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Downregulation of immunomodulator gene expression in LPS-stimulated human polymorphonuclear leukocytes by the proton pump inhibitor lansoprazole

Received: May 14, 2009 / Accepted: August 13, 2009

Abstract Lansoprazole (LPZ) has anti-inflammatory activity and repairs cells damaged by phagocytic cells. In the present study, we evaluated the effects of LPZ on gene expression, especially that of immunomodulator genes, in human polymorphonuclear leukocytes (PMNs) activated by lipopolysaccharide (LPS). Several concentrations of LPZ (final concentrations, 0–10 μg/ml) were added to the PMNs (1 × 10⁶ cells/ml), which were stimulated with LPS (100 ng/ml) and incubated at 37°C for 1 or 3 h. When LPS-stimulated PMNs were treated with LPZ at ≥5.0 μg/ml for 1 h, mRNA expression levels of CXCRI/2 and TNFα were suppressed in a dose-dependent manner. The gene expression level of CD14 was also downregulated by LPZ at ≥0.1 μg/ml, with expression suppressed to 50% by 10 μg/ml LPZ. However, LPZ at 0.01–5.0 μg/ml had no significant effect on the expression of TLR-4 or CD11b/CD18 mRNA. LPZ at 10 μg/ml downregulated the levels of these mRNAs to 80% and 50%, respectively. On the other hand, when the reaction period was extended to 3 h with the same conditions, all mRNA expression levels were downregulated by ≥0.01 μg/ml LPZ, in a dose-dependent manner. LPZ may suppress the biological functions of PMNs, such as chemotaxis and inflammatory chemokine production.

Key words Lansoprazole · Lipopolysaccharide · Polymorphonuclear leukocyte · Tumor necrosis factor α · RT-PCR

Introduction

Gastric epithelial cells produce interleukin (IL)-8, which is a potent chemotactic and activating factor for leukocytes. IL-8 plays a significant role in several types of human gastric injury through the attraction and activation of polymorphonuclear leukocytes (PMNs), and gastric mucosal levels of IL-8 are increased in parallel with the histological severity of gastritis. Prolonged secretion of IL-8 by gastric epithelial cells may result in the recruitment of PMNs to gastric tissues. Infiltrated PMNs may release a number of proinflammatory cytokines, reactive oxygen species (ROS), and chemical mediators, which further contribute to the progression of the inflammatory process.

Proton pump inhibitors (PPIs), such as lansoprazole (LPZ) and omeprazole (OME), which strongly inhibit the release of H⁺ from gastric parietal cells, have been widely used for the treatment of gastric and duodenal ulcers and reflux esophagitis, because of their strong antisecretory effects, and these agents have also been used for the eradication of Helicobacter pylori together with antibiotics such as amoxicillin and clarithromycin. PPIs also inhibit H⁺ and K⁺-adenosine triphosphatase (ATPase), and induce more rapid ulcer healing than histamine-2 receptor antagonists (HRAs) do.

In 1992, Wandall suggested that PPIs inhibit PMN functions such as chemotaxis, superoxide production, and degranulation. In addition, some reports have shown that P-type proton ATPase (P-ATPase) inhibitors counteract inflammation by downregulating the expression of PMN adhesion molecules and the production of ROS. It has been reported recently that LPZ reduces the induction of inflammatory mediators that affect PMNs, vascular endothelial cells, and gastric mucosa, and suppresses interaction between PMNs and vascular endothelial cells. On the other hand, Maity et al. have reported that LPZ induces the protection and repair of damaged gastric mucosa. Thus, targeting proinflammatory cytokines in anti-inflammatory therapy is important, and anti-tumor necrosis factor (TNF) α and anti-IL-6 antibodies have been developed and are already being used clinically.

However, there are few reports on the anti-inflammatory effects of LPZ, other than its inhibition of gastric acid production, and the molecular mechanisms by which LPZ
affects PMN function have not yet been fully elucidated. In the present study, we investigated the influence of LPZ on the biological functions of PMNs, using in vitro gene expression analysis.

**Materials and methods**

**Chemicals**

LPZ (AG-1749) was obtained from Takeda Pharmaceutical (Osaka, Japan). LPZ was resuspended in 100% dimethyl sulfoxide (DMSO) to make a 100-μg/ml stock solution. Reagents were filtered through a 0.22-mm Millex GP Filter Unit (Millipore, Corrigwohill, Ireland) before use. Lipo-poly saccharide (LPS) from *Escherichia coli* O111:B4 was purchased from Sigma-Aldrich Japan (Tokyo, Japan).

**PMN preparation**

Human PMNs were isolated from the peripheral blood of healthy volunteers. Briefly, 20 ml whole blood was mixed with 4.5% dextran solution and allowed to stand for 40 min at room temperature. The leukocyte-rich plasma was centrifuged at 400 g on a Ficoll-Paque Plus gradient (Amersham Bioscience, Milwaukee, WI, USA) for 20 min. To lyse erythrocytes, hypotonic (0.2%) saline was used and the osmolality was restored by hypertonic (1.6%) saline. PMNs were adjusted to a final concentration of 1 × 10⁶ cells/ml in Hanks’ balanced salt solution without Ca, Mg, or phenol red (HBSS(-)). All the volunteers were healthy adults; four men and three women, aged 25–55 years (mean, 35 years). Before the study was undertaken, the volunteers were informed about its purpose, and consent was obtained from all participants.

**LPZ treatment**

Several concentrations of LPZ (final concentrations, 0–10 μg/ml) were added to the PMNs (1 × 10⁶ cells/ml), which were pretreated with LPS (100 ng/ml) at room temperature for 5 min, and incubated at 37°C for 1 or 3 h under 5% CO₂. Nontreated control (NC) PMNs (1 × 10⁶ cells/ml) were not treated with LPS or LPZ. All PMNs were harvested and washed with 1 ml cold phosphate-buffered saline without Ca or Mg (PBS (-)) for further study.

**Cytotoxicity of LPZ**

The trypan blue exclusion test was carried out to determine the cytotoxic activity of LPZ to PMNs. One part of 0.4% trypan blue was mixed with one part of an LPZ-treated PMN suspension. The number of viable PMNs was counted using a Countess Automated Cell Counter (Life Technologies, Carlsbad, CA, USA) within 3 min.

**RNA isolation**

Total RNA was extracted from PMNs using the RNeasy Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer’s instructions. The quantity and quality of total RNA samples were determined with the Agilent 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany).

**Complementary DNA synthesis**

Total RNA was reverse-transcribed to cDNA using Super-Script III First-Strand Synthesis SuperMix for RT-PCR (Life Technologies). Briefly, 1 μg total RNA was incubated with 2.5 μM oligo (dT)₃₀ and 50 ng of random hexamers, including 200 U SuperScript III RT enzyme in a 40-μl reaction volume at 25°C for 10 min, followed by incubation at 50°C for 20 min. Reactions were terminated by heating at 85°C for 5 min, and then the mixture was treated with 2 U *E. coli* RNaseH at 37°C for 20 min, to digest RNA templates.

**Quantitative real-time PCR (qPCR) analysis**

Gene expression levels of *CXCR1/2* (accession no. MN_000634), *CD14* (accession no. MN_000591), *TLR-4* (accession no. MN_138554), *TNFα* (accession no. MN_000594), and *CD11b/CD18* (accession no. MN_000632) in PMNs were quantified using the ABI7300 real-time PCR System (Life Technologies). cDNAs were amplified with SYBR Green using Platinum SYBR Green qPCR SuperMix UDG (Life Technologies). qPCR was performed for *CXCR1/2* and a housekeeping gene, β-actin (accession no. MN_001101). PCR primer sets were designed at the Primer3 web site (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and the sequences of the PCR primer sets are shown in Table 1. The cDNA amplification program was as follows: 50°C for 2 min, and 95°C for 2 min; 95°C for 15 s, 59°C for 1 min, for 40 cycles. All PCR reactions were carried out in 30-μl reaction volumes that contained the following components: 5 μl cDNA solution, 0.9 U Platinum Taq polymerase, 1 × reaction buffer (20 mM Tris/HCl [pH 8.4], 3 mM MgCl₂, 200 μM dVTPs [a mixture of dATP, dCTP, and dGTP], 400 μM dUTP, 500 nM ROX reference dye, 0.6 U uracil DNA glycosylase), and 200 nM primers. *CXCR1/2* mRNA expression levels in PMNs were normalized by gene expression levels of β-actin. Eventually, fold differences in *CXCR1/2* mRNA levels between LPZ-treated PMNs and controls were determined using Sequence Detection System (SDS) software (Life Technologies).

**Statistical analysis**

The expression levels of each gene in the PMNs derived from the seven healthy volunteers were analyzed by two-tailed Student’s t-test and presented as means ± SEM. P <