

# The Therapeutic Potential of Carbon Monoxide for Inflammatory Bowel Disease

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## Key Words

Carbon monoxide · Heme oxygenase · Inflammatory bowel disease · Ulcerative colitis · Crohn's disease

## Abstract

Inflammatory bowel disease (IBD), encompassing ulcerative colitis and Crohn's disease, are chronic, relapsing and remitting inflammatory disorders of the intestinal tract. Because the precise pathogenesis of IBD remains unclear, it is important to investigate the pathogenesis of IBD and to evaluate new anti-inflammatory strategies. Recent accumulating evidence has suggested that carbon monoxide (CO) may act as an endogenous defensive gaseous molecule to reduce inflammation and tissue injury in various organ injury models, including intestinal inflammation. Furthermore, exogenous CO administration at low concentrations is protective against intestinal inflammation. These data suggest that CO may be a novel therapeutic molecule in patients with IBD. In this review, we present what is currently known regarding the therapeutic potential of CO in intestinal inflammation.

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## Introduction

Inflammatory bowel disease (IBD), encompassing ulcerative colitis (UC) and Crohn's disease (CD), is a chronic, relapsing and remitting inflammatory disorder of the intestinal tract. The incidence of IBD is rapidly increasing in eastern countries, including Japan, as well as in North America and Europe [1]. Although the cause of IBD has been extensively investigated over the last few decades, the pathogenesis of IBD is not fully elucidated. Recent research has suggested that a complex interplay between the host genotypes, the immune system and intestinal microbiota plays a crucial role in understanding IBD pathogenesis [2]. At present, the major challenges in the management of IBD include the rapid induction of remission and the prevention of relapse. Medical management, such as the administration of 5-aminosalicylic acid, corticosteroids, immunosuppressive agents and biologic agents, such as anti-tumor necrosis factor (TNF)- $\alpha$  agents, of patients with acute exacerbations of IBD focuses on achieving remission by inhibiting intestinal inflammation and repairing mucosal injury [3]. However, some patients

with IBD do not respond or respond incompletely to these treatments. Therefore, it is important to investigate new anti-inflammatory strategies.

Carbon monoxide (CO), which is an invisible, colorless and odorless gas, is a major product of the incomplete combustion of carbon and carbon-containing compounds. CO is widely known to be a toxic gas because CO avidly binds to hemoglobin with a higher affinity than oxygen and forms carboxyhemoglobin, resulting in interference with the oxygen-carrying capacity of the blood and consequent tissue hypoxia. However, it is well known that CO is endogenously produced. Endogenous CO is one of the three products of heme degradation by heme oxygenase (HO), with the other two being  $\text{Fe}^{2+}$  and biliverdin [4, 5].

Recently, the inducible form of HO, HO-1, has been shown to exert potent cellular protective effects due to its anti-inflammatory, antiapoptotic and antioxidant actions in various settings [6]. Regarding intestinal inflammation, our previous report has shown that HO-1 mRNA and protein are overexpressed in mainly infiltrative inflammatory cells in the intestinal mucosa of patients with active UC, and this upregulated HO-1 might lead to an anti-inflammatory effect [7]. Wang et al. [8] have reported that HO expression increases considerably in rat 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis, which is a well-accepted model of IBD. The authors demonstrated that the inhibition of HO-1 activity exacerbates experimental colitis, indicating that HO-1 plays an important protective role in intestinal inflammation. We have also described the importance of HO-1 in intestinal inflammation by focusing on the BTB and CNC homolog 1 (Bach1), which is a transcriptional repressor of HO-1. Bach1-deficient mice exhibit dramatic increases in HO-1 expression in the gastrointestinal tract, and TNBS-induced colonic inflammation is remarkably attenuated in Bach1-deficient mice, indicating that HO-1 has an important anti-inflammatory function in intestinal inflammation [9, 10].

Although the beneficial roles of HO-1 are not completely understood, these cytoprotective and anti-inflammatory effects of HO-1 are related to the formation of its end product. The pharmacological application of CO can especially mimic the HO-1-dependent cytoprotection and anti-inflammatory effects in many injury models, including colitis models [11]. Hence, CO might be a novel and important molecule in the treatment of intestinal inflammation [12]. This article provides a short overview of the recent advances concerning the therapeutic potential of CO for intestinal inflammation.

## Molecular Mechanism of CO Action

As mentioned above, in addition to the inhalation of exogenous gas, a significant amount of CO is endogenously and physiologically produced in mammalian cells through heme degradation in a rate-limiting step by microsomal HO [4, 5]. For CO action, it is critical for CO to bind the heme moiety and activate heme-containing proteins, such as hemoglobin, myoglobin, cytochrome c [13], cytochrome P450 (CYP) [14], soluble guanylate cyclase (sGC) [15], catalase, NADPH oxidase and the transcription factor neuronal PAS domain protein 2 [16]. The interactions of CO with heme-containing proteins underlie various cellular events that are conducted through the production of mitochondrial reactive oxygen species, such as the promotion of autophagy [17], the inhibition of cytochrome c oxidase leading to a decreased reactive oxygen species generation [18], and the triggering of adaptive responses and cell survival.

The breakdown of CYP, which constitutes a large group of heme proteins, is induced during organ injury by various insults. It has been reported that CO in the organ preservation solution that is used during kidney transplantation can bind to and stabilize renal CYP and prevent CYP degradation and detrimental heme release in renal grafts [19]. In addition, it has been reported that CO-bound red blood cells reduce hepatic ischemia-reperfusion (I-R) injury through the inhibition of CYP destruction [20].

Although CO is a weak activator of sGC in vitro, with a much lower potency and efficacy than NO, endogenous CO is also known to bind and activate sGC [15]. The treatment of different tissues with CO increases cyclic guanosine monophosphate (cGMP) production, the activation of type-I cGMP-dependent protein kinase, and smooth muscle relaxation [5], suggesting that in vivo CO modulates cGMP levels. The activation of cGMP-dependent protein kinase I is one of the targets of CO, resulting in smooth muscle relaxation through direct effects on the contractile machinery as well as by altering  $\text{Ca}^{2+}$  homeostasis and voltage-gated ion channel activity [21]. CO has also been reported to activate  $\text{K}^+$  channels in a variety of tissues [22], including the gastrointestinal tract, because intracellular cGMP activates  $\text{K}^+$  channels and cGMP levels are increased by treatments with exogenous CO [23].

The antiapoptotic effects of CO have been reported in in vitro models of TNF- $\alpha$ -initiated apoptosis. For example, Brouard et al. [24] have shown that TNF- $\alpha$ -initiated apoptosis is inhibited by CO in mouse endothelial cells through the activation of the p38 MAPK pathway. Fur-

**Table 1.** Therapeutic application of CO in intestinal inflammation using in vivo animal colitis models

Type of experimental models	Application of CO	Animal	First author [ref.], year
DSS-induced colitis	CO inhalation (250 ppm)	mouse	Joe [47], 2014
DSS-induced colitis	CO-releasing molecule (CORM-2)	mouse	Uddin [48], 2013
DSS-induced colitis	CO-releasing molecule (CORM-3)	mouse	Fukuda [45], 2014
IL-10-deficient (genetic colitis model)	CO-releasing molecule (ALF186)	mouse	Onyiah [49], 2013
TNBS-induced colitis	CO colonic insufflation (200 ppm)	rat	Takagi [51], 2012
TCR- $\alpha$ -deficient (genetic colitis model)	CO inhalation (250 ppm)	mouse	Sheikh [40], 2011
DSS-induced colitis	CO-releasing molecule (CORM-2)	mouse	Takagi [46], 2011
TNBS-induced colitis	CO inhalation (200 ppm)	mouse	Takagi [41], 2010
IL-10-deficient (genetic colitis model)	CO inhalation (250 ppm)	mouse	Hegazi [39], 2005
Necrotizing enterocolitis	CO inhalation (250 ppm)	rat	Zuckerbraun [42], 2005

thermore, HO-1-derived CO induced NF- $\kappa$ B-dependent antiapoptotic genes to protect against TNF- $\alpha$ -mediated endothelial cell apoptosis [25]. Low-dose pretreatment with CO has been reported to show antiapoptotic effects in several models of disease and tissue injury. Exogenously applied CO inhibited the I-R-induced apoptosis that is associated with the CO-dependent activation of p38 MAPK and the upstream MAPK kinase, and with the suppression of ERK and JNK activation [26]. Recently, CO has been found to inhibit cellular apoptosis through the direct prevention of mitochondrial membrane permeabilization [27, 28].

Anti-inflammatory effects of CO have been observed in cell cultures and animal models of sepsis [29, 30]. TNF- $\alpha$  expression after treatment with lipopolysaccharide is inhibited by CO administration or HO-1 overexpression in RAW264.7 cells. The mechanisms by which CO treatment inhibits the lipopolysaccharide-induced activation of NF- $\kappa$ B have been found to occur by preventing the phosphorylation and degradation of the inhibitory subunit I- $\kappa$ B $\alpha$ , and this mechanism has been associated with granulocyte macrophage colony-stimulating factor (GM-CSF) modulation [31]. GM-CSF is a glycoprotein that reportedly enhances the secretion of proinflammatory cytokines, including TNF- $\alpha$ , interleukin (IL)-1 and interferon (IFN)- $\gamma$  [32]. In several inflammatory models, CO has been shown to inhibit GM-CSF expression, resulting in an attenuation of inflammation.

### Therapeutic Effects of CO in IBD

Numerous previous studies have shown that individuals who smoke are more likely to be protected against the development of UC and are less likely to require colecto-

mies [33]. In CD, smoking is positively correlated with the development of intestinal inflammation. Interestingly, some studies have established a higher rate of ileal complication and a lower prevalence of colonic complication in CD patients who are smokers [34]. These findings suggest that smoking may provide a protective effect to the large intestine. The detailed mechanisms by which smoking exerts these effects remain unclear, but CO, which is one of the components of cigarette smoke, reportedly ameliorates intestinal inflammation. Therefore, CO may be a potent therapeutic molecule in intestinal inflammation.

Potent therapeutic efficacies of CO have been demonstrated in experimental models of several conditions, including lung injuries [35], heart, hepatic and renal I-R injuries [19, 36, 37], as well as inflammation, including arthritis [38], supporting the new paradigm that CO at low concentrations functions as a signaling molecule that exerts significant cytoprotection and anti-inflammatory actions. Similar to what has been observed for the therapeutic effects of CO against various diseases, CO has been reported to mediate potent cytoprotective and anti-inflammatory effects in in vivo colitis models (table 1).

Hegazi et al. [39] reported that CO administration ameliorates chronic intestinal inflammation in IL-10-deficient mice, which is a well-established model of spontaneously developing and T helper 1-mediated colitis. The authors have reported that CO alters IFN- $\gamma$  signaling in macrophages and decreases IFN regulatory factor-8 and IL-12 p40 expression. Sheikh et al. [40] have demonstrated that CO exposure ameliorates chronic T helper 2-mediated colitis in T cell receptor- $\alpha$ -deficient mice and that this CO-mediated amelioration of colitis was associated with increased IL-10 and IL-22 production. We have also reported that CO inhalation ameliorates TNBS-induced

colitis in mice [41]. In that study, the macroscopic damage score, thiobarbituric acid-reactive substances, which are used as an index of lipid peroxidation, and tissue-associated myeloperoxidase activity, which is used as an index of neutrophil infiltration in colonic mucosa, were inhibited by CO treatment. Furthermore, the expression of TNF- $\alpha$  in colonic mucosa and TNF- $\alpha$  production by CD4+ T cells that were isolated from the spleen were significantly inhibited. Because TNF- $\alpha$  has been reported to play a key role in the pathogenesis of IBD and TNF- $\alpha$ -blocking agents have been used as therapeutic agents for treating IBD worldwide [3], our observation that CO regulates TNF- $\alpha$  production may fit newer IBD therapeutic strategies. Zuckerbraun et al. [42] have also demonstrated that inhaled CO protected against the development of intestinal inflammation in a model of experimental necrotizing enterocolitis, and that CO abrogated increased levels of serum TNF- $\alpha$  after the induction of colitis. Thus, CO leads to the inhibition of intestinal inflammation through the regulation of the production of various cytokines.

Recently, transitional metal carbonyls, which are called CO-releasing molecules (CORMs), have been used in biological systems to deliver CO in a controlled manner while keeping carboxyhemoglobin levels stable [43]. CORM-released CO has also been found to inhibit various inflammatory states, including intestinal I-R injury [44]. For the effects of CORMs in colitis models, we have described that CORMs inhibited the development of murine dextran sodium sulfate (DSS)- and TNBS-induced colitis through the inhibition of KC (a functional homolog of human IL-8) and IL-17A production [45, 46]. Recent studies using CORMs have suggested another molecular mechanism by which CO exerts the regulation of intestinal inflammation. It has been demonstrated that CO regulates tristetraprolin, which is an ARE-binding protein that promotes the degradation of a number of inflammatory mediators [47]. In addition, Uddin et al. [48] have demonstrated that CO inhibits glycogen synthase kinase-3 signaling, which is a constitutively active serine/threonine protein kinase mediating NF- $\kappa$ B activity. Interestingly, Onyiah et al. [49] have shown that CORM-derived CO ameliorates intestinal inflammation in IL-10-deficient mice in part by augmenting clearance of enteric microbes that breach the epithelial barrier. Furthermore, the authors found that CO promoted bacterial clearance through the enhancement of the bactericidal activity in macrophages. These data may indicate pleiotropic effects of CO.

The promotion of colonic mucosal healing and the reduction of mucosal inflammation are important therapeutic strategies for controlling the pathogenesis of IBD.

Recently, we have demonstrated that CO promotes colonic epithelial cell restitution through the activation of colonic submucosal myofibroblasts [50]. In brief, CO induces FGF15 expression in mouse colonic myofibroblasts through the inhibition of miR-710, and FGF15 enhances the restitution of mouse colonic epithelial cells. These findings suggest CO as a possible therapeutic agent for IBD through its anti-inflammatory effects and colonic mucosal healing.

Regarding the route of CO application, we have reported that CO gas insufflation into the colonic lumen inhibits TNBS-induced colitis [51]. Importantly, blood CO concentrations were not increased after CO insufflation into the colonic lumen, indicating that the rectal administration of CO gas might be a safe and realistic route for its clinical application in contrast with the inhalation of CO gas, which was highly toxic due to the high blood CO concentration.

## Conclusion

As highlighted above, CO has the ability to produce anti-inflammatory and tissue-protective effects. Although further studies are needed to clarify these novel effects of CO on IBD, CO may have great potential as a new therapeutic molecule for treating IBD.

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