The role of heme oxygenase and carbon monoxide in inflammatory bowel disease

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Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease, is a chronic and recurrent inflammatory disorder of the intestinal tract. Since the precise pathogenesis of IBD remains unclear, it is important to investigate the pathogenesis of IBD and to evaluate new anti-inflammatory strategies. Recent evidence suggests that heme oxygenase-1 (HO-1) plays a critical protective role during the development of intestinal inflammation. In fact, it has been demonstrated that the activation of HO-1 may act as an endogenous defensive mechanism to reduce inflammation and tissue injury in various animal intestinal injury models induced by ischemia-reperfusion, indomethacin, lipopolysaccharide-associated sepsis, trinitrobenzene sulfonic acid or dextran sulfate sodium. In addition, carbon monoxide (CO) derived from HO-1 has been shown to be involved in the regulation of intestinal inflammation. Furthermore, administration of a low concentration of exogenous CO has a protective effect against intestinal inflammation. These data suggest that HO-1 and CO may be novel therapeutic molecules for patients with gastrointestinal inflammatory diseases. In this review, we present what is currently known regarding the role of HO-1 and CO in intestinal inflammation.

Keywords: heme oxygenase, carbon monoxide, inflammatory bowel disease

Introduction

Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease, is a chronic and recurrent intestinal inflammatory disorder. The incidence of IBD is rapidly increasing in Japan as well as in western countries.¹ While certain features of IBD suggest several possible causes, including familial, genetic, infectious, physiological, and immunological factors,^{2,3} the precise pathogenesis of IBD remains unknown. Therefore, it is important to investigate the

pathogenesis of IBD and to evaluate new anti-inflammatory strategies.

Recent clinical and basic science studies have revealed that heme oxygenase (HO) is involved in the pathophysiology of intestinal inflammation.⁴ HO is the rate-limiting enzyme in heme degradation that leads to the generation of biliverdin, free iron and carbon monoxide (CO).⁴⁻⁶ Heme oxygenase-1 (HO-1) is one of three mammalian HO isozymes and is a stress-responsive protein induced by various stimuli including oxidative stress, heat shock, UV radiation, ischemia-reperfusion (I/R), heavy metals, lipopolysaccharide (LPS), cytokines, nitric oxide, and heme (its substrate).^{6,7} Heme oxygenase-2 (HO-2) is constitutively expressed in neurons, endothelia and many other cell types. Heme oxygenase-3 (HO-3) is also involved in heme degradation, but is a less efficient heme catalyst than the other isozymes. Regarding the

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regulation of HO-1 induction, several transcriptional regulators have been shown to be involved in regulation of the expression of HO-1, including nuclear factor erythroid 2-related factor-2 (Nrf2) and the BTB and CNC homolog 1 (Bach1). Nrf2 contains a transcription-activation domain and positively regulates HO-1 transcription, whereas Bach1 competes with Nrf2 and represses transcription.^{8,9}

Recently, the inducible form of HO, HO-1, has been shown to exert potent cellular protective effects due to its anti-inflammatory, anti-apoptotic and antioxidant activities in various settings.^{10,11} Although the beneficial roles of HO-1 are not completely understood, these cytoprotective and anti-inflammatory effects of HO-1 are related to formation of its end-products. Thus, while there is accumulating evidence that the induction of endogenous HO-1 expression can confer cytoprotective and anti-inflammatory effects in various intestinal disease models, the pharmacological application of HO-1 end-products, in particular CO, can mimic HO-1-dependent cytoprotection and anti-inflammation in many injury models. In fact, exogenous CO suppresses the inflammatory response associated with various disease states such as hyperoxia,¹² organ transplantation, I/R injury,^{13–17} and inflammation.^{18,19} Administration of exogenous CO during intestinal inflammation also inhibits the severity of the disease.^{20–22} This article provides an overview of the recent advances concerning the role of HO-1 and its by-product, CO, in intestinal inflammation.

Distribution of HO expression in the gastrointestinal tract

Under normal conditions, HO-1 is expressed at very low levels in the gastrointestinal tract, but its expression is induced by disease, injury and/or inflammation. We investigated the expression of HO-1 mRNA and HO-1 protein in colon specimens obtained from patients with UC.^{23,24} The expression of HO-1 mRNA in inflamed colonic mucosa is strongly increased compared to normal controls. Furthermore, HO-1 protein expression is also increased in the inflamed mucosa of active UC. These results suggest that the increased expression of the HO-1 protein is mainly due to increased transcription of the *ho-1* gene in the inflamed intestine. We confirmed the expression of HO-1 in inflamed intestinal mucosa using histological studies. These studies also indicated that HO-1 is localized in inflammatory cells, mainly mononuclear cells, in the colonic submucosal layer,

but not in epithelial cells. Some of the HO-1 positively stained cells were also stained with anti-CD68. Maestrelli *et al.*²⁵ reported that the majority of HO-1-positive cells in the alveolar spaces are CD68-positive, and Yoshiki *et al.*²⁶ reported that HO-1 expression is localized to CD68-positive macrophages. Thus, the expression of HO-1 has been mainly observed in macrophages in various organs. However, other investigations of the localization of HO-1 in human colonic mucosa have reported that HO-1 is expressed not only in inflammatory cells, but also in epithelial cells.^{27,28}

Regarding HO-2 expression, it has been demonstrated that HO-2 is localized in neuronal cell bodies and fibers in deep muscular plexuses.²⁹ Neurons in the pyloric and ileocecal sphincters have been reported to show particularly high levels of HO-2.³⁰ Non-neuronal cells in the gastrointestinal tract also express HO-2, including populations of cells in the mucosal epithelium, the smooth muscle and endothelium of blood vessels and in the interstitial cells of Cajal.^{31–34}

The role of HO-1 in the small intestine

Many reports have confirmed the anti-inflammatory and cytoprotective effects of HO-1 inducers on small intestinal injuries induced by I/R,^{35–43} indomethacin,^{44,45} LPS,^{46–50} radiation,^{51,52} hemorrhagic shock,^{53–57} or burns^{58,59} (Table 1).

Induction of endogenous HO-1 using various pharmacological approaches can ameliorate intestinal I/R injuries. Treatment with hemin or cobalt protoporphyrin, a substrate and an inducer of HO-1, respectively, resulted in amelioration of I/R injuries in association with the induction of HO-1 expression. Likewise, hemoglobin also induced HO-1 in the intestine and reduced intestinal inflammation, which correlated with the suppression of tumor necrosis factor (TNF)- α production.⁵⁵ Glutamine, the major metabolic fuel for enterocytes, has a protective effect on the intestine, which is associated with HO-1 induction in various intestinal injury models.^{42,50,52,57,60} The induction of HO-1 by glutamine is observed in villous epithelial cells, in crypts and in muscular layers in rats as well as in humans.⁶¹

Non-pharmacological induction of HO-1 is also known to inhibit intestinal injuries. Ischemia preconditioning (IPC) leads to a phenomenon by which pretreatment with brief periods of ischemia reduces subsequent intestinal I/R injuries. Several studies have reported that IPC induces intestinal HO-1 expression.^{38,39,49,56} Although the precise mechanism

Table 1 Therapeutic efficacy of heme oxygenase-1 (HO-1) induction in intestinal diseases using *in vivo* animal models

Type of experimental model	HO-1 induction method	Animal	Reference
Small intestine			
Ischemia-reperfusion (I/R) intestinal injury	Ischemic preconditioning (IPC)	Rat	Mallick <i>et al.</i> (2010) ³⁸
	Cobalt protoporphyrin (CoPP)	Rat	Wasserberg <i>et al.</i> (2007) ⁴³
	Ischemic preconditioning (IPC)	Rat	Mallick <i>et al.</i> (2005) ⁴⁰
	Pyrrolidine dithiocarbamate (PDTTC)	Rat	Mallick <i>et al.</i> (2005) ³⁹
	Hyperthermia	Rat	Sakamoto <i>et al.</i> (2005) ⁴¹
	Hemin	Rat	Attuwaybi <i>et al.</i> (2004) ³⁷
	Hypertonic saline	Rat	Attuwaybi <i>et al.</i> (2004) ³⁵
	Hypothermia	Rat	Attuwaybi <i>et al.</i> (2003) ³⁶
	Glutamine	Rat	Tamaki <i>et al.</i> (1999) ⁴²
	Glutamine	Rat	Umeda <i>et al.</i> (2009) ⁵⁷
Hemorrhagic shock-induced intestinal injury	HO-1-secreting <i>L. lactis</i>	Rat	Pang <i>et al.</i> (2009) ⁵⁴
	Estrogen	Rat	Hsu <i>et al.</i> (2008) ⁵³
	Intestinal preconditioning (IPC)	Rat	Tamion <i>et al.</i> (2002) ⁵⁶
	Hemoglobin	Rat	Tamion <i>et al.</i> (2001) ⁵⁵
	HO-1-secreting <i>L. lactis</i>	Rat	Pang <i>et al.</i> (2008) ⁴⁶
Lipopolysaccharide-induced intestinal injury	Intestinal preconditioning (IPC)	Rat	Tamion <i>et al.</i> (2007) ⁴⁹
	Hemin	Rat	Tamion <i>et al.</i> (2006) ⁴⁸
	Glutamine	Rat	Uehara <i>et al.</i> (2005) ⁵⁰
	Heme	Rat	Ping <i>et al.</i> (2000) ⁴⁷
	Glutamine	Rat	Giris <i>et al.</i> (2006) ⁵²
Radiation-induced intestinal injury	Octreotide	Rat	Abbasoglu <i>et al.</i> (2006) ⁵¹
Indomethacin-induced intestinal injury	Bach 1-deficient	Mouse	Harusato <i>et al.</i> (2009) ⁴⁴
	Lansoprazole	Rat	Higuchi <i>et al.</i> (2009) ⁴⁵
Others			
Postoperative ileus	CO-releasing molecule (CORM)	Mouse	De Backer <i>et al.</i> (2009) ⁹⁴
Ethanol- and burn-induced intestinal injury	Cobalt protoporphyrin (CoPP)	Rat	Li <i>et al.</i> (2008) ⁵⁸
Burn injury-induced impaired intestinal transit	Hemin	Rat	Gan <i>et al.</i> (2007) ⁵⁹
Large intestine			
DSS-induced colitis	Hemin	Mouse	Zhong <i>et al.</i> (2010) ⁶⁷
	Tranilast	Mouse	Sun <i>et al.</i> (2010) ⁶⁶
	Cobalt protoporphyrin (CoPP)	Mouse	Paul <i>et al.</i> (2005) ²⁸
	Cobalt protoporphyrin (CoPP)	Mouse	Berberat <i>et al.</i> (2005) ⁶⁵
	5-Aminosalicylic acid	Rat	Horvath <i>et al.</i> (2008) ⁶⁹
TNBS-induced colitis	Heme, cadmium	Rat	Varga <i>et al.</i> (2007) ⁷²
	Chalcone	Mouse	Lee <i>et al.</i> (2007) ⁷¹
	Glutamine	Rat	Giris <i>et al.</i> (2007) ⁶⁰
	Octreotide	Rat	Erbil <i>et al.</i> (2007) ⁶⁸
	Glutathione	Mouse	Jun <i>et al.</i> (2006) ⁷⁰
	Carbon monoxide (CO)	Mouse	Hegazi <i>et al.</i> (2005) ²⁰
IL-10 deficient (genetic colitis model)	Carbon monoxide (CO)	Mouse	Hegazi <i>et al.</i> (2005) ²⁰

that underlies the protective effect of IPC is unclear, induction of HO-1 may contribute to this protective effect.

Sakamoto *et al.*⁴¹ demonstrated that whole-body hyperthermia induces the production of HO-1 in intestinal mucosa and inhibits intestinal I/R injuries. On the other hand, Attuwaybi *et al.*³⁶ have shown that hypothermia induced the production of HO-1 and inhibited intestinal I/R injuries. In both studies, the administration of HO-1 inhibitors abrogated these protective effects. Regarding indomethacin-induced intestinal mucosal injury, Higuchi *et al.*⁴⁵ showed that lansoprazole inhibits indomethacin-induced intestinal injury in rats and that this inhibition is reversed by tin-protoporphyrin, an HO-1 inhibitor. We have also investigated the role of HO-1 in indomethacin-induced intestinal mucosal injury using mice lacking the gene for

Bach1. These mice have dramatic increases in HO-1 expression in the heart, lung, liver, and gastrointestinal tract. Indomethacin-induced intestinal injury is remarkably reduced in Bach1-deficient mice, and the indomethacin-induced increase in expression of inflammatory chemokines and myeloperoxidase activity in the intestinal mucosa is also suppressed in Bach1-deficient mice.⁴⁴

Pang *et al.*^{46,54} used live *Lactococcus lactis*, which secrete bioactive HO-1, to treat intestinal mucosal injury induced by lipopolysaccharide in rats. Intragastric administration of the HO-1-secreting *L. lactis* strain led to delivery of bioactive HO-1 to the intestinal mucosa and significantly decreased mucosal damage, myeloperoxidase activity, bacterial translocation, and TNF- α levels when compared with rats treated with the wild-type strain.

Role of HO-1 in large intestinal diseases

Wang *et al.*⁶² used a rat model of inflammatory bowel disease induced by TNBS to investigate whether the expression of HO-1 is an endogenous host defense mechanism against inflammatory injury in colonic tissue. They demonstrated that HO activity and HO-1 gene expression strongly increased after TNBS treatment, and that administration of tin-mesoporphyrin, an HO inhibitor, potentiated this colonic damage and also decreased HO-1 activity. These results indicate that HO-1 plays a protective role in the colonic damage induced by a 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.⁶³ Co-administration of DSS with zinc-mesoporphyrin, an HO inhibitor, also enhanced intestinal inflammation and increased the disease activity index as determined by a score that was calculated based on changes in body weight, stool consistency and intestinal bleeding. In that study, it was shown that, during the inflammatory condition induced by DSS in mice, HO inhibition significantly increased TNF- α and interferon (IFN)- γ in the colonic mucosa and decreased interleukin (IL)-4 and IL-10 levels, whereas it did not do so under physiological conditions. These data indicated that HO may play a role in regulating the balance between Th1 and Th2 cytokines in inflamed intestinal mucosa.⁶⁴

Recent investigation has shown that up-regulation of HO-1 by several HO-1 inducers significantly reduces the colitis induced by DSS^{28,65–67} or TNBS^{60,68–72} (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 inhibition of the production of NF- κ B-dependent pro-inflammatory cytokines. To assess further these HO-1 anti-inflammatory mechanisms, Zhong *et al.*⁶⁷ investigated whether hemin enhances the proliferation of Treg cells and suppresses the production of IL-17 in a DSS-colitis model. Flow cytometric analysis revealed that hemin markedly expands the CD4⁺CD25⁺Foxp3⁺ Treg population and attenuates the production of IL-17. It also has been demonstrated that HO-1 exerts immunoregulatory effects by modulating Treg cell function,⁷³ and that HO-1 activity in antigen-presenting cells is important for suppression mediated by Tregs. These data provide an explanation for the apparent defect in immune regulation in HO-1-

deficient mice.⁷⁴ Taken together, these findings indicate that HO-1 is an inducible protein that is responsible for host defense against intestinal inflammation and which may be a novel therapeutic molecule for IBD.

In this respect, it has been shown that 5-aminosalicylate (5-ASA), which is a conventional agent for the treatment of IBD, has the ability to induce HO-1.⁶⁹ However, since 5-ASA exerts anti-inflammatory effects through various other functional activities including by scavenging reactive oxygen species (ROS) and by inhibiting NF- κ B as well as T-cell proliferation, its therapeutic importance as a HO-1 inducer remains unclear. Interestingly, recent investigations have demonstrated that treatment of UC patients with 5-ASA compounds can prevent UC-associated colon carcinogenesis.⁷⁵ This result suggests that at least one of the agents that are used for IBD treatment can provide an excellent therapeutic effect by inducing HO-1 expression. In the near future, we anticipate the development of novel IBD therapeutic strategies that are based on the regulation of HO-1 expression.

Mechanism of CO action

CO is an invisible, chemically inert, colorless and odorless gas. It is a major product of the incomplete combustion of carbon and carbon-containing compounds. CO is widely known to be toxic at high concentrations, because CO has a higher binding affinity for hemoglobin than oxygen, resulting in interference with the oxygen-carrying capacity of the blood and consequent tissue hypoxia. As described above, CO is also endogenously and physiologically generated in mammalian cells via the catabolism of heme in the rate-limiting step of HO catalysis.⁵ It has been reported that endogenous CO regulates neurotransmission, smooth muscle tone and response to cellular injury, and also shows cellular protective effects such as anti-apoptotic and anti-oxidant actions against oxidative stress.⁷⁶

The strong affinity of CO for the heme moiety of heme-containing proteins is important for CO effects. Thus, CO binds to the heme moiety of mitochondrial cytochrome-*c* oxidase, thereby substantially decreasing mitochondria-derived ROS.⁷⁷ Additionally, this inhibition of mitochondria-derived ROS can result in an overall decrease in ROS generation and trigger adaptive responses and cell survival. These effects constitute a novel mechanism to explain redox signaling by CO.^{78,79} Breakdown of cytochrome P450

(CYP), which constitutes a large group of heme proteins, is induced during organ injury induced by various insults. Recently, Nakao *et al.*⁸⁰ demonstrated that CO, present in the organ preservation solution used during kidney transplantation, can bind to and stabilize renal CYP and prevent CYP degradation and detrimental heme release in renal grafts. This effect of CO results in potent protection from transplantation-induced tissue injury.

Endogenous CO is also known to be an activator of soluble guanylate cyclase (sGC).⁸¹ Although CO is a weak activator of sGC *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,^{5,82} suggesting that CO does modulate cGMP levels *in vivo*. The cGMP-dependent protein kinase I is one of the targets of CO, and CO-activation of its kinase activity results in smooth muscle relaxation due to direct effects of the kinase on the contractile machinery as well as by altering Ca²⁺ homeostasis and voltage-gated ion channel activity.⁸³ CO also has been reported to activate K⁺ channels in a variety of tissues, including the gastrointestinal tract. This activation is due to the fact that exogenous CO increases the level of cGMP, and intracellular cGMP activates K⁺ channels.⁸⁴

The anti-apoptotic effect of CO was first reported using an *in vitro* model that assayed apoptosis initiated by TNF- α . Brouard *et al.*⁸⁵ reported that CO inhibits TNF- α -initiated apoptosis in mouse endothelial cells through activation of the p38 MAPK pathway. Furthermore, HO-1-derived CO induces the synthesis

of NF- κ B-dependent anti-apoptotic genes, which protect against TNF- α -mediated endothelial cell apoptosis.⁸⁶ Pretreatment with low doses of CO has been reported to show anti-apoptotic effects in several models of disease and tissue injury. Exogenously applied CO inhibits I/R-induced apoptosis associated with CO-dependent activation of p38 MAPK and the upstream MAPK kinase (MKK3), and with the suppression of ERK and JNK activation.⁸⁷

Anti-inflammatory effects of CO have been reported using cell culture and animal models of sepsis.⁸⁸ CO administration or HO-1 overexpression in RAW 264.7 cells inhibits TNF- α expression after treatment with LPS. One of the mechanisms that underlie these findings is reportedly that CO treatment inhibits LPS-induced activation of NF- κ B by preventing the phosphorylation and degradation of the inhibitory subunit I- κ B α . This proposed mechanism has been associated with CO/HO-1 modulation of granulocyte-macrophage colony stimulating factor (GM-CSF).⁸⁹ GM-CSF is a glycoprotein that reportedly enhances the secretion of pro-inflammatory cytokines, including TNF- α , IL-1 and IFN- γ .⁹⁰ In several inflammatory models, CO has been reported to inhibit GM-CSF expression, resulting in attenuation of inflammation.

Role of CO in the small intestine

Potent therapeutic effects of CO have been demonstrated using experimental models of many conditions, including lung hyperoxia,¹² heart, hepatic, renal, and intestinal I/R injury,^{13–16,80} and inflammation.^{20–22} These

Table 2 Therapeutic application of carbon monoxide (CO) in intestinal diseases using *in vivo* animal models

Type of experimental models	Application of CO	Animal	Reference
Small intestine			
Lipopolysaccharide-induced intestinal injury	CO intraperitoneal administration (continuous)	Rat	Liu <i>et al.</i> (2010) ⁹¹
Lipopolysaccharide-induced intestinal injury	CO intraperitoneal administration (single)	Rat	Liu <i>et al.</i> (2010) ⁹²
Ischemia-reperfusion intestinal injury	CO-releasing molecule (CORM)	Mouse	Katada <i>et al.</i> (2010) ¹¹⁰
Postoperative ileus	CO-releasing molecule (CORM)	Mouse	De Backer <i>et al.</i> (2009) ⁹⁴
Thermal intestinal injury	CO-releasing molecule (CORM)	Mouse	Liu <i>et al.</i> (2008) ¹¹¹
Thermal intestinal injury	CO-releasing molecule (CORM)	Mouse	Sun <i>et al.</i> (2007) ¹⁰⁴
Lipopolysaccharide-induced intestinal injury	CO inhalation	Rat	Liu <i>et al.</i> (2007) ⁹³
Intestinal transplantation	CO-bubbled solution	Rat	Nakao <i>et al.</i> (2006) ¹⁰⁰
Postoperative ileus	CO-bubbled solution	Mouse	Nakao <i>et al.</i> (2006) ⁹⁷
Postoperative ileus	CO inhalation	Rat, swine	Moore <i>et al.</i> (2005) ⁹⁶
Postoperative ileus	CO inhalation	Mouse	Moore <i>et al.</i> (2003) ⁹⁵
Intestinal transplantation	CO inhalation	Rat	Nakao <i>et al.</i> (2003) ⁹⁹
Intestinal transplantation	CO inhalation	Rat	Nakao <i>et al.</i> (2003) ⁹⁸
Intestinal transplantation	CO inhalation	Rat	Nakao <i>et al.</i> (2003) ¹⁵
Large intestine			
TNBS-induced colitis	CO inhalation	Mouse	Takagi <i>et al.</i> (2010) ²¹
IL-10 deficient (genetic colitis model)	CO inhalation	Mouse	Hegazi <i>et al.</i> (2005) ²⁰
Necrotizing enterocolitis	CO inhalation	Rat	Zuckerbraun <i>et al.</i> (2005) ²²

effects support the new paradigm that, at low concentrations, CO functions as a signaling molecule that exerts significant cytoprotective and anti-inflammatory actions.

Similar to the effects observed with HO-1, CO has been reported to mediate potent cytoprotective and anti-inflammatory effects against intestinal I/R injuries, LPS-induced intestinal injury,^{91–93} or post-operative ileus^{94–97} (Table 2). Nakao *et al.*^{15,98,99} have shown the efficacy of CO gas inhalation for the prevention of cold intestinal I/R injury using a small intestinal transplantation model. Treatment of rats with CO at a concentration of 250 ppm added 1 h before and 24 h after reperfusion inhibited intestinal injuries associated with the suppression of inflammatory cytokines. Interestingly, they also reported that CO treatment exerted anti-apoptotic activities in endothelial and intestinal epithelial cells. This anti-apoptotic activity involved down-regulation of the pro-apoptotic molecule, Bax, and up-regulation of the anti-apoptotic molecule, Bcl-2.¹⁵

Furthermore, Nakao *et al.*¹⁰⁰ have shown that cold storage of tissues in a preservation solution that was bubbled with 5% CO significantly reduced intestinal I/R injury. They also reported that a single intraperitoneal dose of a solution containing dissolved CO ameliorated murine postoperative ileus.⁹⁷

Recently, transitional metal carbonyls, termed CO-releasing molecules (CORMs), have been used in biological systems to deliver CO in a controlled manner, while keeping carboxyhemoglobin levels stable.^{101,102} CORM-released CO has also been found to inhibit various inflammatory states, including those that occur following damage to the heart,¹⁰³ kidney,^{104,105} or liver^{106,107} following thermal lung injury¹⁰⁸ or pancreatitis,¹⁰⁹ as well as those following thermal or intestinal I/R injury^{108,110,111} or in the postoperative ileus.⁹⁴

Role of CO in the large intestine

Previous studies have shown that cigarette smoking has protective effects against the development of UC.^{112–114} The detailed mechanisms of this protective effect remain unclear. However, CO, one of the components of cigarette smoke, reportedly ameliorates colonic inflammation. Therefore, CO may be a potent therapeutic molecule for intestinal inflammation.

We reported that CO inhalation ameliorates TNBS-induced colitis in mice.²¹ CO treatment decreased the macroscopic damage score, inhibited the production

of thiobarbituric acid (TBA)-reactive substances, which are used as an index of lipid peroxidation, and decreased tissue-associated myeloperoxidase (MPO) activity, which is an index of neutrophil infiltration into colonic mucosa. Furthermore, the expression of TNF- α in colonic mucosa and TNF- α production by CD4⁺ T-cells isolated from the spleen were significantly inhibited. These results are consistent with those of previous studies. Heagzi *et al.*²⁰ reported that CO administration ameliorates chronic intestinal inflammation in IL-10-deficient mice, which is a well-established model of spontaneous (and T helper (Th) 1-mediated) colitis. They also reported that CO altered IFN- γ signalling in macrophages, and decreased IFN regulatory factor (IRF)-8 and IL-12 p40 expression. Zuckerbraun *et al.*²² also demonstrated that inhaled CO protects against the development of intestinal inflammation in a model of experimental necrotizing enterocolitis, and CO also abrogated elevated serum TNF- α levels after the induction of colitis.

Promotion of colonic mucosal healing and reduction of mucosal inflammation are important therapeutic strategies for controlling the pathogenesis of IBD.¹¹⁵ Effects of CO on colonic epithelial cell restitution have been reported.¹¹⁶ That study showed that CO induces FGF15 expression in mouse colonic myofibroblasts via inhibition of mir710, and the induced FGF15 then enhances the restitution of mouse colonic epithelial cells.

Although these findings suggest that CO is a possible therapeutic agent for IBD that might have beneficial anti-inflammatory effects, as well as be of benefit for colonic mucosal healing, further studies are needed to clarify the newly reported effects of CO on IBD. Currently, there are several on-going human clinical trials that are testing the potential therapeutic effects of inhaled CO for various pathophysiological disease states. In these trials, CO is being administered at concentrations similar to those used in animal transplantation models.^{117,118} Interestingly, the inhalation of CO by patients with chronic obstructive pulmonary disease (COPD) was shown to be feasible and led to anti-inflammatory effects.

Conclusions

As highlighted above, the ability of HO-1 and CO to induce anti-inflammatory and tissue-protective effects is in no doubt. Although further studies are warranted to unravel the mechanisms involved in these phenomena in more detail, we anticipate the development of novel IBD therapeutic strategies that

are based on the anti-inflammatory and tissue-protective activities of HO-1 and CO.

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