49). Activation of Janus protein tyrosine kinases JAK1, associated with α-chain, and JAK3, associated with γc-chain of the receptor, occurs by transphosphorylation and leads to the recruitment of STAT proteins, which become phosphorylated at critical tyrosine residues (28, 29, 49). Of the several known STAT proteins, only STAT5 and STST6 are involved in IL-4 induced intracellular signaling, and gene regulation by these proteins appears to be of central importance to IL4-governed responses (26, 29, 49). The phosphorylated STAT factors then dimerize, translocate to the cell nucleus, and modulate transcription of target genes leading to such cellular events as suppression of apoptosis and blocking the synthesis and processing of metalloproteinases (25, 28, 29, 50). Conversely, tyrosine dephosphorylation of JAK-STAT prpteins is a key for down regulation of IL-4 signaling. There are at least two types of tyrosine phosphatase (PTP) involved in the negative regulation of JAK-STAT signaling. The activated JAKs and cytokine receptors are inactivated by an upstream phosphatase PTP-x, whereas PTP-y dephosphorylates activated STSTs in the nucleus (49).

From the results of this study, it is apparent that a decrease in the mucosal level of the regulatory cytokine IL-4 during ulcer onset may well be a key factor in causing up-regulation of ECE expression, dysregulation of ET-1

production, induction of TNF-α, and triggering the apoptotic events that affect the efficiency of mucosal repair. Hence, it is reasonable to conclude that sucralfate by eliciting rapid induction in IL-4 expression is capable of efficient suppression of the mucosal apoptotic events the cessation of which determines the efficiency of the healing process.

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