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OMEPRAZOLE FAILS TO SUPPRESS UP-REGULATION OF GASTRIC MUCOSAL ENDOTHELIN-CONVERTING ENZYME-1 BY *HELICOBACTER PYLORI* LIPOPOLYSACCHARIDE

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Background: Endothelin-1, a key mediator of inflammatory processes, is produced from its biologically inactive precursor, big ET-1 by the action of endothelin converting enzyme-1(ECE-1). In this study, we applied the animal model of *H. pylori* lipopolysaccharide-induced gastritis to assess the effect of three different types of antiulcer agents on the gastric mucosal expression of ECE-1 activity.
Methods: Rats, pretreated twice daily for 3 days with proton pump inhibitor, omeprazole at 40 mg/kg, gastroprotective agent, sulglycotide at 200 mg/kg, H2-receptor antagonist, ebrotidine at 100 mg/kg or the vehicle, were subjected to intragastric application of *H. pylori* lipopolysaccharide at 50 μg/animal, and after 2, and 4 additional days on the drug or vehicle regimen their mucosal tissue used for histologic and biochemical assessment.
Results: In the absence of antiulcer agents, *H. pylori* lipopolysaccharide elicited a pattern of mucosal inflammatory responses resembling that of acute gastritis which reached a maximum by the 4th day and were accompanied by a 4.1-fold increase in the mucosal expression of ECE-1 activity and an 8.8-fold enhancement in TNF-α. Treatment with sulglycotide led to a 56.7% reduction in the extent of mucosal inflammatory involvement, the mucosal expression of ECE-1 activity fell by a 40.5% and the level of TNF-α declined by a 69%. Ebrotidine produced a 50.9% decrease in the extent of mucosal inflammatory involvement, a 33.6% decrease in the expression of ECE-1 activity and a 64.1% decline in TNF-α, whereas omeprazole elicited a 37.6% reduction in the extent of mucosal inflammatory involvement and a 29.5% decrease in TNF-α, but had no effect on the lipoploysaccharide-induced increase in the mucosal expression of ECE-1 activity.
Conclusions: The findings implicate up-regulation of ECE-1 in triggering the induction of TNF-α and propagation of gastric mucosal inflammatory responses to *H. pylori*. We also show that omeprazole, in contrast to sulglycotide and ebrotidine, fails to counter the enhancement in the mucosal expression of ECE-1 caused by *H. pylori*- lipopolysaccharide.

INTRODUCTION

Infection with *Helicobacter pylori* is a primary factor responsible for eliciting mucosal inflammatory changes that characterize gastritis and duodenal ulcers, and the product of particular significance to the virulent action of the bacterium is its cell wall lipopolysaccharide (1—4). 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 massive epithelial cell apoptosis (5—7).

The 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 and involve the formation of NFκB p50/p65 dimers for the enhanced TNF-α expression (8, 9). The process apparently involves the activation of a specific ET receptor by ET-1 followed by a rapid degradation of IκB in the cytosol and translocation of NFκB into the nucleus (9). Indeed, ET-1 has been shown to increase the formation of NFκB complexes as well as stimulation of NFκB DNA binding, and the enhanced mucosal levels of ET-1 accompany local and systemic inflammations characterized by a massive up-regulation of proinflammatory cytokine production (10—12).

The bioactive form of ET-1 is a cleavage product of prepro ET-1 by as yet not defined enzyme to yield a 39 amino acid peptide, termed big ET-1, which is subsequently cleaved at $Tryp^{21}$-Val^{22} bound by a specific protease that removes 18 amino acids from its carboxyl terminal (13—15). This membrane-bound metallopeptidase is known as endothelin-converting enzyme-1 or ECE-1 (13, 15). Recently, we have established the presence of ECE-1 activity in gastric mucosa and demonstrated that the increase in the enzyme expression characterizes gastric mucosal inflammatory responses to *H. pylori* lipopolysaccharide (16).

The most successful treatment strategies for *H. pylori* eradication include combination of proton pump inhibitors or H2 blockers with gastroprotective agents and antibiotics (17, 18). The literature data, however, indicate that the treatment with proton pump inhibitor, omeprazole, leads to aggravation of *H. pylori* gastritis and results in the progression of glandular atrophy (19—21). Moreover, omeprazole exhibits no discernible effect on the *H. pylori*-induced mucosal release of ET-1 (7, 22), known for its stimulatory effect on proinflammatory cytokine generation, microcirculatory perturbations, and the alterations in cell cycle progression and apoptotic cell death (8, 23, 24).

In the study presented herein, we assessed the effect of proton pump inhibitor, omeprazole, H2 blocker, ebrotidine, and gastroprotective agent, sulglycotide, on the mucosal expression of ECE-1 activity and TNF-α level during the course of acute gastritis elicited in rats by intragastric application of *H. pylori* lipopolysaccharide.

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 either with omeprazole at 40 mg/kg, sulglycotide at 200 mg/kg, ebrotidine at 100 mg/kg or the vehicle, and then 12 h later were subjected to intragastric surface epithelial application of *H. pylori* lipopolysaccharide at 50 μg per animal (5, 6), and maintained on the drug or vehicle regimen for an additional 2 and 4 days. The rats in each group were killed 16 h after the last dose and 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 (5). The morphological pattern of gastritis was graded in accordance with the Sydney system (24), and the changes in mucosal histology were quantified on the basis of the scoring system of Rauws *et al.* (25) as described earlier (5, 6). All specimens were examined by a person unaware of the type of treatment received by the animals.

H. pylori lipopolysaccharide

H. pylori ATCC No. 4350 clinical isolate was used for lipopolysaccharide preparation (5). The bacterium was washed with water, treated with ethanol and acetone, dried and homogenized with liquid phenol-chloroform-petroleum ether (26). 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 $100,000 \times g$ for 4 h, and the resulting lipopolysaccharide sediment subjected to lyophilization (5). Analysis indicated that the lipopolysaccharide preparation was essentially free of nucleic acids and its protein content was less than 0.15% (27).

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 substrate solution and TNF-α quantitated spectrophotometrically (7, 27).

ECE-1 preparation

The minced specimens of gastric mucosal scrapings were suspended in ice-cold solution, consisting of 0.25 M sucrose in 0.15 M Tris-HCl buffer, pH 7.4, and containing 1 mM PMSF, 20 μM leupeptin, and homogenized for 1 min in a Polytron tissuemizer (28). The homogenate was centrifuged at 1000 g for 10 min and the resulting supernatant was centrifuged at 10,000 g to sediment the crude mitochondrial fraction. Centrifugation of the resulting supernatant at 100,000 g for 1 h produced the microsomal pellet (16, 28). The pellet was solubilized by stirring at 4°C for

30 min with 0,25 M buffered sucrose, pH 7.0, containing 0.5% Triton X-100, the mixture was centrifuged at 100,000 g for 1 h, and the supernatant used as an enzyme source.

ECE-1 activity assay

Reaction mixtures for ECE-1 assay, incubated at 37°C for 60 min in a total volume of 100 μl, consisted of 0—30 g enzyme protein, 0,5 μM big ET-1 (Sigma), and 100 mM phosphate buffer, pH 6.8, containing 0.5 M NaCl (16). The reaction was terminated by addition of 100 mM EDTA and the mixture was boiled for 5 min, centrifuged, and the resulting supernatant applied to a Sep-Pack C-18 cartridge (27). After initial washing with 0.1% trifluoroacetic acid, the adsorbed ET-1 was eluted from the cartridge with methanol-water-trifluoroacetic acid (90:10:0.1, v/v/v).

ET-1 quantitization

The eluates containing purified ET-1 were dried under vacuum, reconstituted in the assay buffer, and subjected to immunometric ET-1 quantitization with a double-antibody sandwich technique in accordance with the manufacturer's (Alexis Corporation) instruction. The sample aliquots were applied to the microtiter wells coated with ET-1 capture antibody and the complex was incubated at 4°C for 16 h. After washing, the wells were probed with Ellman reagent, incubated at room temperature for 2 h, and ET-1 was quantiated spectrophotometrically at 412 nm (7, 27).

Antiulcer drugs

The antiulcer agents, omeprazole and ebrotidine was kindly donated by Ferrer Internacional, S.A., Barcelona, Spain, while the sulglycotide was provided by Crinos Industria, Villa Guardia, Italy. The drugs were stored in at 4°C in the dark and was 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 $\pm$ 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, ebrotidine and sulglycotide, on the course of events associated with gastric mucosal inflammatory reaction to *H. pylori* infection was studied over the period of 4 days using rats subjected to intragastric application of *H. pylori* lipopolysaccharide. 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 which reached a maximum by the 4th day and then declined by a 41.5% by the tenth day (16), and were 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 (*Fig. 1*). Treatment with proton pump inhibitor, omeprazole, produced no significant changes in the severity of mucosal inflammatory responses within the first 2 days, but a 37.6% reduction in the severity pattern occurred by the 4th day. The animals subjected to H2 blocker, ebrotidine, showed a 24.5% reduction in the severity of mucosal changes on the 2nd day following the lipopolysaccharide and a 50.9% reduction by the 4th day, while the animals treated with gastroprotective agent, sulglycotide, showed a 27.5% reduction in the severity of mucosal involvement on the 2nd day following the lipopolysaccharide and a 56.7% reduction by the 4th day (*Fig. 1*).
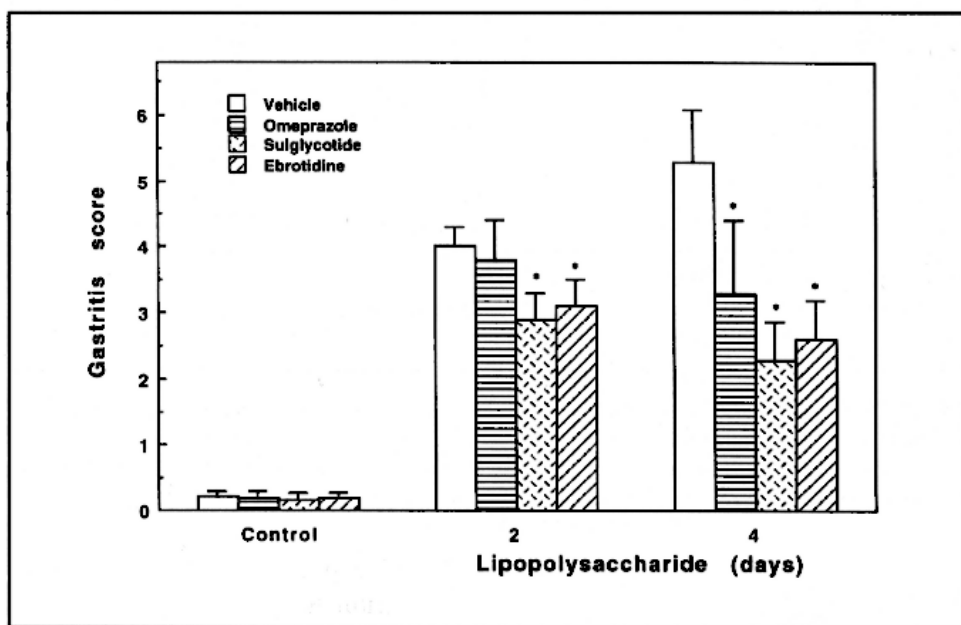

Fig. 1. Effect of antiulcer agents, sulglycotide, ebrotidine and omeprazole on the course of acute gastritis elicited in rats by intragastric surface epithelial application of *H. pylori* lipopolysaccharide (LPS). Values represent the means ± SD of duplicate analyses performed on eight animals in each group. *P < 0.05 compared with that of vehicle.

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 24% by the 4th day (*Fig. 2*). Treatment with omeprazole elicited by the 2nd day following the lipopolysaccharide a 19.7% drop in the mucosal expression of TNF-α, a 39.6% reduction was achieved with ebrotidine, and a 52% reduction with sulglycotide. By the 4th day, the lipopolysaccharide-induced increase in the mucosal expression of TNF-α fell

by 29.5% in the group treated with omeprazole, ebrotidine evoked a 64.1% reduction, and a 69.2% decrease in TNF-α was achieved with sulglycotide (*Fig. 2*).
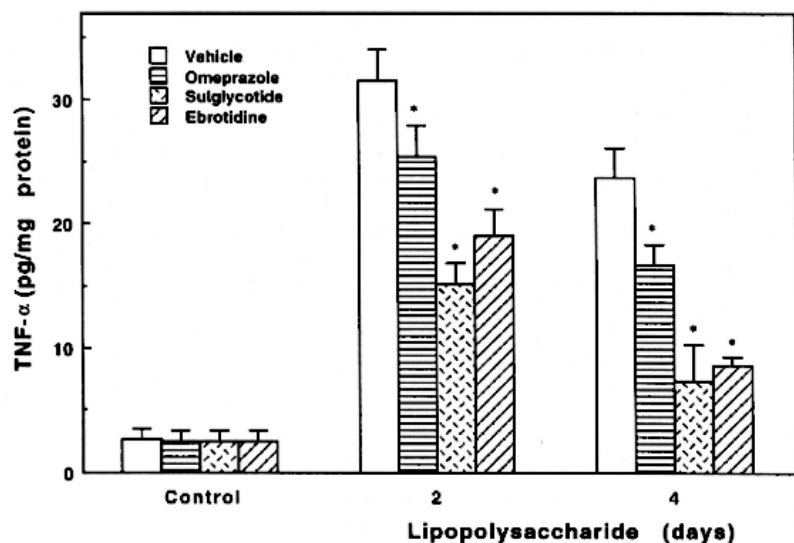

Fig. 2. Effect of antiulcer agents, sulglycotide, ebrotidine and omeprazole on the expression of gastric mucosal tumor necrosis factor-α (TNF-α) during the course of acute gastritis elicited in rats by intragastric surface epithelial application of *H. pylori* lipopolysaccharide (LPS). Values represent the means ± SD of duplicate analyses performed on eight animals in each group. *P < 0.05 compared with that of vehicle.

The pattern of changes in gastric mucosal expression of ECE-1 activity following *H. pylori* lipopolysaccharide application is depicted in *Fig. 3*. The lipopolysaccharide evoked a 3.7-fold increase in ECE-1 by the 2nd day and a 4.1-fold increase in ECE-1 expression was observed on the 4th day following the application. Compared to the vehicle controls, administration of ebrotidine caused a 41.6% decline in *H. pylori* lipopolysaccharide-induced ECE-1 expression on the 2nd day and a 33.6% reduction was attained on the 4th day, while treatment with sulglycotide decreased the lipopolysaccharide-induced ECE-1 expression by a 44% on the 2nd day and by a 40.5% on the 4th day. On the other hand, administration of omeprazole did not produce any significant reduction in the lipopolysaccharide-induced mucosal expression of ECE-1 activity during the course of treatment (*Fig. 3*).
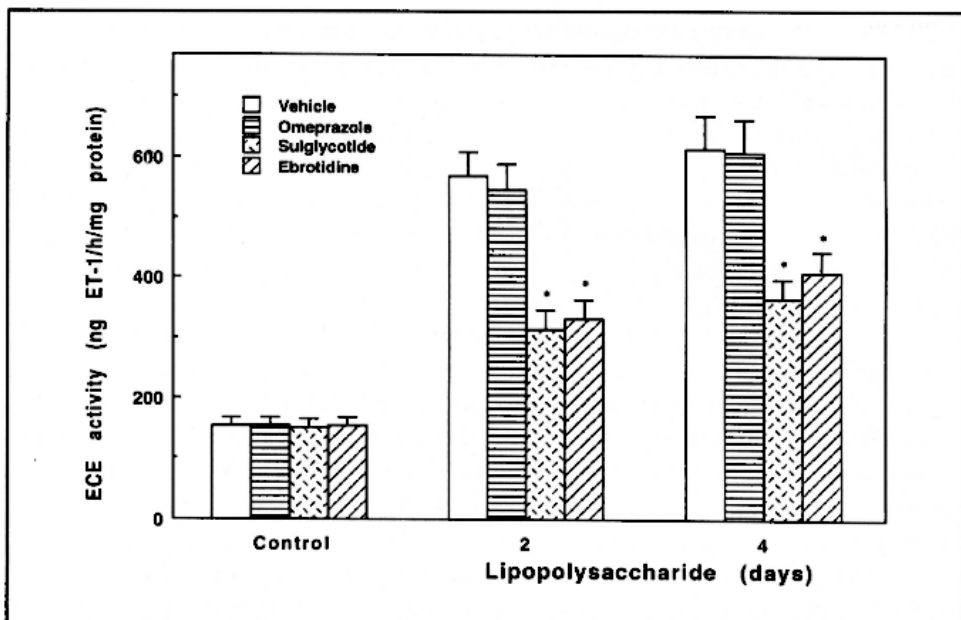
Fig. 3. Effect of antiulcer agents, sulglycotide, ebrotidine and omeprazole on the expression of gastric mucosal ECE-1 activity during the course of acute gastritis elicited in rats by intragastric surface epithelial application of *H. pylori* lipopolysaccharide (LPS). Values represent the means $\pm$ SD of duplicate analyses performed on eight animals in each group. $*P < 0.05$ compared with that of vehicle.

DISCUSSION

The increased awareness of pathologic consequences of *H. pylori* infection and the demonstration that the bacterium is a causative factor of gastric mucosal inflammatory changes that characterize gastritis and duodenal ulcers have brought to the forefront the importance of *H. pylori* eradication for the success of therapeutic intervention. The treatment strategies involve dual and triple combination therapies of proton pump inhibitors or H2 blockers with gastroprotective agents and antibiotics, and the most widely used therapy is that involving omeprazole (17, 18, 29). Studies show, however, that aside from the apparent differences in *H. pylori* eradication rates between the regiments, the treatment with proton pump inhibitors leads to aggravation of *H. pylori* gastritis of the corpus mucosa and results in the progression of glandular atrophy (19—21). Moreover, with the exception of one fervidly contested report (30), there are clear indications that even short-term acid-suppressive maintenance therapy with omeprazole in *H. pylori* infected patients increases inflammation activity of the gastric mucosa (19—21, 29). This is supported by

the studies with an animal model of *H. pylori*-induced gastritis which revealed that omeprazole exhibits relatively low efficacy in countering the mucosal inflammatory responses propagated by the rise in proinflammatory cytokine expression and has no discernible effect on the *H. pylori* lipopolysaccharide-induced increase in gastric mucosal level of a potent vasoactive peptide, ET-1 (7, 22, 31). The peptide is known for its diverse biological activities associated with microcirculatory perturbations, cell cycle progression, inflammatory cytokine generation, and the process of programmed cell death (8, 23, 24).

As the formation of ET-1 from its biologically inactive precursor, big ET-1, is controlled by a specific membrane-bound protease, ECE-1 (13, 15), the expression of which correlates with the extent of mucosal inflammatory involvement (16), in this study we assessed the capacity of three different types of antiulcer agents in countering the *H. pylori* lipopolysaccharide-induced rise in gastric mucosal expression of ECE-1 activity. The results obtained revealed that in the absence of antiulcer agents, *H. pylori* lipopolysaccharide elicited a pattern of mucosal inflammatory responses resembling that of acute gastritis which reached a maximum by the 4th day and were accompanied by a 4.1-fold increase in the mucosal expression of ECE-1 activity and an 8.8-fold enhancement in TNF-α. Treatment with sulglycotide led to a 56.7% reduction in the extent of mucosal inflammatory involvement elicited by the lipopolysaccharide, the mucosal expression of ECE-1 activity fell by a 40.5% and the level of TNF-α declined by a 69%, while a 50.9% decrease in the extent of mucosal inflammatory involvement and a 33.6% decrease in the expression of ECE-1 activity, and a 64.1% decline in TNF-α was achieved with ebrotidine. On the other hand, treatment with omeprazole caused a 37.6% reduction in the extent of mucosal inflammation and a 29.5% decline in TNF-α, but had no effect on the lipopolysaccharide-induced increase in the mucosal expression of ECE-1 activity. These findings, together with the data on the existence of a close relationship between the mucosal expression of ET-1 and the extent of gastric mucosal inflammatory involvement in response to *H. pylori* lipopolysaccharide (7, 27), and the evidence indicating that *H. pylori* causes induction of proinflammatory cytokine expression (27, 22, 33), point to a role played by ECE-1 in triggering the prolonged mucosal inflammatory reaction to the bacterium. Indeed, the up-regulation of ECE-1 expression and elevated level of ET-1 are also prominent features in pathogenesis of a number of pathologic wound healing diseases, including pulmonary and cardiac fibrosis, and hepatic cirrhosis (15, 34, 35).

Our findings that the antiulcer agents, sulglycotide and ebrotidine, exerting inhibitory effect on the lipopolysaccharide-induced rise in the mucosal expression of ECE-1 were also more potent then omeprazole in countering the extent of mucosal inflammatory involvement elicited by *H. pylori* provide an important clue as to the mechanism underlying the apparent limitations of

omeprazole therapy in the treatment of corpus gastritis in *H. pylori*-infected patients. In this interpretation, the lack of omeprazole effect in countering *H. pylori*-induced up-regulation of ECE-1 expression would lead in *H. pylori*-infected patients to rise in the mucosal ET-1 level and subsequent enhancement in proinflammatory TNF-α formation, thus causing exacerbation of the mucosal inflammatory involvement. The above course of events is supported by the data demonstrating that up-regulation of ET-1 expression leads to the enhanced formation of NFκB p50/65 dimers for the enhanced expression of TNF-α (8, 9). Moreover, ET-1 acting through its specific receptor, has been shown to cause a rapid degradation of IκB in the cytosol, thus causing translocation of NFκB to the nucleus and induction of TNF gene for TNF-α production (8, 9, 24).

In conclusion, our results demonstrate that gastroprotective agent, sulglycotide (1, 36), and ebrotidine, an H2 blocker combining acid suppressant activity with gastroprotective properties (1, 37—39), are capable of exerting modulatory effect on the *H. pylori*-induced rise in gastric mucosal ECE-1 expression, and markedly decrease the extent of mucosal inflammatory involvement caused by *H. pylori*. The proton pump inhibitor, omeprazole, which fails to counter the *H. pylori*-induced rise in the mucosal expression of ECE-1, is also known to increase the inflammation activity of the gastric mucosa in *H. pylori* infected patients (19—21, 29). The results of our study also point to ECE-1 as a target in suppressing the mucosal inflammatory reaction to *H. pylori* infection.

REFERENCES

1. Piotrowski J. Lipopolysaccharide a virulence factor of *Helicobacter pylori*: effect of antiulcer agents. *J Physiol Pharmacol* 1998; 49: 3—24.
2. Stolte M, Edit S. *Helicobacter pylori* and the evolution of gastritis. *Scand J Gastroenterol* 1996; 31 Suppl 214: 13—16.
3. Rune SJ. History of *Helicobacter* infection. *Scand J Gastroenterol* 1996; 31 Suppl 214: 2—4.
4. El-Omar EM, Oien K, El-Nujumi A *et al*. *H. pylori* infection and chronic gastric hypersecretion. *Gastroenterology* 1997; 113: 15—24.
5. Piotrowski J, Piotrowski E, Skrodzka D, Slomiany A, Slomiany BL. Induction of acute gastritis and epithelial apoptosis by *Helicobacter pylori* lipopolysaccharide. *Scand J Gastroenterol* 1997; 32: 203—11.
6. Slomiany BL, Piotrowski J, Slomiany A. Induction of caspase-3 and nitric oxide synthase-2 during gastric mucosal inflammatory reaction to *H. pylori* lipopolysaccharide. *Biochem Mol Biol Int* 1998; 46: 1063—1070.
7. Slomiany BL, Piotrowski J, Slomiany A. Involvement of endothelin-1 in up-regulation of gastric mucosal inflammatory responses to *Helicobacter pylori* lipopolysaccharide. *Biochem Biophys Res Commun* 1999; 258: 17—20.
8. Ruetten H, Thiemermman C. Endothelin-1 stimulates the biosynthesis of tumor necrosis factor in macrophages: Et-receptors, signal transduction and inhibition by dexamethasone. *J Physiol Pharmacol* 1997; 48: 675—688.

9. Gallois C, Habib A, Tao J et al. Role of NFκB in the antiproliferative effects of endothelin-1 and tumor necrosis factor-α in human hepatic stellate cells. J. Biol. Chem. 1998; 273: 23183—23190.

10. Michida T, Kawano S, Nagano K, Fusamoto H, Kamada T, Sugimoto T. Endothelin-1 in the gastric mucosa in stress ulcers of critically ill patients. Am J Gastroenterol 1997; 92: 1177—1181.

11. Hassan M, Kashimura H, Nakahara A et al. Gastric mucosal injury induced by local ishemia-reperfusion in rats. Role of endogenous endothelin-1 and free radical. Dig Dis Sci 1997; 42: 1375—1380.

12. Slomiany BL, Piotrowski J, Slomiany A. Role of endothelin-1 and constitutive nitric oxide synthase in gastric mucosal resistance to indomethacin injury: Effect of antiulcer agents. Scand J Gastroenterol 1999; 34: 459—464.

13. Xu D, Emoto N, Giaid A, Slaughter C et al. ECE-1: a membrane-bound metalloprotease that catalyzes the proteolytic activation of big endothelin-1. Cell 1994; 78: 473—485.

14. Matsuura A, Kawashima S, Yamochi W et al. Vascular endothelial growth factor increases endothelin-converting enzyme expression in vascular endothelial cells. Biochem Biophys Res Commun 1997; 235: 713—716.

15. Ehrenreich H, Loffler BM, Hasselblatt M et al. Endothelin converting enzyme activity in primary rat astrocytes is modulated by endothelin B receptors. Biochem Biophys Res Commun 1999; 261: 149—155.

16. Slomiany BL, Piotrowski J, Slomiany A. Up-regulation of endothelin-converting enzyme-1 in gastric mucosal inflammatory responses to Helicobacter pylori lipopolysaccharide. Biochem Biophys Res Commun 2000; 267: 801—805.

17. Gabryelewicz A. Critical evaluation of the treatment of peptic ulcer disease associated with H. pylori infection. J Physiol Pharmacol 1966; 47: 51—58.

18. Pounder RE. New developments in Helicobacter pylori eradication therapy. Scand J Gastroenterol 1997; 32 (Suppl 223): 43—45.

19. Kupiers EJ, Lundell L, Klinkenberg-Knol EC et al. Atrophic gastritis and Helicobacter pylori infection in patients with reflux esophagitis treated with omeprazole or fundoplication. N Engl J Med 1996; 334: 1018—1022.

20. Pounder RE, Williams MP. Omeprazole and accelerated onset of atrophic gastritis. Gastroenterology 2000; 118; 238—239.

21. Stolte M, Meining, A. Lack of effect of acid suppression therapy on gastric atrophy. Gastroenterology 2000; 118: 242—243.

22. Slomiany BL, Piotrowski J, Slomiany A. Up-regulation of endothelin-1 in gastric mucosal inflammatory responses to Helicobacter pylori lipopolysaccharide. J Physiol Pharmacol 2000; 51: 179—192.

23. Xin X, Cai Y, Matsumoto K, Agui T. Endothelin-induced interleukin-6 production by rat aortic endothelial cells. Endocrinology 1995; 136: 132—137.

24. Price A.B. Sydney system: histological division. J Gastroenterol Hepatol 1991; 6: 209—222.

25. Rauws EAJ, Langenberg W, Houthoff H, Zanen MC, Tytgat GNJ. Campylobacter pylori-associated chronic active antral gastritis: a prospective study of its prevalence and the effect of antibacterial and antiulcer treatment. Gastroenterology 1988; 94: 33—40.

26. Galanos C, Luderlitz O, Westphal O. A new method for the extraction of R lipopolysaccharide. Eur J Biochem 1969; 9: 245—249.

27. Slomiany BL, Piotrowski J, Slomiany A. Effect of ebrotidine on Helicobacter pylori lipopolysaccharide-induced up-regulation of endothelin-1 in gastric mucosa. J Endotox Res 1999; 5: 279—285.

28. Carter SR, Slomiany A, Gwozdzinski K, Liau YH, Slomiany BL. Effect of ethanol on mucus glycoprotein sulfotransferase from gastric mucosa. J Biol Chem 1988; 22: 1179—1183.

29. Unge P. Current indications for Helicobacter pylori eradication threapy. Scand J Gastroenterol 1996; 31(Suppl 215): 70—75.

30. Lundell L, Miettinen P, Myrvold HE *et al.* Lack of acid suppression therapy on gastric atrophy. *Gastroenterology* 1999; 117: 319—326.

31. Slomiany BL, Piotrowski J, Slomiany A. Gastric mucosal inflammatory responses to *Helicobacter pylori* lipopolysaccharide: suppression of caspase-3 and nitric oxide synthase-2 by omeprazole and sucralfate. *Inflammopharmac* 1999; 7: 163—177.

32. Okazawa M, Shiraki T, Ninomiya H, Kobayashi S, Masaki T. Endothelin-induced apoptosis of A375 human melanoma cells. *J Biol Chem* 1998; 273: 12584—12592.

33. Chen W, Shu D, Chadwick VS. *Helicobacter pylori* infection in interleukin-4-deficient and transgenic mice. *Scand J Gastroenterol* 1999; 34: 987—992.

34. Pollock DM, Keith TL, Highsmith RF. Endothelin receptors and calcium signaling. *FASEB J* 1995; 9: 1196—1204.

35. Shao RS, Yan W, Rockey DC. Regulation of endothelin-1 synthesis by endothelin-converting enzyme-1 during wound healing. *J Biol Chem* 1999; 274: 3228—3234.

36. Slomiany BL, Piotrowski J, Slomiany A. Gastric mucosal inflammatory responses to *Helicobacter pylori* lipopolysaccharide: down-regulation of nitric oxide synthae-2 and caspase-3 by sulglycotide. *Biochem Biophys Res Commun* 1999; 261: 15—20.

37. Konturek PC, Brzozowski T, Konturek SJ, Marquez M, Torres J, Ortiz JA. Studies on the cytoprotective and antisecretory activity of ebrotidine. *Arzneim Forsch Drug Res* 1997; 47: 578—589.

38. Slomiany BL, Piotrowski J, Slomiany A. Effect of ebrotidine on gastric mucosal inflammatory responses to *Helicobacter pylori* lipopolysaccharide. *J Physiol Pharmacol* 1999; 50: 391—404.

39. Slomiany BL, Piotrowski J, Slomiany A, Konturek JW, Domschke WW. Enhancement in the protective qualities of gastric mucus with combination therapy of ebrotidine and amoxicillin for *H. pylori* eradication. *Gen Pharmacol* 1998; 31: 227—231.

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