Anti-inflammatory Effects Of Lansoprazole By Suppressing Ros Production In Murine Macrophage Raw 264.7 Cells

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Introduction: Aberrantly activated macrophages overproduce inflammatory mediators such as nitric oxide (NO) and prostaglandin E2 (PGE2), both of which are involved in the pathogenesis of many inflammatory diseases, including rheumatoid arthritis, arteriosclerosis, asthma, and pulmonary fibrosis. Agents that target macrophages, inhibiting the secretion of multiple inflammatory mediators, may provide a breakthrough as novel therapy for patients with inflammatory disorders. Proton pump inhibitors (PPIs), including lansoprazole (LPZ), are widely used clinically to treat gastrointestinal mucosal disorders caused by gastric acid hypersecretion, such as gastric ulcer and reflux esophagitis. The mucosal-protective effects of PPIs are due primarily to their inhibition of P-ATPase, thus reducing acid secretion. More recently, however, PPIs were shown to have local pleiotropic effects in the gastric mucosa, including having anti-apoptotic effects, inhibiting the generation of reactive oxygen species (ROS), and inhibiting the expression of macrophage adhesion molecules. PPIs have shown clinical effects not only in gastric mucosal disorders, but in Crohn’s disease, an intestinal disorder. In Crohn’s disease, abnormal activation of macrophages leads to chronic intestinal inflammation, suggesting that PPIs have anti-inflammatory effects on macrophages. The purpose of this study is to investigate the effects of LPZ on activated macrophages and the mechanisms by which LPZ inhibits the production of inflammatory mediators such as NO and PGE2.

Methods: RAW264.7 murine macrophages were obtained from the Riken Cell Bank (Ibaragi, Japan). The cells were incubated with various concentrations of LPZ for 24 h. The cell was evaluated by MTS assay. The cells were treated with 0, 20, 50, and 100 µM LPZ in the presence or absence of µg/ml lipopolysaccharide (LPS), and the concentrations of NO in each supernatant was measured using the Griess reagent system (Promega) and the concentration of PGE2 was measured using an enzyme-linked immunosorbent assay (ELISA) kit (R & D, Minneapolis, MI) according to the manufacturer’s instruction. Antibodies specific for iNOS and COX-2 and β-actin expression were analysed. Chemiluminescence was visualized using an ECL plus Western blotting detection kit (Amersham). We used RT-PCR to determine whether P-ATPase mRNA is expressed in RAW264.7 cells to analyze whether the anti-inflammatory effects of LPZ are related to P-ATPase. We measured intracellular ROS concentrations of LPZ-stimulated RAW264.7 cells by flow cytometry using the probe 2, 7-dichlorodihydrofluorescein diacetate (DCFH-DA) in the presence or absence of 100µM LPZ. All data are expressed as means±standard deviation (SD) and compared by analysis of variance (ANOVA). Post hoc testing was performed using the Turkey-Kramer test. P<0.05 was defined as statistically significant.

Results: LPZ, at concentrations of 0-100µM, had no effects on the viability of RAW264.7 cells, thus allowing experiments to be performed using LPZ concentrations as high as 100µM. LPZ inhibited the LPS-induced production of NO in a concentration-dependent manner, with 100µM LPZ treated cells showing the same level of NO production as unstimulated cells. In addition, 50 and 100µM LPZ inhibited the LPS-induced secretion of PGE2 into culture supernatants. To evaluate whether the ability of LPZ to inhibit LPS-induced NO and PGE2 production was mediated by expression of iNOS and COX-2 proteins, we performed Western blot analysis using antibodies specific to these two proteins. LPZ inhibited the expression of both iNOS and COX-2. Expression of iNOS and COX-2 was also suppressed by LPZ at a concentration of 100 µM. P-ATPase expression in mouse gastric mucosal tissue, our positive control, but no in RAW264.7 cells. Moreover, cells incubated with both LPZ and LPS failed to show P-ATPase expression. LPZ alone, in the absence of LPS, did not alter ROS levels, whereas preincubation with LPZ prior to LPS stimulation significantly reduced the levels of ROS.

Discussion: The anti-inflammatory effects of LPZ may be mediated through macrophages, but the mechanisms by which LPZ exerts anti-inflammatory effects have not been clarified. We found that LPZ not only inhibited NO and PGE2 production by LPS-stimulated RAW264.7 cells but also suppressed iNOS and COX-2 expression by these cells. This results suggest that LPZ exerts anti-inflammatory effects by suppressing the expression of iNOS and COX-2. ROS have been shown to play important roles in inflammatory responses including the regulation of cell growth and differentiation and the control of cell responses to cytokines and stress. We found that LPS stimulation of RAW264.7 cells increased the production of both ROS and NO. These findings suggest that, in macrophages, LPS stimulation increases ROS production and is associated with inflammatory responses by inducing NO production. LPZ is already being used clinically in patients, but safe application for other than gastrointestinal disorders has not been reported. Since the safety of LPZ in humans has been established, the hurdle for clinical application is
low. Our findings, showing that LPZ suppressed the inflammatory activity of activated macrophages in vitro, suggest that LPZ may be promising in the treatment of inflammatory diseases involving activated macrophages, including rheumatoid arthritis and Crohn's disease. Studies in animal models may show these therapeutic effects.

**Significance:** LPZ may be useful in the treatment of many inflammatory diseases associated with activated macrophages.

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**References:**
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