Pleotropic Effects of Proton Pump Inhibitors
Guest Editor: Yuji Naito

The Expression of Heme Oxygenase-1 Induced by Lansoprazole

Tomohisa Takagi, Yuji Naito*, and Toshikazu Yoshikawa

Molecular Gastroenterology and Hepatology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto 602-8566, Japan

Received 23 March, 2009; Accepted 7 April, 2009

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.

* To whom correspondence should be addressed.
Tel: +81-75-251-5508  Fax: +81-75-251-0710
E-mail: ynaito@koto.kpu-m.ac.jp

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
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.

**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.

**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.
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.

References

[1] Maines, M.D.: The heme oxygenase system: a regulator of second messenger gases. Annu. Rev. Pharmacol. Toxicol., 37, 517–554, 1997.
[2] Sassa, S.: Biological implications of heme metabolism. J. Clin. Biochem. Nutr., 38, 138–155, 2006.
[3] Shibahara, S.: Regulation of heme oxygenase gene expression. Semin. Hematol., 25, 370–376, 1988.
[4] Nakao, A., Kaczorowski, D.J., Sugimoto, R., Billiar, T.R., and McCurry, K.R.: Application of heme oxygenase-1, carbon monoxide and biliverdin for the prevention of intestinal ischemia/reperfusion injury. J. Clin. Biochem. Nutr., 42, 78–88, 2008.
[5] Takahashi, T., Shimizu, H., Morimatsu, H., Maeshima, K., Inoue, K., Akagi, R., Matsumi, M., Katayama, H., and Morita, K.: Heme Oxygenase-1 is an essential cytoprotective component in oxidative tissue injury induced by hemorrhagic shock. J. Clin. Biochem. Nutr., 44, 28–40, 2009.
[6] Alam, J., Stewart, D., Touchard, C., Boinanpally, S., Choi, A.M., and Cook, J.L.: Nrf2, a Cap’n’Collar transcription factor, regulates induction of the heme oxygenase-1 gene. J. Biol. Chem., 274, 26071–26078, 1999.
[7] Ishii, T., Itoh, K., Takahashi, S., Sato, H., Yanagawa, T., Katoh, Y., Bannai, S., and Yamamoto, M.: Transcription factor Nrf2 coordinately regulates a group of oxidative stress-inducible genes in macrophages. J. Biol. Chem., 275, 16023–16029, 2000.
[8] Itoh, K., Wakabayashi, N., Katoh, Y., Ishii, T., Igarashi, K., Engel, J.D., and Yamamoto, M.: Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. Genes Dev., 13, 76–86, 1999.
[9] Itoh, K., Wakabayashi, N., Katoh, Y., Ishii, T., O’Connor, T., and Yamamoto, M.: Keap1 regulates both cytoplasmic-nuclear shuttling and degradation of Nrf2 in response to electrophiles. Genes Cells, 8, 379–391, 2003.
[10] Wild, A.C., Moinova, H.R., and Mulcahy, R.T.: Regulation of gamma-glutamylcysteine synthetase subunit gene expression by the transcription factor Nrf2. J. Biol. Chem., 274, 33627–33636, 1999.
[11] Jeong, S.O., Oh, G.S., Ha, H.Y., Soon Koo, B., Sung Kim, H., Kim, Y.C., Kim, E.C., Lee, K.M., Chung, H.T., and Pae, H.O.: Dimethoxycurcumin, a Synthetic Curcumin Analogue, Induces Heme Oxygenase-1 Expression through Nrf2 Activation in RAW264.7 Macrophages. J. Clin. Biochem. Nutr., 44, 79–84, 2009.
[12] Ryter, S.W. and Choi, A.M.: Heme oxygenase-1: redox regulation of a stress protein in lung and cell culture models. Antioxid. Redox Signal, 7, 80–91, 2005.
[13] Sekhar, K.R., Yan, X.X., and Freeman, M.L.: Nrf2 degradation by the ubiquitin proteasome pathway is inhibited by KIAA0132, the human homolog to InNrf2. Oncogene, 21, 6829–6834, 2002.
[14] He, C.H., Gong, P., Hu, B., Stewart, D., Choi, M.E., Choi, A.M., and Alam, J.: Identification of activating transcription factor 4 (ATF4) as an Nrf2-interacting protein. Implication for heme oxygenase-1 gene regulation. J. Biol. Chem., 276, 20858–20865, 2001.
[15] Bloom, D.A. and Jaiswal, A.K.: Phosphorylation of Ser80 of the transcription factor Nrf2 at Ser40 by protein kinase C in response to antioxidants leads to the release of Nrf2 from InNrf2, but is not required for Nrf2 stabilization/accumulation in the nucleus and transcriptional activation of antioxidant response element-mediated NAD(P)H:quinone oxidoreductase-1 gene expression. J. Biol. Chem., 278, 44675–44682, 2003.
[16] Huang, H.C., Nguyen, T., and Pickett, C.B.: Phosphorylation of Nrf2 at Ser-40 by protein kinase C regulates antioxidant response element-mediated transcription. J. Biol. Chem., 277, 42769–42774, 2002.
[17] Numazawa, S., Ishikawa, M., Yoshida, A., Tanaka, S., and Yoshida, T.: Atypical protein kinase C mediates activation of

J. Clin. Biochem. Nutr.
Lansoprazole Induced HO-1

NF-E2-related factor 2 in response to oxidative stress. Am. J. Physiol. Cell Physiol., 285, C334–C342, 2003.

[18] Garnett, W.R.: Lansoprazole: a proton pump inhibitor. Ann. Pharmacother., 30, 1425–1436, 1996.

[19] Langtry, H.D. and Wilde, M.I.: Omeprazole. A review of its use in Helicobacter pylori infection, gastro-oesophageal reflux disease and peptic ulcers induced by nonsteroidal anti-inflammatory drugs. Drugs, 56, 447–486, 1998.

[20] Wolfe, M.M. and Sachs, G.: Acid suppression: optimizing therapy for gastroduodenal ulcer healing, gastroesophageal reflux disease, and stress-related erosive syndrome. Gastroenterology, 118, S9–S31, 2000.

[21] Zimmermann, A.E. and Katona, B.G.: Lansoprazole: a comprehensive review. Pharmacotherapy, 17, 308–326, 1997.

[22] Fellenius, E., Berglindh, T., Sachs, G., Olbe, L., Elander, B., Sjöstrand, S.E., and Wallmark, B.: Substituted benzimidazoles inhibit gastric acid secretion by blocking (H+ + K+) ATPase. Nature, 290, 159–161, 1981.

[23] Yoshida, N., Uchiyama, K., Kuroda, M., Sakuma, K., Kokura, S., Ichikawa, H., Naito, Y., Takemura, T., Yoshikawa, T., and Okanoue, T.: Interleukin-8 expression in the esophageal mucosa of patients with gastroesophageal reflux disease. Scand. J. Gastroenterol., 39, 816–822, 2004.

[24] Suzuki, M., Suzuki, H., and Hibi, T.: Proton pump inhibitors and gastritis. Clin. Biochem. Nutr., 42, 71–75, 2008.

[25] Ichikawa, H., Yoshida, N., Takagi, T., Tomatsuri, N., Katada, K., Isozaki, Y., Uchiyama, K., Naito, Y., Okanoue, T., and Yoshikawa, T.: Lansoprazole ameliorates intestinal mucosal damage induced by ischemia-reperfusion in rats. World J. Gastroenterol., 10, 2814–2817, 2004.

[26] Kuroda, M., Yoshida, N., Ichikawa, H., Takagi, T., Okuda, T., Naito, Y., Okanoue, T., and Yoshikawa, T.: Lansoprazole, a proton pump inhibitor, reduces the severity of indomethacin-induced rat enteritis. Int. J. Mol. Med., 17, 89–93, 2006.

[27] Hendriks, J.J., Kester, A.D., Donckerwolcke, R., Forget, P.P., and Wouters, E.F.: Changes in pulmonary hyperinflation and bronchial hyperresponsiveness following treatment with lansoprazole in children with cystic fibrosis. Pediatr. Pulmonol., 31, 59–66, 2001.

[28] Becker, J.C., Grosser, N., Waltke, C., Schulz, S., Erdmann, K., Domschke, W., Schröder, H., and Pohle, T.: Beyond gastric acid reduction: proton pump inhibitors induce heme oxygenase-1 in gastric and endothelial cells. Biochem. Biophys. Res. Commun., 345, 1014–1021, 2006.

[29] Isomoto, H., Nishi, Y., Kanazawa, Y., Shikuwa, S., Mizuta, Y., Inoue, K., and Kohno, K.: Immune and Inflammatory Responses in GERD and Lansoprazole. Clin. Biochem. Nutr., 41, 84–91, 2007.

[30] Henda, O., Naito, Y., Takagi, T., Shimozawa, M., Kokura, S., Yoshida, N., Matsui, H., Cepinskas, G., Kvietys, P.R., and Yoshikawa, T.: Tumor necrosis factor-alpha-induced cytokine-induced neutrophil chemoattractant-1 (CINC-1) production by rat gastric epithelial cells: role of reactive oxygen species and nuclear factor-kappaB. J. Pharmacol. Exp. Ther., 309, 670–676, 2004.

[31] Yoshida, N., Yoshikawa, T., Tanaka, Y., Fujita, N., Kassai, K., Naito, Y., and Kondo, M.: A new mechanism for anti-inflammatory actions of proton pump inhibitors—hibitory effects on neutrophil-endothelial cell interactions. Aliment. Pharmacol. Ther., 14, S74–S81, 2000.

[32] Naito, Y.: Anti-inflammatory and anti-oxidant properties of proton pump inhibitors. Clin. Biochem. Nutr., 41, 82–83, 2007.

[33] Takagi, T., Naito, Y., Okada, H., Ishii, T., Kajikawa, H., Mizushima, K., Akagiri, S., Adachi, S., Handa, O., Kokura, S., Ichikawa, H., Itoh, K., Yamamoto, M., Matsui, H., and Yoshikawa, T.: Lansoprazole, a proton pump inhibitor, mediates anti-inflammatory effect in gastric mucosal cells through the induction of heme oxygenase-1 via activation of Nrf2 and oxidation of keap1. J. Pharmacol. Exp. Ther., 2009, in preparation.