

Lansoprazole, a Proton Pump Inhibitor, to Reduce Gastrointestinal Inflammation via Heme Oxygenase-1 Induction

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Abstract

Lansoprazole, a proton pump inhibitor, inhibits acute inflammatory reactions as well as intestinal mucosal injuries induced by ischemia-reperfusion or indomethacin administration in rats. These cytoprotective and anti-inflammatory actions induced by lansoprazole are demonstrated to be independent of acid inhibition. In *in vitro* study, lansoprazole can induce the expression of heme oxygenase-1 (HO-1) in epithelial as well as inflammatory cells, and exerts anti-inflammatory effect dependent on HO-1 expression. Furthermore, NF-E2-related factor-2 (Nrf2)/keap-1 system plays an important role in HO-1 expression induced by lansoprazole. In this article, we have focused on lansoprazole-induced HO-1 expression, its anti-inflammatory action, and the role of Nrf2 in its expression.

Keywords: Lansoprazole; Heme Oxygenase-1; NF-E2-related factor-2; Inflammation; Proton pump

Introduction

Proton pump inhibitors (PPIs) such as lansoprazole have dramatically influenced the management of acid-peptic disorders in recent years (1-7). They are extensively used to treat acid-related disorders, including gastroesophageal reflux and peptic ulcer caused by stress, non-steroidal anti-inflammatory drugs and *Helicobacter pylori* infection,

due to their strong anti-secretory effects by inhibiting H⁺/K⁺-adenosine triphosphatase (H⁺K⁺-ATPase) of parietal cells (1-7). Lansoprazole, 2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]methyl]sulfinyl]-1H-benzimidazole, is a strong anti-secretory agent that acts on gastric H⁺/K⁺ ATPase of parietal cells. In addition to its acid-suppressing effects, lansoprazole have been shown to modulate the inflammatory status, reduce oxidative stress, and ameliorate mucosal injuries in the esophagus (8-10), intestine (11-13), and lung (14) as well as in stomach (15). Our recent study using a DNA microarray clearly showed that lansoprazole induced several genes, including phase II detoxifying enzyme (NADPH-ubiquinone oxidoreductase, glutathione S-transferase) and antioxidant stress proteins [heme oxygenase-1 (HO-1), thioredoxin reductase, and superoxide dismutase] in gastric epithelial cells (16). Becker et al. (17) also demonstrated that PPIs protected gastric epithelial cells against oxidative stress, and this protection was abrogated in the presence of an HO-1 inhibitor. These data indicated that lansoprazole-induced HO-1 induction might account for the cytoprotective and anti-inflammatory effects of lansoprazole independent of acid-secretion inhibition.

HO-1 is highly inducible by a vast array of stimuli, including oxidative stress, heat shock, ultraviolet radiation, ischemia-reperfusion, heavy metals, bacterial lipopolysaccharide (LPS), cytokines, nitric oxide, and its substrate, heme (18, 19). This strong adaptive response of HO-1 to various stimuli suggests an entirely new paradigm by which HO-1 could play a significant role in protection against inflammatory processes and oxidative tissue injury. Recent studies have extensively investigated the

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transcriptional factors and regulatory regions that are responsible for induction of the *ho-1* gene. Several signaling molecules (e.g., mitogen-activated protein kinases (MAPK)) and transcriptional regulators (activator protein-1, NF-E2-related factor-2 (Nrf2), hypoxia-inducible factor-1, Bach-1) participate in the regulation of the *ho-1* gene (20-23). However, it remains unclear whether lansoprazole is involved in the transcriptional regulation of *ho-1* gene, or in the subsequent induction of HO-1. Recently our study shows that lansoprazole up-regulates HO-1 expression in rat gastric epithelial cells, and the up-regulated HO-1 had anti-inflammatory effects, and that lansoprazole-induced HO-1 induction is mediated by the activation, phosphorylation and nuclear translocation of Nrf2 in association with the dissection of oxidized Kelch-like ECH associating protein 1 (Keap1) (24).

Lansoprazole Reduces Inflammation in the Gastrointestinal Tract

In 2004, Yoshida et al. (8) demonstrated that the expression of interleukin-8 (IL-8) mRNA was correlated with the endoscopic grade of reflux esophagitis or with inflammatory cell infiltration in patients with gastroesophageal reflux disease (GERD). Furthermore, administration of lansoprazole decreased both IL-8 mRNA and protein levels in the esophageal mucosa, suggesting that IL-8 in the esophageal mucosa could be involved in the pathogenesis of esophageal inflammation in GERD patients, and that lansoprazole might exert an anti-inflammatory action via the inhibition of IL-8 expression. Isomoto et al. (9) also demonstrated that the presence of intraepithelial neutrophils and eosinophils, also indicating reflux esophagitis, was associated with high IL-8 mRNA levels and regulated on activation normal T-cell expressed and presumably secreted (RANTES), respectively. Furthermore, the IL-8 levels were found to be significantly lower after lansoprazole treatment. These two reports indicate that chemokine production locally in the esophageal mucosa may be involved in the development and progression of reflux esophagitis and that gastric acid may play a role in the induction of esophageal inflammation.

We have firstly demonstrated that lansoprazole inhibits acute inflammatory reactions as well as small intestinal mucosal injuries induced by ischemia-reperfusion (11) or by indomethacin administration in rats (12). These intestinal injuries induced by ischemia-reperfusion or indomethacin are significantly inhibited by lansoprazole at a dose

of 5 mg/kg together with significant suppression of the increased levels of thiobarbituric acid-reactive substances, an index of lipid peroxidation, myeloperoxidase activities and cytokine-induced neutrophil chemoattractant-1 (CINC-1) and indices of acute inflammation in the small bowel. Furthermore, the increased CINC-1 mRNA expression after ischemia-reperfusion or indomethacin administration is also inhibited by the treatment with lansoprazole. This protective effect of lansoprazole against small intestine is recently confirmed by Higuchi et al. (13). They have demonstrated that lansoprazole ameliorates indomethacin-induced small intestinal ulceration through upregulation of HO-1. These results strongly suggest that the action of lansoprazole could be dependent on its anti-inflammatory or anti-oxidative responses, independent of acid suppression.

It has been also demonstrated by in vitro studies that lansoprazole inhibits the increased expression of vascular adhesion molecules, the activation of neutrophils, the production of pro-inflammatory cytokines from activated endothelial cells (25, 26), and the activation of macrophages/monocytes (27-29). In 2000, Yoshida et al. (25) firstly demonstrated that lansoprazole blocked the trans-endothelial migration of neutrophils attracted by IL-8, and that lansoprazole inhibited both the *Helicobacter pylori* water extract-induced expression of CD11b and CD18 surface adhesion molecules on neutrophils and the interleukin-1 β (IL-1 β)-induced expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) in human umbilical vein endothelial cells (HUVEC). These results suggested that both lansoprazole may inhibit neutrophil trans-endothelial migration by inhibiting both IL-8-dependent mechanism and adhesion molecules-dependent mechanism. To further investigate the mechanism, by which lansoprazole inhibited neutrophil trans-endothelial migration, Handa et al. (26) measured the cytosolic calcium concentration in neutrophils in response to fMLP, which was a well-known bacteria-derived chemotactic oligopeptide and upregulated CD11b/CD18 expression in neutrophils. They have shown that lansoprazole significantly inhibited the fMLP-induced elevation of the cytosolic calcium concentration of neutrophils, and thus possibly suppressed CD11b/CD18 expression and the subsequent neutrophil trans-endothelial migration.

In addition to neutrophils, macrophages and mononuclear cells play a pivotal role in gastrointestinal inflammation by the production of

proinflammatory cytokines such as tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β). In response to interaction with pathogenic bacteria, these cells can produce proinflammatory cytokines, which activate further inflammatory processes and promote inflammation. Tanigawa et al. (29) have demonstrated that lansoprazole inhibits production of TNF- α and IL-1 β by the monocytic cell line THP-1 stimulated by lipopolysaccharide or *Helicobacter pylori* water extract via inhibition of nuclear factor (NF)- κ B and extracellular signal-regulated kinase (ERK) activation. Moreover, Nakamura et al. (30) reported that uptake of lansoprazole was observed in inflammatory cells including polymorphonuclear cells and macrophages in the colonic mucosa of rats with dextran sodium sulfate-induced colitis. These findings suggest that lansoprazole may exert anti-inflammatory effects in gastrointestinal inflammation via suppression of production of

proinflammatory cytokines from inflammatory cells stimulated by pathogenic bacteria.

Role of HO-1 in Anti-inflammatory Effects by Lansoprazole

HO is involved in heme catabolism, a process in which the oxidation of heme leads to the production of iron, biliverdin and carbon monoxide. Three mammalian HO isozymes have been identified, one of which, namely HO-1, is a stress-responsive protein. HO-1 is highly inducible by a vast array of stimuli, including oxidative stress, heat shock, ultraviolet radiation, ischemia-reperfusion, heavy metals, bacterial lipopolysaccharide, cytokines, nitric oxide, and its substrate, heme. This strong adaptive response of HO-1 to various stimuli suggests an entirely new paradigm by which HO-1 could play a significant role in protection against inflammatory processes and oxidative tissue injury.

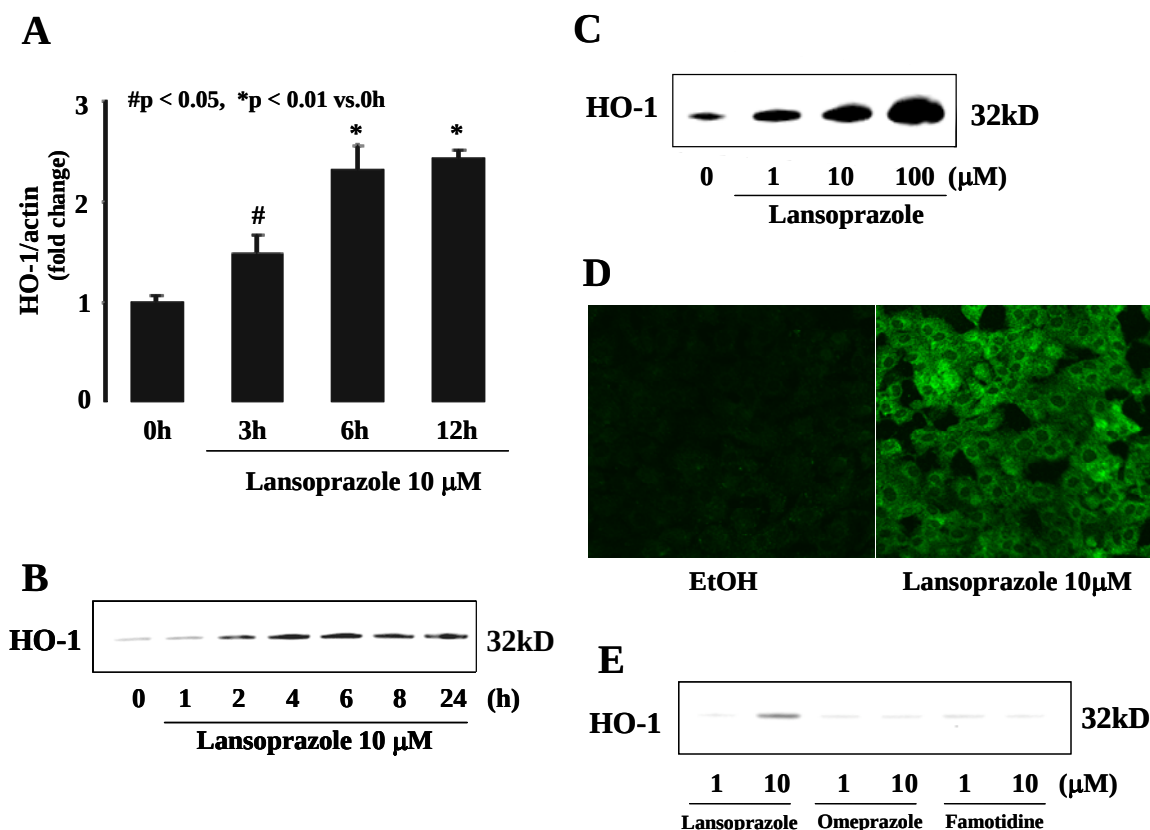


Figure 1. Up-regulation of HO-1 by lansoprazole in RGM-1 cells. RGM-1 cells were incubated with lansoprazole. (A) Ho-1 gene expression was measured using real-time PCR. The mRNA level of β -actin was determined simultaneously, and HO-1/ β -actin ratio was calculated for each sample. Values represent the mean $\pm$ S.E.M. (n=6). #p < 0.05 and *p < 0.01 compared to no stimulation (0 h). (B) and (C) Whole-cell extracts were prepared and analyzed by immunoblotting with an antibody against HO-1. (D) The expression and localization of HO-1 in RGM-1 cells 6 h after lansoprazole treatment was investigated using confocal microscopy. (E) Omeprazole, another PPI, and famotidine, H2-receptor antagonists induced the faint expression of HO-1 compared to HO-1 expression induced by lansoprazole.

To test the expression of HO-1 induced by lansoprazole, we checked the HO-1 gene and protein expression using real-time PCR and Western blotting. Lansoprazole stimulation significantly increased the expression of the *ho-1* gene in RGM-1 cells, a rat normal gastric mucosal cell line, in a time- and a concentration-dependent manner (Figure 1). In association with the induction of *ho-1* gene expression, the expression of the HO-1 protein was significantly increased in a time-dependent manner after lansoprazole treatment, and confocal microscopy revealed that the HO-1 protein was localized to the cytoplasmic fraction. Furthermore, we checked whether another anti-acid secretory agents also induced the expression of HO-1 and noted that omeprazole (another PPI) and famotidine (an H_2 -receptor antagonist) induced a faint expression of HO-1 compared to HO-1 expression induced by lansoprazole (Figure 1E).

To investigate the contribution of lansoprazole-induced HO-1 up-regulation to chemokine production by RGM-1 cells, we measured whether the production of CINC-1 induced by IL-1 β was inhibited by lansoprazole. CINC-1, a counterpart of the human GRO (a member of IL-8 family), has been suggested to play a critical role as a mediator of neutrophil infiltration in rats during inflammation. Our previous report demonstrated that RGM-1 cells produced CINC-1 in response to various pro-inflammatory cytokines, such as TNF- α , IL-1 β , and bacterial LPS (31). Pretreatment with lansoprazole significantly inhibited the production of CINC-1 from stimulated RGM-1 cells. In addition, the inhibition was reversed by co-treatment with the HO-1 inhibitor SnPP. *In vivo* experiment also demonstrated that anti-inflammatory action induced by lansoprazole in a model of indomethacin-induced intestinal injury was reversed by the treatment with SnPP at a dose of 30 mg/kg (13). Recently our data supports the significance of HO-1 induction in indomethacin-induced intestinal injury. Intestinal inflammation induced by indomethacin is markedly inhibited in mice highly expressing HO-1 by the deletion of BTB and CNC homolog 1 (BACH1), a negative repressor of HO-1 (32). These data indicate that the anti-inflammatory effect of lansoprazole is mediated through the induction of HO-1.

Role of Keap1/Nrf2 System in HO-1 Up-regulation by Lansoprazole

Among many transcriptional molecules that regulate *ho-1* gene expression, accumulating data implicate Nrf2 as a key regulator of the adaptive

response to oxidative stress (33-36) and of the transcriptional activation of *ho-1* (37). As for the mechanism of lansoprazole-induced up-regulation of HO-1, we have shown using an siRNA approach and Nrf2-deficient fibroblasts that the transcriptional factor Nrf2 plays a critical role in the up-regulation of HO-1 in RGM-1 cells (24). The treatment of RGM-1 cells with Nrf2-siRNA decreased the constitutive *ho-1* mRNA levels and abolished the lansoprazole-induced *ho-1* mRNA and HO-1 protein expression, suggesting a pivotal role of Nrf2 in the regulation of HO-1 in RGM-1 cells. The involvement of Nrf2 in the HO-1 up-regulation induced by lansoprazole was also confirmed by the findings obtained from Nrf2-deficient fibroblasts. The expression of *ho-1* mRNA and HO-1 protein was weak in Nrf2-deficient fibroblasts with/without lansoprazole incubation. These experiments demonstrated a direct correlation between Nrf2 and HO-1 expression and supported the contention that lansoprazole-mediated up-regulation of HO-1 was Nrf2-dependent.

Extensive analysis has identified a common mechanism involving the stress response element (StRE)/Nrf2 transcription factor pathway for gene regulation in response to a diverse array of HO-1 inducers. The StREs mediate transcriptional activation in response to multiple agents, including heme, heavy metals, TPA, arsenite, hydrogen peroxide, hyperoxia, lipopolysaccharide, and various electrophiles (38). These motifs in StRE are targets for multiple members of the basic-leucine zipper (bZIP) superfamily of sequence-specific DNA-binding proteins, including the AP-1, CREB/ATF, Maf, and CNC-bZIP classes of transcription factors. Alam J. et al. (33) have also described that the induction of HO-1 by StREs in L929 fibroblasts demonstrated potent trans-activation of the E1 enhancer by CNC-bZIP factors, particularly Nrf2. In our recent study, EMSA reactions using nuclear protein fractions from RGM-1 cells were carried out to identify DNA-binding proteins potentially responsible for lansoprazole-mediated *ho-1* up-regulation (24). Lansoprazole treatment of RGM-1 cells significantly increased the levels of the StRE-protein complex in a concentration-dependent manner. In addition, the increased complex band disappeared following the addition of anti-Nrf2 polyclonal antibody. These data indicated that activation of the *ho-1* gene by lansoprazole occurred, at least in part, as a consequence of increased binding of Nrf2 to the StREs. Moreover, in a luciferase reporter assay using HO-1 enhancer-luciferase reporter gene containing mutant StREs, lansoprazole-induced HO-

1 reporter gene activity was markedly decreased by the mutation. On the other hand, lansoprazole-induced reporter activity was also diminished by the co-transfection of dominant negative Nrf2 expression plasmids. Together, these results confirmed that lansoprazole activated HO-1 enhancer activity via Nrf2.

Role of MAP Kinases in HO-1 Up-regulation by Lansoprazole

Multiple protein kinase pathways, such as MAP kinase, protein kinase C, and p38 MAP kinase, have been proposed to play a role in HO-1 induction. The present study demonstrated that lansoprazole promoted the phosphorylation of ERK, but not of p38 MAPK or JNK in RGM-1 cells, and that lansoprazole-induced HO-1 up-regulation was suppressed by U01263, a specific inhibitor for ERK. These data indicate that HO-1 up-regulation by lansoprazole was at least partly mediated by the ERK pathway. The role of the ERK pathway in the induction of HO-1 is controversial; ERK has been found to regulate HO-1 expression in some studies (39, 40) but not in others (41). This discrepancy may reflect cell type- and/or species-specific differences in the regulatory mechanism of HO-1.

It has recently been described that phosphorylated forms of Nrf2 modified after chemically induced oxidative stress play a potential role in Nrf2 activation and degradation (42). Furthermore, electrophilic- or reactive oxygen species-induced nuclear Nrf2 accumulation is shown to be highly dependent on protein kinase CK2 activity. In our study, the detection of phosphorylated protein was assayed using Pro-Q® Diamond phosphoprotein stain. Consequently, the phosphoproteins in RGM-1 cells were markedly increased by treatment with lansoprazole. The purification of these phosphoproteins induced by lansoprazole was performed using a Pro-Q® Diamond Phosphoprotein Enrichment kit, and Nrf2 was clearly detected in the purified phosphoproteins. The increased phosphorylated forms of Nrf2 might play a role in its activation or nuclear accumulation.

Oxidation of the Keap1 Protein in RGM-1 Cells Treated with Lansoprazole

Keap1 is rich in cysteine residues and this fact has indicated that Keap1 is a sensor protein of xenobiotic and oxidative stress. Earlier studies have proposed that Keap1 oxidation leads to release of Nrf2, allowing it to enter the nucleus and bind to ARE-containing genes (36, 43). In agreement with

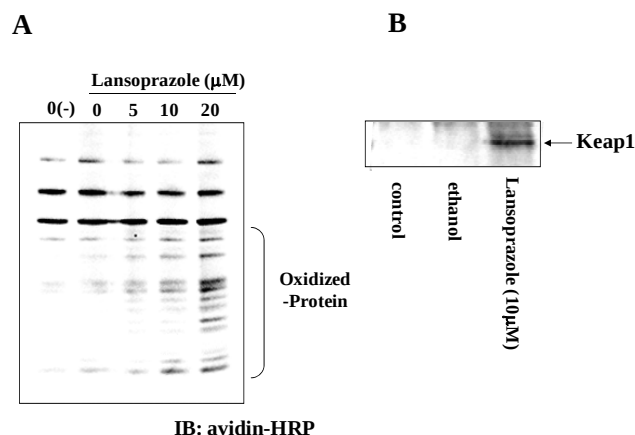


Figure 2. The detection and identification of S-oxidized proteins in RGM-1 cells exposed to lansoprazole. (A) Biotin-cysteine-protein adducts were detected by Western blotting. RGM-1 cells were incubated with 100 μ M biotin-cysteine for 15 min and then treated with 10 μ M lansoprazole for 45 min. Total cell lysate was separated by 10% SDS-PAGE followed by Western blot analysis. (B) To isolate the biotin-cysteine-modified protein, cell lysates were mixed with Streptavidin MicroBeads, and subsequently the MicroBeads-labeled proteins were separated using μ Columns. The collected samples were analyzed by Western blotting using anti-Keap1 antibody.

these studies, *in vitro* oxidation of Keap1 has been shown to cause a change in the affinity of Keap1 for the Neh2 domain, releasing Nrf2 (44). Recent *in vivo* studies have reported that oxidation does release the Nrf2/Keap1 complex (45). To detect cellular proteins that undergo cysteine-target oxidation by lansoprazole stimulation, biotin-cysteine was utilized as molecular probe. This probe rapidly crosses the plasma membrane and can be used to detect, quantify, purify, and identify proteins susceptible to oxidation in all compartments of cells (46). By this technique, Ishii et al. (46) have reported that alpha-enolase is modified by intracellular oxidative stress at the S-oxidation sites of two cysteine residues, Cys-118 and Cys-388, which may play a role in the regulation of the biological activities of the protein and may be regulated by a reversible S-oxidation/thiolation reaction. RGM-1 cells exposed to biotin-cysteine were treated with 10 μ M lansoprazole, and the biotin-cysteine-modified proteins were analyzed by Western blotting with streptavidin-HRP. Several proteins were found to be S-oxidized in response to lansoprazole in a dose-dependent manner. Moreover, the incorporation of biotin-cysteine into endogenous Keap1 was confirmed by using pull-down of Streptavidin beads and μ columns followed by Western blotting with an anti-Keap1 antibody (Figure 2) (24). The Keap1 protein was detected among the biotin-cysteine

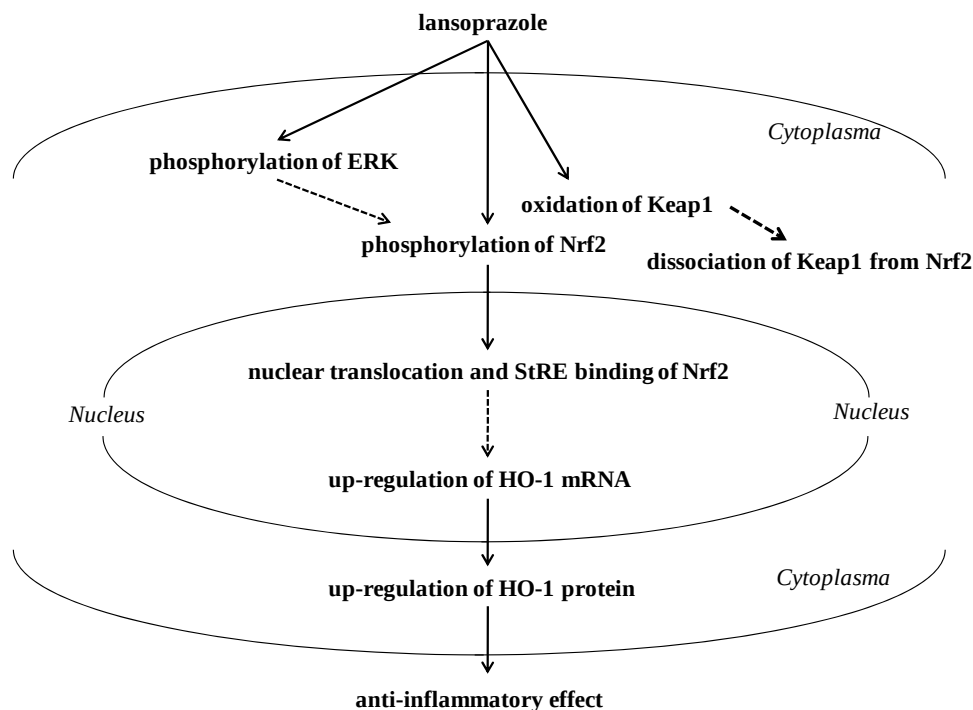


Figure 3. Lansoprazole exerts anti-inflammatory effect through up-regulation of HO-1 expression in rat gastric epithelial cells. Phosphorylation of ERK and Nrf2, activation and nuclear translocation of Nrf2, and oxidation of Keap1 are all involved in the lansoprazole-induced HO-1 up-regulation.

modified proteins, indicating that endogenous Keap1 was oxidatively modified during the lansoprazole stimulation. Although we could not investigate the target sites of Keap1 protein by oxidative stress, a recent investigation clearly demonstrated that Keap1 appeared to utilize multiple cysteine residues differentially as acceptors of electrophiles and oxidants to achieve its functional conversion from the active to the inactive state (47).

Conclusion

In summary, our data show that lansoprazole up-regulated HO-1 expression in rat gastric epithelial cells, and the up-regulated HO-1 has anti-inflammatory effects. It appears that lansoprazole-induced HO-1 production is mediated by the activation, phosphorylation and nuclear translocation of Nrf2 in association with the oxidized Keap1 (Figure 3).

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Conflicts of Interest

No potential conflicts of interest to disclose.

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