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Endothelin-1-dependent leptin induction in gastric mucosal inflammatory responses to *Helicobacter pylori* lipopolysaccharide

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Abstract

Leptin, a multifunctional hormone that regulates food intake and energy expenditure, has emerged recently as an important modulator of gastric mucosal responses to *Helicobacter pylori* infection. We applied the animal model of *H. pylori* LPS-induced gastritis to investigate the role of endothelin-1 (ET-1) in the mucosal leptin production. We show that the histologic pattern of inflammation reached a maximum on the fourth day following the LPS and was reflected in a marked increase in the mucosal level of ET-1 and leptin. Therapeutic administration of phosphoramidon, an inhibitor of ECE-1 activity, led to a 61.2% decline in the mucosal ET-1 level and a 64.1% reduction in leptin, while the severity of mucosal inflammatory involvement increased by 28.6%. A drop in the level of leptin and the increase in severity of the inflammatory involvement elicited by the LPS was also attained in the presence of ET_A receptor antagonist BQ610, but not the ET_B receptor antagonist BQ788. Moreover, administration of ERK inhibitor, PD98059, in the presence of ET_B receptor antagonist, but not the ET_A receptor antagonist, caused reduction in the mucosal leptin level. Our findings are the first to implicate ET-1 as a key factor in up-regulation of gastric mucosal leptin-associated *H. pylori* infection. We also show that the effect of ET-1 on leptin production is a consequence of ET_A receptor activation.

Keywords: *Helicobacter pylori* LPS; Acute gastritis; Leptin; ET-1; ET_A receptor

Leptin is a pleiotropic 16 kDa peptide hormone secreted predominantly by adipocytes and recognized for its role in the maintenance of body weight, angiogenesis, modulation of β-cell insulin secretion, and regulation of immune responses [1–3]. The biological activities of leptin are mediated through the interaction with its specific membrane receptor, OB-R, which exists in several variant forms differing in the length of transmembrane coding regions [1]. While the hypothalamic site of leptin action is well recognized, the rapidly accumulating data indicate that the peptide is also involved in the processes of mucosal defense and repair, control of gastric secretion, and the regulation of the extent of mucosal inflammatory reaction [4–6]. Leptin and leptin receptors have been identified in gastric and intestinal mucosa, and leptin released locally within the gastric mucosa has been implicated in the interaction with proinflammatory cytokines to control local inflammations [5–8].

Indeed, the increased mucosal levels of leptin accompany acute gastric mucosal injury as well as characterize gastric mucosal inflammatory responses to *Helicobacter pylori* infection, and the exogenous leptin has been demonstrated to affect the extent of inflammatory responses to *Escherichia coli* LPS [2,4,6,9,10]. Moreover, it has been suggested recently that vascular factors such as endothelin-1 (ET-1) may play a role in the regulation of local leptin release [11]. There are also data showing that leptin and ET-1 production are both activated by insulin and inhibited by PPARγ agonists, and that stimulation of endothelial cells with leptin leads to the increase in ET-1 promoter activity [11–13].

Although initially identified in vascular tissue, the ET-1 is now recognized to play a major role in the pathogenesis

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of gastric mucosal injury induced by stress, nonsteroidal anti-inflammatory drugs, and *H. pylori* infection [14–16]. At present, the existence of three active isoforms of ET (-1, -2, and -3), and two distinct receptors, ET_A and ET_B, is well documented [18]. The active form of ET-1 arises from biologically inactive big-ET-1 through the action of a specific protease that removes 18 amino acids from its carboxyl terminal. This membrane-bound metallopeptidase, characterized by its sensitivity to phosphoramidon, is known as endothelin-converting enzyme-1 (ECE-1) [17,18], and the increase in its expression has been singled out as a primary factor responsible for the enhanced ET-1 levels observed in local and systemic inflammations as well as normal wound repair [19–21].

The role of *H. pylori* infection in etiology of gastric disease is well established, and its cell wall LPS is recognized as a primary virulence factor responsible for eliciting mucosal inflammatory responses that characterize gastritis and duodenal ulcers [4,22,23]. Indeed, gastric mucosal responses associated with *H. pylori* infection in humans as well as those characterizing mucosal inflammatory changes in the animal model of *H. pylori* LPS-induced gastritis are manifested by the increase in proinflammatory cytokine production, excessive NO and prostaglandin generation, massive rise in epithelial cell apoptosis, and a marked up-regulation in gastric mucosal ET-1 level [14,16,24].

In this study, we applied the animal model of *H. pylori* LPS-induced gastritis to examine the role of ET-1 in the mucosal leptin production.

Materials and methods

Animals. The study was conducted with Sprague–Dawley rats in compliance with the Institutional Animal Care and Use Committee. The animals were deprived of food 16 h before the experiment and water was withheld 2 h before the procedure. All experiments were carried out with groups consisting of five animals per treatment, and the animals were subjected to intragastric surface epithelial application of *H. pylori* LPS at 50 μg per animal. This was followed 4 h later by intragastric administration of ECE-1 inhibitor, phosphoramidon (Sigma), at 0–40 mg/kg, or ET_A receptor antagonist, BQ610 (Sigma), at 0–30 mg/kg, or ET_B receptor antagonist, BQ788 (Sigma), at 0–30 mg/kg, or ERK1/2 inhibitor, PD98059 (Sigma), at 0–30 mg/kg, or vehicle consisting of 5% gum arabic in saline, and the animals were maintained on the twice daily regimen of the agents or the vehicle for 4 days. The dose of *H. pylori* LPS used in the experiments was based on the results of earlier studies which demonstrated that the LPS applied intragastrically at 50 μg per animal was sufficient to elicit within 2 days a pattern of gastric mucosal inflammatory responses resembling that of acute gastritis and persisting for at least 10 days [23,25]. The rats in each group were killed 16 h after the last dose, their stomachs dissected, and the mucosal tissue used for histologic and biochemical measurements.

Helicobacter pylori LPS. Helicobacter pylori used for LPS preparation was cultured from clinical isolates obtained from ATCC No. 43050 [23]. The bacterium was homogenized with liquid phenol–chloroform–petroleum ether, centrifuged, and the LPS contained in the supernatant was precipitated with water, washed with 80% phenol solution, and dried with ether. The dry residue was dissolved in water at 45 °C, centrifuged at 10,000*g* for 4 h, and the resulting LPS sediment was subjected to lyophilization.

Mucosal histology. The sections of gastric mucosa were cut into 4 μm strips, fixed in 10% buffered formalin, and stained with hematoxylin and eosin [23]. The morphological pattern of gastritis was graded in accordance with the Sydney system and the changes in mucosal histology were quantified, as described earlier [23,25].

ET-1 and leptin quantification. ET-1 assays were carried out on the individual specimens of gastric mucosal tissue following homogenization with 4 volumes of 1 M acetic acid [16,25]. The homogenates were heated for 5 min at 100 °C, centrifuged, the supernatants were applied to a Sep-Pack C-18 cartridges, and the adsorbed ET-1 was eluted with methanol–water–trifluoroacetic acid (90:10:0.1, v/v/v). The eluates were dried, reconstituted in the assay buffer, and subjected to ET-1 quantification using a double-antibody sandwich technique in accordance with the manufacturer's (Alexis) instructions. For leptin measurements, the specimens of gastric mucosa were homogenized at 4 °C in phosphate-buffered saline, pH 7.4, centrifuged for 10 min at 800*g*, and the resulting supernatant was used for leptin quantification. Leptin assays were carried out using mouse leptin enzyme-linked immunometric assay as instructed by the manufacturer (Calbiochem). The protein content of samples was measured with the BCA protein assay kit (Pierce).

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

Results

The role of ET-1 in up-regulation of gastric mucosal leptin production associated with gastric mucosal inflammatory reaction to *H. pylori* infection was investigated in the animal model over the period of 10 days, using rats subjected to *H. pylori* LPS-induced gastritis [23,25]. The LPS was applied intragastrically at a dose of 50 μg per animal produced within 2 days a pattern of inflammatory changes resembling that of acute gastritis 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 the mucosal pathological condition reached a maximum by the fourth day (mean score of 5.3) and showed a decline (mean score of 3.1) in severity by the 10th day (Fig. 1). Moreover, the extent of gastric mucosal inflammatory involvement was reflected by a marked increase in the mucosal levels of ET-1 and leptin. By the second day following the LPS application, the mucosal level of ET-1 increased 3.2-fold, reached a maximum of 3.6-fold increase by the fourth day, and then declined by 57% on the 10th day (Fig. 1). The mucosal level of leptin showed a 3.7-fold increase by the second day following the LPS application, attained a maximum increase of 4.2-folds by the fourth day, and showed a decline (27.5%) by the 10th day (Fig. 1).

We next examined the changes in gastric mucosal inflammatory involvement in responses to *H. pylori* LPS in the presence of phosphoramidon, a potent inhibitor of ECE-1 activity. As depicted in Fig. 2, administration of phosphoramidon commenced 4 h following the LPS application and continued with two daily doses for up to 4 days, resulted in a concentration-dependent reduction (up to 61.2% at 20 mg/kg) in the mucosal level of ET-1 induced by the LPS, and was accompanied by the enhanced (28.6%) severity of mucosal inflammatory involvement.