Heme Oxygenase-1 and Carbon Monoxide in Vascular Pathobiology: Focus on Angiogenesis
Jozef Dulak, Jessy Deshane, Alicja Jozkowicz, and Anupam Agarwal

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Carbon monoxide (CO) is a colorless, tasteless, and odorless gas that, when inhaled, enters the bloodstream and replaces the oxygen on hemoglobin to form carboxyhemoglobin. Increasing levels of carboxyhemoglobin can result in a wide range of symptoms from mild cognitive impairment, including reduction in visual perception and driving performance, to more severe effects like headache, weakness, gastrointestinal symptoms, and finally progressive confusion, collapse, and coma. Indeed, thoughts that come to mind when CO is being discussed relate mostly to accidental deaths from malfunctioning home appliances, suicides in closed garages, and assisted suicides. Although the toxicity of CO has been studied extensively, it is now also being explored for its physiological effects and potential therapeutic benefits.1

Since the realization that the poisonous gas nitric oxide (NO) has a significant biological role in physiology and pathophysiology, CO, which is a structurally similar gas, has gained much attention as a molecule with many analogous chemical and biological properties (Table 1). Like NO, CO is produced endogenously during cellular metabolism, primarily from the degradation of heme by the heme oxygenase (HO) enzyme system.2 Endogenous CO formation has been measured in several biological systems, and normal human adults have been shown to exhale ≈12 mL of CO per day.3 The major exogenous source for CO is generated from the incomplete burning of carbon from solid, liquid, and gaseous fuels. The predominant endogenous source of CO is through the degradation of heme via the HO enzyme system (Table 2).

HO catalyzes the rate-limiting step in heme degradation, leading to the generation of equimolar amounts of iron, biliverdin, and CO (Figure 1).2 Biliverdin is then converted to bilirubin by biliverdin reductase. HO exists in 2 distinct isoforms, an inducible (HO-1) and a constitutive form (HO-2). HO-1 is a 32-kDa protein that is highly upregulated in mammalian tissues by a wide variety of stimuli including heme, heavy metals, growth factors (eg, transforming growth factor-β [TGF-β], platelet-derived growth factor [PDGF]), vascular endothelial growth factor [VEGF]), stromal cell–derived factor-1, NO, peroxynitrite, modified lipids, hypoxia, hyperoxia, cytokines, and others.4 HO-2 is a 36-kDa protein that is constitutively expressed in distinct locations including in the brain, endothelium, and testis.

Although the role of HO in heme degradation was recognized by Tenhunen and colleagues5 in 1968, the protective properties of HO-1 were first demonstrated in an animal model of heme protein–induced kidney injury by Nath and colleagues5 in 1992. Previous in vitro studies had shown the remarkable inducibility of HO-1 by a wide range of oxidant stimuli in assorted cell types,7 but the functional significance of such induction was not reported. Subsequent work demonstrated that the induction of ferritin along with HO-1 contributed to the protective effects of HO-1 in endothelial cells in vitro.8 These pivotal studies played a major role in fostering an enormous expansion of the HO field, attracting numerous investigators from different areas to the study of this enzyme system. The products of HO-mediated heme degrad...
 degradation (biliverdin, bilirubin, CO, and ferrous iron) regulate important biological processes including oxidative stress, inflammation, apoptosis, cell proliferation, fibrosis, and angiogenesis. Several recent reviews and editorials have highlighted the biological effects of the reaction product(s) and the importance of HO-1 as a potent cytoprotective enzyme in diverse conditions. A list of vascular diseases associated with HO-1 and CO is shown in Table 3. The purpose of this article is to provide an overview of the role of HO-1 and CO in vascular biology, specifically as it relates to angiogenesis.

Angiogenesis and Vasculogenesis
The sprouting of endothelial cells from preexisting vessels along with their subsequent migration and proliferation for the generation of tubelike structures is termed angiogenesis. The de novo formation of blood vessels from bone marrow–derived precursor cells, a population that possesses great plasticity, is the process of vasculogenesis. Aside from its critical role in fetal development, vasculogenesis also has been shown recently to play a major role in adult neovascularization. Angiogenesis is necessary for the development of several physiological and pathological processes, including endometrial proliferation and placental development, wound healing, cancer, and postschematic repair. Studies by Asahara and colleagues have elaborated the versatility of bone marrow–derived endothelial precursors in neovascularization in ischemia, wound healing, and cancer. The mobilization of these potentially therapeutic cell populations from the bone marrow into circulation and further colonization at angiogenic sites are influenced by modulators including VEGF, SDF-1, and other factors.

### Table 1. Similarities Between NO and CO

<table>
<thead>
<tr>
<th>Features</th>
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<tr>
<td>Gaseous modulators</td>
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<tr>
<td>Bind to hemoglobin</td>
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<tr>
<td>Formed by inducible and constitutive isoforms</td>
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<tr>
<td>Regulate intracellular cGMP</td>
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<tr>
<td>Neurotransmitter actions</td>
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<tr>
<td>Dose-dependent effects</td>
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<tr>
<td>Effects on leukocyte and platelet function</td>
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<td>Vasodilatory actions</td>
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### Table 2. Sources of CO

<table>
<thead>
<tr>
<th>Exogenous</th>
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<tbody>
<tr>
<td>Automobile exhaust</td>
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<tr>
<td>Cigarette smoke</td>
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<tr>
<td>Unvented kerosene and gas space heaters</td>
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<tr>
<td>Leaking chimneys and furnaces</td>
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<tr>
<td>Gas water heaters, wood stoves, and fireplaces</td>
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<table>
<thead>
<tr>
<th>Endogenous</th>
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<tr>
<td>Heme degradation via heme oxygenase</td>
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<tr>
<td>Lipid peroxidation</td>
</tr>
<tr>
<td>Photo-oxidation of organic compounds</td>
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<tr>
<td>Auto-oxidation of phenols, flavonoids, and halomethanes</td>
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### Table 3. Vascular Disorders Associated With HO/CO

<table>
<thead>
<tr>
<th>Aneurysms</th>
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<tr>
<td>Arteriovenous fistula restenosis</td>
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<tr>
<td>Atherosclerosis</td>
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<tr>
<td>Cancer</td>
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<tr>
<td>Hypertension</td>
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<tr>
<td>Impaired wound healing</td>
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<tr>
<td>Ischemia/reperfusion injury</td>
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<tr>
<td>Peripheral vascular disease</td>
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<tr>
<td>Preeclampsia</td>
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<tr>
<td>Psoriasis</td>
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<tr>
<td>Sickle cell disease</td>
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<tr>
<td>Transplant-related arteriosclerosis</td>
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<tr>
<td>Vascular restenosis</td>
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### Physiological Angiogenesis
Constant exchange of nutrients, respiratory gases, and waste products across the placenta is critical for proper fetal growth and development and is primarily dependent on the exchange of blood between maternal and fetal tissues. The rate of blood flow is determined by angiogenesis in the placenta and is necessary for the development of viable offspring. The role of HO in pregnancy has been reviewed recently. In preeclamptic patients, HO-1 protein levels in the placenta and exhaled CO (an indicator of HO activity) are significantly lower than in healthy pregnant women. It has also been reported that women who smoke during their pregnancies have significantly less incidence of developing preeclampsia, suggesting that exposure to cigarette smoke, an exogenous source for CO, may be protective in preeclampsia. Recent studies showing that HO-1 and CO block the release of antiangiogenic mediators of preeclampsia, soluble fms-like tyrosine kinase-1 (sFlt1) and soluble endoglin (sEng), provide further evidence for a protective role of HO-1/CO in pregnancy.

### Tumor Angiogenesis
Several human tumors, including renal cell and prostate cancer, express high levels of HO-1. HO-1 may promote tumor cell survival, hindering the effectiveness of anticancer therapies. In contrast, inhibition of HO has been shown to enhance tumor regression in animal models, suggesting that the HO-1 pathway may be a therapeutic target in carcinogenesis. The balance of endothelial cell proliferation
and apoptosis is critical in mediating tumor angiogenesis and affects growth, invasion, and metastasis of tumors. Several angiogenic factors promote angiogenesis as well as survival of endothelial cells. In addition, HO-1 and CO enhance endothelial cell survival through antiapoptotic mechanisms. VEGF promotes endothelial cell survival not only in embryonic vasculogenesis but also in tumor angiogenesis. In the initial stages of tumor growth, a process resembling chemotaxis occurs toward the already existing host vasculature before tumor angiogenesis. These vessels then regress because of apoptosis of the resident endothelial cells, followed by induction of specific signals, nutrient gradients, growth factors, and chemokines that are secreted by the host vessels. Proangiogenic bone marrow cells including subsets of hematopoietic cells, which are known to provide vascular support as well as endothelial progenitor cells (EPC), are known to differentiate into functional vascular cells, which contribute to tumor vasculature.

Recent evidence indicates that the chemokine SDF-1 has a major role in the recruitment and retention of CXCR4+ bone marrow–derived cells to the neoangiogenic niches, supporting revascularization of not only ischemic tissue but also areas of tumor growth. SDF-1 also promotes tumor cell growth, migration, and invasion and has profound effects on the tumor microenvironment. CXCR4, the receptor for SDF-1, is implicated in the cross talk between tumor cells and the tumor microenvironment. Inhibition of the SDF-1/CXCR4 axis attenuates tumor growth in vivo by inhibiting angiogenesis in a VEGF-independent manner.

**Wound Healing**

The replacement of damaged capillaries and reestablishment of a steady supply of oxygen to a wound are accomplished by neovascularization. The phases in wound healing including a coagulation phase (characterized by endothelial dysfunction and platelet activation), early extracellular matrix deposition, release of factors by platelets, inflammatory phase, and the resulting granulation are all events that rely on angiogenesis. Growth factors including VEGF, chemokines like SDF-1, and hypoxia-inducible factors (HIFs) also coordinate the multifaceted events involved in wound healing. Interestingly, compared with wild-type littermate mice, HO-1–deficient mice exhibit impaired wound healing due, in part, to reduced recruitment of EPC and capillary formation at the site of injury. It would be of interest to examine the effects of CO in reversing the defective wound repair in HO-1 knockout mice.

**Mediators of Angiogenesis**

Proangiogenic chemokines, such as SDF-1, and growth factors, such as VEGF, are essential elements in angiogenesis in the context of ischemic injury. Ischemia results in an increase in SDF-1 levels that leads to increased EPC number and formation of new blood vessels in the injured tissue. Overexpression of SDF-1 in ischemic tissues enhances EPC recruitment from peripheral blood to induce neovascularization. Although an influential role of VEGF and a potential synergy between VEGF and SDF-1 in therapeutic neovascularization have been suggested, VEGF-independent effects have also been clearly demonstrated. Pathological retinal neovascularization associated with proliferative diabetic retinopathy results from an imbalance in proangiogenic and antiangiogenic factors such as VEGF and SDF-1 as well as changes in other chemokines, cytokines, adhesion molecules, and immune cells. Notably, HO-1–deficient EPC are unable to reendothelialize the retinal vasculature after ischemic injury compared with wild-type EPC.

There is considerable evidence to support the inverse correlation between the number of circulating EPC and risk factors for atherosclerosis. In patients with diabetes mellitus, the circulating EPC number and the ability to form tubes correlate with glycemic control. Cytoskeletal alterations dictate the impaired mobility of EPC for the purpose of vascular repair in the diabetic milieu. The presence of vasodilators, such as NO, seems to reverse these defects and mobilizes this population into the circulation for repair. Defects in EPC function and signaling and peripheral tissue responses to hypoxia have also been shown to be associated with diabetes. Smoking has also been linked to decreased EPC numbers, and cessation of smoking reverses this phenomenon. EPC number also correlates with the extent of ischemia in stroke or myocardial infarction.

The therapeutic efficacy of EPC as a mode of cell therapy has drawn much attention. Reports from preclinical studies indicate that transplantation of EPC improved not only neovascularization and blood flow recovery but also reduced limb necrosis in models of hind limb ischemia, even with subtherapeutic doses of EPC. Initial clinical trials testing the efficacy of EPC in patients with coronary artery disease reported promising results. However, more recent, larger randomized controlled studies have shown only modest short-term benefits in the setting of coronary artery disease. Whether these results could be improved by engineering EPC with protective genes such as HO-1 would be of potential interest.

**Role of HO-1 and CO in Angiogenesis**

The first link of HO-1 in angiogenesis was suggested by Abraham and coworkers, who showed that overexpression of HO-1 in endothelial cells enhanced their proliferation. Subsequent experiments confirmed that HO-1 promotes endothelial cell cycle progression. Inhibition of HO-1 by antisense strategies decreased endothelial cell proliferation and capillary formation in vitro, an effect that was associated with increase in p21 and p27, inhibitors of the cell cycle. The studies supporting a role for HO-1 in angiogenesis have shown that proangiogenic factors such as VEGF and, more recently, SDF-1 activate HO-1 expression in endothelial cells. Furthermore, local HO inhibition with zinc protoporphyrin blocks angiogenesis and tumor growth in vivo. In parallel, in vivo studies have linked enhanced expression of HO-1 in tumor-infiltrating macrophages to accentuated angiogenesis in human gliomas. Recent studies performed in vitro have shown that the proangiogenic properties of HO-1 are attributable to CO. The well-recognized vasodilatory, antiinflammatory, and antiapoptotic effects of CO also contribute to the potentiation of angiogenesis.

**Cross Talk Between NO and CO in Angiogenesis**

NO is recognized as a mediator of angiogenesis. It induces the synthesis of VEGF and also potentiates its effect on
endothelial cells. Production of VEGF in response to NO occurs in vascular smooth muscle cells overexpressing inducible NO synthase, and the effect is mimicked by NO donors. A similar influence of NO has been observed in tumor cells, keratinocytes, and several other cell types.

Endogenous NO and NO donors are potent inducers of HO-1. It can therefore be hypothesized that HO-1 and its by-products are also involved in NO-dependent angiogenic events. Indeed, enhancement of VEGF expression in vascular smooth muscle cells treated with interleukin-1β is partially dependent on HO activity. In these cells, induction of inducible NO synthase was accompanied by increased expression of HO-1, and inhibition of HO activity attenuated the effect of interleukin-1β. Similar interactions have been demonstrated in a rat model of adjuvant arthritis, in which tin protoporphyrin IX (SnPPIX) attenuated NO-dependent VEGF production. Also in tumor cells, treatment with NO donors enhanced VEGF expression in an HO-1–dependent manner. Thus, HO-1 is a mediator of NO-induced VEGF synthesis in various cells.

**VEGF and HO-1**

Treatment of cells by numerous activators of HO-1, such as heme, cobalt protoporphyrin, prostaglandin J₂, hydrogen peroxide, NO, or hypoxia, induces VEGF expression in an HO-1–dependent manner in a variety of cell types (Figure 2). Treatment of macrophages with prolactin and endothelial cells with interleukin-6 also enhances VEGF expression in an HO-1–dependent manner. Accordingly, genetic overexpression of HO-1 leads to the stimulation of VEGF synthesis. The angiogenic effect of heme, the “classic” activator of HO-1 expression, can be considered in relation to conditions associated with release of large amounts of heme from damaged erythrocytes, e.g., after tissue injury and in hemorrhagic tumors. In human HaCaT keratinocytes, short exposure to heme induces VEGF expression, but longer treatment attenuates its production, an effect probably related to the released iron. This observation may explain the discrepant results in some studies wherein no induction of VEGF on heme exposure has been reported.

Potentially all 3 by-products of HO-1 activity can affect the synthesis of VEGF. In smooth muscle cells and human microvascular endothelial cells, CO, but not biliverdin or bilirubin, enhances VEGF synthesis. Treatment of vascular smooth muscle cells with 1% CO gas or endothelial cells with CORM-2, a CO-releasing molecule, induces VEGF production. Accordingly, inhibition of CO action, either by scavenging with oxyhemoglobin or by attenuation of soluble guanylyl cyclase activity, abolishes the stimulatory effect of prostaglandin J₂ (PGJ₂) on VEGF synthesis. HO-1 can exert its effect by stimulating transcription factors, such as nuclear factor-κB or activator protein-1, which regulate VEGF synthesis.

Induction of HO-1 expression by oxidative stress suggests that HO-1 can be particularly involved in the angiogenesis linked to inflammatory conditions. Indeed, the demonstration of enhanced vascularization in tumors overexpressing HO-1, the link between HO-1 and angiogenesis in rheumatoid arthritis, and the significance of HO-1 in wound healing confirm such a supposition. HO-1 induction can also be elicited by overexpression of other genes, particularly oncogenes. In an elegant study, Marinissen et al demonstrated that herpes virus-8–derived G-protein coupled receptor induces potent HO-1 upregulation and concomitant VEGF synthesis in fibroblast and endothelial cells. It would be worthwhile to investigate the involvement of HO-1 in mediating the angiogenic effects of other oncogenes.

**HO-1 as a Downstream Mediator of Angiogenic Stimuli**

The findings that NO is a mediator of both upstream VEGF synthesis and downstream response of endothelial cells to VEGF stimulation suggest that HO-1/CO can also be involved in a similar fashion. Indeed, stimulation of endothelial cells with VEGF in the presence of SnPPIX, a blocker of HO activity, attenuates their proliferation and differentiation in angiogenic assays in vitro. Endothelial cells from HO-1 knockout mice do not proliferate on VEGF stimulation. Conversely, overexpression of HO-1 enhances endothelial cell sprouting in response to VEGF exposure. The effect appears to be mediated by CO because cells treated with CORM-2 are more permissive to VEGF treatment.

VEGF is capable of inducing HO-1 expression in endothelial cells. Compared with HO-1 inducers like hemin, the increase in HO-1 protein after exposure to VEGF is delayed and occurs after 24 to 48 hours in human umbilical vein endothelial cells and microvascular endothelial cells. In contrast, treatment with fibroblast growth factor-1 (acidic fibroblast growth factor) does not induce HO-1 expression in endothelial cells, although murine endothelial cells devoid of HO-1 are insensitive to fibroblast growth factor-2 (basic fibroblast growth factor) stimulation. Recent work by Siner and colleagues has shown that lung-specific overexpression of VEGF results in marked induction of HO-1 in the lung and is protective in an in vivo model of hyperoxia-induced lung injury through the HO-1 pathway. HO-1 has been also implicated in the regulation of synthesis and activity of other growth factors known to be involved in angiogenesis indirectly. For example, upregulation of HO-1 was observed in various cells on treatment with TGF-β, PDGF, hepatocyte growth factor, nerve growth factor, and interleukin-6. POSM.
factor, fibroblast growth factor-1, and fibroblast growth factor-2, although such an effect has not been demonstrated as yet in endothelial cells. It remains to be elucidated whether these growth factors, known to upregulate VEGF production, can do so via an HO-1–dependent mechanism. Induction of HO-1 in endothelial cells, resulting in increased proliferation, is observed on exposure to prolactin, which stimulates endothelial tube formation on Matrigel. Overexpression of thymidine phosphorylase or treatment with its catalytic product 2-deoxy-D-ribose-1-phosphate and downstream 2-deoxy-D-midine phosphorylase or treatment with its catalytic product facilitates repair. The importance of SDF-1 in angiogenesis is referred to as CXCL12) binds to a high-affinity receptor, CXCR4, and is the predominant chemokine that mobilizes hematopoietic stem cells and EPC to sites of injury and is implicated in EPC migration. SDF-1–inhibited HO-1. Treatment of EPC with CORM-2 was able to phosphorylate VASP in the absence and presence of HO-1. Treatment of EPC with CORM-2 also resulted in a rapid redistribution of VASP to the filopodia (Figure 3). A hypothetical model for the mechroscopic role for CO in promoting SDF-1–mediated postischemic neovascularization was also explored. The addition of CORM-2, but not bilirubin, restored responsiveness of HO-1−/− aortic rings to SDF-1, providing direct evidence for CO-dependent modulation of the effects of SDF-1. Because CO has been implicated to modestly activate soluble guanylate cyclase and affect downstream cellular targets, the phosphorylation status of vasodilator-stimulated phosphoprotein (VASP) at the protein kinase G preferred site at Ser 239 by SDF-1 and CORM-2 was investigated in HO-1−/− and HO-1+/− endothelial cells. VASP is a cytoskeletal-associated protein that is abundant in microfilaments and is implicated in EPC migration. SDF-1–induced phosphorylation of VASP was dependent on HO-1 because no phosphorylation was observed in HO-1−/− cells. However, CORM-2 was able to phosphorylate VASP in the absence and presence of HO-1. Treatment of EPC with CORM-2 also resulted in a rapid redistribution of VASP to the filopodia (Figure 3). A hypothetical model for the mechanistic link between HO-1 and SDF-1 in vascular repair is shown in Figure 4. Other potential mechanisms including effects of CO on cell cycle regulatory proteins such as p21, cell proliferation, and recruitment of bone marrow–derived cells could also contribute to the SDF-1–mediated proangiogenic effects of HO-1 and need further exploration.
downregulation has been observed.82 The expression of HO-1 is not induced by hypoxia, and even interestingly, in many human cells, particularly endothelial, sequence has been found in the promoter of murine HO-1, but, HO-1 gene expression. The hypoxia response element sequence has been identified in HO-1, which increases HIF-1 stability, also strongly induces HO-1 and stimulates VEGF synthesis through HIF-1 but not HO-1. In these microvascular endothelial cells, hypoxia does not induce HO-1 but stimulates VEGF through HIF-1.

Cross Talk Between Hypoxia, Hypoxia-Inducible Factor-1, and HO-1 in Angiogenesis

HIF-1 is a transcriptional activator that is potently induced in hypoxia and drives the expression of >100 genes (reviewed in Pouyssegur et al79). Active HIF-1 consists of HIF-1α and HIF-1β, both of which are constitutively generated in cells; however, under normoxic conditions HIF-1α is immediately degraded. The process is dependent on hydroxylation of proline residues and is performed by a group of specific prolyl hydroxylases, oxygen, α-ketoglutarate, and iron-dependent enzymes.79 The capability to hydroxylate prolines disappears during low oxygen tension, resulting in the stabilization of the HIF-1α subunit. HIF-1 is a potent inducer of VEGF expression.79 Interestingly, the stability of HIF-1α can be increased in normoxia on exposure to NO80 or reactive oxygen species.81 HIF-1 is also linked to the upregulation of HO-1 gene expression. The hypoxia response element sequence has been found in the promoter of murine HO-1, but, interestingly, in many human cells, particularly endothelial, the expression of HO-1 is not induced by hypoxia, and even downregulation has been observed.82

The stimulatory effect of hypoxia (1% oxygen) on VEGF in rat vascular smooth muscle cells can be reverted by inhibitors of HO activity.57 In human HaCaT keratinocytes, hypoxic induction of HIF upregulates VEGF in an HO-1–dependent manner, as the effect is blunted by inhibitors of HO-1 activity or HO-1 siRNA.64 The production of VEGF, induced by cobalt chloride, in human microvascular endothelial cells is dependent on reactive oxygen species–driven stabilization of HIF-1 but independent of HO-1, although cobalt chloride concomitantly induces HO-1 expression (Figure 5).83

CO treatment can activate HIF-1 by inducing systemic hypoxia. However, in vitro studies show that hypoxic induction of HIF-1 is attenuated by CO.83 The same effect is exerted by NO,64 indicating that the influence of CO and NO on HIF can vary depending on the oxygen availability. Treatment with CO or NO in hypoxia attenuates VEGF expression in vascular smooth muscle cells,63 and CO also downregulates hypoxic induction of PDGF.84 Interestingly, induction of VEGF by hypoxia is also attenuated by PGJ2,85 which is a potent inducer of HO-1 expression via the Nrf2 transcription factor;86 and in normoxic conditions PGJ2 enhances VEGF expression in an HO-1–dependent manner.60 However, inhibition of VEGF production in hypoxia by PGJ2 is further attenuated by blocking of HO-1, suggesting that possibly other HO-1–independent pathways are responsible for the variable effects of PGJ2 under different oxygen tensions.

On exposure of human microvascular endothelial cells to cobalt protoporphyrin IX (CoPPIX), VEGF production is augmented in an HO-1–dependent manner, but HIF-1 transcription factor is not activated (Figure 5).83 In contrast, CO treatment stabilizes HIF-1 in murine macrophages.87 The effect is dependent on reactive oxygen species production by mitochondria and leads to increased synthesis of TGF-β. However, in another study, retroviral overexpression of HO-1 attenuates TGF-β production in rat endothelial cells, whereas it concomitantly upregulates VEGF,88 suggesting that the stimulatory effect of HO-1/CO on TGF-β may be cell-type specific.

Angiogenesis in HO-1 Deficiency

Animals lacking the functional HO-1 gene do not demonstrate any visible phenotype suggestive of defective angiogenesis. However, the impairment of pregnancy in HO-1−/− homozygotes and the significant mortality of HO-1−/− embryos89 suggest an effect of HO-1 on prenatal angiogenesis. It would be interesting to test whether CO could normalize pregnancy outcomes in HO-1−/− mice. The role of HO-1 in development is supported by observations showing that overexpression of HO-1 enhances VEGF in placenta, and an increased pup size is noted in such animals.90

The absence of HO-1 disrupts not only the production of VEGF on stimulation with H2O2, hemin, lysophosphatidylcholine, and PGJ2,91 but also the response of endothelial cells to angiogenic stimuli. The proliferation of murine endothelial cells stimulated with VEGF or fibroblast growth factor-2 is almost completely blunted by HO-1 inactivation.65 SDF-1–induced proliferation and migration of endothelial cells or EPC from HO-1−/− mice is also deranged.94 This defect is also seen in vivo because the formation of blood vessels during wound healing is impaired in HO-1 knockout mice.34 Diminished expression of HO-1 may also impede vascularization by increasing production of antiangiogenic mediators in vivo. In agreement with this are the recent findings that plasma of HO-1 knockout animals contains higher levels of sFlt1 and sEng than plasma of their wild-type counterparts.20

HO-1–Dependent and –Independent Regulation of Angiogenic Mediators

Data on the effect of HO-1 on the production of other angiogenic mediators are limited. Transcriptome analysis revealed several potential candidates in tumor cells overexpressing HO-1.23 The synthesis of interleukin-8 (IL-8), a member of the chemokine family with important roles in tumor growth, angiogenesis, and metastasis, can be regulated by HO-1. Accordingly, human umbilical vein endothelial cells treated with S-nitroso-penicillamine (SNAP), a NO donor, expressed HO-1 and produced more VEGF and IL-8.92

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![Figure 5. HO-1–dependent and –independent regulation of VEGF and IL-8 synthesis. In human microvascular endothelial cells, induction of VEGF synthesis by CoPPIX and PGJ2 is dependent on HO-1. However, the same stimuli enhance IL-8 expression independently of HO-1. Cobalt chloride (CoCl2), which increases HIF-1 stability, also strongly induces HO-1 and stimulates VEGF synthesis through HIF-1 but not HO-1. In these microvascular endothelial cells, hypoxia does not induce HO-1 but stimulates VEGF through HIF-1.](http://circ.ahajournals.org/)
When HO-1 expression was attenuated by transfection of specific siRNA or antisense oligonucleotides, the production of VEGF and IL-8 was blunted. With the use of VEGF neutralizing antibodies, SNAP-induced IL-8 synthesis was attenuated, whereas IL-8 neutralizing antibodies had no effect on VEGF production. On the other hand, the production of IL-8 by human microvascular endothelial cells, induced by CoPPIX, a potent activator of HO-1, is independent of HO-1 induction. In addition, synthesis of VEGF induced by CoPPIX was abolished by SnPPIX, indicating the existence of HO-1-dependent and –independent pathways regulating the production of VEGF and IL-8, respectively (Figure 5). Furthermore, PGJ2 enhances VEGF synthesis in an HO-1–dependent manner, and the production of IL-8 is not blocked in the presence of inhibitors of HO activity (Figure 5).

Monocyte chemotactic protein-1 is a mediator of inflammatory angiogenesis, stimulating macrophage recruitment and promoting inflammation in atherosclerosis, cancer, or rheumatoid arthritis. Interestingly, hemin induced monocyte chemotactic protein-1 expression in kidney proximal tubular epithelial cells through both HO-1–dependent and –independent pathways. Whether such a mechanism is relevant to the proangiogenic activity of monocyte chemotactic protein-1 remains to be established.

**Dual Role of HO-1 in Angiogenesis**

The role of HO-1 in angiogenesis may vary depending on the underlying conditions. Bussolati and coworkers demonstrated that VEGF-induced angiogenesis required HO-1 activity, whereas inflammation-induced blood vessel formation was attenuated by overexpression of HO-1. In lipopolysaccharide-induced angiogenesis, blood vessel formation is secondary to leukocyte infiltration, which can be attenuated by prior induction of HO-1 expression, which prevented leukocyte invasion into Matrigel plugs and subsequent angiogenesis. Conversely, in VEGF-induced noninflammatory angiogenesis, pharmacological inhibition of HO-1 induced marked leukocytic infiltration, which further enhanced VEGF-induced angiogenesis. However, blocking of HO-1 with interruption of leukocytic infiltration by anti-CD18 antibodies inhibited VEGF-induced angiogenesis. In addition, inhibition of HO enzyme activity with SnPPIX significantly decreased angiogenesis induced by agonistic antibodies against CD40. Thus, it is hypothesized that during chronic inflammation, HO-1 (1) inhibits leukocytic infiltration and (2) facilitates tissue repair by promoting VEGF-driven noninflammatory angiogenesis. Such an interaction may also operate in portal hypertensive rats, in which HO-1 attenuates oxidative stress and inflammation in the splanchnic circulation, whereas it concomitantly induces VEGF production.

This complex interaction indicates that when HO-1 expression occurs in the environment free of the inflammatory reactions, the products of HO-1 activity can be proangiogenic. In contrast, when blood vessel formation is driven by lipopolysaccharide-induced inflammation, the expression of HO-1 may inhibit new vessel formation. However, in inflammatory conditions associated with cancer, rheumatoid arthritis, or wound healing, HO-1 may also be proangiogenic.

**Pharmacological Modulation of HO-1 in Angiogenesis**

HO-1 expression can be enhanced by numerous compounds, including several drugs that are in clinical use in cardiovascular diseases (eg, statins, rapamycin, erythropoietin, probucol), but not in endothelial cells. However, the physiological relevance of such an effect is not obvious because induction of HO-1 expression occurs particularly at high, micromolar concentrations of statins, which are not attained in patients (reviewed in Stocker and Perrella and Dulak and Jozkowicz). Statins affect angiogenesis in a dual way, being proangiogenic at low, nanomolar concentrations and antiangiogenic at higher concentrations. However, higher doses of statins are also cytotoxic. This complicates the issue because pharmacological levels of these agents appear to be modestly HO-1 inducers in endothelial cells. Therefore, it remains to be established whether proangiogenic or antiangiogenic effects of statins via HO-1 operate in physiological conditions.

**Neovascularization by HO-1 Gene and Cell Therapy**

The beneficial effect of HO-1 gene transfer in vascular diseases in vivo has been linked to increased angiogenesis. In a rat hind limb ischemia model, adenoviral delivery of HO-1 enhances angiogenesis in the ischemic muscles through production of VEGF, an effect that is abrogated by inhibition of HO activity. Agel-related and disease-linked impairment of EPC may be due to a loss of antioxidative defense. EPC express antioxidative enzymes, like catalase, glutathione peroxidase-1 (GPx-1), and manganese superoxide dismutase, which make them resistant to oxidative stress. Experimental overexpression of manganese superoxide dismutase enhances EPC protection, whereas knockout of GPx-1 gene diminishes viability and impairs vasulogenic potency of EPC. Our recent work has shown that the same may apply to HO-1 because the function of EPC in vitro and in vivo is impaired by the lack of HO-1. Therefore, it is possible that enhanced expression of HO-1 in EPC can improve neovascularization in postnatal vasculogenesis.

In a recent study, rabbit EPC, modified with retroviral vectors harboring either green fluorescent protein, endothelial NO synthase, or HO-1, were delivered to denuded arteries. As expected, the instillation of progenitor cells enhances the process of reendothelialization. Additionally, overexpression of endothelial NO synthase in EPC significantly improved endothelial regeneration in comparison to green fluorescent protein–transduced cells. HO-1 transduction, however, did not affect the capacity of EPC. The reason for such an insufficiency of HO-1 is not clear. Although HO enzyme activity and CO concentrations were not assessed in these studies, the authors suggest that the level of HO-1 expression may not have been sufficiently high to generate CO in amounts required to enhance endothelial cell proliferation. Hypoxia-regulated vectors harboring HO-1 have recently been used to modify murine mesenchymal stem cells. HO-1 overexpression enhances the tolerance of engrafted mesen-
chymal stem cells to hypoxia-reoxygenation injury in vitro and improves their viability in ischemic hearts.112 Further examination of engineered stem cells or EPC with HO-1 in models of angiogenesis in vivo would be of immense interest.

**Perspectives and Therapeutic Implications**

HO-1 is as an inducer of VEGF synthesis and is a downstream mediator of the activity of 2 major angiogenic growth factors, VEGF and SDF-1. HO-1 enhances endothelial cell and EPC proliferation, migration, and differentiation. The angiogenic response, in part, may also be dependent on the salutary effect of HO-1 on endothelial cell apoptosis. HO-1 induces the synthesis of VEGF, which in turn enhances HO-1 expression. Such a positive feedback can be of particular importance in EPC, which are known to be permissive not only to proangiogenic mediators but also are capable of generating large amounts of growth factors. It is possible that the presence of HO-1 in such cells can facilitate their survival in a noxious environment. Expression of HO-1 can protect EPC from oxidative injury, and it can also mediate EPC migration.34 Several of these HO-1–dependent processes are due, at least in part, to CO. The beneficial effects of CO as an inhaled gas or through the use of CO-releasing molecules have been demonstrated in cell culture and animal models of a number of diseases (reviewed by Wu and Wang,1 Durante et al.,113 Kim et al.,114 and Motterlini et al115). As the results of inhaled CO treatment from clinical trials116 become available, the utility of this therapy to facilitate angiogenesis in ischemia/reperfusion and wound healing will need to be established.

Interaction between HO-1 and angiogenesis, although crucial for proper wound healing and neovascularization of ischemic heart and peripheral muscles, will have obvious detrimental outcomes in diseases, in which new blood vessel formation is undesirable. Indeed, overexpression of HO-1 is linked to enhanced tumor neovascularization.23,57,59,117,118 Therefore, blocking HO activity can be considered an additional strategy to enhance the efficiency of antitumor therapy. Additionally, the role of HO-1 in lymphangiogenesis remains to be explored. These data are not as yet available, although 1 study demonstrated the lack of an effect of HO-1 on expression of VEGF-C,59 one of the VEGF receptors that regulates lymphatic vessel growth. Further studies should also elucidate the detailed mechanisms of induction of HO-1 expression by VEGF and SDF-1. More work is required to envisage the role of HO-1 in the growth of atherosclerotic plaques. It remains to be established whether interactions between HO-1 and VEGF, and probably other growth factors, play any role in the progression of atherosclerosis.

Human HO-1 expression is dependent partially on a GT repeat length polymorphism in the proximal promoter.119 Epidemiological studies have linked long GT repeats (>30) with aggravation of human diseases, whereas short repeats (<25) provide a higher level of HO-1 expression in response to oxidative stress120 and have been claimed to protect against numerous pathologies, including restenosis, emphysema, and graft rejection (reviewed in Exner et al121). However, several recent studies in large populations have shown no effect of the GT repeat polymorphism in vascular restenosis and other disorders.122,123 It remains to be established whether such a polymorphism is of significance in terms of EPC function in patients with vascular diseases.

The role of HO-2, the constitutive isofrom of HO, in angiogenesis is also emerging. A recent study demonstrated that in animals lacking HO-2, corneal wound closure was impaired, and this was characterized by enhanced neovascularization.124 Exaggerated inflammation, represented by an increased number of leukocytes, superoxide, cyclooxygenase-2 expression, and elevated levels of KC chemokine, was observed, whereas HO-1 was downregulated in the HO-2–deficient mice. It can be hypothesized that decreased expression of HO-1 in HO-2+/− animals is responsible for the aggravated inflammation-dependent angiogenesis, which appears to be downregulated by HO-1, supporting the dual role of HO-1 in angiogenesis.96

It is not known whether the lack of or a decrease in HO-1 expression can be linked to any human angiogenesis-related pathologies, although the natural silencing of HO-1 in the single human case involved endothelial cell injury.125 The real significance of the upregulation of HO-1 and CO in cardiovascular diseases remains to be assessed in human subjects. Is it merely a marker of an oxidative injury or a prerequisite mediator of endogenous protection against more severe insults? In the latter case, finding potent and safe pharmacological agent(s) may allow us to employ the HO-1/CO pathway for the prevention of disease initiation and progression and modulation of neovascularization in an effective way.

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None.

**References**


