ANTIOXIDANTS & REDOX SIGNALING
Volume 4, Number 2, 2002

Forum Review

Carbon Monoxide and Human Disease

DANIELLE MORSE AND JIGME SETHI

ABSTRACT

Carbon monoxide is produced endogenously in humans through the breakdown of hemoglobin by heme oxygenase. Although originally thought to be a superfluous by-product of heme catabolism, carbon monoxide is now known to play a central role in many aspects of human health and disease. The functions of carbon monoxide that have been described to date are myriad, including blood pressure regulation, maintenance of organ-specific vascular tone, neurotransmission, stress response, platelet activation, and smooth muscle relaxation. This review outlines what is known to date about carbon monoxide as it relates to human disease.
Antioxid. Redox Signal. 4, 331–338.

INTRODUCTION

THE ERA OF THE GASEOUS MOLECULE IN MEDICINE is upon us. In 1990, when nitric oxide (NO) was celebrated as the "molecule of the decade," the concept of an evanescent gaseous molecule playing a central role in processes as diverse as smooth muscle relaxation, platelet aggregation, neurotransmission, and host response to infection was truly novel. This represented a major shift in thinking for the medical scientific community. Throughout the 1990s, as the NO success story continued to unfold at a remarkable pace, a question arose in the minds of investigators: "If nitric oxide is so important to so many systems, could a structurally similar molecule, carbon monoxide, be equally important?" The answer is proving to be a resounding "Yes."

HISTORY

The carbon monoxide (CO) that has occupied the thinking of physicians since the time of Aristotle is derived from exogenous sources. Aristotle is credited with the first recognition of CO poisoning in the third century BC: "Coal fumes lead to heavy head and death." In 18th century England, Priestley was the first to describe CO as a combustible gas that burned with a bright blue flame. It was not until the 19th century that a French physician, Leblanc, verified Aristotle's observation by identifying CO as the toxic substance in coal gas. Numerous case reports of the ill effects of CO exposure followed this recognition. The concept of CO as a scourge became entrenched with good reason: CO has taken the lives of countless individuals over the centuries, and it remains the most common cause of poison-related deaths in the U.S.

The harmful effects of CO on humans have been extensively studied. CO competitively binds to the oxygen-carrying heme moiety of hemoglobin, dissociating oxygen and depriving tissues of their oxygen supply. The affinity of hemoglobin for CO is ~240 times that for oxygen. The binding of two CO molecules to hemoglobin also causes a change in the allosteric conformation of the hemoglobin molecule, preventing oxygen at the other two binding sites from being easily released. This results in a leftward shift of the hemoglobin dissociation curve and further exacerbates tissue hypoxia. It is possible that CO also causes detrimental effects in humans by binding to cytochrome P450, cytochrome *c* oxidase, or myoglobin, although this would likely occur only at very high levels of CO exposure (27, 60, 83).

It was undoubtedly a great surprise to the medical community when Sjorstrand reported in 1949 that CO is produced endogenously in humans (69). The source of endogenous CO remained occult for almost 20 years, until it was discovered to be derived from heme degradation (11, 14, 33, 82). The first rate-limiting step of heme degradation is accomplished by heme oxygenase (HO) as it cleaves the α-meso carbon

Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, University of Pittsburgh Medical Center, MUH 628NW, 3459 Fifth Ave, Pittsburgh, PA 15213.

bridge of heme molecules to yield equimolar quantities of biliverdin IXa, free iron, and CO (Fig. 1). Three isoforms of HO, each the product of a separate gene, have been reported: HO-1, HO-2, and HO-3. HO-1 is inducible by many stimuli, whereas HO-2 is constitutively synthesized. HO-3 is structurally similar to HO-2, but is a less efficient heme catalyst.

As might be expected, HO is abundant in sites responsible for heme breakdown, such as the spleen. Perhaps less obviously, HO is also highly expressed in the brain and testes, and its activity has been detected in every organ system where it has been sought. In fact, both HO and its substrate, heme, are highly conserved molecules across almost all forms of life. Heme is a fundamental component of numerous enzymes, including nitric oxide synthase (NOS), peroxidase, guanylate cyclase, respiratory chain cytochromes, and cytochrome P450s. HO has been detected in almost all species and all tissues. Molecules that are so evolutionarily conserved and ubiquitous generally serve a necessary and fundamental purpose. When HO was discovered in the 1960s, however, this purpose was obscure. The destruction of heme as an end in itself could not be easily explained, particularly as its resynthesis is complex and energy-consuming for the organism.

Clues to ulterior purposes for HO arose in the 1980s when Keyse and Tyrell (28, 29) demonstrated that HO serves as a cytoprotectant against oxidative stress. This led to increased interest in HO and its catabolic products. In particular, the similarity between NO and CO prompted investigators to seek a physiologic role for CO. In 1991, CO was shown to relax blood vessels (18). In 1993, Maines and Verma *et al.* reported that CO might bind to activate guanylate cyclase in a similar manner to NO (39, 77). In the subsequent decade, our understanding of the role of CO in human health and disease has further expanded to include the regulation of blood pressure (13, 50), the maintenance of vascular tone and blood flow to several organs (48, 57), uterine contractility (1), neurotransmission, and reponse to stress, among other things. CO has also been measured as a marker of disease activity. What follows is a description of some of the human disease states in which CO has been found to play a role.

CO AND THE CARDIOVASCULAR SYSTEM

Vascular tone

The effect of CO on coronary blood flow was noted in 1984, when McGrath and Smith exposed isolated perfused rat hearts to exogenous CO (10%) and noted an increase in coronary blood flow (43, 44). They concluded that CO caused dilation of the coronary arteries and that the effect was reversible because coronary flow returned to control levels when the CO was removed. They noted that CO also reversed the vasoconstrictive effects of the α-agonist methoxamine and, importantly, that myocardial oxygen consumption did not change significantly when the hearts were perfused with CO. A direct relaxing effect of CO on coronary vascular smooth muscle was further demonstrated on isolated porcine coronary artery and vein (20), rabbit aorta (9, 18), and rat thoracic aorta (36). A concentration-dependent relaxation of rat-tail artery tissues precontracted with phenylephrine was also demonstrated, with vasorelaxation occurring at a threshold CO concentration of 1 μM (81). This effect was independent of the presence of an intact endothelium. Convincing evidence of the regulation of vascular resistance by CO came from experiments on the isolated perfused rat liver, where all three HO isoforms are expressed constitutively. Suematsu *et al.* demonstrated that inhibition of HO activity, but not NOS activity, increased hepatic vascular resistance, which was reversed by exogenous CO (1 μM) (72).

The vasorelaxation caused by CO may not be universal to all vascular tissues. One study found that the vascular tone of basilar and middle cerebral arteries from rabbits was not affected by CO (10^{-6} to 3×10^{-4} M), whereas that of the aorta was. Furthermore, the same cerebral arteries were sensitive to the vasodilating effects of NO (9). The reason for this heterogeneity of response is not clear. Differential effects in various organ systems may be important to the endogenous regulatory role of CO.

The physiologic importance of the vascular effects of CO remains to be fully elucidated. Ischemia–reperfusion injury is one condition in which HO-1 is up-regulated. In the rat kidney, ischemia–reperfusion results in an eight- to 10-fold increase in HO-1 mRNA and a corresponding increase in HO-1 protein level and total HO activity (40). This may provide a defense against oxidant injury, but the CO generated by HO-1 may also serve to increase blood supply to the kidney. Similarly, hypoxia is known to increase significantly the transcription of HO-1 gene (49); this may represent the body's attempt to restore blood flow and oxygenation. In ischemic myocardium, endogenous CO production appears to be increased (42). CO appears to play a role in the regulation of blood pressure as well. Heme, which is a potent inducer of HO-1, lowers blood pressure in spontaneously hypertensive rats (35). The inhibition of HO activity by administration of zinc deuteroporphyrin-2,4-bisglycol (an HO inhibitor) increases mean arterial blood pressure and total peripheral resistance in rats (25). The effects of CO on blood pressure are both central and peripheral: In the central nervous system, CO apparently acts in the nucleus tractus solitarii, promoting changes in glutamatergic neurotransmission and lowering blood pressure (26). In the periphery, CO can directly promote vasodilation, as described above.

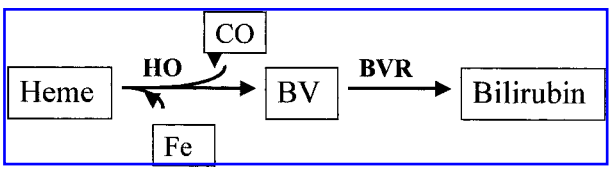

FIG. 1. HO cleaves the α-meso carbon bridge of heme molecules to yield equimolar quantities of biliverdin IXa, free iron, and CO. BV, biliverdin; BVR, biliverdin reductase; Fe, free iron.

Atherosclerosis

Increasing interest in the role of oxidants in the pathogenesis of atherosclerosis has led to an examination of the significance HO and CO in this disease process. HO-1 is induced

in endothelial and smooth muscle cells by proatherogenic agents, such as heme, oxidized low-density lipoprotein (LDL), lipid metabolites, peroxynitrite, hypoxia, heavy metals, proinflammatory cytokines, and angiotensin II (68). HO-1 is highly expressed in the endothelium and foam cells of intimal lesions in both humans and apolipoprotein E-deficient mice (80). The induction of HO-1 expression and activity in LDL receptor knockout mice inhibits the formation of atherosclerotic lesions (24). Shear stress, which is believed to be involved in the pathogenesis of atherosclerosis, has been shown to increase HO-1 expression in vascular smooth muscle cells. The CO produced by this induction of HO-1 inhibits platelet aggregation via activation of guanylyl cyclase *in vitro* (79).

There is mounting evidence that HO-1 and CO are important factors in vascular remodeling. Vascular smooth muscle cell-derived CO has been shown to inhibit endothelial cell platelet-derived growth factor (PDGF) and endothelin-1 expression, and thus inhibit smooth muscle cell growth (31). CO has also been shown to inhibit hypoxia-induced vascular endothelial growth factor induction in smooth muscle cells (37). In vivo, the induction of HO by hemin administration has been demonstrated to reduce neointimal thickness and medial wall thickness in the carotid arteries of balloon-injured rats (75).

CO AND REPRODUCTION

Pregnancy

It has long been known that smoking protects pregnant women against preeclampsia. A recent systematic review concluded that women who smoke cigarettes during pregnancy have a 32% decrease in risk of preeclampsia (12). Given the known vasorelaxant properties of CO, Baum *et al.* hypothesized that CO might mediate the effect of smoking (6). They measured exhaled CO levels in nonsmoking pregnant women with and without pregnancy-induced hypertension and found CO production to be significantly lower in the hypertensive group than in the control group (6). This suggests a role for CO in the maintenance of normal blood pressure during pregnancy.

Others have sought evidence for the activity of HO and CO in the placenta and umbilical cord. Production of CO by human umbilical cord tissues has been demonstrated by gas chromatography (78), and HO has been localized in the placenta by immunohistochemistry. McLean *et al.* described a wide distribution of HO in the human term placenta, including the syncytiotrophoblast layer of placental villi, the endothelium and smooth muscle cells of the umbilical–placental blood vessels, and all layers of the fetal membranes (45). Lyall *et al.* compared the expression of HO-1 and HO-2 in the placenta at 8–19 weeks gestation and at term and found that the expression was dynamic over time (38). HO-2 immunostaining increased in endothelial cells toward term, whereas that in the syncytiotrophoblast diminished toward term. HO-1 immunostaining was low in the placenta, but intense in the invading cytotrophoblast cells. Placental perfusion studies demonstrated a dose-dependent increase in perfusion pressure in the presence of zinc protoporphyrin, an inhibitor of HO (38). In support of the hypothesis that HO is important in the pathogenesis of preeclampsia, Ahmed *et al.* demonstrated that HO-1 protein expression is significantly reduced in placentae from pregnancies complicated with preeclampsia compared with normal pregnancies (3). More recently, Navarra *et al.* described evidence for CO modulation of corticotropin-releasing hormone release from trophoblast cells (52). All of these findings strongly suggest a role for CO in placental function and development.

As CO appears to be important in regulating vascular smooth muscle cell tone, it also likely regulates uterine muscle tone. Acevedo *et al.* demonstrated that the expression of both HO-1 and HO-2 was >15-fold higher in pregnant myometrium compared with nonpregnant myometrium, and that the activation of HO completely inhibited spontaneous contractility and oxytocin-stimulated contractions (1). They further demonstrated that progesterone, but not estradiol-17β, stimulated increased HO synthesis and CO production in term myometrial explants. They concluded that the HO–CO pathway maintains the uterus in a quiescent state during pregnancy (1).

Reproductive hormones

There is some evidence that CO may be important in endocrine aspects of reproduction. In *in vitro* studies, the formation of CO within the hypothalamus has been associated with inhibition of the release of hormones such as corticotropin-releasing hormone, arginine vasopressin, and oxytocin involved in hypothalamo–pituitary–adrenal axis activation. Conversely, CO stimulates luteinizing hormone-releasing hormone release (41). This suggests that CO may prevent overexuberant activation of the hypothalamo–pituitary–adrenal axis and prevent the inhibition of reproductive processes within the hypothalamus during stress, although these findings have not been confirmed *in vivo*.

Male reproductive system

In the male reproductive system, HO has been demonstrated in the endothelium lining penile arteries, and CO has been shown to relax noradrenaline-contracted preparations of human corpus cavernosum and spongiosum, suggesting a role in penile erection (22). HO has also been demonstrated in Sertoli cells in the human testis. Induction of HO-1 in isolated seminiferous tubules with sodium arsenite or hematin results in increased cyclic guanosine monophosphate (cGMP), whereas the HO inhibition with zinc protoporphyrin IX reduces tubular cGMP generation (46).

CO AND THE LIVER

The liver is a good candidate organ for the study of HO. As a site for degradation of senescent erythrocytes and catabolism of hemoglobin, the liver is rich in HO. Furthermore, hepatic cytochrome P450 is a good substrate for HO and, by virtue of containing ferroheme under normal metabolic conditions, cytochrome P450 is also susceptible to regulation by CO.

The isozymes of HO have distinct topographic patterns in the liver: HO-1 is prominent in Kupffer cells, whereas HO-2

is more abundant in hepatocytes (19). CO appears to be important in the maintenance of hepatic perfusion. Blocking HO in isolated rat livers with a competitive inhibitor (zinc protoporphyrin) decreases CO production and increases baseline vascular resistance (72). Oxyhemoglobin, which captures CO, produces the same vasoconstrictor effect as zinc protoporphyrin, whereas methemoglobin, which captures only NO, does not cause vasoconstriction. When the oxyhemoglobin is liposome-encapsulated and therefore restricted to the sinusoids, vasoconstriction does not take place, suggesting that the locus of the CO activity is extrasinusoidal (71).

CO produced in the liver may be important in bile excretion as well. Elimination of constitutive CO production with zinc protoporphyrin has been shown to stimulate bile acid-dependent bile flow (71) and to increase the contractility of the bile canaliculus (67). The addition of exogenous CO (1 μM) reverses these changes.

HO-1 appears to be important in the hepatic reaction to stress. Partial ligation of the portal vein in portal hypertensive rats causes HO-1 induction in the liver (16). In HO-1-deficient mice, the liver has heightened susceptibility to injury by endotoxin (61). The only identified human case of HO-1 deficiency demonstrated endothelial cell damage and iron accumulation in the liver (84). In a model of compensated hemorrhagic shock, Rensing *et al.* demonstrated that HO-1 is markedly up-regulated at 5–11 h after resuscitation, and that administration of a pharmacologic inhibitor of HO increases centrilobular necrosis in the liver (62). The administration of an antioxidant (Trolox) failed to reverse this effect, and the authors postulated that the protection by HO in this model may be mediated by the vasodilatory properties of CO (62). More recently, Kyokane *et al.* examined the role of CO in protection against hepatobiliary dysfunction in endotoxin-treated rat liver (32). Isolated perfused livers of endotoxemic rats were treated with either oxyhemoglobin or methemoglobin as in the study by Suematsu above (71). The livers treated with oxyhemoglobin evidenced vasoconstriction and cholestasis not seen in the livers treated with methemoglobin. This effect was not seen with the addition of a NO inhibitor alone, but was duplicated by the addition of the HO inhibitor zinc protoporphyrin IX. CO supplementation reversed the vasoconstriction and cholestasis.

CO AND THE NEUROLOGIC SYSTEM

In the late 1980s, a neural role for NO was discovered (7, 8), and this led to the investigation of CO as a potential neural messenger. The localization of HO-2 in the brain is strikingly similar to that of soluble guanylyl cyclase (77), suggesting that CO and NO bind guanylyl cyclase in a like fashion. The function of NO as a neurotransmitter is well established in the myenteric plexus of the intestine, and there is good evidence that CO also acts as a neurotransmitter in the myenteric plexus (85). The intestines of HO-2 gene-deleted mice have impaired nonadrenergic, noncholinergic relaxation, as do those of neuronal NOS gene-deleted mice. HO inhibitors cause intestinal contraction in the neuronal NOS-deficient mice, and NOS inhibitors do the same in the HO-2-deficient mice (85). Based on these observations, it would appear that NO and CO function as cotransmitters in the same neurons.

In the brain, NO and CO appear to act on the hypothalamus to increase adrenocorticotropic hormone (ACTH) levels in response to stress. Kim and Rivier demonstrated that intracerebroventricular injection of NOS or HO inhibitors decreased NOS and HO activity and inhibited the production of ACTH in response to electroshocks. The action of the inhibitors appeared to be specific to the brain and not to the pituitary gland (30).

There is evidence that oxidative stress is central to the pathogenesis of Alzheimer's disease and Parkinson's disease. HO-1 immunoreactivity has been shown to be greatly enhanced in regions with senile plaques and neurofibrillary tangles in subjects with Alzheimer's disease and in astrocytes in the nigra of patients with Parkinson's disease (65). The amyloid precursor protein, which is postulated to participate in the neurotoxicity of Alzheimer's disease, has been shown to bind to, and inhibit, HO. Moreover, transgenic mice expressing mutant amyloid precursor protein have lower HO activity (as measured by bilirubin production) and more oxidative neurotoxicity than wild-type mice (74). Paradoxically, it seems that individuals with Alzheimer dementia have lower plasma and cerebrospinal fluid HO-1 protein and lymphocyte HO-1 mRNA levels than control elderly subjects (66). The mechanism of involvement of HO-1 in these aging-related neurodegenerative disorders has not been fully explored, and it is not clear what role CO may play in these disorders, if any.

CO AND THE BLOOD

NO is known to induce inhibitory effects on platelet activation and neutrophil migration via cGMP (47). It has now been demonstrated that CO plays a similar role. Van Uffelen *et al.* demonstrated that exogenous CO enhances random migration of human neutrophils, and that CO causes an increase in cGMP. The enhancing effect on migration was largely blocked by inhibitors of cGMP accumulation (76). In platelets, CO has been shown to decrease platelet aggregation, an effect mediated by cGMP (10).

There is evidence that platelet and vascular smooth muscle cell interactions involve CO. PDGF can increase the expression of HO-1 in vascular smooth muscle cells. Furthermore, incubation of platelets with PDGF-treated smooth muscle cells results in an increase in platelet cGMP concentration. This effect is reversed by treatment of the smooth muscle cells with an inhibitor of HO-1 (tin protoporphyrin-IX) or by the addition of a CO scavenger (hemoglobin) to the platelets (15).

CO AND THE LUNG

A great deal of work has been done to investigate the cytoprotective effects of HO-1 in the lung. As an organ that provides an interface between the environment and the circulation, the lung affords access for measurement of CO production, for gene transfer, and potentially for administration of CO. The lung is also an ideal environment for studying the effects of oxidative stress.

Stress response

One common model of oxidative stress is exposure to hyperoxia. Increased HO-1 activity has been observed in the lungs of rodents exposed to hyperoxic conditions (34). Overexpression of HO-1 in isolated pulmonary epithelial cells (34) and rat fetal lung cells (73) has been shown to confer protection against hyperoxic cell injury. This same protective phenomenon has been demonstrated *in vivo* with adenoviral transfer of HO-1 into rat lungs (54); rats overexpressing HO-1 demonstrated marked resistance to hyperoxic lung injury and a longer survival time in a hyperoxic environment. There may, however, be a threshold effect for cytoprotection by HO-1. Suttner *et al.* observed that whereas moderate overexpression of HO-1 in fibroblasts conferred protection against oxidative injury, higher levels of HO-1 were detrimental (73).

Postulating that CO may be mediating the protective effect of HO-1, Otterbein *et al.* tested the effect of CO on hyperoxic lung injury (55). Rats were exposed to hyperoxia in the presence of a low concentration of CO (250 ppm). The protective effect of CO alone proved to be equivalent to that of HO-1 overexpression (55). The mechanism by which CO provides cytoprotection has not been entirely elucidated, but down-regulation of proinflammatory cytokines, such as tumor necrosis factor-α and interleukin-1β, along with augmentation of the antiinflammatory cytokine interleukin-10 appears to play a role (56).

Exhaled marker

As CO is excreted in the breath, disease processes affecting organs remote from the lung can potentially be monitored through the measurement of breath CO. Indeed, it has been demonstrated that exhaled CO levels are increased in asthma (23, 86), cystic fibrosis (59), diabetes (58), and critically ill patients (64). It is presumed that the exhaled CO reflects increased activity of HO due to a disease process. It has been noted that during exacerbations of chronic disease, CO production may increase over what is already an elevated baseline. For instance, Antuni *et al.* demonstrated that although cystic fibrosis patients have an elevated baseline exhaled CO level, the level rises even further with episodes of worsening respiratory symptoms and lung function (4). They also demonstrated that the CO levels decrease toward baseline levels after treatment with intravenous antibiotics (4). Similarly, Paredi *et al.* studied a small group of diabetic patients versus normal controls and discovered that not only were their levels of exhaled CO higher, but that the levels correlated with the incidence of glycemia and the duration of the disease (58). In addition, five normal control subjects underwent oral glucose tolerance testing and were found to have elevated breath CO coinciding with maximal serum glucose levels. The authors concluded that oxidative stress due to advanced glycation end products was inducing HO in the diabetic subjects.

There is growing interest in the measurement of exhaled CO levels in diverse conditions as it may allow for prediction of disease exacerbations and evaluation of therapeutic interventions.

CO AND THE KIDNEY

There has been one reported case of congenital human HO-1 deficiency, and this child manifested persistent proteinuria and hematuria. Renal biopsies revealed tubulointerstitial injury, interstitial fibrosis, and inflammatory cell infiltration, as well as endothelial swelling and subendothelial deposits in the glomeruli (53). HO-1 is induced in the kidney in several models of injury, including rhabdomyolysis (51), glycerol-induced acute renal failure (51), and cisplatin nephrotoxicity (2). Chemical inhibitors of HO-1 worsen tubular injury in these models (2, 51). The mechanism by which CO mediates these protective effects is unclear.

CO AND TRANSPLANTATION

HO-1 expression has been convincingly linked to the survival of transplanted organs. Hancock *et al.* demonstrated that the induction of protective genes such as HO-1 protects allografts from chronic rejection (21). Soares *et al.* have further shown that when hearts from HO-1 gene-deficient mice are transplanted into rats immunosuppressed with cyclosporin A and cobra venom factor, the xenografts undergo acute vascular rejection, whereas hearts from wild-type mice survive long-term (70). They went on to show that the ability of HO-1 to suppress the rejection of mouse-to-rat cardiac grafts depends on the generation of CO. By inhibiting HO-1 activity with tin protoporphyrin, the investigators were able to provoke acute graft rejection. This effect was completely reversed with the addition of exogenous CO, restoring long-term graft survival. The mechanism of graft protection by CO appears to involve the inhibition of platelet aggregation and the suppression of endothelial cell apoptosis (63).

CONCLUSION

There is now ample evidence that CO is a vital molecule in human health and disease. Every passing week brings new knowledge about the systems in which CO acts and the mechanisms by which it performs its myriad functions. Although the toxicological effects of high concentrations of exogenous CO fueled most of the research for nearly a century, observations over the last decade of the effects of lower concentrations have led to a remarkable rehabilitation of this gas, from poison to physiological effector and, perhaps, pharmacological agent. Nevertheless, the gaps in our current knowledge are large and will provide ample work for investigators; as Barinaga commented in *Science* in 1993, ". . . this gas is likely to provide fuel to run plenty of labs" (5).

ABBREVIATIONS

ACTH, adrenocorticotropic hormone; cGMP, cyclic guanosine monophosphate; CO, carbon monoxide; HO, heme oxygenase; LDL, low density lipoprotein; NO, nitric oxide; NOS, nitric oxide synthase; PDGF, platelet-derived growth factor.

REFERENCES

1. Acevedo CH and Ahmed A. Hemeoxygenase-1 inhibits human myometrial contractility via carbon monoxide and

is upregulated by progesterone during pregnancy. *J Clin Invest* 101: 949–955, 1998.
2. Agarwal A, Balla J, Alam J, Croatt AJ, and Nath KA. Induction of heme oxygenase in toxic renal injury: a protective role in cisplatin nephrotoxicity in the rat. *Kidney Int* 48: 1298– 1307, 1995.
3. Ahmed A, Rahman M, Zhang X, Acevedo CH, Nijjar S, Rushton I, Bussolati B, and St. John J. Induction of placental heme oxygenase-1 is protective against TNF alpha-induced cytotoxicity and promotes vessel relaxation. *Mol Med* 6: 391–409, 2000.
4. Antuni JD, Kharitonov SA, Hughes D, Hodson ME, and Barnes PJ. Increase in exhaled carbon monoxide during exacerbations of cystic fibrosis. *Thorax* 55: 138–142, 2000.
5. Barinaga M. Carbon monoxide: killer to brain messenger in one step. *Science* 259: 309, 1993.
6. Baum M, Schiff E, Kreiser D, Dennery PA, Stevenson DK, Rosenthal T, and Seidman DS. End-tidal carbon monoxide measurements in women with pregnancy-induced hypertension and preeclampsia. *Am J Obstet Gynecol* 183: 900–903, 2000.
7. Bredt DS and Snyder SH. Nitric oxide mediates glutamate-linked enhancement of cGMP levels in the cerebellum. *Proc Natl Acad Sci U S A* 86: 9030–9033, 1989.
8. Bredt DS, Hwang PM, and Snyder SH. Localization of nitric oxide synthase indicating a neural role for nitric oxide. *Nature* 347: 768–770, 1990.
9. Brian JE Jr, Heistad DD, and Faraci FM. Effect of carbon monoxide on rabbit cerebral arteries. *Stroke* 25: 639–643, 1994.
10. Brune B and Ullrich V. Inhibition of platelet aggregation by carbon monoxide is mediated by activation of guanylate cyclase. *Mol Pharmacol* 32: 497–504, 1987.
11. Coburn RF, Williams WJ, White P, and Kahn SB. Production of carbon monoxide from hemoglobin in vivo. *J Clin Invest* 46: 346–356, 1967.
12. Conde-Agudelo A, Althabe F, Belizan JM, and Kafury-Goeta AC. Cigarette smoking during pregnancy and risk of preeclampsia: a systematic review. *Am J Obstet Gynecol* 181: 1026–1035, 1999.
13. Dos Santos EA, Yamaguishi GA, and Heimann JC. Effect of the heme/heme oxygenase pathway on the relationship between salt consumption and blood pressure. *J Hypertens* 16: 1965–1969, 1998.
14. Drabkin DL. The hemophagous organ of the placenta and in vivo studies of endogenous carbon monoxide production. *Ann N Y Acad Sci* 174: 49–63, 1970.
15. Durante W, Peyton KJ, and Schafer AI. Platelet-derived growth factor stimulates heme oxygenase-1 gene expression and carbon monoxide production in vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 19: 2666–2672, 1999.
16. Fernandez M and Bonkovsky HL. Increased heme oxygenase-1 gene expression in liver cells and splanchnic organs from portal hypertensive rats. *Hepatology* 29: 1672–1679, 1999.
17. Freibe A, Mullershausen F, Smolenski A, Walter U, Schultz G, and Koesling D. YC-1 potentiates nitric oxide- and carbon monoxide-induced cyclic GMP effects in human platelets. *Mol Pharmacol* 54: 962–967, 1998.
18. Furchgott RF and Jothianandin S. Endothelium-dependent and -independent vasodilation involving cGMP: relaxation induced by nitric oxide carbon monoxide and light. *Blood Vessels* 28: 52–61, 1991.
19. Goda N, Suzuki K, Naito M, Takeoka S, Tsuchida E, Ishimura Y, Tamatani T, and Suematsu M. Distribution of heme oxygenase isoforms in rat liver: topographic basis for carbon monoxide-mediated microvascular relaxation. *J Clin Invest* 101: 604–612, 1998.
20. Graser T, Vedernikov YP, and Li DS. Study on the mechanism of carbon monoxide induced endothelium-independent relaxation in porcine coronary artery and vein. *Biomed Biochim Acta* 4: 293–296, 1990.
21. Hancock WW, Buelow R, Sayegh MH, and Turka LA. Antibody-induced transplant arteriosclerosis is prevented by graft expression of anti-oxidant and anti-apoptotic genes. *Nat Med* 4: 1392–1396, 1998.
22. Hedlund P, Ny L, Alm P, and Andersson KE. Cholinergic nerves in human corpus cavernosum and spongiosum contain nitric oxide synthase and heme oxygenase. *J Urol* 164 (3 part 1): 868–875, 2000.
23. Horvath I, Donelly LE, Paredi P, Kharitonov SA, and Barnes PJ. Elevated levels of exhaled carbon monoxide are associated with an increased expression of heme oxygenase-1 in airway macrophages in asthma: a new marker of chronic inflammation. *Thorax* 53: 668–672, 1998.
24. Ishikawa K, Wang XP, Qiao JH, Navab M, Fogelman AM, and Lusis AJ. Heme oxygenase-1 inhibits atherosclerotic lesion formation in LDL receptor knockout mice. *Circulation* 8SS: 619, 1997.
25. Johnson RA, Lavesa M, Askari B, Abraham NG, and Nasjletti A. A heme oxygenase product presumably carbon-monoxide mediates a vasodepressor function in rats. *Hypertension* 25: 166–169, 1995.
26. Johnson RA, Kozma F, and Colombari E. Carbon monoxide: from toxin to endogenous modulator of cardiovascular functions. *Braz J Med Biol Res* 32: 1–14, 1999.
27. Keilin D and Hartree EF. Cytochrome and cytochrome oxidase. *Proc R Soc Lond [Biol]* 27: 167, 1939.
28. Keyse SM and Tyrrell RM. Both near ultraviolet radiation and the oxidizing agent hydrogen peroxide induce a 32-kDa stress protein in normal human skin fibroblasts. *J Biol Chem* 262: 14821–14825, 1987.
29. Keyse SM and Tyrrell RM. Heme oxygenase is the major 32-kDa stress protein induced in human skin fibroblasts by UVA radiation, hydrogen peroxide, and sodium arsenite *Proc Natl Acad Sci U S A* 86: 99–103, 1989.
30. Kim CK and Rivier CL. Nitric oxide and carbon monoxide have a stimulatory role in the hypothalamic–pituitary–adrenal response to physico-emotional stressors in rats. *Endocrinology* 141: 2244–2253, 2000.
31. Kourembanas S, Morita T, Liu Y, and Christou H. Mechanisms by which oxygen regulates gene expression and cell–cell interaction in the vasculature. *Kidney Int* 51: 438–443, 1997.
32. Kyokane T, Norimizu S, Taniai H, Yamaguchi T, Takeoka S, Tsuchida E, Naito M, Nimura Y, Ishimura Y, and Suematsu M. Carbon monoxide from heme catabolism protects against hepatobiliary dysfunction in endotoxin-treated rat liver. *Gastroenterology* 120: 1227–1240, 2001.

33. Landaw SA, Callahan EW Jr, and Schmid R. Catabolism of heme in vivo: comparison of the simultaneous production of bilirubin and carbon monoxide. *J Clin Invest* 49: 914–925, 1970.
34. Lee PJ, Alam J, Sylvester SL, Inamdar N, Otterbein L, and Choi AM. Regulation of heme oxygenase-1 expression in vivo and in vitro in hyperoxic lung injury. *Am J Respir Cell Mol Biol* 14: 556–568, 1996.
35. Levere RD, Martasek P, Escalante B, Schwartzman ML, and Abraham NG. Effect of heme arginate administration on blood pressure in spontaneously hypertensive rats. *J Clin Invest* 86: 213–219, 1990.
36. Lin H and McGrath JJ. Vasodilating effects of carbon monoxide. *Drug Chem Toxicol* 11: 371–385, 1988.
37. Liu Y, Christou H, Morita T, Laughner E, Semenza GL, and Kourembanas S. Carbon monoxide and nitric oxide suppress the hypoxic induction of vascular endothelial growth factor gene via the 5′ enhancer. *J Biol Chem* 273: 15257–15262, 1998.
38. Lyall F, Barber A, Myatt L, Bulmer JN, and Robson SC. Hemeoxygenase expression in human placenta and placental bed implies a role in regulation of trophoblast invasion and placental function. *FASEB J* 14: 208–219, 2000.
39. Maines MD. Carbon monoxide: an emerging regulator of cGMP in the brain. *Mol Cell Neurosci* 4: 389–397, 1993.
40. Maines MD, Mayer RD, Ewing JF, and McCoubrey WK Jr. Induction of kidney heme oxygenase-1 (HSP32) mRNA and protein by ischemia/reperfusion: possible role of heme as both promotor of tissue damage and regulator of HSP32. *J Pharmacol Exp Ther* 264: 457–462, 1993.
41. Mancuso C, Preziosi P, Grossman AB, and Navarra P. The role of carbon monoxide in the regulation of neuroendocrine function. *Neuroimmunomodulation* 4: 225–229, 1997.
42. Maulik N, Engelman DT, Watanabe M, Engelman RM, and Das DK. Nitric oxide—a retrograde messenger for carbon monoxide signalling in ischemic heart. *Mol Cell Biochem* 157: 75–86, 1996.
43. McFaul SJ and McGrath JJ. Studies on the mechanism of carbon monoxide-induced vasodilation in the isolated perfused rat heart. *Toxicol Appl Pharmacol* 87: 464–473, 1987.
44. McGrath JJ and Smith DL. Response of rat coronary circulation to carbon monoxide and nitrogen hypoxia. *Proc Soc Exp Biol Med* 177: 132–136, 1984.
45. McLean M, Bowman M, Clifton V, Smith R, and Grossman AB. Expression of the heme oxygenase–carbon monoxide signalling system in human placenta. *J Clin Endocrinol Metab* 85: 2345–2349, 2000.
46. Middendorff R, Kumm M, Davidoff MS, Holstein AF, and Muller D. Generation of cyclic guanosine monophosphate by heme oxygenases in the human testis—a regulatory role for carbon monoxide in Sertoli cells? *Biol Reprod* 63: 651–657, 2000.
47. Moncada S and Higgs A. The L-argenine-nitric oxide pathway. *N Engl J Med* 329: 2002–2012, 1993.
48. Montecot C, Seylaz J, and Pinard E. Carbon monoxide regulates cerebral blood flow in epileptic seizures but not in hypercapnia. *Neuroreport* 9: 2341–2346, 1998.
49. Morita T, Perrella MA, Lee ME, and Kourembanas S. Smooth muscle cell-derived carbon monoxide is a regulator of vascular cGMP. *Proc Natl Acad Sci U S A* 92: 1475–1479, 1995.
50. Motterlini R, Gonzales A, Foresti R, Clark JE, Green CJ, and Winslow RM. Heme oxygenase-1-derived carbon monoxide contributes to the suppression of acute hypertensive responses in vivo. *Circ Res* 83: 568–577, 1998.
51. Nath KA, Balla G, Vercellotti GM, Balla J, Jacob HS, Levitt MD, and Rosenberg ME. Induction of heme oxygenase is a rapid protective response in rhabdomyolysis in the rat. *J Clin Invest* 90: 267–270, 1992.
52. Navarra P, Miceli F, Tringal G, Minici F, Pardo MG, Lanzone A, Mancuso S, and Apa R. Evidence for a functional link between the heme oxygenase–carbon monoxide pathway and corticotropin-releasing hormone release from primary cultures of human trophoblast cells. *J Clin Endocrinol Metab* 86: 317–323, 2001.
53. Ohta K, Yachie A, Rujimoto K, Kaneda H, Wada T, Toma T, Seno A, Kasahara Y, Yokoyama H, Seki H, and Koizumi S. Tubular injury as a cardinal pathologic feature in human heme oxygenase-1 deficiency. *Am J Kidney Dis* 35: 863–870, 2000.
54. Otterbein LE, Kolls JK, Mantell LL, Cook JL, Alam J, and Choi AM. Exogenous administration of heme oxygenase-1 by gene transfer provides protection against hyperoxia-induced lung injury. *J Clin Invest* 103: 1047–1054, 1999.
55. Otterbein LE, Mantell LL, and Choi AM. Carbon monoxide provides protection against hyperoxic lung injury. *Am J Physiol Lung Cell Mol Physiol* 276: L688–L694, 1999.
56. Otterbein LE, Bach FH, Alam J, Soares M, Tao Lu H, Wysk M, Davis RJ, Flavell RA, and Choi AM. Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. *Nat Med* 6: 422–428, 2000.
57. Pannen BH and Bauer M. Differential regulation of hepatic arterial and portal venous vascular resistance by nitric oxide and carbon monoxide in rats. *Life Sci* 62: 2025–2033, 1998.
58. Paredi P, Biernacki W, Invernizzi G, Kharitonov SA, and Barnes PJ. Exhaled carbon monoxide levels elevated in diabetes and correlated with glucose concentration in blood: a new test for monitoring the disease? *Chest* 116: 1007–1011, 1999.
59. Paredi P, Kharitonov SA, Leak D, Shah PL, Cramer D, Hodson ME, and Barnes PJ. Exhaled ethane is elevated in cystic fibrosis and correlates with carbon monoxide levels and airway obstruction. *Am J Respir Crit Care Med* 161(4 part 1): 1247–1251, 2000.
60. Piantadosi CA. Toxicity of carbon monoxide: hemoglobin *vs* histotoxic mechanisms. In: *Carbon Monoxide,* edited by Penney DG. Boca Raton, FL: CRC Press, 1996, pp. 163–186.
61. Poss KD and Tonegawa S. Heme oxygenase-1 is required for mammarian iron reutilization. *Proc Natl Acad Sci U S A* 94: 10919–10924, 1997.
62. Rensing H, Bauer I, Datene V, Patau C, Pannen BH, and Bauer M. Differential expression pattern of heme oxygenase-1/heat shock protein 32 and nitric oxide synthase-II and their impact on liver injury in a rat model of hemorrhage and resuscitation. *Crit Care Med* 27: 2766–2775, 1999.
63. Sato K, Balla J, Otterbein L, Smith RN, Brouard S, Lin Y, Csizmadia E, Sevigny J, Robson SC, Vercellotti G, Choi

AM, Bach FH, and Soares MP. Carbon monoxide generated by heme oxygenase-1 suppresses the rejection of mouse-to-rat cardiac transplants. *J Immunol* 166: 4185–4194, 2001.
64. Scharte M, Bone HG, Van Aken H, and Meyer J. Increased carbon monoxide in exhaled air of critically ill patients. *Biochem Biophys Res Commun* 267: 423–426, 2000.
65. Schipper HM. Heme oxygenase-1: role in brain aging and neurodegeneration. *Exp Gerontol* 35: 821–830, 2000.
66. Schipper HM, Chertkow H, Mehindate K, Frankel D, Melmed C, and Bergman H. Evaluation of heme oxygenase-1 as a systemic biological marker of sporadic AD. *Neurology* 54: 1297–1304, 2000.
67. Shinoda Y, Suematsu M, Wakabayashi Y, Goda N, Suzuki T, Saito S, Yamaguchi T, and Ishimura Y. Carbon monoxide as a regulator of bile canalicular contractility in cultured rat hepatocytes. *Hepatology* 28: 286–295, 1998.
68. Siow RCM, Sato H, and Mann G. Heme oxygenase–carbon monoxide signalling pathway in atherosclerosis: anti-atherogenic actions of bilirubin and carbon monoxide? *Cardiovasc Res* 41: 385–394, 1999.
69. Sjorstrand T. Endogenous formation of carbon monoxide in man under normal and pathological conditions. *Scand J Clin Lab Invest* 1: 201–214, 1949.
70. Soares MP, Lin Y, Anrather J, Csizmadia E, Takigami K, Sato K, Grey ST, Covin RB, Choi AM, Poss KD, and Bach FH. Expression of heme oxygenase-1 can determine cardiac xenograft survival. *Nat Med* 4: 1073–1077, 1998.
71. Suematsu M and Ishimura Y. The heme oxygenase–carbon monoxide system: a regulator of hepatobiliary function. *Hepatology* 31: 3–6, 2000.
72. Suematsu M, Goda N, Sano T, Kashiwagi S, Egawa T, Shinoda Y, and Ishimura Y. Carbon monoxide: an endogenous modulator of sinusoidal tone in the perfused rat liver. *J Clin Invest* 96: 2431–2437, 1995.
73. Suttner DM, Sridha K, Lee CS, Tomura T, Hansen TN, and Dennery PA. Protective effects of transient HO-1 overexpression on susceptibility to oxygen toxicity in lung cells. *Am J Physiol Lung Cell Mol Physiol* 276: L443–L451, 1999.
74. Takahashi M, Dore S, Ferris CK, Tomita T, Sawa A, Wolosker H, Borchelt DR, Iwatsubo T, Kim SH, Thinakaran G, Sisodia SS, and Snyder SH. Amyloid precursor proteins inhibit heme oxygenase activity and augment neurotoxicity in Alzheimer's disease. *Neuron* 28: 461– 473, 2000.
75. Tulis DA, Durante W, Peyton K, Evans A, and Schafer A. Heme oxygenase-1 attenuates vascular remodeling following balloon injury in rat carotid arteries. *Atherosclerosis* 155: 113–122, 2001.
76. Van Uffelen BE, de Koster BM, Van Steveninck J, and Elferink JG. Carbon monoxide enhances human neutrophil migration in a cyclic GMP-dependent way. *Biochem Biophys Res Commun* 226: 21–26, 1996.
77. Verma A, Hirsch DJ, Glatt CE, Ronnett GW, and Snyder SH. Carbon monoxide: a putative neural messenger. *Science* 259: 381–384, 1993.
78. Vreman HJ, Wong RJ, Kim EC, Nabseth DC, Marks GS, and Stevenson DK. Haeme oxygenase activity in human umbilical cord and rat vascular tissues. *Placenta* 21: 337–344, 2000.
79. Wagner CT, Durante W, Christodoulides N, Hellums JD, and Schafer AI. Hemodynamic forces induce the expression of heme oxygenase in cultured vascular smooth muscle cells. *J Clin Invest* 100: 589–596, 1997.
80. Wang LJ, Lee TS, Lee FY, Pai RC, and Chau LY. Expression of HO-1 in atherosclerotic lesions. *Am J Pathol* 152: 711–720, 1998.
81. Wang R, Wang ZZ, and Wu L. Carbon monoxide-induced vasorelaxation and the underlying mechanisms. *Br J Pharmacol* 121: 927–934, 1997.
82. White P. Carbon monoxide production and heme catabolism. *Ann N Y Acad Sci* 174: 23–31, 1970.
83. Wittenberg BA and Wittenberg JB. Transport of oxygen in muscle. *Annu Rev Physiol* 51: 857–878, 1989.
84. Yachie A, Niida Y, Wada T, Igarashi N, Kaneda H, Toma T, Ohta K, Kasahara Y, and Koizumi S. Oxidative stress causes enhanced vascular endothelial cell injury in human heme oxygenase-1 deficiency. *J Clin Invest* 103: 129–135, 1999.
85. Zakhary R, Poss KD, Jaffrey SR, Ferris CD, Tonegawa S, and Snyder SH. Targeted gene deletion of heme oxygenase 2 reveals neural role for carbon monoxide. *Proc Natl Acad Sci U S A* 94: 14848–14853, 1997.
86. Zayasu K, Sekizawa K, Okinaga S, Yamaya M, and Sasaki H. Increased carbon monoxide in exhaled air of asthmatic patients. *Am J Respir Crit Care Med* 156: 1140–1143, 1997.

Address reprint requests to:
Jigme Sethi
Division of Pulmonary, Allergy and Critical Care Medicine
Department of Medicine
University of Pittsburgh Medical Center
MUH 628NW
3459 Fifth Ave
Pittsburgh, PA 15213

E-mail: SethiJM@msx.upmc.edu

Received for publication June 15, 2001; accepted August 15, 2001.

This article has been cited by:

1. Kelly Decaluwé, Bart Pauwels, Charlotte Boydens, Johan Van de Voorde. 2012. Divergent Molecular Mechanisms Underlay CO- and CORM-2-Induced Relaxation of Corpora Cavernosa. *The Journal of Sexual Medicine* **9**:9, 2284-2292. [CrossRef]
2. Yeonsoo Joe, Min Zheng, Seul-Ki Kim, Sena Kim, Jamal MD Uddin, Tae Sun Min, Do Gon Ryu, Hun Taeg Chung. 2011. The role of carbon monoxide in metabolic disease. *Annals of the New York Academy of Sciences* **1229**:1, 156-161. [CrossRef]
3. Young-Myeong Kim , Hyun-Ock Pae , Jeong Euy Park , Yong Chul Lee , Je Moon Woo , Nam-Ho Kim , Yoon Kyung Choi , Bok-Soo Lee , So Ri Kim , Hun-Taeg Chung . 2011. Heme Oxygenase in the Regulation of Vascular Biology: From Molecular Mechanisms to Therapeutic Opportunities. *Antioxidants & Redox Signaling* **14**:1, 137-167. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
4. H Smithline. 2003. Whole body oxygen consumption and critical oxygen delivery in response to prolonged and severe carbon monoxide poisoning. *Resuscitation* **56**:1, 97-104. [CrossRef]
5. Augustine M.K. Choi , Leo E. Otterbein . 2002. Emerging Role of Carbon Monoxide in Physiologic and Pathophysiologic States. *Antioxidants & Redox Signaling* **4**:2, 227-228. [Abstract] [Full Text PDF] [Full Text PDF with Links]