Review

Heme oxygenase-1: a novel therapeutic target for gastrointestinal diseases

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Heme oxygenase-1 (HO-1) is the rate-limiting enzyme in the catabolism of heme, followed by production of biliverdin, free iron and carbon monoxide (CO). HO-1 is a stress-responsive protein induced by various oxidative agents. Recent studies demonstrate that the expression of HO-1 in response to different inflammatory mediators may contribute to the resolution of inflammation and has protective effects in several organs against oxidative injury. Although the mechanism underlying the anti-inflammatory actions of HO-1 remains poorly defined, both CO and biliverdin/bilirubin have been implicated in this response. In the gastrointestinal tract, HO-1 is shown to be transcriptionally induced in response to oxidative stress, preconditioning and acute inflammation. Recent studies suggest that the induction of HO-1 expression plays a critical protective role in intestinal damage models induced by ischemia-reperfusion, indomethacin, lipopolysaccharide-associated sepsis, trinitrobenzene sulfonic acid, and dextran sulfate sodium, indicating that activation of HO-1 may act as an endogenous defensive mechanism to reduce inflammation and tissue injury in the gastrointestinal tract. In addition, CO derived from HO-1 is shown to be involved in the regulation in gastro-intestinal motility. These *in vitro* and *in vivo* data suggest that HO-1 may be a novel therapeutic target in patients with gastrointestinal diseases.

***Key Words*: Bach1, bilirubin, carbon monoxide, heme oxygenase, indomethacin, Nrf2, ulcerative colitis**

Heme oxygenase (HO) is the rate-limiting enzyme in heme catabolism, a process which leads to the generation of equimolar amounts of biliverdin, free iron and carbon monoxide (CO).[1] Heme oxygenase-1 (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 (NO), and its substrate, heme.[2] Heme oxygenae-2 (HO-2) is a constitutive gene, expressed in neurons, endothelium and many other cell types. Although both HO-1 and HO-2 catalyze the identical biochemical reaction, there are some fundamental differences between the two in genetic origin, primary structure, and molecular weight. HO-1, once expressed under various pathological conditions, has an ability to metabolize high amounts free heme to produce high concentrations of its enzymatic by-products that can influence various biological events, and has recently been the focus of considerable medical interest.[3] HO-1 expression can confer cytoprotection and anti-inflammation in gastric and intestinal disease models. The cytoprotective effects of HO-1 are related to end-products formation. The pharmacological application of CO and biliverdin/bilirubin can mimic the HO-1-dependent cytoprotection and anti-inflammation in many injury models. In this review, we provide a comprehensive overview on the molecular mechanisms underlying the regulation and function of HO-1 and its possible clinical implications, especially in gastrointestinal diseases.

Regulation of HO-1 Expression

The transcriptional upregulation of the *ho-1* gene, and subsequent de novo synthesis of the corresponding protein, occurs in response to elevated levels of its natural substrate heme and to a multiplicity of endogenous factors including NO, cytokines, heavy metals, heat shock, ultraviolet radiation, ischemia-reperfusion, and growth factors.[4,5] Many agents that induce HO-1 are associated with oxidative stress in that they (i) directly or indirectly promote the intracellular generation of reactive oxygen species (ROS), (ii) fall into a class of electrophilic antioxidant compounds that includes plant-derived polyphenolic substances, or (iii) form complexes with intracellular reduced glutathione and other thiols. Two enhancer regions located at approximately –4 and –10 kb relative to the *ho-1* transcriptional start site have been identified in the mouse gene. The dominant sequence element of the enhancers is the stress-responsive elements (StRE), which is structurally and functionally similar to the Maf-response element (MARE) and the antioxidant-response element (ARE).[6] Several transcriptional regulators bind these sequences, including nuclear factor erythroid 2-related factor-2 (Nrf2) and BTB and CNC homolog 1 (Bach1) (Fig. 1). Nrf2 contains a transcription-activation domain and positively regulates HO-1 transcription, whereas Bach1 competes with Nrf2 and represses transcription.[7–9] Under normal conditions, Nrf2 localizes in the cytoplasm, where it interacts with the actin-binding protein, Kelch-like ECH associating protein 1 (Keap1), and is rapidly degraded by the ubiquitin-proteasome pathway, which results in a lower accumulation of Nrf2 in the nucleus and reduced transcription of the HO-1 gene.[10] 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 stress- or antioxidant-response elements (StRE/ARE). 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.[11] Furthermore, it has been demonstrated that the oxidation of Keap1 cysteine residues causes a change in the affinity of Keap1 with Nrf2, easily releasing Nrf2.[12,13] Thus the Nrf2-Keap1 system is considered a major defense mechanism that plays a key role in the induction of HO-1.

Bach1 under baseline condition forms a heterodimer with small maf proteins that represses transcription of the *ho-1* gene by binding to MARE in the 5'-untranslated region of the *ho-1* promoter. Under conditions of excess heme, increased heme binding

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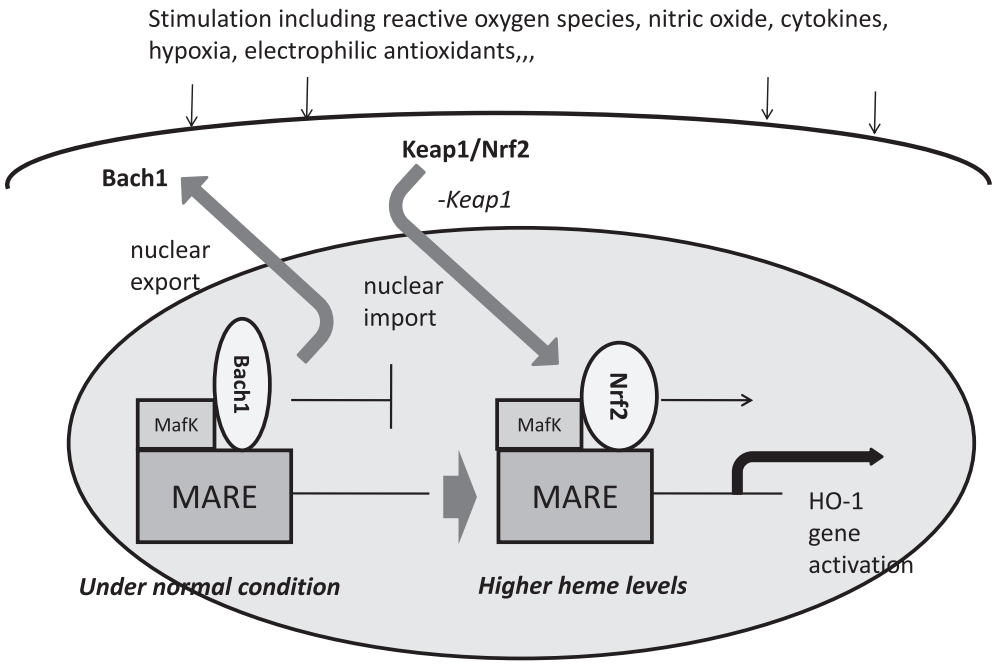

Fig. 1. Model describing the regulation of *ho-1* or other target genes by Bach1 and heme. Besides MafK, other Maf-related factors may also serve as partners for Bach1. Bach1 occupies MARE enhancers to repress transcription of *ho-1* gene under normal conditions. An increase in heme levels alleviates Bach1-mediated repression through inhibition of its DNA-binding activity and subsequent nuclear export, making MAREs available for activating Maf complexes including Nrf2.

to Bach1 causes a conformational change and a decrease in DNA-binding activity followed by nuclear export of Bach1, which in turn leads to transcriptional activation of the *ho-1* gene through MARE. Heme also induced nuclear translocation of Nrf2, a partner molecule for the family, and promotes stabilization of Nrf2. Thus, an intracellular heme concentration displaces Bach1 from the MARE sequences by heme binding, which then permits Nrf2 binding to a member of small maf proteins, ultimately resulting in transcriptional activation of *ho-1* genes.

The mitogen-activated protein kinase (MAPK)-activated signaling pathway was also recognized as able to mediate the induction of HO-1 by extracellular stimuli. The phosphatidylinositol 3 kianse (PI3K)/Akt signaling pathway is also involved in HO-1 regulation.[14] Akt can directly phosphorylate HO-1 protein at Ser-188 and modulate its activity. In addition to the transcriptional regulation, a recent study has shown that HO-1 is subjected to post-translational regulation by the ubiquitin-proteasome system through an ER-associated degradation pathway.[15]

Reaction Products of HO-1 and Their Roles

It is most likely that the many properties including anti-inflammation and cytoprotection afforded by HO-1 may be attributed not only its own action but also to other actions of three by-products of HO-1 activity. Especially in anti-inflammation, the degradation of the pro-oxidant heme by HO-1 itself, the signaling action of CO, the antioxidant properties of biliverdin/bilirubin, and the sequestration of free iron by ferritin could all concertedly contribute to the anti-inflammatory effects observed with HO-1.

Carbon monoxide (CO). CO is known to be an activator of soluble guanylate cyclase (GC). Though CO is a weak activator of GC *in vitro* with much lower potency and efficacy than NO, application of CO to a number of different tissues results in increased cGMP production, activation of type I cGMP-dependent protein kinase and smooth muscle relaxation,[1] suggesting that *in vivo* CO does modulate cGMP levels. The activation of cGMP-dependent protein kinase I is one of the target of CO that acts as smooth muscle relaxation by direct effects on the contractile machinery as well as by altering Ca^{2+} homeostasis and voltage-gated ion channel activity.[16] CO has also reported to activate K^+ channels in a variety of tissues, including gastrointestinal tract. Intracellular cGMP activate K^+ channel and cGMP level is increased by the treatment of exogenous CO. The antiapoptotic potential of CO has been reported. Tumor necrosis factor-α (TNF-α) induced apoptosis in mouse fibroblasts[17] and endothelial cells[18] were inhibited by exogenous CO treatment. This anti-apoptotic effect of CO is reported to depend on p38 MAPK pathway[18] and its upstream MAPK kinase (MKK3).[19] On the other hand, in Jurkatt T cells, CO treatment increased Fas/CD95-induced apoptosis. Furthermore, HO-1 or CO cooperated with NF-κB-dependent antiapoptotic genes to protect against TNF-α-mediated endothelial cell apoptosis.[20] Anti-inflammatory effect of CO has been reported using cell culture and animal models of sepsis.[21] In macrophages, CO inhibited the production of pro-inflammatory cytokines, such as TNF-α, interleukin-1β (IL-1β), and macrophage inflammatory protein-1, through modulation of p38 MAPK activation.[21] In human T cells, CO suppressed IL-2 secretion and clonal expansion via inhibition of ERK pathway.[22] CO also inhibited the expression of pro-inflammatory enzymes, such as inducible NO synthase (iNOS) and cyclo-xygenase-2, in macrophages via the regulation of C/EBP and NF-κB activation.[23] In human colonic epithelial cells, the inhibitory effects of CO on iNOS expression and IL-6 secretion were dependent on the modulation of NF-κB, activator protein-1 (AP-1), C/EBP activation, and MAPK pathway.[24] Our group has recently shown the beneficial effect of CO to colonic epithelial cell restitution.[25] It has been suggested that submucosal myofibroblast has a crucial role of epithelial cell restitution via TGF-β secretion. In our experiments, CO induces fibroblast growth factor-15 (FGF15) expression in mouse colonic myofibroblast via inhibition of mir710, and FGF15 enhances the restitution of mouse colonic epithelial cells.[25]

Biliverdin/bilirubin. Biliverdin and bilirubin both act as

antioxidants *in vitro* and *in vivo*[26–28] and their increased local concentrations after HO induction may be beneficial in protecting several types of cells from injury. Bilirubin can scavenge peroxyl radicals *in vitro* as effectively as α-tocopherol, which is regarded as the most potent antioxidant against lipid peroxidation.[27] Several epidemiological studies indicate that mild to moderately elevated serum bilirubin levels are associated with a better outcome in diseases involving oxidative stress.[29] High plasma bilirubin levels in the general population are correlated with a reduced risk of coronary heart disease.[30] Ossola *et al.*[31,32] demonstrated that the administration of bilirubin completely inhibited HO induction as well as oxidative stress parameters such as glutathione and thiobarbituric acid-reactive substances in rat liver exposed to ultraviolet A and copper sulfate. These results suggest that bilirubin is a major contributor to cytoprotective activities against oxidative stress. Otani *et al.*[33] demonstrated for the first time that oxidative stress in sepsis quickly induced HO-1 in intestinal mucosa and that the bilirubin produced subsequently acted as an antioxidant. They showed that the concentration of bilirubin in the intestinal mucosa increased to slightly more than twice control values at 3 and 5 h after LPS injection, then peaked at 4.3-fold control values at 10 h. Vachharajani *et al.*[34] demonstrated that biliverdin is as effective as hemin in attenuating LPS-induced expression of endothelial selectins in the small and large intestine, indicating that biliverdin itself or its subsequent metabolite bilirubin may be more important than CO production in mediating the beneficial anti-inflammatory effects of HO-1 in a model of LPS-induced selectin upregulation. Hayashi *et al.*[35] showed that the effects of HO-1 induction on leukocyte adhesion could be mimicked by bilirubin, suggesting that this product of HO reaction is an important contributor to the anti-inflammatory effects of HO. However, no report has appeared on the measurement of tissue levels of biliverdin/bilirubin in human gastrointestinal tract, and even experimental models have not clarified the role of the biliverdin-bilirubin pathway in gastrointestinal diseases.

Lessons from HO-1, Nrf2, and Bach1-Deficient Mice

In 1999, Yachie *et al.*[36,37] firstly reported the first case of human HO-1 deficiency. This patient suffered persistent hemolytic anemia and an abnormal coagulation/fibrinolysis system, which were associated with elevated thrombomodulin and von Willebrand factor, indicating persistent endothelial damage. Mice with a HO-1 null mutation have been shown to develop anemia associated with hepatic and renal iron overload[38] and right ventricular infarction after chronic hypoxia exposure.[39] Absence of HO-1 exacerbates ischemia and reperfusion injury,[40,41] atherosclerotic lesion and vascular remoldeling,[42] chronic renovascular hypertension and acute renal failure,[43] and end-organ damage and mortality after lipopolysaccharide injection.[44] These findings provide strong evidence to support that HO-1 has important functions in normal physiology and pathophysiology, especially associated with oxidative stress.

Nrf2 regulates the inducible expression of a group of detoxication enzymes, such as glutathione S-transferase and NAD(P)H: quinone oxidoreductase, via antioxidant response elements. In addition, Ishii *et al.*[45] have shown that Nrf2 also controls the expression of a group of electrophile- and oxidative stress-inducible proteins and activities, which includes HO-1, A170, and peroxiredoxin using peritoneal macrophages from Nrf2-deficient mice. Nrf2-deficient mice have an increased susceptibility to dextran sulfate sodium-induced colitis.[46] It has been reported that anti-inflammatory and anti-oxidative properties with the HO-1 induction by transforming growth factor-b1 or 15-deoxy-$D^{(12,14)}$-prostaglandin J_2 are clearly cancelled in Nrf2-deficient mice.[47,48]

Mice lacking the gene for Bach1 have dramatic increases in HO-1 expression in the heart, lung, liver, and gastro-intestinal tract, indicating a role for Bach1 in tonic suppression of HO-1 transcription. Bach1 deficiency ameliorates lipopolysaccharide-induced hepatic injury,[49] hyperoxic lung injury,[50] myocardial injury induced by ischemia-reperfusion,[51] hypertensive cardiopathy,[52] spinal cord injury,[53] indomethacin-induced intestinal injury,[54] and atherosclerosis in apolipoprotein E.[55] Recently, we have investigated the role of Bach1 in the pathogenesis of indomethacin-induced intestinal injury using Bach1-deficient mice, which are kindly presented from Prof. Igarashi (Tohoku University, Japan).[56] We have shown that the indomethacin-induced intestinal injury is remarkably improved in Bach1-deficient mice (Fig. 2), and that the increased expression of inflammatory chemokines and myeloperoxidase activity in the intestinal mucosa is suppressed in Bach1-deficient mice, respectively.[54] In addition, these beneficial effects observed in Bach1-deficient mice are reversed by the cotreatment with an HO-1 inhibitor, SnPP, indicating that these effects are mediated by the HO-1 activity.

HO-1 Expression in Gastrointestinal Tract

We have investigated the expression of *ho-1* mRNA and HO-1 protein in colon specimens obtained from patients with ulcerative colitis.[57] The expression of *ho-1* mRNA in inflamed colonic mucosa is remarkably increased compared with normal controls. Furthermore, in the inflamed mucosa of active ulcerative colitis, HO-1 protein expression was also increased. These results suggest that the increased expression of HO-1 protein is mainly derived from the increase in transcription of *ho-1* in the inflamed intestine. In the histological study, we have confirmed the expression of HO-1 in inflamed intestinal mucosa and that it was localized in the inflammatory cells, mainly mononuclear cells in the colonic submucosal layer, but not in epithelial cells. Some of the HO-1 positively stained cells were positively stained CD68 cells. Maestrelli *et al.*[58] have reported that the majority of HO-1-positive cells in the alveolar spaces were CD68-positive cells, and Yoshiki *et al.*[59] have reported that HO-1 expression localized in CD68-positive macrophages. Thus, the expression of HO-1 has been observed mainly in macrophages in various organs. However, other reports regarding the localization of HO-1 in human colonic mucosa have described HO-1 expression not only in inflammatory cells but also in the epithelial cells.[60,61]

Recent studies showed that glutamine, the major fuel for enterocytes, induces HO-1 in intestinal mucosa of rats[62] as well as humans.[63] Substantial expression of HO-1 after glutamine administration is observed in villous epithelial cells, crypts and muscular layers. In rats, the protective effect of glutamine on the intestine is associated with HO-1 induction in a model of ischemia-reperfusion injury.[62] In human duodenal mucosa, HO-1 is constitutively expressed in nearly all types of intestinal epithelial cells and approximately 10% of lamina propria cells from the villi core, whereas its expression is minimal in deep mucosa. Glutamine increases intestinal HO-1 expression in both intestinal epithelial cells and lamina propria cells, and this histological finding is correlated with an increase in mRNA levels for HO-1. These data suggest that the modulation of HO-1 expression by glutamine may contribute to its protective effect on intestinal injury, together with the previously reported reduction of proinflammatory cytokines production.[64] Further investigation is required as to whether glutamine may affect HO-1 expression under conditions of intestinal inflammation, including inflammatory bowel disease. As one example, HO-1 mRNA expression was reported to be not affected in pouchitis.[65]

Roles of HO-1 in Gastric Diseases

It has been demonstrated that gastric cytoprotection induced by polaprezinc,[66] eupatilin,[67] and ketamine[68] against noxious

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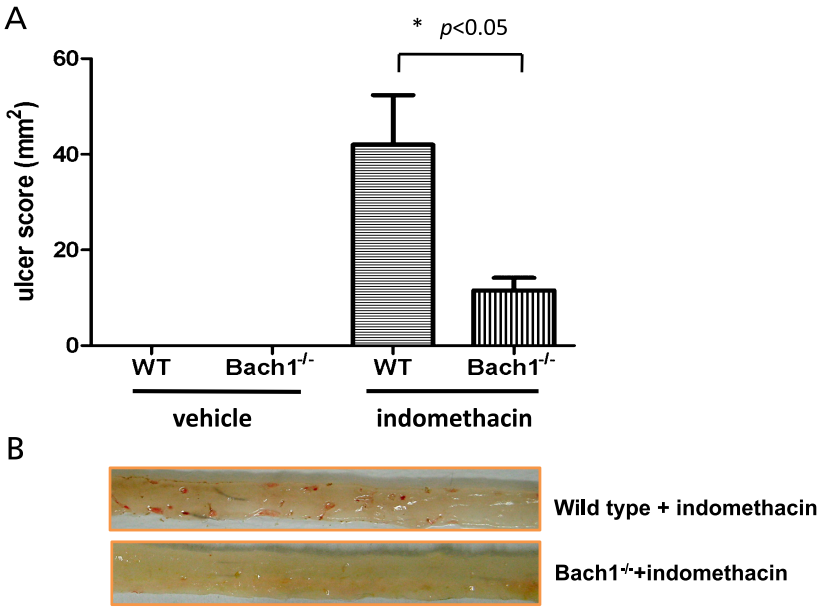

Fig. 2. (A) Effect of Bach1 deficiency on ulcer index in the intestinal mucosa treated with indomethacin. Data are expressed as means ± SEM of five to seven mice. (B) Macroscopic findings of the small intestine in mice treated with indomethacin. The administration of indomethacin provoked multiple erosions in the small intestine in wild type mice. On the other hand, in Bach1-deficient mice, the number and the severity of legions were clearly diminished.

agents is mediated by HO-1 induction (Table 1). Recent our study shows that lansoprazole, a gastric H^+/K^+ ATPase inhibitor, up-regulates HO-1 expression in rat gastric epithelial cells, and the up-regulated HO-1 has anti-inflammatory effects, and that lansoprazole-induced HO-1 induction is mediated by the activation, phosphorylation and nuclear translocation of Nrf2 in accompaniment with the dissection of oxidized Keap1.[69,70] In this study, we firstly demonstrated that oxidation of Keap1 protein is crucial in the induction of HO-1 by lansoprazole.

In addition to cytoprotection by HO-1, it has been shown that HO-1 exerts a modulatory role on gastric smooth muscle excitability via CO production.[71,72] Using a diabetic gastroparesis model, Choi *et al.*[72,73] have demonstrated that Kit expression in interstitial cells of Cajal is lost during diabetic gastroparesis due to increased levels of oxidative stress caused by low levels of HO-1, and that CD206(+) M2 macrophages that express HO1 appear to be required for prevention of diabetes-induced delayed gastric emptying. Because lansoprazole induces HO-1 in macrophages,[74] it should be investigated whether lansoprazole could reverse delayed gastric emptying in diabetic mice via the induction of HO-1.

Roles of HO-1 in Small Intestinal Diseases

Three reports are available on the use of HO-1 inducers (lansoprazole, sulforafane, and Bach1-deficiency) in indomethacin-induced gastric mucosal injury[54,75,76] (Table 1). Higuchi *et al.*[75] have shown that lansoprazole inhibits indomethacin-induced intestinal injury in rats and that the inhibition is reversed by SnPP, an HO-1 inhibitor. Because CORM, a CO donor, also ameliorates these injury, cytoprotective effects of HO-1, in part, exerts via CO-dependent manner. Many reports have confirmed the anti-inflammatory and cytoprotective effects of HO-1 inducers on small intestinal injuries induced by ischemia-reperfusion,[77–83] lipopolysaccharide,[84–86] radiation,[87–89] and burn shock.[90–93] Pang *et al.*[86,94] have used live *Lactococcus lactis* secreting bioactive HO-1 to treat intestinal mucosal injury induced by lipopolysaccharide in rats. Intragastric administration of HO-1-secreting *Lactococcus lactis* strain led to bioactive delivery of HO-1 at intestinal mucosa and significantly decreased mucosal damage, myeloperoxidase activity, bacterial translocation, and tumor necrosis factor-α levels when compared with rats treated with the wild-type strain.

Among products of HO-1, CO may be an important mediator of the host defense response to sepsis.[95] Chung *et al.*[95] have demonstrated that targeting HO-1 to smooth muscle cells and myofibroblasts of blood vessels and bowel ameliorates sepsis-induced death associated with *Enterococcus faecalis* in association with enhancement of bacterial clearance by increasing phagocytosis and the endogenous antimicrobial response, and that injection of a CO-releasing molecule into wild type mice increases phagocytosis and rescues HO-1-deficient mice from sepsis-induced lethality. More interstingly, it has been reported that CO-releasing molecule ameliorates postoperative ileus and muscularis inflammation, and that these protective effects are, at least in part, mediated through induction of HO-1, in a p38-dependent manner, as well as reduction of ERK1/2 activation. These findings shown here may be of significant importance in clinical small bowel transplantation, post-operative condition for small intestine, or sepsis-related intestinal failure.

Role of HO-1 in Large Intestinal Diseases

Wang *et al.*[96] used a rat model of inflammatory bowel disease induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS) to investigate whether the expression of HO-1 is an endogenous mechanism responsible for host defense against inflammatory injury in colonic tissue. They demonstrated that HO activity and HO-1 gene expression increased markedly after TNBS induction, and that administration of tin mesoporphyrin (SnMP), an HO inhibitor, potentiated the colonic damage as well as decreased HO-1 activity. These results indicate that HO-1 plays a protective role in the colonic damage induced by TNBS enema. Using a dextran sulfate sodium (DSS)-induced colitis model of mice, we have demonstrated that HO-1 mRNA is markedly induced in inflamed colonic tissue, whereas HO-2 mRNA is constitutively expressed.[97] Co-administration with ZnPP, an HO inhibitor, also enhanced

Table 1. Induction of heme oxygenase-1 inhibits gastrointestinal diseases

Type of experimental models	HO-1 induction	Mechanism	References
Stomach			
gastroparesis in diabetic mice	CD206(+)/HO1(+) M2 macrophage	protection for Kit cells	Choi *et al.* 2010
HCl-induced acute gastric mucosal lesions	polaprezinc	cytoprotection	Ueda *et al.* 2009
gastric mucosal cells	lansoprazole	anti-inflammatory	Takagi *et al.* 2009
endothelial cells/macrophages	lansoprazole	phosphatidylinositol 3-kinase dependecnt cytoprotection	Schulz-Geske *et al.* 2009
diabetic gastroparesis	hemin	protection for Cajal cells	Choi *et al.* 2008
H_2O_2-induced cell injury	eupatilin	cytoprotection	Choi *et al.* 2008
LPS-induced gastricl injury	ketamine	NF-kappaB ↓, AP-1 ↓, iNOS ↓	Helmer *et al.* 2006
gastric smooth muscle excitability	CoCl2	CO-dependent	Kadinov *et al.* 2002
Small intestine			
Ischemia-reperfusion	ischemic preconditioning		Mallick *et al.* 2010
indomethacin-induced intestinal injury	Bach1$^{-/-}$	inflammatory chemokine ↓, MPO ↓	Harusato *et al.* 2009
indomethacin-induced cell injury	sulforaphane	cytoprotection	Yen *et al.* 2009
indomethacin-induced intestinal injury	lansoprazole	cytoprotection	Higuchi *et al.* 2009
hemorrhagic shock-induced intestinal injury	glutamine	anti-inflammatory, cytoprotection	Umeda *et al.* 2009
postoperative ileus	CORM	p38-dependent pathway ↑, ERK1/2 ↓	De Backer *et al.* 2009
hemorrhagic shock-induced gastric mucosal injury	HO1-secreting Lactococcus lactis	anti-inflammatory, cytoprotection	Pang *et al.* 2009
feline ileal smooth muscle cells	eupatilin	ERK and Nrf2 signaling	Song *et al.* 2008
lipopolysaccharide-induced intestinal injury	HO1-secreting Lactococcus lactis	anti-inflammatory, intestinal barrier ↑	Pang *et al.* 2008
neutrophil-mediated intestinal damage	cobalt protoporphyrin IX chloride	neutrophil O2- production ↓	Li *et al.* 2008
trauma-hemorrhage-induced intestinal injury	estrogen	p38 MAPK-dependent pathway	Hsu *et al.* 2008
sepsis	HO-1 Tg mice	CO-dpendent host defense response ↑	Chung *et al.* 2008
ischemia-reperfusion injury	Cobalt protoporphyrin (CoPP)	cytoprotection	Wasserberg *et al.* 2007
LPS-induced intestinal injury	Intestinal preconditioning	bilirubin-dependent cytoprotection	Tamion *et al.* 2007
burn injury-induced impaired intestinal transit	hemin	iNOS, COX-2, IL-1β ↓	Gan *et al.* 2007
endotoxin-shock model	hemin	anti-inflammatory	Tamion *et al.* 2006
ischemia-reperfusion injury	pyrrolidine dithiocarbamate	leucocyte-endothelial interactions ↓	Mallick *et al.* 2006
radiation-induced intestinal damage	glutamate	NF-kappaB ↓	Giris *et al.* 2006
radiation-induced intestinal damage	octreotide	anti-inflammatory	Abbasoglu *et al.* 2006
ischemia-reperfusion injury	hypothermia	cytoprotection	Sakamoto *et al.* 2005
ischemia-reperfusion injury	hemin	MPO ↓	Attuwaybi *et al.* 2004
impaired intestinal transit after gut I/R	Hypertonic saline	anti-inflammatory	Attuwaybi *et al.* 2004
	hemin	intestinal cell cycle progression	Uc *et al.* 2003
ischemia-reperfusion injury	hypothermia	cytoprotection	Attuwaybi *et al.* 2003
ischemia-reperfusion injury	preconditioning	cytoprotection	Tamion *et al.* 2002
Large intestine			
DSS-induced colitis	hemin (i.p.)	Treg ↑, IL-17 ↓, apoptosis ↓	Zhong *et al.* 2010
DSS-induced colitis	tranilast (enema)	IFN-g ↓, IL-6 ↓	Sun *et al.* 2010
colitis-related colon carcinogenesis	4'-geranyloxy-ferulic acid	modulating proliferation, oxidative stress ↓	Miyamoto *et al.* 2008
TNBS-induced colitis	heme, cadmium chloride	damage ↓, MPO ↓	Varga *et al.* 2007
TNBS-induced colitis	2',4',6'-Tris(methoxymethoxy) chalcone	nuclear translocation of NF-kappaB ↓	Lee *et al.* 2007
TNBS-induced colitis	glutamine	antioxidant, antiapoptotic, anti-inflammatory	Giris *et al.* 2007
TNBS-induced colitis	octreotide	NF-kappaB ↓	Erbil *et al.* 2007
TNBS-induced colitis	gliotoxin	NF-kappaB ↓	Jun *et al.* 2006
DSS-induced colitis	cobalt-protoporphyrin	biliverdin-dependent	Berberat *et al.* 2005
TNBS-induced colitis	CO	anti-inflammatory	Hegazi *et al.* 2005
TNBS-induced colitis	bolinaquinone (BQ) petrosaspongiolide M (PT)	NF-kappaB ↓	Busserolles *et al.* 2005

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intestinal inflammation and increased the disease activity index as determined by a calculated score based on changes in body weight, stool consistency, and intestinal bleeding.

Recent investigation has shown that upregulation of HO-1 by several HO-1 inducers significantly reduces the intestinal injuries induced by DSS[98–100] or TNBS[101–107] (Table 1). In these studies, HO-1 inducers increase HO-1 expression in intestinal mucosa, and ameliorate mucosal injury as well as inflammatory cell accumulation by decreasing infiltrating neutrophils and lymphocytes via the inhibition of NF-κB-dependent proinflammatory cytokines. To further assess the anti-inflammatory mechanisms, Zhong *et al.*[100] have examined whether hemin enhanced the proliferation of Treg cells and suppressed the production of interleukin (IL)-17 in a DSS-colitis model. Flow cytometry analysis has revealed that hemin markedly expands the $CD4^+$ $CD25^+$ $Foxp3^+$ Treg population and attenuates IL-17 and TH17-related cytokines. It has been also demonstrated that HO-1 exerts immunoregulatory effects by modulating Treg cell function,[108] and that HO-1 activity in antigen-presenting cells is important for Treg-mediated suppression, providing an explanation for the apparent defect in immune regulation in HO-1-deficient mice.[109]

Conclusion

The biological significance of HO-1 up-regulation in gastrointestinal inflammation remains to be fully elucidated. However, there is no doubt that CO derived from HO-1 exerts significant effects on many pathways of cellular metabolism. In inflamed intestinal cells CO may inhibit the inflammatory response, by consequently influencing the synthesis of cytokines, expression of adhesion molecules, and cell proliferation. Although the mechanisms underlying HO-1 activity on gene expression are not well known, the results obtained in recent years have demonstrated its importance in modulation of the inflammatory reaction. Recent experimental studies clearly demonstrated that HO-1 expression is a self-defense mechanism against inflammation. These data suggest that HO-1 is a possible therapeutic target in several kinds of gastrointestinal diseases.

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