**Pleotropic Effects of Proton Pump Inhibitors**
Guest Editor: Yuji Naito

# The Expression of Heme Oxygenase-1 Induced by Lansoprazole

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***Summary*** **Our previous studies have demonstrated that lansoprazole inhibits acute inflammatory reactions as well as intestinal mucosal injuries induced by ischemia-reperfusion or indomethacin administration in rats. Thus, proton pump inhibitors such as lansoprazole have been demonstrated to prevent gastrointestinal mucosal injury by mechanisms independent of acid inhibition. In our *in vitro* study, lansoprazole induced the expression of heme oxygenase-1 (HO-1) on rat gastric epithelial cells (RGM-1 cells), and exerted anti-inflammatory effect on the dependent of HO-1 expression. Furthermore, NF-E2-related factor-2 (Nrf2) played an important role in HO-1 expression induced by lansoprazole. In this review, we focused on lansoprazole-induced HO-1 expression, its anti-inflammatory action, and the role of Nrf2 in its expression.**

***Key Words*: Lansoprazole, Heme Oxygenase-1 (HO-1), NF-E2-related factor-2 (Nrf2)**

## Introduction

Heme oxygenase (HO) is involved in heme catabolism, a process in which the oxidation of heme leads to the production of iron, biliverdin and carbon monoxide [*1*]. Three mammalian HO isozymes have been identified, one of which, 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 (LPS), cytokines, nitric oxide, and its substrate, heme [*2–5*]. 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.

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Recent studies have extensively investigated the 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 (HIF1) and Bach-1) participate in the regulation of the *ho-1* gene. In these molecules, accumulating data implicate Nrf2 as a key regulator of the adaptive response to oxidative stress [*6–11*] and of the transcriptional activation of *ho-1* [*12*]. Under normal conditions, Nrf2 localizes in the cytoplasm, where it interacts with Kelch-like ECH associating protein 1 (Keap1), and is rapidly degraded by the ubiquitin-proteasome pathway [*13*]. Namely, Keap1 acts as negative regulator of Nrf2. Various stimuli, including electrophiles and oxidative stress, liberate Nrf2 from Keap1, allowing Nrf2 to translocate into the nucleus and to bind to antioxidant-response elements (ARE) [*14*]. Nuclearly translocated Nrf2 provides immediate transactivation of regulated encoding genes. In this sequence of Nrf2 activation, the phosphorylation of

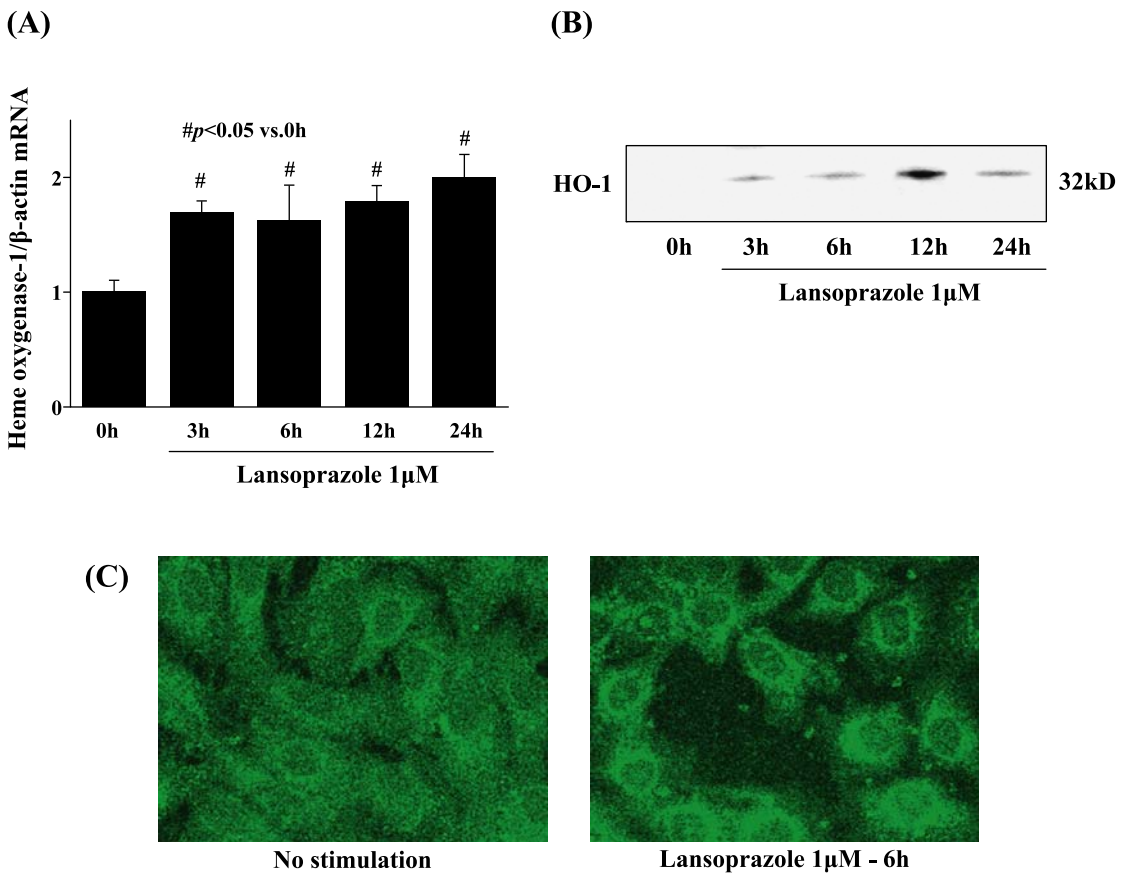


Fig. 1. The expression of HO-1 induced by lansoprazole.
RGM-1 cells were incubated with lansoprazole. (A) *ho-1* gene expression was measured using real-time PCR. The mRNA level of β-*actin* was determined simultaneously, and *HO-1*/β-*actin* ratio was calculated for each sample. Values represent the mean ± SEM ($n = 4$). $^{\#}p<0.05$ compared to No stimulation (0 h) (B) Whole cell extracts were prepared and analyzed by immunoblotting with an antibody against HO-1. (C) The expression and the localization of HO-1 in RGM-1 cells 6 h after lansoprazole treatment was investigated using confocal microscopy.

Nrf2 is an important event in the dissociation of Nrf2 from Keap1 [*15–17*]. Thus the translocation of Nrf2 is considered a major defense mechanism that plays a key role in the induction of HO-1.

In this review, we focused on the expression of HO-1 associated with Nrf2 pathway by lansoprazole.

## Anti-inflammatory Effects by Lansoprazole

Proton pump inhibitors (PPIs) such as lansoprazole have dramatically influenced the management of acid-peptic disorders in recent years, and are extensively used to treat acid-related disorders, including gastroesophageal reflux disease and peptic ulcer disease caused by stress, non-steroidal anti-inflammatory drugs and *Helicobacter pylori* infection [*18–21*]. Lansoprazole is a strong anti-secretory agent that acts on gastric $H^+/K^+$-adenosine triphosphatase ($H^+/K^+$ ATPase) of parietal cells [*22*]. 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 [*23*, *24*], intestine [*25*, *26*], and lung [*27*], in addition to the stomach [*28*, *29*]. It has been also demonstrated by *in vitro* studies that lansoprazole inhibits the increased expression of vascular adhesion molecules, the activation of neutrophils, and the production of pro-inflammatory cytokines from activated endothelial cells [*30*, *31*]. We recently demonstrated using *in vivo* models that lansoprazole inhibits acute inflammatory reactions as well as intestinal mucosal injuries induced by ischemia-reperfusion [*25*] or indomethacin administration in rats [*26*]. These intestinal injuries induced by ischemia-reperfusion or indomethacin were significantly inhibited by lansoprazole at a dose of 5 mg/kg together with significant suppression of the increased levels of thiobarbituric acid-reactive substances, myeloperoxidase activities and cytokine-induced neutrophil chemoattractant-1 (CINC-1) in the small bowel. Furthermore, the increased CINC-1 mRNA expression after ischemia-reperfusion or indomethacin administra-

tion was also inhibited by the treatment with lansoprazole. These results suggest that lansoprazole administered exogenously prevents the small intestine against ischemia-reperfusion or indomethacin-induced damage, the action being dependent on its anti-inflammatory and anti-oxidative responses. These data indicate the possibility that lansoprazole may prevents intestinal mucosal injury by mechanisms independent of acid inhibition.

## HO-1 Expression Induced by Lansoprazole

Our recent study using a DNA microarray clearly showed that lansoprazole induces several genes, including phase II detoxifying enzyme (NADPH-ubiquinone oxidoreductase, glutathione S-transferase) and antioxidant stress proteins (HO-1, thioredoxin reductase, and superoxide dismutase) in gastric epithelial cells (Naito, JCBN2007, http://www2.kpu-m.ac.jp/%7Efirstmed/GeneChip.html) [*32*]. As shown in Figure 1, we confirmed that lansoprazole induced HO-1 up-regulation in rat gastric epithelial cells. Incubation with lansoprazole (1 μM) induced expression of the *ho-1* gene in the early phase within 3 h of lansoprazole addition. 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 cytoplasm fraction. Becker *et al.* [*28*] also demonstrated that PPIs protect gastric epithelial cells against oxidative stress, and this protection is abrogated in the presence of an HO-1 inhibitor. Exposure to lansoprazole resulted in a strong induction of HO-1 expression on mRNA and protein level, and led to an increased activity of this enzyme. These data indicate that lansoprazole-induced HO-1 induction might account for the cytoprotective and anti-inflammatory effects of lansoprazole independent of acid-secretion inhibition.

## Anti-Inflammatory Effect throughout HO-1 Induction by Lansoprazole

Rat CINC-1, a counterpart of the human growth-regulated oncogene (GRO), has been suggested to participate in neutrophil recruitment in an experimental model of gastritis in rat. Our previous report demonstrated that rat gastric epithelial cells (RGM-1 cells) produced CINC-1 in response to various pro-inflammatory cytokines, such as TNF-α, IL-1β, and bacterial LPS [*30*]. To confirm the anti-inflammatory effect of lansoprazole-induced HO-1 on RGM-1 cells, we measured the production of CINC-1 on RGM-1 cells using ELISA. As shown in Table 1, pretreatment with lansoprazole significantly inhibited the production of CINC-1 from stimulated RGM-1 cells with IL-1β. In addition, the inhibition was reversed by co-treatment with the HO-1 inhibitor SnPP. These data indicate that the anti-inflammatory effect of lansoprazole is mediated through the induction of HO-1. On this basis, the up-regulation of HO-1 by lansoprazole contributes to the inhibition of chemokine production from stimulated gastric mucosal cells.

Table 1. The inhibition of CINC-1 production in RGM-1 cells treated with lansoprazole

| Treatment | CINC-1 production (pg/ml) |
|---|---|
| Normal | 13.4 ± 0.08 |
| IL-1β (1 ng/ml) | 48.7 ± 8.72* |
| IL-1β (1 ng/ml) + Lansoprazole 1 μM | 22.7 ± 1.21$^{\#}$ |
| IL-1β (1 ng/ml) + Lansoprazole 1 μM + SnPP 1 μM | 46.6 ± 4.71 |

Data represent the mean + SEM ($n$ = 3). *$p$<0.05 compared to normal group. $^{\#}p$<0.05 compared to IL-1β stimulation group.

Table 2. The effect of Nrf2-siRNA on HO-1 expression

| Treatment | HO-1 mRNA expression (HO-1/β-actin) |
|---|---|
| Normal (Control RNAi) | 1 |
| Lansoprazole 1 μM (Control RNAi) | 1.62 ± 0.08* |
| Nrf2 RNAi | 0.85 ± 0.08 |
| Nrf2 RNAi + Lansoprazole 1 μM | 1.19 ± 0.04$^{\#}$ |

Data represent the mean + SEM ($n$ = 3). *$p$<0.05 compared to normal group. $^{\#}p$<0.05 compared to Lansoprazole 1 μM (Control RNAi) group.

## Role of Nrf2 in HO-1 Up-Regulation by Lansoprazole

We used a small interfering RNA (siRNA) approach to determine if lansoprazole-mediated up-regulation of HO-1 was dependent on Nrf2 in RGM-1 cells. RGM-1 cells were transiently transfected with either control siRNA or siRNA directed against Nrf2. Thirty hour after transfection, cells were exposed to lansoprazole for 6 h, and then *ho-1* mRNA and HO-1 protein expression were examined by real-time PCR and Western blotting, respectively. Under these conditions, the treatment of RGM-1 cells with Nrf2-siRNA decreased the constitutive *ho-1* mRNA level and abolished the lansoprazole-induced *ho-1* mRNA (Table 2) and HO-1 protein expression, suggesting a pivotal role of Nrf2 in the regulation of HO-1 in RGM-1 cells. These experiments demonstrate a direct correlation between Nrf2 and HO-1 expression and support the contention that lansoprazole-mediated up-regulation of HO-1 is Nrf2-dependent.

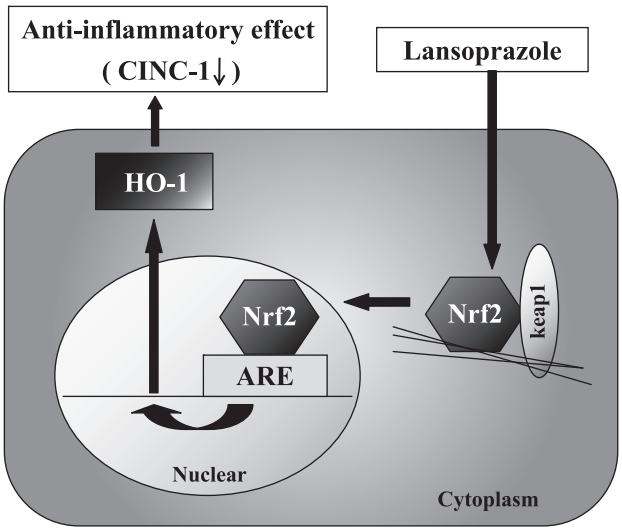


Fig. 2. Lansoprazole induced HO-1 expression throughout the translocation of Nrf2.

## Conclusion

In summary, lansoprazole up-regulated HO-1 expression throughout Nrf2 in rat gastric epithelial cells, and the up-regulated HO-1 had anti-inflammatory effects (Fig. 2) [*33*]. Further studies will be needed to clarify the mechanisms involved in this phenomenon in greater detail.

## Abbreviations

PPI, proton pump inhibitor; HO-1, heme oxygenase-1; MAPK, mitogen-activated protein kinases; Nrf2, NF-E2-related factor-2; HIF-1, hypoxia-inducible factor-1; Keap1, Kelch-like ECH associating protein 1; CINC-1, cytokine-induced neutrophil chemoattractant-1; siRNA, small interfering RNA.

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