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UP-REGULATION OF ENDOTHELIN-1 IN GASTRIC MUCOSAL INFLAMMATORY RESPONSES TO *HELCOBACTER PYLORI* LIPOPOLYSACCHARIDE: EFFECT OF OMEPRAZOLE AND SUCRALFATE

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**Background:** *Helicobacter pylori* is recognized as a primary etiologic factor in the development of gastric disease and the product of particular significance to the virulent action of the bacterium is its cell wall lipopolysaccharide. We applied the animal model of *H. pylori* lipopolysaccharide-induced acute gastritis to study the effect of antiulcer agents, omeprazole and sucralfate, on the course of mucosal inflammatory responses by analyzing the interplay between the extent of epithelial cell apoptosis and the mucosal expression of endothelin-1 (ET-1), tumor necrosis factor-α (TNF-α), and the activity of constitutive (cNOS) and inducible (NOS-2) nitric oxide synthase. **Methods:** Rats pretreated twice daily for 3 consecutive days with omeprazole at 40 mg/kg, sucralfate at 100 mg/kg or the vehicle, were subjected to intragastric application of *H. pylori* lipopolysaccharide at 50 μg/animal, and after 2, 4, and 10 additional days on the antiulcer drug or vehicle regimen their mucosal tissue used for histologic and biochemical assessment. **Results:** In the absence of antiulcer agents, *H. pylori* lipopolysaccharide elicited within 2 days a pattern of acute mucosal inflammatory responses accompanied by a massive epithelial cell apoptosis, a 2.9-fold increase in the mucosal expression of ET-1, an 11.7-fold enhancement in TNF-α, and a 9.3-fold increase in NOS-2, while cNOS activity showed a 5.5-fold decrease. The extent of mucosal inflammatory involvement reached a maximum by the 4th day and showed a decline by the 10th day. This was reflected in a marked reduction in epithelial cell apoptosis, decrease in the mucosal expression of ET-1, TNF-α and NOS-2, and the recovery in cNOS activity. Comparing to the vehicle controls, treatment with proton pump inhibitor, omeprazole, led at the end of a 10 day period to a 48.3% reduction in the extent of mucosal inflammatory involvement elicited by *H. pylori* lipopolysaccharide, while a 74.2% reduction in the mucosal inflammatory involvement was achieved with gastroprotective agent, sucralfate. Moreover, this advantage of sucralfate over omeprazole in countering the lipopolysaccharide-induced changes was reflected at the end of 10 day treatment period in a 20.4% greater decrease in apoptosis, a 47.5% greater reduction in TNF-α and a 50.7% greater reduction in ET-1. However, both agents exerted similar influence on the restoration of gastric mucosal cNOS activity and showed a comparable effect at the end of a 10 day treatment in countering the lipopolysaccharide-induced increase in the expression of NOS-2. **Conclusions:** The findings suggest that an increase in the mucosal ET-1 level elicited by *H. pylori* lipopolysaccharide, combined with a decline in cNOS may be responsible for the
induction of TNF-α and triggering the inflammatory process. We also show that succinylate exhibits greater efficacy than omeprazole in suppressing the H. pylori-induced mucosal inflammatory responses. This property of succinylate may well be due to its ability to suppress the mucosal rise in ET-1.

Key words: Helicobacter pylori, lipopolysaccharide, acute gastritis, apoptosis, ET-1, TNF-α, cNOS, NOS-2.

INTRODUCTION

Infection with Helicobacter pylori is a primary factor in the etiology of gastric disease, and the relationship between the bacterium and gastric mucosal inflammatory changes that characterize gastritis and duodenal ulcers is well established (1—4).

The product of particular significance to the virulent action of H. pylori is its cell wall lipopolysaccharide (5—8). The pathogenic effects of H. pylori lipopolysaccharide are manifested by a marked up-regulation in gastric mucosal ET-1 level and proinflammatory cytokine expression, excessive nitric oxide generation, repression of regulatory cytokine production, apoptotic caspase activation, and a marked enhancement in gastric epithelial cell apoptosis (5, 8—10). Other responses observed with Gram-negative bacterial lipopolysaccharide involve stimulation transcriptional factor NFκB nuclear translocation, and the disturbances in nitric oxide synthase (NOS) activity responsible for NO generation (11—15).

The initial phase of lipopolysaccharide-induced NFκB activation involves the glycosphatidylinositol-anchored surface membrane glycoprotein, known as mCD14, which lacks transmembrane and cytoplasmic regions required for signal transduction (12, 13, 16). A second form of CD, present in serum and referred as solucic, is involved in conferring responsiveness to lipopolysaccharide upon cells that lack mCD as in the case of epithelial cells (13). The complex formed between CD and lipopolysaccharide is then recognized by an extracellular domain of a specific, only recently identified, Toll-like transmembrane receptors (TLRs), members of the IL-1 receptor family (17—19). Of the five members of Toll-receptor family characterized by an extracellular domains that contain multiple leucine-rich repeats, two proteins, TLR2 and TLR4, so far have been implicated in the lipopolysaccharide signaling. Both proteins are highly expressed in cells that respond to LPS, such as leukocytes, macrophages and monocytes, and are potent activators of NFκB as well as the induction of mRNA for several proinflammatory cytokines, including TNFα, IL1 and IL-6. Furthermore, it has been demonstrated that LPS-induced NFκB activation requires a region in the intracellular domain of TLRs that is homologous to the intracellular region of the IL-1 receptor implicated in the activation of the IL-1 receptor-associated kinase (18, 19).
The sustained activation of NOS-2 has been identified as a culprit of transcriptional disturbances leading to apoptosis, while the cNOS appears to play an active role in the inhibition of apoptogenic signals through S-nitrosylation of cysteine residue on the catalytic site of the major executioner caspase, caspase-3 (14, 15, 20—22). Induction of apoptosis by lipopolysaccharide of Gram-negative bacteria is mediated by cytokines of the TNF family, which remain under stimulatory control by ET-1 (23). The process apparently involves the activation by ET-1 of ETA receptor which, in turn, leads to the activation of tyrosine kinase intracellular cascade and ultimately culminates in translation of TNF-α gene (23, 24). The enhanced mucosal levels of ET-1 accompany local and systemic inflammations, and the increase in gastric mucosal expression of this potent vasoactive peptide is a characteristic feature of stress ulcer and plays a major role in pathogenesis of gastric mucosal injury (25, 26).

In this study, we assessed the effect of antiulcer agents, omeprazole and sucralfate, on the course of acute gastritis elicited in rats by intragastric surface epithelial application of H. pylori lipopolysaccharide by analyzing the extent of gastric epithelial cell apoptosis, mucosal expression of ET-1 and TNF-α, and the activity of cNOS and NOS-2.

**MATERIALS AND METHODS**

**Animals**

The study was conducted with Sprague-Dawley rats weighing 180 to 200 g, and cared for by the professional personnel of the Research Animal Facility of UMDNJ. All experiments were conducted with groups of eight animals per treatment. The animals received twice daily for 3 consecutive days the intragastric pretreatment with either omeprazole at 40 mg/kg or sucralfate at 100 mg/kg or the vehicle, and were then subjected to intragastric surface epithelial application of H. pylori lipopolysaccharide at 50 μg per animal, and maintained on the drug or vehicle regimen for an additional 10 days (6, 10). The rats in each group were killed 2, 4 and 10 days after the lipopolysaccharide administration, their stomachs dissected.

**Mucosal histology**

The sections of gastric mucosa were cut into 4-μm strips, fixed in 10% buffered formalin, and stained with hematoxylin and eosin (6). The morphological pattern of gastritis was graded in accordance with the Sydney system (27), and the changes in mucosal histology were quantified on the basis of the scoring system of Rauws et al. (28) as described earlier (6, 10). All specimens were examined by a person unaware of the type of treatment received by the animals.

**Apoptosis assay**

Measurements of apoptosis was carried out with epithelial cells prepared from gastric mucosal scrapings (10). The cells were incubated in the lysis buffer in accordance with the manufacturer’s (Boehringer Mannheim) instruction, centrifuged, and the diluted supernatant containing the
cytoplasmic histone-associated DNA fragments reacted in the microtitrator wells with immobilized anti-histone antibody. Following washing, the retained complex was reacted with anti-DNA peroxidase, and probed with 2,2'-azino-di[3-ethylbenzthiazoline sulfonate] (ABTS) reagent for spectrophotometric quantification (10).

**TNF-α expression assay**

TNF-α was quantitated with an enzyme-linked immunosorbent assay according to the manufacturer's (Genzyme) instructions. The wells were precoated with monoclonal anti-TNF-α to capture TNF-α from the mucosal homogenates, and, after washing, the retained complex was probed with horseradish peroxidase-conjugated anti-TNF-α. The complex was then incubated with tetramethylbenzidine (TMB) reagent for TNF-α quantitization (10).

**ET-1 expression assay**

ET-1 assays were carried out on the individual specimens of gastric mucosa following lyophilization and homogenization with 4 volumes of 1 M acetic acid containing 10 µg/ml of pepstatin (9). The homogenates were heated for 5 min at 100°C, centrifuged, and the resulting supernatants applied to a Sep-Pack C-18 reverse phase cartridges. After initial 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 manufacturer's (Alexis Corporation) instruction.

**NOS activity assay**

Gastric mucosal activity of eNOS and NOS-2 was measured using a NOS-detect kit (Stratagene). The individual specimens of gastric mucosa were homogenized in a sample buffer containing either 10 mM EDTA (NOS-2) or 6 mM CaCl₂, (eNOS), and centrifuged at 800µg for 10 min (8). The aliquots of the resulting supernatants were incubated for 30 min at 25°C in the presence of 50 µCi/µL L-[2,3,4,5-³H]arginine, 10 mM NADPH, 5 µM tetrahydrobiopterin, and 50 µM Tris-HCl buffer, pH 7.4, in a final volume of 250 µL. The reaction was terminated by adding to each sample a 0.4 mL of stop buffer followed by 0.1 mL of Dowex-50W (Na⁺) resin. The mixtures were transferred to spin cups, centrifuged, and the formed L-[³H]citrulline contained in the flow through was quantitated by scintillation counting (8).

**H. pylori lipopolysaccharide**

*H. pylori* ATCC No. 4350 clinical isolate was used for lipopolysaccharide preparation (6). The bacterium was washed with water, treated with ethanol and acetone, dried and homogenized with liquid phenol-chloroform-petroleum ether (6). The resulting suspension was centrifuged, and the lipopolysaccharide contained in the supernatant was precipitated with water, washed with 80% phenol solution, and dried with ether. The dry residue was dissolved in a small volume of water at 45°C, centrifuged at 100,000 × g for 4 h, and the resulting lipopolysaccharide sediment subjected to lyophilization (10).

**Antiulcer drugs**

The antiulcer agent omeprazole was kindly donated by Ferrer Internacional, S.A., Barcelona, Spain, while sucralfate was provided by Chugai Pharmaceutical Co. Ltd., Tokyo, Japan. The drugs
were stored in at 4°C in the dark and were suspended in saline shortly before experimentation. The drugs or vehicle were given in a volume of 1 ml.

**Data analysis**

All experiments were carried out in duplicate, and the results are expressed as the means ± SD. The significance level was set at p < 0.05. The tests were performed using Soft Stat, STATISTICA, software. The protein content of samples was measured with BCA protein assay kit (Pierce).

**RESULTS**

The effect of antiulcer agents, omeprazole and sucralfate, on the course of events associated with gastric mucosal inflammatory reaction to *H. pylori* infection was assessed in the animal model, using rats subjected to intragastric surface epithelial application of *H. pylori* lipopolysaccharide (6, 10). The results of histologic examination revealed that the lipopolysaccharide applied in a dose of 50 µg per animal produced within 2 days a pattern of inflammatory responses resembling that of acute gastritis with the mean grade of the mucosal pathologic condition of 4.0 (Fig. 1), and characterized by the infiltration of lamina propria with lymphocytes and plasma cells, edema, hyperemia, and epithelial hemorrhage extending from the lamina propria to the surface of the mucosa. The extent of mucosal inflammatory involvement reached a maximum by the 4th day and showed a decline (mean score of 3.1) by the 10th day (Fig. 1). Treatment with proton pump inhibitor, omeprazole, produced no significant changes in the severity of mucosal inflammatory responses within the first 2 days following the lipopolysaccharide. However, a 37.6% reduction in the severity pattern occurred by the 4th day of treatment and a 48.3% greater reduction in the mucosal inflammatory involvement was observed by the 10th day of treatment (Fig. 1). On the other hand, the animals treated with sucralfate showed a 32.5% reduction in the severity of mucosal changes on the 2nd day following the lipopolysaccharide, a 62.3% reduction was observed by the 4th day, and a 74.2% on the 10th day of treatment (Fig. 1).

The results of apoptotic DNA fragmentation assays conducted with the epithelial cells isolated from gastric mucosa of the animals following exposure for up to 10 days to *H. pylori* lipopolysaccharide in the absence and the presence of antiulcer agents are summarized in Fig. 2. In the absence of antiulcer agent treatment, the animals subjected to *H. pylori* lipopolysaccharide application showed the greatest increase (13.4-fold) in gastric epithelial cells DNA fragmentation by the 2nd day, which then declined 13.2% by the 4th day and 38.1% by the 10th day. Comparing to the vehicle controls, the group treated with omeprazole exhibited a 34.2% greater reduction in the extent of epithelial cell apoptosis by the 2nd day and a 75.7% reduction occurred by the 4th day. In the case of sucralfate, a 67.8% reduction
Fig. 1. Effect of treatment with omeprazole and sucralfate on the course of acute gastritis elicited in rats by intragastric surface epithelial application of *H. pylori* lipopolysaccharide (LPS). Values represent the means ± SD of duplicate analyses performed on eight animals in each group. *P* < 0.05 compared with that of vehicle.

in the lipopolysaccharide-caused epithelial cells apoptosis occurred on the 2nd day and an 85.8% reduction was observed on the 4th day. However, by the 10th day of treatment the reduction in the extent of gastric epithelial cell apoptosis attained with omeprazole (80.3%) was comparable to that achieved (84.3%) with sucralfate (Fig. 2).
The analysis of gastric mucosal expression of TNF-α revealed that by the 2nd day following *H. pylori* lipopolysaccharide application its level increased 11.7-folds, and then declined a 63.8% by the 10th day (Fig. 3). After 2 days of treatment with omeprazole, the lipopolysaccharide-induced increase in the mucosal expression of TNF-α fell by 19.7% and a 13.2% decrease was observed on the 10th day. On the other hand, by the 2nd day of sucralfate treatment the lipopolysaccharide-induced mucosal TNF-α expression fell by 42.1%, and was a 54.3% lower on the 10th day of treatments (Fig. 3).

![Fig. 3. Effect of treatment with omeprazole and sucralfate on the expression of gastric mucosal tumor necrosis factor-α (TNF-α) during the course of acute gastritis elicited in rats by intragastric surface epithelial application of *H. pylori* lipopolysaccharide (LPS). Values represent the means ± SD of duplicate analyses performed on eight animals in each group. *P < 0.05 compared with that of vehicle.](image)

The time course of gastric mucosal ET-1 expression in response to *H. pylori* lipopolysaccharide challenge is presented in Fig. 4. The results of assays revealed that the mucosal inflammatory reaction to the lipopolysaccharide was associated with a marked increase in the ET-1 level, which reached a maximum of a 3.1-fold enhancement over that of controls by the 4th day and then fell 56.8% by the 10th day. Treatment with omeprazole produced only negligible (4—6%) reduction in the mucosal ET-1 level during the entire 10 day course of the study. Compared to the vehicle controls, treatment with sucralfate decreased the lipopolysaccharide-induced mucosal ET-1 level by a 39.4% on the 2nd day, a 37.5% reduction in ET-1 was obtained on the 4th day, and a 32.4% on 10th day of treatment.
Fig. 4. Effect of treatment with omeprazole and sucralfate on the expression of gastric mucosal endothelin-1 (ET-1) during the course of acute gastritis elicited in rats by intragastric surface epithelial application of *H. pylori* lipopolysaccharide (LPS). Values represent the means±SD of duplicate analyses performed on eight animals in each group. *P < 0.05 compared with that of vehicle.

The data on the time-course expression of gastric mucosal activity of cNOS during *H. pylori* lipopolysaccharide-induced mucosal inflammatory reaction are shown in Fig. 5. In the absence of antiulcer agents, the lipopolysaccharide evoked a maximum of 5.5-fold decrease in gastric epithelial expression of cNOS activity by the 2nd day, followed thereafter by a slow recovery. However, its level even by the 10th day of study was still about 2 times lower than that of the controls. Treatment with both antiulcer agents countered the lipopolysaccharide-induced mucosal decrease in cNOS activity to a similar extent. In the case of omeprazole, the mucosal expression of cNOS activity showed an 83.2% increase over that of the vehicle control by the 4th day, and an 80% by the 10th day treatment, while sucralfate elicited a 93% increase in cNOS activity by the 4th day of treatment and an 82% by the 10th day.

The pattern of changes in gastric mucosal expression of NOS-2 activity following *H. pylori* lipopolysaccharide application is depicted in Fig. 6. The lipopolysaccharide evoked a maximum increase (9.3-fold) in NOS-2 by the 2nd day following the application and its level still remained about 4-fold greater than that of the controls on the 10th day of study. Treatment with omeprazole caused a 46.7% decline in *H. pylori* lipopolysaccharide-induced NOS-2 activity by the 4th day of treatment, while at the end of 10 days of treatment the NOS-2 activity showed a 52.3% decline. In the case of sucralfate treatment, the lipopolysaccharide induced mucosal expression of NOS-2 decreased by a 53% on the 4th day and by 59.5% by the 10th day of treatment.
Fig. 5. Effect of treatment with omeprazole and sucralfate on the expression of gastric mucosal constitutive nitric oxide synthase (cNOS) activity during the course of acute gastritis elicited in rats by intragastric surface epithelial application of H. pylori lipopolysaccharide (LPS). Values represent the means ± SD of duplicate analyses performed on eight animals in each group.

*P < 0.05 compared with that of vehicle.

Fig. 6. Effect of treatment with omeprazole and sucralfate on the expression of gastric mucosal inducible nitric oxide synthase (NOS—2) activity during the course of acute gastritis elicited in rats by intragastric surface epithelial application of H. pylori lipopolysaccharide (LPS). Values represent the means ± SD of duplicate analyses performed on eight animals in each group.

*P < 0.05 compared with that of vehicle.
Our previous studies with animal model demonstrated that intragastric surface epithelial application of *H. pylori* lipopolysaccharide elicits acute mucosal inflammatory responses that are histologically similar to those observed in patients with *H. pylori*-associated gastritis (1—6). In the work presented herein, we investigated further the course of events associated with gastric mucosal inflammatory responses over a period of 10 days following the lipopolysaccharide application by analyzing the interplay between the extent of mucosal pathology, epithelial cell apoptosis, and the mucosal expression of ET-1, TNF-α and the activity of cNOS and NOS-2. Furthermore, the efficacy of two antiulcer agents, omeprazole and sucralfate, in countering the effects of *H. pylori* lipopolysaccharide was assessed.

The results revealed that *H. pylori* lipopolysaccharide produced within 2 days a pattern of inflammatory responses resembling that of acute gastritis and characterized by the infiltration of lamina propria, hyperemia, and epithelial hemorrhage. This was accompanied by a massive epithelial cell apoptosis, a 2.9-fold increase in the mucosal expression of ET-1, an 11.7-fold enhancement in TNF-α, and a 9.3-fold increase in NOS-2, while cNOS activity showed a 5.5-fold decrease. The extent of mucosal inflammatory involvement reached a maximum by the 4th day and showed a decline by the 10th day. This was reflected in a marked reduction in apoptosis, decrease in the mucosal expression of ET-1, TNF-α and NOS-2, and the recovery in cNOS activity. Treatment with omeprazole led at the end of a 10 day study period to a 48.3% reduction in the mucosal inflammatory changes elicited by *H. pylori* lipopolysaccharide, while a 74.2% reduction in the extent of mucosal inflammatory involvement was achieved with sucralfate. Moreover, this advantage of sucralfate over omeprazole in countering the lipopolysaccharide-induced changes was reflected at the end of 10 day treatment period in a 20.4% decrease in apoptosis, a 47.5% greater reduction in TNF-α and a 50.7% greater reduction in ET-1. Both agents, however, exerted similar influence on the restoration of gastric mucosal cNOS activity and showed a comparable effect in countering the lipopolysaccharide-induced increased in the expression of NOS-2. These findings, together with the data on the enhanced mucosal expression of apoptotic caspase-3 in response to the lipopolysaccharide (8), and the results indicating that *H. pylori* causes up-regulation of proinflammatory cytokine expression (10, 29, 30), underscore the role played by ET-1 and NOS in triggering the prolonged mucosal inflammatory reaction to the bacterium that interferes with cessation of apoptotic events required for mucosal repair.

Indeed, the enhanced expression of NOS-2 results in the formation of NO-related species which exert a direct inhibitory effect on NFκB causing transcriptional disturbances that lead to apoptosis, while the cNOS plays an
active role in the inhibition of apoptogenic signals through S-nitrosylation of cysteine residue on the catalytic site of caspase-3 and interference with the activation of ET\textsubscript{A} receptor by ET-1 which leads to the suppression of TNF-\textalpha biosynthesis (31–33). It is noteworthy that the in vivo existence of caspase-3zymogens in S-nitrosylated form has been confirmed recently (34), and the mechanism of caspase S-nitrosylation/denitrosylation is an integral component of Fas-induced apoptotic signaling pathway. Moreover, eNOS appears to be involved in the inhibition of the caspase enzymes through a cGMP mechanism, associated with phosphorylation-dephosphorylation events, that function on the level of caspase zymogen activation that requires cleavage adjacent to aspartates (35). Thus, the observed suppression in eNOS and the induction of NOS-2 activity by \textit{H. pylori} lipopolysaccharide may be an important factor in \textit{H. pylori} cytotoxicity associated with mucosal inflammatory conditions that characterize gastritis in patients infected with this bacterium (2, 4).

While the exact molecular mechanism of the lipopolysaccharide-mediated events remains obscure, the available data point toward essential role of proteins of the NF\textsuperscript{x}B family consisting of c-Rel, NF\textsuperscript{x}B1, NF\textsuperscript{x}B2, RelA, and RelB, and forming a variety of homo- and heterodimers (12, 13, 16, 36). These proteins have been shown to be essential for the induction of NOS-2 gene expression in macrophages exposed to \textit{E. coli} lipopolysaccharide as well as in astrocytes responding to challenge by lipopolysaccharide from \textit{S. typhimurium} (12, 37). Under normal conditions, NF\textsuperscript{x}B exists in the cytoplasm in inactivated form through association with members of a family of inhibitory proteins known as I\textsuperscript{x}Bs (inhibitors of nuclear factor xB) (12, 13, 38). Upon exposure to lipopolysaccharide (or proinflammatory cytokine, TNF-\textalpha), the I\textsuperscript{x}B proteins undergo rapid phosphorylation at two critical N-terminal serine residues, Ser\textsuperscript{32} and Ser\textsuperscript{36} in the case of I\textsuperscript{x}B\textalpha and Ser\textsuperscript{19} and Ser\textsuperscript{23} in I\textsuperscript{x}B\textbeta, which serve as a signal targeting I\textsuperscript{x}Bs for degradation by the ubiquitin-proteosome pathway consisting of a multicatalytic high molecular weight protease system (39, 40). The initial step of the pathway involves the interaction of I\textsuperscript{x}Bs with \beta-TrCP protein which triggers the formation of a ubiquitin-ligase complex that adds ubiquitin groups to I\textsuperscript{x}B on lysine residues (Lys\textsuperscript{21} and Lys\textsuperscript{22}), thus targeting the complex to the 26S proteosome for degradation. The removal of the inhibitor I\textsuperscript{x}B allows the nuclear translocation of the liberated NF-\textalpha B dimer and initiates its selective binding to the affinity site in the promoters of inducible genes to activate the expression of NOS-2 and nitric oxide release (or genes coding for proinflammatory cytokine TNF-\alpha, or IL-1 and IL-8, as well as its inhibitor, I\textsuperscript{x}B\textalpha) (12, 37, 41, 42).

The termination of NF\textsuperscript{x}B activity occurs upon I\textsuperscript{x}B resynthesis, which leads to its accumulation in the cytoplasm as well as in the nucleus, where it leads to dissociation of DNA-bound NF\textsuperscript{x}B and its sequestration from the nuclear
compartment and redistribution back into the cytoplasm (38). Thus the kinases responsible for the inducible phosphorylation of IkBα proteins are clearly the main players in this cascade of events. Indeed, studies indicate that a high molecular weight complex capable of IκB phosphorylation (IKK) consists of two catalytic subunits, referred to as IKKα, IKKβ, and a regulatory subunit, IKKγ. The IKKα and IKKβ, interact through their leucine-zipper domain to form a heterodimeric complex that in response to TNF-α phosphorylates IκBα and activates NFκB, while the IKKγ, which does not contain catalytic kinase domain, associates with IKKβ and is required for the activation of IKKα—IKKβ heterodimers in response to proinflammatory cytokine TNFα and IL1 (39, 40). Moreover, there are most recent indications that IKKα and IKKβ heterodimers are in turn activated through phosphorylation by the MAPK/ERK kinase kinase complex (43).

The results our study suggest that an increase in the mucosal ET-1 level elicited by *H. pylori* lipopolysaccharide, combined with a decline in eNOS may be responsible for the induction of TNF-α and triggering the gastric mucosal inflammatory process. Moreover, we also show that sucralfate exhibits greater efficacy than omeprazole in suppressing the *H. pylori*-induced mucosal inflammatory reaction. This effect of sucralfate may well stem from its ability to counter the mucosal rise in ET-1 elicited by the bacterium.

REFERENCES


43. Zhao Q, Lee FS. Mitogen-activated protein kinase/ERK kinase kinase2 and 3 activate nuclear factor-$\kappa$B through IkB kinase-$\alpha$ and IkB kinase-$\beta$. *J Biol Chem* 1999; 274: 8355—8358.

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