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J. Cell. Mol. Med. Vol 10, No 3, 2006 pp. 672-686

Role of carbon monoxide in cardiovascular function

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Received: May 4, 2006; Accepted: August 7, 2006

Abstract

Carbon monoxide (CO) is an endogenously derived gas formed from the breakdown of heme by the enzyme heme oxygenase. Although long considered an insignificant and potentially toxic waste product of heme catabolism, CO is now recognized as a key signaling molecule that regulates numerous cardiovascular functions. Interestingly, alterations in CO synthesis are associated with many cardiovascular disorders, including atherosclerosis, septic shock, hypertension, metabolic syndrome, and ischemia-reperfusion injury. Significantly, restoration of physiologic CO levels exerts a beneficial effect in many of these settings, suggesting a crucial role for CO in maintaining cardiovascular homeostasis. In this review, we outline the actions of CO in the cardiovascular system and highlight this gas as a potential therapeutic target in treating a multitude of cardiovascular disorders.

Keywords: carbon monoxide • heme oxygenase-1 • vascular smooth muscle • endothelium • atherosclerosis • restenosis • ischemia-reperfusion • blood pressure

Introduction

Carbon monoxide (CO) is a colorless, odorless, and tasteless diatomic gas that has long been considered a toxic byproduct of environmental and industrial processes. The toxic effect of CO is well known and resides in its strong affinity for hemoglobin, which is nearly 245 times that of oxygen [1]. In addition, partial occupation of CO at the heme binding sites inhibits the release of O_2 from the remaining heme groups, shifting the O_2 dissociation curve to the left. These actions of CO reduce the O_2 carrying capacity and delivery potential leading to tissue hypoxia. Higher concentrations of CO also bind to cytochromes P450,

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Reprinted from:
Journal of Cellular and Molecular Medicine
doi:10.2755/jcmm010.003.11

cytochrome c, and myoglobin further amplifying the detrimental actions of CO [2]. Paradoxically, studies in the mid 20th century reported that CO is also generated endogenously in humans, and that under specific pathophysiological conditions CO production is greatly increased [3, 4]. The daily production of CO in the human body is quite substantial; approaching nearly 20 μmoles/hour [5]. The predominant biological source of CO (> 86%) is from the degradation of heme by the enzyme heme oxygenase (HO) with minor amounts formed by photo-oxidation, lipid peroxidation, and xenobiotic metabolism [4]. HO cleaves the α-meso carbon bridge of heme yielding equimolar amounts of biliverdin, iron, and CO (Fig. 1). This oxidative reaction serves as the first and rate-limiting step in heme catabolism and is catalyzed by two distinct isoforms of HO: HO-1 is a ubiquitously distributed isoform that is strongly induced by biochemical and biophysical stress while HO-2 is constitutively expressed and concentrated in specific organs such as the brain and testes [6]. HO-1 and HO-2 are expressed in the heart and blood vessels, and both proteins are found in vascular endothelium and smooth muscle. Moreover, HO is catalytically active in cardiovascular tissue as reflected by the HO-1-mediated production of bilirubin and CO [see 7].

Although long considered an obscure byproduct of heme metabolism with potential toxicological implications, the finding that another structurally similar poisonous gas, nitric oxide (NO) plays a significant role in human health, raised the possibility that CO may also serve an important physiological function [8]. Studies in the past decade have clearly established the biological significance of CO in numerous organ systems. In fact, many of the cytoprotective actions resulting from the induction of HO-1 are attributable to the generation of CO. In addition, studies employing the exogenous application of CO have confirmed the protective properties of this gas in several pathological conditions. However, emerging evidence suggests that in some instances an overproduction of CO may have deleterious effects. In this article, we will focus on the effects of CO in the cardiovascular system and emphasize the potential therapeutic approaches that target this gas in treating specific cardiovascular diseases.

CO in atherosclerosis and vascular injury

Atherosclerosis and its cardiovascular complications are the major cause of morbidity and mortality of the industrialized world. Considerable evidence suggests that the HO-1/CO system plays a beneficial role in this disorder. HO-1 is highly expressed in the endothelium and foam cells of atherosclerotic lesions in both humans and animals [9]. Moreover, oxidized low density lipoprotein, a major determinant in the pathogenesis of atherosclerosis, is a potent inducer of HO-1 in vascular cells [10]. In addition, the first human case of HO-1 deficiency displayed early atherosclerotic changes in the vasculature as reflected by the presence of fatty streaks and fibrous plaque [11, 12]. Interestingly, a long (GT)*n* microsatellite polymorphism in the human HO-1 promoter that is linked to reduced expression is associated with susceptibility to coronary artery disease in some patient populations, suggesting that the induction of HO-1 is a protective response in humans [13–15]. Several animal models of atherosclerosis have also identified HO-1 as an important modulator of atherosclerosis. Inhibition of HO enzyme activity increases lesion formation in Watanabe heritable hyperlipidemic rabbits and low density lipoprotein (LDL)-receptor knockout mice fed a high fat diet [16, 17]. Alternatively, pharmacological induction of HO-1 or adenovirus-mediated gene transfer of HO-1 decreases lesion formation in murine models of atherosclerosis whereas the inhibition of HO-1 promotes lesion development [18]. In addition, transgenic mice deficient in HO-1 in an apolipoprotein E null background exhibit accelerated and more advanced atherosclerotic lesion formation in response to a western diet compared to control animals, despite similar elevations in total plasma cholesterol levels [19].

A role for CO in promoting the anti-atherogenic property of this enzyme was recently established in models of transplant atherosclerosis. Continuous exposure to a relatively low concentration of CO (250 ppm) for 56 days retards the development of atherosclerotic lesions following the transplantation of aortic segments of Brown Norway rats into Lewis rats [20]. CO exposure significantly reduces intimal hyperplasia as well as the accumulation of leukocytes in the adventitia of aortas transplanted

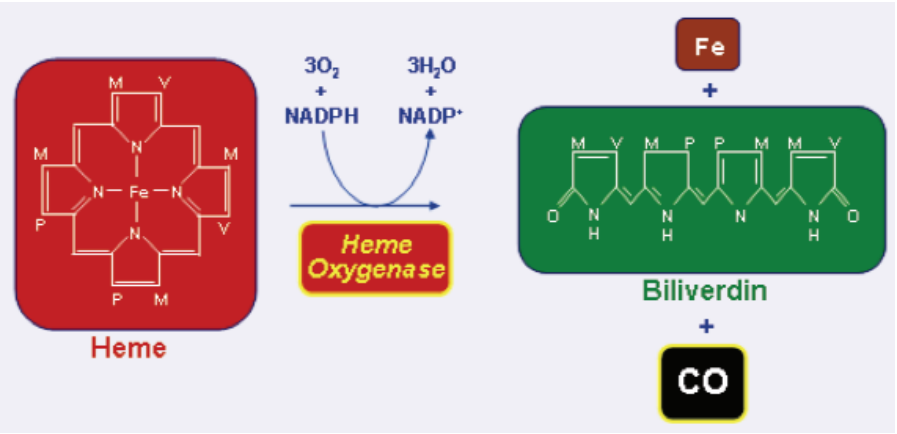

Fig. 1 Heme oxygenase degrades heme to equimolar amounts of iron, biliverdin, and carbon monoxide. (M, methyl (CH_3); P, propionate (CH_2-CH_2-COOH); Fe, iron; V, vinyl ($CH{=}CH_2$; CO, carbon monoxide).

into recipients. The ability of CO to inhibit graft infiltration by activated leukocytes is dose-dependent and associated with diminished expression of pro-inflammatory genes, including the major histocompatibility class II antigens and intracellular adhesion molecule-1. The capacity of CO to block transplant atherosclerosis may explain its ability to improve both allograft and xenograft survival following organ transplantation [21–23]. While CO can protect against transplant atherosclerosis and rejection, it is not known whether it can influence the development of atherosclerosis in genetic and dietary animal models.

Recent work also indicates that the HO-1/CO system exerts a salutary effect on the pathologic remodeling response following balloon angioplasty. Several studies have demonstrated that prior induction of HO-1 by hemin attenuates vascular neointima formation following balloon injury of rat carotid arteries, while inhibition of HO activity exacerbates lesion formation [24–26]. In addition, localized adenovirus-mediated HO-1 gene delivery immediately following arterial injury ameliorates neointima formation in rat carotid and pig femoral arteries [27, 28]. Moreover, HO-1 deficient mice exhibit exaggerated neointima formation following wire induced arterial injury and robust SMC proliferation in a murine model of vein graft stenosis [19, 27]. Finally, it appears that HO-1 may also modulate the vascular response to injury in humans since a HO-1 promoter polymorphism connected to impaired inducibility is associated with enhanced restenosis in patients undergoing percutaneous transluminal angioplasty in femoropopliteal arteries and with angiographic restenosis and adverse cardiac events after coronary stenting [29, 30].

More recently, CO has been directly demonstrated to modify the vascular response to injury. Inhalation of CO (250 ppm) for one hour prior to balloon injury of rat carotid arteries is sufficient to block intimal thickening by approximately 60% [20]. Similarly, we found that incubation of vessel segments with a saturated solution of CO (~875 μM) immediately after balloon injury leads to a marked decrease in neointima formation [31]. CO does not alter the negative inward remodeling response following injury since the circumferential length of both the inner and outer elastic laminae is unaffected. However, arteries transiently exposed to CO demonstrate significantly reduced DNA synthesis leading to a diminished intimal cell population. While CO clearly inhibits lesion formation following arterial injury in rats, additional studies examining whether CO can blunt the remodeling response in larger, non-rodent species are needed to further validate the therapeutic potential of this gas.

Several potential mechanisms may contribute to the vasoprotective actions of CO (Fig. 2). Since excessive vascular smooth muscle cell (SMC) proliferation following endovascular injury is a major determinant of neointima formation, the ability of CO to inhibit SMC growth is highly relevant. Inhibition of CO synthesis or CO scavenging with hemoglobin promotes the growth of SMC while delivery of CO attenuates cell growth in response to several mitogens [20, 25, 27, 32, 33]. The antiproliferative action of CO is mediated by the soluble guanylate cyclase/cGMP pathway since inhibitors of guanylate cyclase or protein kinase G restores SMC growth [20, 27]. Flow cytometry studies demonstrate that HO-1 overexpression or the exogenous administration of CO arrests cultured

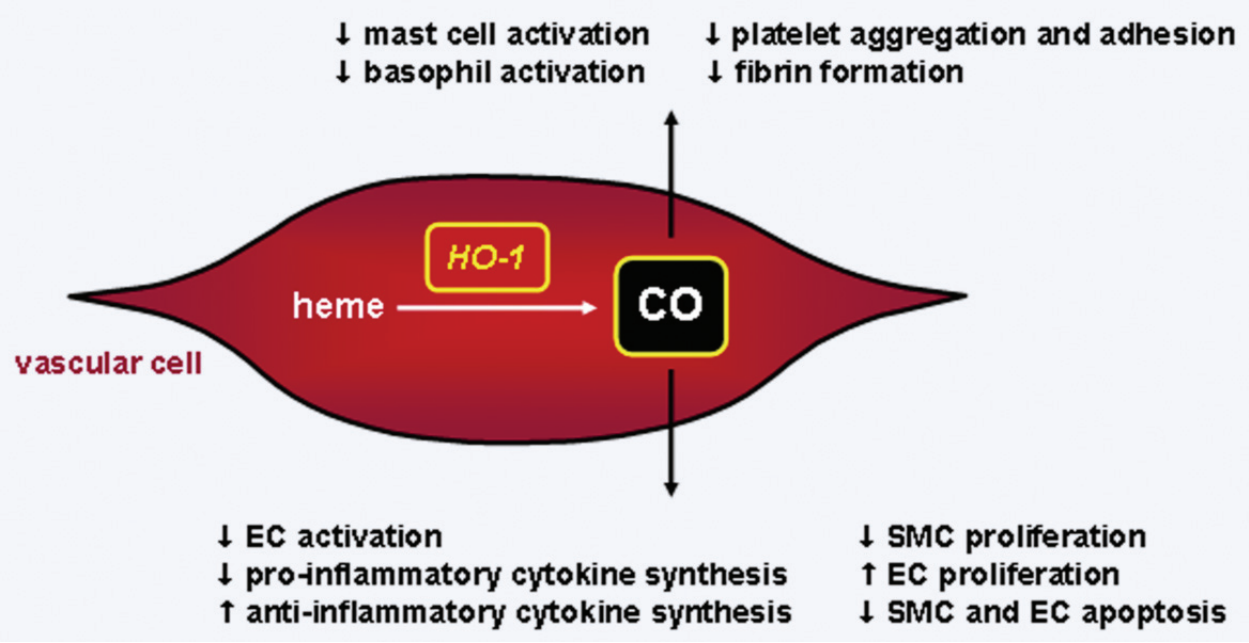

Fig. 2 Vasoprotective actions of heme oxygenase-1 (HO-1)-derived carbon monoxide (CO). CO preserves vascular homeostasis via multiple mechanisms. CO inhibits smooth muscle cell (SMC) growth, endothelial cell (EC) activation, SMC and EC apoptosis, and the generation of pro-inflammatory cytokines, and stimulates the synthesis of anti-inflammatory cytokines. In addition, CO blocks the activation of mast cells and basophils, the aggregation and adhesion of platelets, and the formation of fibrin.

SMC in the G_0/G_1 phase of the cell cycle [20, 27, 32]. This inhibition of cell cycle progression is associated with a marked decrease in cyclin-dependent kinase 2 (cdk2) activity, a critical event required for S-phase entry and DNA synthesis [32]. The ability of CO to block cdk2 activity is likely mediated *via* its ability to modulate the expression of key regulatory proteins. In particular, CO suppresses the expression of the cdk2 activators, cyclin A and D1, while stimulating the expression of the cdk2 inhibitor, p21 [20, 32, 34]. Consistent with these *in vitro* findings, we recently reported that CO-treated vessels display reduced expression of the G1 cyclins, cyclin E and A [28].

Aside from activating soluble guanylate cyclase, CO modulates the activity of other mitogenic signaling pathways. CO blocks the phosphorylation and activation of the mitogen-activated protein kinase (MAPK), ERK1/2, *via* its inhibitory effect on the mitochondrial respiratory chain [34]. Interestingly, CO also blocks NAD(P)H oxidase and this contributes to the inhibition of cyclin D1 expression [34], raising the possibility that CO-mediated alterations in redox signaling may underlie some of the antiproliferative actions of this gas. Furthermore, CO stimulates p38 MAPK activity in a cGMP-dependent manner and this has been implicated in the suppression of cyclin A and D1 expression along with the induction of p21 [17]. More recently, the activation of p38 MAPK by CO was shown to increase caveolin-1 expression in cultured SMC and in neointimal lesions of injured arteries [35]. Significantly, genetic depletion of caveolin-1 abolishes the antiproliferative effect of CO. Collectively, these findings suggest that CO inhibits SMC growth *via* multiple, overlapping signaling pathways that converge to arrest SMC in the G_0/G_1 phase of the cell cycle.

Interestingly, HO-1 stimulates cell cycle progression and proliferation in vascular endothelium [see 36]. Transduction of the HO-1 gene into endothelial cells (EC) promotes their growth and the development of capillary-like tube structures while inhibition of HO activity blocks cell growth, and tube formation. In addition, overexpression of HO-1 facilitates angiogenesis in human pancreatic cancer and in a rat model of hindlimb ischemia [37, 38]. The mechanism(s) by which HO-1 is able

to stimulate the growth of vascular endothelium and angiogenesis is not known, but may be related to the production of vascular endothelial growth factor [36]. Recently, evidence for a role of CO in promoting endothelial growth and capillary sprouting has been provided [39, 40]. However, additional studies are needed to directly determine whether CO influences angiogenesis and the underlying mechanism(s) of action. The ability of CO to stimulate EC regrowth at sites of arterial injury would provide another mechanism to limit lesion formation since the reendothelialization of the vessel wall is believed to maintain the underlying SMC in a quiescent state.

In addition to regulating vascular cell growth, CO modulates cell survival. The exogenous application of CO inhibits apoptosis in both SMCs and ECs [41–44]. The anti-apoptotic actions of CO are mediated *via* several discrete mechanisms. CO blocks the cytokine-mediated mitochondrial release of cytochrome c in vascular cells and this is associated with a decrease in the expression and activity of the pro-apoptotic proteins p53 and Bid, respectively. In addition, CO suppresses the expression of Fas/Fas ligand and the downstream activation of caspase 8 by cytokines. A role for heat shock protein-70 has also been implicated in the anti-apoptotic actions of CO [45]. More recently, we found that CO also inhibits SMC apoptosis during endoplasmic reticulum stress and this is associated with decreased expression of the pro-apoptotic transcription factor, GADD153 [43]. The capacity of CO to block the death receptor (extrinsic), mitochondrial (intrinsic), and endoplasmic reticulum pathways of apoptosis likely contributes to its ability to inhibit apoptosis in numerous pathological conditions. In the vasculature, inhibition of apoptosis by CO may suppress plaque formation by preventing the development of the acellular lipid necrotic core.

Another major mechanism by which CO exerts an anti-atherogenic effect is by arresting inflammation. CO inhibits the lipopolysaccharide-mediated expression of pro-inflammatory cytokines such as tumor necrosis factor-α, interleukin-1β and macrophage inflammatory protein-1β while simultaneously increasing the expression of the anti-inflammatory cytokine interleukin-10 in both EC and macrophages [46]. Furthermore, CO downregulates the inflammatory response by blocking the release of NO from inducible NO synthase and the expression of granulocyte-macrophage colony stimulating factor from macrophages and SMC [47, 48]. Both the activation of soluble guanylate cyclase and p38 MAPK have been implicated in suppression of inflammatory cytokines by CO [46, 47]. Other anti-inflammatory actions of CO include desensitization of the adhesive response of leukocytes following endotoxin administration, the inhibition of histamine release from mast cells, and the prevention of immunological activation of basophils [49, 50]. Thus, CO is able to control the inflammatory response in a multifold manner in several distinct cell types.

Emerging studies indicate that CO may also exert important protection against thrombosis. Both endogenously derived and exogenously applied CO inhibits platelet aggregation by stimulating the activation of soluble guanylate cyclase [51, 52]. In addition, CO mitigates platelet adhesion to venular endothelium in response to inflammation [49]. Furthermore, CO inhibits platelet aggregation and thrombosis following organ transplantation, and may contribute to the inhibition of platelet-dependent thrombosis following the induction of HO-1 in a rodent artery injury model [21, 53]. Significantly, inhalation of CO rescues mice from lethal ischemic injury by preventing microvascular thrombosis and the accumulation of fibrin [54]. This protection is driven by the activation of soluble guanylate cyclase and the suppression of plasminogen activator inhibitor-1 expression. These findings suggest that CO may play a fundamental role in preserving blood fluidity under various inimical conditions by inhibiting platelet activation and fibrin formation.

However, not all studies show a cytoprotective role for CO in the vasculature. Acute exposure of bovine pulmonary aortic EC to CO induces apoptosis [55]. This toxic effect is believed to arise from the CO-mediated increase in peroxynitrite formation secondary to the displacement of NO from heme proteins. In addition, high concentrations of CO induce oxidative stress [see 2]. CO can bind to mitochondrial heme proteins and disrupt the mitochondrial electron transport chain leading to the generation of superoxide. Furthermore, CO has been associated with the conversion of xanthine dehydrogenase to xanthine oxidase leading to the generation of reactive oxygen species. Thus, under certain conditions, CO may adversely affect vascular viability and function by stimulating nitrosative and/or oxidative stress.

CO and myocardial ischemia-reperfusion injury

Considerable evidence supports a protective role for the HO-1/CO system against coronary artery ischemia-reperfusion injury. Pharmacological induction of HO-1 significantly reduces infarct size and the incidence of reperfusion arrythmias following myocardial ischemia-reperfusion, whereas cardiac tissue damage is exacerbated by HO inhibitors [56–58]. Similarly, cardiac-specific overexpression of HO-1 protects against ischemia-reperfusion-induced cardiac dysfunction and apoptosis in isolated-perfused heart preparations [59, 60]. In addition, isolated hearts from heterozygote HO-1 knockout mice demonstrate an increased susceptibility to ischemia-reperfusion injury relative to wild type controls [61]. A maladaptive response consisting of enhanced ventricular dilatation, infarction, and thrombosis has also been reported in HO-1 null mice during hypoxia [62]. Finally, gene delivery of HO-1 by adeno-associated virus several weeks in advance of coronary ligation leads to marked myocardial protection in a rat model of acute ischemia-reperfusion injury [63]. Moreover, the pre-emptive delivery of HO-1 inhibits postmyocardial infarct remodeling and restores ventricular function following ischemia-reperfusion [64].

Recent studies indicate that CO can also confer cytoprotective actions in the heart. Treatment of isolated cardiac cells or hearts with a CO-releasing molecule preserves cell viability and myocardial performance against hypoxia-reoxygenation damage [65]. Similarly, the administration of a CO-releasing compound at the time of reperfusion reduces infarct size in an *in vivo* murine model of coronary occlusion [66]. Interestingly, CO causes the heart to shift to a preconditioned phenotype. Mice receiving a short infusion of CO are protected against myocardial infarction for up to 72 hours, which is equivalent to the protection afforded by ischemic preconditioning [67]. Inhalation of CO also protects against myocardial ischemia-reperfusion injury in rats, safeguards the heart during reperfusion after cardiopulmonary bypass in pigs, and attenuates ischemia-reperfusion injury following cardiac transplantation [68–70]. Interestingly, a recent report found that CO exerts a biphasic effect on cardiac performance following ischemia-reperfusion in isolated-perfused rat hearts [71]. While very low concentrations of CO (0.001–0.01%) in the perfusion buffer improves post-ischemic recovery of hemodynamic parameters and reduces infarct size and ventricular fibrillation, a higher concentration of CO (0.1%) led to severe ventricular fibrillation. Thus, the cardioprotection mediated by CO may be strictly related to the concentration of the gas that is used.

CO and blood pressure regulation

Studies in the past decade have established that CO is an important regulator of vasomotor tone. Exogenously administered CO relaxes isolated vessels from numerous tissues and animal species [see 72]. Furthermore, infusion of CO dilates resistance vessels in several organs, including liver, heart, kidney, and lung. The CO-induced vasodilation is reversible, not a consequence of tissue hypoxia, and is independent of any adrenergic effect or the presence of the endothelium. Similar to NO, CO relaxes numerous vascular tissues by activating soluble guanylate cyclase in vascular smooth muscle leading to the production of cGMP. However, unlike NO, which forms a pentacoordinate complex with the heme moiety of the enzyme, CO forms a hexacoordinate complex and this likely contributes to the lower potency of CO for soluble guanylate cyclase activation and vasodilation [73]. Although cGMP appears to play a major role in CO-induced dilation in large vessels such as the aorta, CO promotes relaxation in resistance vessels by stimulating calcium-activated potassium channels in vascular SMC. This mode of action by CO was first identified in rat tail arteries, where CO was shown to directly interact with histidine residues in the channel to increase their open probability [74] and has subsequently been extended to include renal interlobular arteries and porcine cerebral arterioles [see 72].

However, dilation in response to CO is not a universal finding. CO has no effect on the contractile status of canine and rabbit cerebral vessels [72]. More recently, we demonstrated that physiologically relevant concentrations of CO also fail to dilate rodent middle cerebral arteries [75]. Significantly, we found that exogenously applied or endogenously derived CO promotes vasoconstriction of isolated skeletal muscle arterioles [76].

This response is abolished by removal of the endothelium and converted to dilation by NO synthase inhibition, suggesting that CO may evoke its constrictor effect by inhibiting endothelial NO formation. Consistent with this hypothesis, biochemical studies have shown that CO inhibits NO synthase activity by directly binding to the heme moiety of the enzyme [77]. Moreover, CO-mediated inhibition of endothelial NO synthesis and impaired vasodilation have recently been reported in renal and cerebral microvessels [78, 79], raising the possibility that CO may promote endothelial dysfunction in some vascular beds.

Previous studies by our laboratory and others demonstrate that endogenously-formed CO contributes to central nervous system (CNS) mediated blood pressure regulation. Systemic administration of the heme oxygenase inhibitor, zinc deuteroporphyrin 2,4-bis ethylene glycol (ZnDPBG) blocks brain HO activity and increases blood pressure in Spargue-Dawley rats by increasing CNS sympathetic outflow [80]. ZnDPBG administration directly into the nucleus of the tractus solitarius (NTS) of rats also increases blood pressure and this effect is reversed by ipsilateral microinjection of CO into the NTS. Furthermore, the systemic pressor effect of ZnDPBG can also be reversed by CO administration directly into the NTS [81]. Collectively, these findings suggest that endogenous CO production in the NTS helps to maintain normal blood pressure by suppressing the activity of the sympathetic nervous system.

The HO-1/CO system appears to be a key regulator of blood pressure and alterations in this pathway have been linked to the pathogenesis of hypertension. However, the role of HO-1 in blood pressure regulation varies with different animal models and experimental settings. Pharmacological induction of HO-1 or the administration of HO substrates normalizes blood pressure in spontaneously hypertensive rats (SHR) [80, 82, 83]. Moreover, a single intracardiac injection of a retroviral vector containing human HO-1 in SHR is able to produce widespread transgene expression and this is associated with a significant decrease in blood pressure [84]. The antihypertensive effect of HO-1 is likely mediated by CO since the administration of biliverdin does not significantly alter systemic blood pressure [80]. Interestingly, HO-1 expression in vascular tissues is lower in SHR at a pre-hypertensive stage but similar to age-matched control animals during the established stage of hypertension [85]. Moreover, the activities of the downstream targets of CO, including soluble guanylate cyclase, are also reduced in pre-hypertensive SHR rats. These finding indicate that suppression of the HO-1/CO/cGMP signaling pathway precedes the development of hypertension and may contribute to the rise in blood pressure in SHR.

A beneficial role for the HO-1/CO system has also been proposed in angiotensin II-induced hypertension. Chronic administration of angiotensin II induces hypertension in rats and this is accompanied by the induction of HO-1 in various tissues, including blood vessels. The upregulation of HO-1 in this model is believed to serve a protective compensatory mechanism by attenuating the pressor response to constrictor stimuli [86]. In addition, HO-1 affords protection in the one kidney-one clip model of renovascular hypertension. In this model, HO-1-null mice demonstrate more severe hypertension and renal injury compared to wild-type animals [87]. Significantly, the antihypertensive action of HO-1 is not restricted to the systemic circulation. Induction of HO-1 expression and CO production inhibits the structural remodeling of pulmonary arteries and the development of pulmonary hypertension in response to chronic hypoxia [88]. Similar protection against pulmonary hypertension is noted in transgenic mice overexpressing HO-1 [89].

However, excessive production of CO may also be detrimental. In particular, overproduction of CO has been implicated in the vascular collapse during septic shock. Endotoxemia results in the extensive induction of HO-1 within the SMC and EC of large vessels and arterioles [90]. Moreover, the administration of the HO inhibitor, zinc protoporphyrin-IX abrogates endotoxin-induced hypotension, suggesting that CO may contribute to the decrease in systemic blood pressure. Interestingly, while HO-1 null mice are better able to maintain their systemic blood pressure relative to wild type animals, they exhibit increased oxidative stress, end organ damage, and mortality during endotoxemia [91]. Consistent with these findings, the exogenous administration of CO has been demonstrated to blunt the deterioration of lung, kidney, and liver function during septic shock [92]. Thus, while CO may participate in the hypotensive response to sepsis it may play an important role in preserving organ function.

Surprisingly, CO promotes endothelial dysfunction and hypertension in salt-sensitive forms of hypertension. Studies in our laboratory found that placing Dahl salt-sensitive rats on a high salt diet for 4 weeks leads to an increase in blood pressure that is associated with a significant increase in vascular HO-1 protein expression and CO production [93]. In addition, isolated gracilis muscle arterioles from salt-treated animals demonstrate a reduced response to the endothelium-dependent vasodilator, acetylcholine, and to the NO synthase inhibitor, L-NAME. Furthermore, flow-induced dilations are abolished in these rats [93]. However, all these responses are fully restored by acutely treating blood vessels with a HO inhibitor. Moreover, the co-application of CO prevents the restoration of flow-induced dilation by HO inhibition [94]. Significantly, we observed that the administration of the HO inhibitor, zinc deuteroporphyrin 2,4-*bis* glycol, lowers blood pressure in Dahl rats treated with a high salt diet but not in low salt control animals [94]. Collectively, these results indicate that HO-1-derived CO mediates endothelial dysfunction and contributes to hypertension in these animals fed a high salt diet. Similarly, we found that increased CO synthesis contributes to the development of endothelial dysfunction in deoxycorticosterone acetate-salt hypertensive rats [95]. However, endogenous CO formation is not upregulated in SHR rats indicating that increases in CO production in salt-sensitive animals is not a consequence of high blood pressure *per se* but may be, rather associated with the salt-sensitive trait [95].

Interestingly, the vascular effect of CO in hypertension varies depending on the vascular bed. While increased CO production promotes skeletal muscle arteriolar endothelial dysfunction in Dahl salt-sensitive rats, elevated HO-1 expression and CO synthesis in the coronary arteries of the same animal helps maintain cardiac perfusion during salt-induced hypertension [96]. The reason(s) for the divergent action by CO in different vascular beds is not known but may be related to differences in soluble guanylate cyclase content, calcium-activated potassium channel density, and/or NO bioavailability in different blood vessels.

More recently, we found that CO may also promote hypertension and endothelial dysfunction in metabolic syndrome [97]. Obese Zucker rats, a well established genetic model of obesity and metabolic syndrome, demonstrate increased respiratory CO excretion relative to lean control animals. This is consistent with a previous report in patients with type 2 diabetes showing increased respiratory CO levels [98]. However, the administration of a HO inhibitor lowers CO excretion and normalizes blood pressure in the obese animals. Furthermore, HO inhibition restores endothelial function in resistance vessels from these animals without any change in metabolic parameters. These findings suggest that approaches targeting the endogenous production of CO may provide a novel therapeutic approach in treating vascular disease in patients with metabolic syndrome.

Further support for a role for HO-1 in promoting hypertension is provided by genetic studies demonstrating that transgenic overexpression of HO-1 in vascular SMC elevates blood pressure in mice [99]. Since the ability of CO to activate soluble guanylate cyclase is less than that for NO, it was suggested that overproduction of CO in these transgenic animals impairs NO-mediated increases in cGMP. Thus, CO may elicit a hypertensive response by decreasing the synthesis and vascular response to NO.

In summary, CO evokes a complex and, at times, opposing set of actions to regulate blood pressure (Fig. 3). This regulation occurs at multiple levels and involves several organ systems. CO exerts an antihypertensive effect by directly relaxing SMC via the activation of soluble guanylate cyclase and/or calcium-activated potassium channels. Beyond its direct vasodilating effect, CO may also inhibit vascular tone by regulating the synthesis of vasoactive compounds. In this respect, CO has been shown to inhibit the synthesis of the potent vasoconstrictor, endothelin-1 and may block the cytochrome P-450-dependent synthesis of endogenous vasoconstrictor substances [82, 83, 100]. In addition, CO may stimulate the vascular release of NO from internal stores [78]. Finally, CO may also lower blood pressure by depressing sympathetic outflow from the CNS and by decreasing cardiac contractility [80, 81]. However, in certain circumstances, these actions that promote a reduction in blood pressure are counterbalanced by the ability of CO to inhibit both the synthesis and vascular response to NO, and marginally increase circulating renin activity [101–102]. Thus, the relative importance and role that CO plays in modulating blood pressure will likely vary depending on the underlying physiological state and the amount of CO being generated.

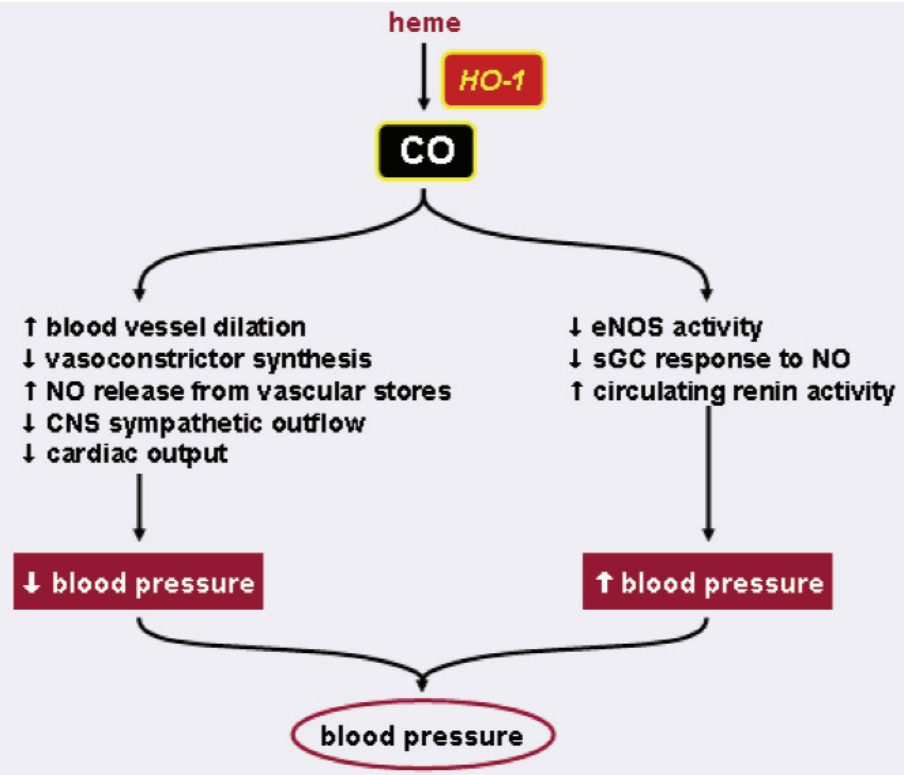

Fig. 3 Divergent regulatory effects on blood pressure by HO-1-derived carbon monoxide (CO). CO can exert an antihypertensive effect by inhibiting vasomotor tone, the generation of vasoconstrictors, central nervous system (CNS) sympathetic outflow, and cardiac output, while stimulating nitric oxide (NO) release from vascular stores. Alternatively, CO can promote hypertension by blocking endothelial NO synthase (eNOS) activity and the response of soluble guanylate cyclase (sGC) to NO, and by increasing circulating renin activity.

Therapeutic application of CO to cardiovascular disease

CO has recently emerged as a potential therapeutic modality for the treatment of cardiovascular disease. Several strategies have been proposed for targeting HO-1 or CO to cardiovascular tissue (Table 1). The use of pharmacological inducers of HO-1 offers a promising approach. Many potent inducers of HO-1 have been identified and shown to exert beneficial effects in the circulation. Heme and its synthetic analogues are strong inducers of HO-1 and have been demonstrated to protect against cardiovascular disease in numerous animal models. In addition to inducing HO-1, heme may also promote CO synthesis by providing additional substrate for the enzyme. We previously demonstrated that CO synthesis is likely substrate-limited in vascular cells, and that endogenous application of heme results in an immediate increase in the rate of CO synthesis [103]. However, heme and its derivatives possess pro-oxidant properties and will require caution in their use. Natural antioxidants and dietary supplements offer an alternative approach to stimulate HO-1 expression that may not provoke tissue damage [104]. Interestingly, there is increasing recognition that many of the vanguard drugs used to treat cardiovascular disease, including aspirin, statins, nitrovasodilators, rapamycin, and paclitaxol, are effective inducers of HO-1 and exert their clinical benefits, at least in part, through the release of CO [see 105]. A possible concern with the use of pharmacological inducers of HO-1 relates to the GT length polymorphism in the HO-1 promoter that may make such an approach difficult in patients with the long GT repeats that are more resistant to HO-1 induction. Increasing HO-1 expression *via* viral-mediated delivery of HO-1 circumvents this problem and provides for a more selective approach in targeting this gene to specific tissues [18, 27,28, 63, 64, 84]. Gene therapy approaches with HO-1 have proven highly effective in animal studies, and the recent development of inducible and/or tissue specific vectors will allow for a more refined pattern of HO-1 expression. However, current limitations in human gene therapy are well known and will require further improvements in vector design and certification of clinical safety and efficacy.

The administration of CO provides a direct approach in delivering the gas. Inhalation of CO has been demonstrated to be effective in several animal models of cardiovascular disease [20, 68–70, 92].

Table 1 Therapeutic strategies targeting CO in cardiovascular disease

A	**Enhancing endogenous CO synthesis and/or activity** • pharmacological induction of HO-1 • gene deliver of HO-1 • increasing substrate availability • application of CO-sensitizing compounds
B	**Exogenous delivery of CO** • inhalation of CO • CO-containing solutions • use of prodrugs to generate CO • use of CO-releasing compounds
C	**Inhibition of endogenous CO synthesis** • use of metalloporphyrins or imidazole-dioxolane compounds • HO-1 antisense technology • HO-1 small interference RNA technology

However, reports on tolerance to CO inhalation are contradictory and require further investigation [106–108]. The use of prodrugs to generate CO provides another route for the systemic administration of CO. In particular, dichloromethane is readily metabolized by cytochrome P450 isozymes to CO and CO_2. Interestingly, the production of CO following the oral ingestion of dichloromethane markedly attenuates intimal thickening in a model of chronic allogeneic aorta rejection in rats, demonstrating the feasibility of this technique to convey biologically relevant concentrations of CO [109]. More recently, the generation of novel CO-releasing compounds (CORMs) provides another alternative for the delivery of CO. Several CORMs with various solubility and release kinetics have been synthesized and their biological activity validated in both vascular and cardiac tissue [see 110]. These compounds may allow for a more controlled delivery of CO and could easily be impregnated onto various medical devices, including coronary stents. Finally, the combined use of CO with CO-sensitizing agents, such as 3-(5'-hydroxymethyl-2-furyl)-1-benzyl indazole (YC-1), may circumnavigate the possible development of tissue hypoxia by decreasing the amount of CO required to exert its therapeutic effect [111].

Since emerging studies suggest that CO may in certain instances promote cardiovascular dysfunction by stimulating the production of reactive oxygen species and/or by inhibiting heme-containing proteins (*e.g.* endothelial NO synthase), approaches that prevent the formation of CO may also be of therapeutic relevance. Metalloporphyrins are well recognized and potent inhibitors of HO that have been widely employed to block the endogenous formation of CO. These pharmacological inhibitors resemble heme in their porphyrin structure, but the iron core is substituted by a heavy metal such as zinc, tin, or cobalt. These substituted porphyrins compete with heme for binding to HO and can be used to block HO activity both acutely and chronically [28, 80]. However, at high doses these metalloporphyrins are not selective for HO and can paradoxically induce the expression of HO-1 [see 7]. In addition, some metalloporphyrins are photosensitive and can undergo non-enzymatic degradation to release CO. Interestingly, recent work has identified novel non-porphyrin inhibitors of HO. Several imidazole-dioxolane derivatives have been demonstrated to be potent inhibitors of HO [114, 115]. Significantly, these compounds have no effect on NO synthase or soluble guanylate cyclase activity, and a subset of these derivatives exhibit high selectively for HO-1 relative to HO-2. These later compounds may serve as important pharmacological tools to further define the role of HO-1 and CO in the cardiovascular system. Finally, CO production

may be blocked by inhibiting the expression of HO-1. Molecular approaches targeting HO-1 mRNA using both antisense and small interference RNA technology have been successfully employed and may provide a more specific approach in downregulating CO synthesis [112, 113].

Conclusion

Studies in the past few decades have defined the molecular pathways responsible for the endogenous production of CO and have highlighted the many biological effects of this gas in the cardiovascular system. This work has clearly established CO as a physiologically relevant signaling gas, and has implicated alterations in endogenous CO synthesis in the development of cardiovascular disease. Strategies targeting CO represent a novel therapeutic modality in treating a myriad of cardiovascular disorders.

Acknowledgements

This work was supported by National Institutes of Health grants R01 HL59976 (WD), R01 HL74966 (WD), R01 HL76187 (RAJ), the Juvenile Diabetes Research Foundation (WD), and an Institutional Development Award (IdeA) Program P20 RR017659 (FKJ) from the National Center for Research Resources. Due to the nature of this article and space limitations, we apologize if certain relevant papers have been excluded.

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