Targeting Heme Oxygenase

Therapeutic Implications for Diseases of the Cardiovascular System

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Abstract: Heme oxygenase (HO) is important in attenuating the overall production of reactive oxygen species through its ability to degrade heme and to produce carbon monoxide, biliverdin/bilirubin, and release of free iron. Excess free heme catalyzes the formation of reactive oxygen species, which leads to endothelial cell (EC) dysfunction as seen in numerous pathologic vascular conditions including systemic hypertension and diabetes, as well as in ischemia/reperfusion injury.

The up-regulation of *HO-1* can be achieved through the use of pharmaceutical agents such as metalloporphyrins and statins. In addition, atrial natriuretic peptide and nitric oxide donors are important modulators of the heme-HO system, either through induction of *HO-1* or the increased biologic activity of its products. Gene therapy and gene transfer, including site- and organ-specific targeted gene transfer have become powerful tools for studying the potential role of the 2 isoforms of HO, *HO-1*/*HO-2*, in the treatment of cardiovascular disease, as well as diabetes. *HO-1* induction by pharmacological agents or the in vitro gene transfer of human *HO-1* into ECs increases cell cycle progression and attenuates angiotensin II, tumor necrosis factor-α, and heme-mediated DNA damage; administration in vivo corrects blood pressure elevation after angiotensin II exposure. Delivery of human *HO-1* to hyperglycemic rats significantly lowers superoxide levels and prevents EC damage and sloughing of vascular EC into the circulation. In addition, administration of human *HO-1* to rats in advance of ischemia/reperfusion injury considerably reduces tissue damage.

The ability to up-regulate *HO-1* either through pharmacological means or through the use of gene therapy may offer therapeutic strategies for the prevention of cardiovascular disease in the future. This review discusses the implications of *HO-1* delivery during the early stages of cardiovascular system injury or in early vascular pathology, and suggests that pharmacological agents that regulate HO activity or *HO-1* gene delivery itself may become powerful tools for preventing the onset or progression of various cardiovascular diseases.

Key Words: heme oxygenase, carbon monoxide, bilirubin, diabetes, circulating endothelial cells, hypertension

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The heme-heme oxygenase (HO) system may be regarded as a regulator of endothelial cell (EC) integrity and oxidative stress. Heme is the prosthetic group of numerous enzymes and is important to EC function through regulation of the activity of soluble guanylate cyclase (sGC), nitric oxide synthase (NOS), cytochrome P450 monooxygenases, cyclooxygenase (COX), and catalase.[1] HO has been reported to exist as isoenzymes *HO-1*, *HO-2*, and *HO-3*;[2] however, *HO-3* has no activity and is not expressed in humans (G. Scapagnini, personal communication). *HO-2* is the constitutive isoform,[3–5] whereas *HO-1* is the isoform that is induced by a broad spectrum of pharmaceutical agents. We have shown previously that overexpression of HO can be achieved through site- and organ-specific gene delivery by means of adenoviral, retroviral, and liposome-based vectors.[6–11]

HO-1 and *HO-2* play a major role in heme breakdown[1,12] and are alike in terms of mechanism of heme oxidation, cofactor and substrate specificities, and susceptibility to inhibition by synthetic metalloporphyrins. In the latter, the central iron atom of heme is replaced, for example, by zinc, tin, chromium, or other metals.[1] Synthetic heme analogues have been used in animals and in humans to either suppress or induce HO activity and to inhibit development of severe hyperbilirubinemia in newborns.[13–15] The recognition that *HO-1* is induced by both oxidant stress and its substrate, heme, in conjunction with the robust ability of *HO-1* to protect against oxidative insult,[7,16–19] has led to an examination of the antioxidant nature of *HO-1* and *HO-2*.[1,7,18,20] Antioxidant effects arise from the capacity of *HO-1* to degrade heme from destabilized heme proteins[21] and from the formation of biliverdin and bilirubin, degradation products with potent antioxidant properties (Fig. 1).[22,23]

Carbon monoxide (CO), also a heme degradation product, is not an antioxidant,[24] but causes the induction of antioxidant genes. It decreases superoxide (O_2^-) levels,[25,26] increases glutathione levels,[27] and has an antiapoptotic effect.[28] Furthermore, CO is a vasodilator and has been shown to regulate basal and constrictor-induced vascular tone in blood vessels.[29–34] Up-regulation of bilirubin and CO, through the induction of *HO-1* and increase in HO activity, has shown promise in protection against oxidative stress and injury, whereas the absence of *HO-1* in mice resulted in accelerated atherosclerotic lesion formation and vein-graft disease as well as elevated blood pressure, cardiac hypertrophy, and acute renal failure. In addition, organ damage and mortality are more frequent in *HO-1* null mice.[35] Induction of *HO-1* also prevents cell death attributable to augmentation of ion efflux and exportation of iron-binding protein.[36] The protective actions of *HO-1* and/or CO are not confined to overtly oxidant processes. Conners et al[37] reported that induction of *HO-1* has an anti-inflammatory effect, which has been confirmed by other studies.[38,39] Furthermore, *HO-1* induction is cytoprotective in atherosclerosis,[40–42] sepsis,[24] diabetes,[26,43] lung injury,[44] occlusive vascular disease, and ischemia.[45–47]

In this review, we will discuss the normal functioning of the HO system, how alterations in the system can influence cardiovascular pathology, and what therapeutic approaches exist for augmenting HO activity to prevent and treat cardiovascular disease. A major component of cardiovascular disease is vascular injury and dysfunction. This review will show how *HO-1* induction has different functions on ECs compared with vascular smooth muscle cells (VSMC), emphasizing that all actions can be regarded as beneficial. In ECs, *HO-1* facilitates endothelial repair and increases NO production by accelerating cell proliferation and inducing eNOS via an increase in (phosphorylated AMP kinase [pAMPK]). Antioxidant protection is increased while decreasing the expression of cell adhesion molecules, thereby decreasing the number of inflammatory

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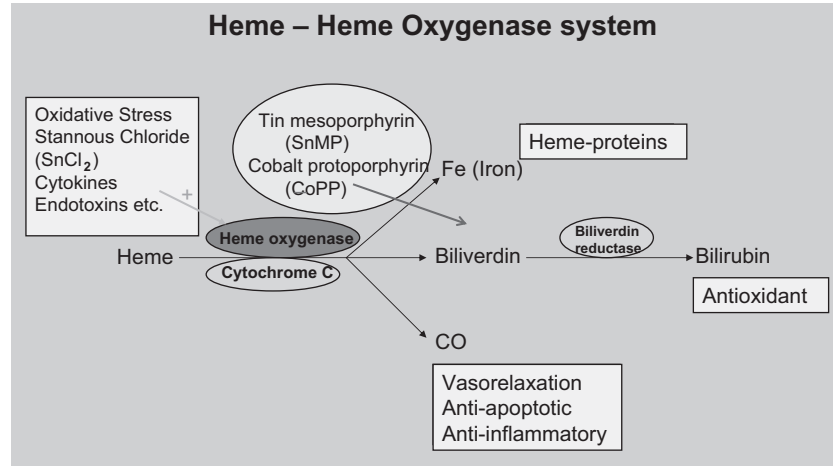

FIGURE 1. The heme-HO system, including the metabolism of heme and effects of HO on its final products. Bilirubin is an antioxidant with properties allowing reduction of reactive oxygen species. Carbon monoxide (CO) is an antiapoptotic agent with the capability of reducing cytokines and chemokines, which are proinflammatory proteins.

cells in the vascular wall. In VSMC, *HO-1* decreases cell proliferation, increases apoptosis, relaxation, and antioxidant protection. In essence, the HO system is a critical defense system of the human body. Humans deficient in *HO-1* have died prematurely due to vascular dysfunction and renal failure.[48] Thus, the HO system presents an excellent prospective target for beneficial therapeutic manipulation in cardiovascular disease.

ANTIOXIDANT EFFECT OF HO-DERIVED BILIRUBIN

Biliverdin, one of the products of heme degradation, is rapidly converted into bilirubin by the cytosolic enzyme biliverdin reductase at the expense of nicotinamide adenine dinucleotide phosphate (NADPH). Unconjugated bilirubin efficiently scavenges singlet oxygen and serves as a reducing agent for certain peroxidases, including horseradish peroxidase and prostaglandin H synthase, in the presence of hydrogen peroxide or organic hydroperoxides. Biliverdin and bilirubin are reducing species and hence potential antioxidants.

An adult human produces about 300 mg of bilirubin per day.[49,50] Some 80% to 85% of the bilirubin produced in vivo is derived by heme catabolism of hemoglobin released by aging or damaged erythrocytes.[49,50] The biologic actions of bilirubin may be particularly relevant to prevention of the oxidant-mediated vasoconstrictive actions of tumor necrosis factor (TNF) and angiotensin II (Ang II).[51,52] Bilirubin, in low concentrations, scavenges reactive oxygen species in vitro, reduces oxidant-induced cellular injury, and attenuates oxidant stress in vivo.[22,53–62] The antioxidant and cytoprotective effects of bilirubin have been shown to attenuate Ang II-mediated EC DNA damage and cell death.[63] Ang II significantly stimulates O_2^- formation in monocytes, and exogenously applied bilirubin suppresses not only O_2^- formation but also Ang II-enhanced chemotactic activity in monocytes.[64]

Bilirubin has been shown to inhibit NADPH oxidase[65] and protein kinase activity[66]; both enzymes mediate Ang II-induced vascular injury.[67,68] Recently, biliverdin and bilirubin have been shown to preserve EC integrity[69] and to prevent EC death and sloughing, resulting in the enhancement of vascular reactivity in diabetic rats[26,70] and in the prevention of postangioplasty restenosis.[71,72] Bilirubin has also been implicated in reducing oxidative stress in experimental diabetes, in part by increasing the bioavailability of NO required for EC integrity. Bilirubin-mediated inhibition of protein kinase-C and NADPH oxidase may be one mechanism by which *HO-1* attenuates the diabetes-mediated generation of oxidants and the uncoupling of EC eNOS. Glucose enhances EC O_2^- production, leading to increased vascular formation of the NO/O_2^- reaction product, peroxynitrite. Peroxynitrite oxidizes the active NOS cofactor, tetrahydrobiopterin, to cofactor inactive molecules, such as dihydrobiopterin. This uncouples the enzyme, which then preferentially increases O_2^- production over NO production.[73] Functional eNOS expression, rather than dysfunctional uncoupled eNOS (Fig. 2) may be increased by increased *HO-1* gene expression. Peroxynitrite stimulates *HO-1* in nondiabetic pathologic conditions,[74] but not in diabetes,[25,26] due to a glucose suppressive effect on *HO-1* gene expression.[75] Thus, the *HO-1* gene expression-mediated increase in extracellular superoxide dismutase may protect eNOS from uncoupling, which in turn attenuates the diabetes-mediated generation of oxidants.[76,77]

In summary, *HO-1* derived bilirubin has important cytoprotective properties in the cardiovascular system.[62,78] This is manifested by the higher serum bilirubin levels seen in individuals with Gilbert syndrome, which is associated with a decreased risk for coronary artery disease. In addition, free and albumin-bound bilirubin has been shown to inhibit oxidation of low density lipoprotein (LDL).[54]

VASODILATORY, ANTIHYPERTENSIVE, AND ANTIAPOPTOTIC PROPERTIES OF HO-DERIVED CO

There are 2 major sources of CO in biologic systems: 1 is heme-dependent (80%), the other is heme-independent (~20%).[48] However, the rapid increase in CO that occurs in vivo is solely due to the induction of *HO-1*. Before CO was found to have an antihypertensive effect, induction of *HO-1* served to attenuate hypertension in spontaneously hypertensive rats (SHR).[7] This finding provided a crucial lead in defining the potential therapeutic benefits of regulating HO activity in the treatment of hypertension. Later, it was reported that CO and NO have similar properties,[79–81] both gases behaving as messenger and signaling molecules. CO and NO are capable of inducing the relaxation of blood vessels through vasodilation and inhibiting the proliferation of VSMC.[82] Like NO, HO-derived CO influences the sGC and cyclic guanosine monophosphate (cGMP) pathways, which serve to regulate both blood pressure and vascular contractility.[83] sGC increases cGMP, which in turn serves as a vasodilator to lower blood pressure levels. Upregulating the HO system in young (8-week-old) SHR cause sGC and cGMP levels to rise, leading to a significant reduction in blood pressure.[83,84] Conversely, by inhibiting *HO-1* activity, the blood pressure of rats significantly increases.[85] Moreover, CO has been shown to inhibit platelet aggregation and to stimulate angiogenesis.[86] Interestingly, CO regulates blood pressure cooperatively with NO in hypertensive rats, and by impairing the function and produc-

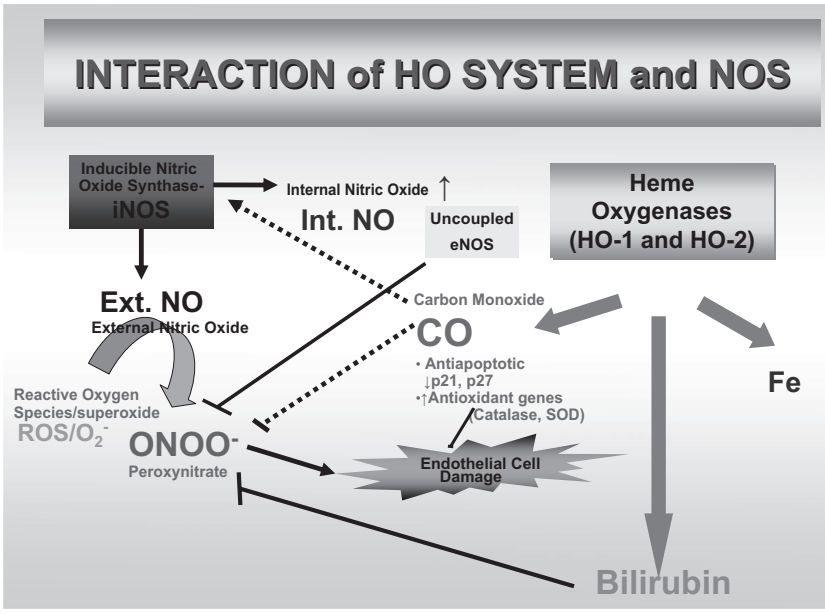

FIGURE 2. The effects of hypertension, diabetes and oxidants in a form of free radicals and peroxynitrate on endothelium along with ameliorative effects of HO system in a form of antioxidative properties of bilirubin and CO.

tion of NO, HO gene expression is increased and the effect of CO in the regulation of blood pressure is, in turn, augmented.[87]

CO, BILIRUBIN, AND BALLOON ANGIOPLASTY

CO, in addition to being a vasodilator, protects against ischemic tissue damage through antiapoptotic mechanisms.[46] Inducing *HO-1* during postischemic myocardial dysfunction results in myocardial function improvement after total ischemia and reperfusion.[88,89] Hemin was injected into rats, before inducing ischemia, to up-regulate *HO-1*.[90] It was demonstrated that during ischemia and reperfusion left ventricular pressure decreased, while end diastolic pressure, coronary perfusion pressure and coronary resistance increased. The products of heme degradation are protective in rodent models of ischemia-reperfusion injury, allograft and xenograft survival, intimal hyperplasia after balloon injury, and chronic graft rejection.[89] *HO-1* expression is increased in human atherosclerotic lesions.[42,91] as well as in vascular EC and SMC exposed to oxidized low-density lipoprotein (oLDL).[41]

CO generated through HO inhibits mitogen-induced proliferation of VSMCs, which in turn inhibits neointimal formation.[88] The pharmacological induction of *HO-1* also attenuates neointimal formation after balloon angioplasty-induced injury.[92] Furthermore, *HO-1* gene transfer attenuated the remodeling response to vascular injury by stimulating medial wall SMC apoptosis and inhibiting medial wall DNA replication.[92] Others have shown that adenovirus-mediated *HO-1* overexpression inhibits the growth of SMCs in vitro and in vivo,[44,92,93] suggesting the importance of *HO-1* in vascular wall remodeling. Overexpression of human *HO-1* in rabbit and rat ECs renders the cells resistant to hemoglobin toxicity and highlights not only the important metabolic and cytoprotective roles of the *HO-1* gene,[7,17] but its importance in cell cycle progression.[7,19,93]

Discovery of the differential effects of HO-derived products on ECs and SMCs opens an avenue toward understanding the basic and clinical problems of neointimal formation.[93] Three key findings substantiate this statement. The first is that increased HO activity in both ECs and SMCs,[51,93,94] as well as other mammalian cells,[95,96] regulates cell cycle progression. The second is that, in SMCs, up-regulation of *HO-1* gene expression is associated with a decrease in cell cycle progression. In ECs, unlike SMCs, overexpression of *HO-1* increases cell cycle progression and DNA distribution in the S and G_2/M phases.[25,93] The third is that inhibition of HO activity results in enhancement of cell cycle progression in SMCs. In contrast, cell cycle progression is reduced by inhibition of HO activity in ECs.

HO-1 has a differential effect on cell cycle proliferation in ECs and VSMCs.[71] Inhibition of *HO-1* led to VSMC proliferation, thus, causing stenosis in the lumen (while induction of *HO-1* stimulated neointimal formation compared with control) (Fig. 3). Transplantation of circulating endothelial progenitor cells overexpressing eNOS and *HO-1* to balloon-induced injured vessels served to enhance the vasculoprotective properties of the reconstituted endothelium, which in turn led to inhibition of neointimal hyperplasia.[71,97] These findings are of particular relevance because the differential role of *HO-1* could be a critical factor in vascular wall remodeling in different clinical situations, such as occurs in hypertension or VSMC hyperplasia, or in the recovery of the vascular wall after mechanical injury, such as restenosis.

CYTOPROTECTIVE EFFECTS OF HO-DERIVED CO AND BILIRUBIN AFTER MYOCARDIAL INFARCTION

The induction of *HO-1* may also have therapeutic benefits during chronic heart failure. Up-regulation of *HO-1* during heart failure serves to mitigate pathologic left ventricular remodeling and reduce myocardial hypertrophy, oxidative stress, and inflammatory activation.[98] Up-regulating *HO-1* has the potential of attenuating cardiac hypertrophy in genetically hypertensive rats.[99] More recently, studies in mice suggest that *HO-1* confers significant antioxidant and cytoprotective effects in the heart.[100] *HO-1* is induced by β-adrenergic receptor stimulation and may protect against β-adrenergic receptor-mediated apoptosis.[100] Further, induction of *HO-1* increases adult cardiomyocyte tolerance to ischemia after in vivo transplantation.[101] Autologous atrial cardiomyocytes are a readily available cell source for infarct repair; however, they readily undergo apoptosis, precluding their use as cellular repair grafts. Kawamoto et al[101] demonstrate that preconditioning with *HO-1* acts to retain functional viability in vivo in adult cardiomyocyte cellular grafts after implantation. This, in turn, may prove effective in repairing infarcted myocardium. This has been confirmed in mesenchymal stem cells.[102] Hypoxia-regulated *HO-1* vector modifica-

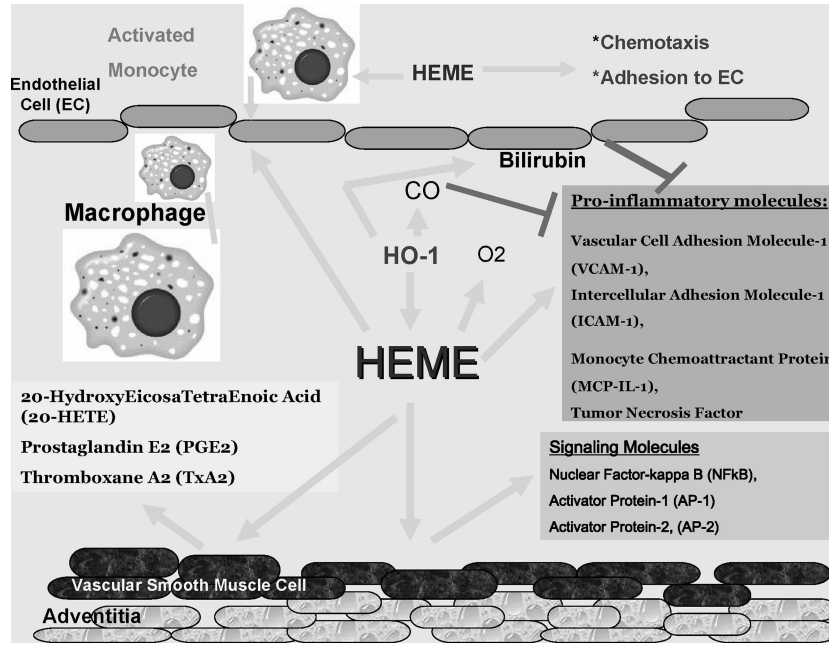

FIGURE 3. Heme and its pathologic properties exerted on ECs. Activation of monocytes, macrophages along with stimulation of chemotaxis and adhesion to EC caused by heme. Activation of *HO-1* after blockade of proinflammatory molecules, activated by heme. The effects of heme-HO system on oxidized LDL, VCAM-1, and ICAM-1.

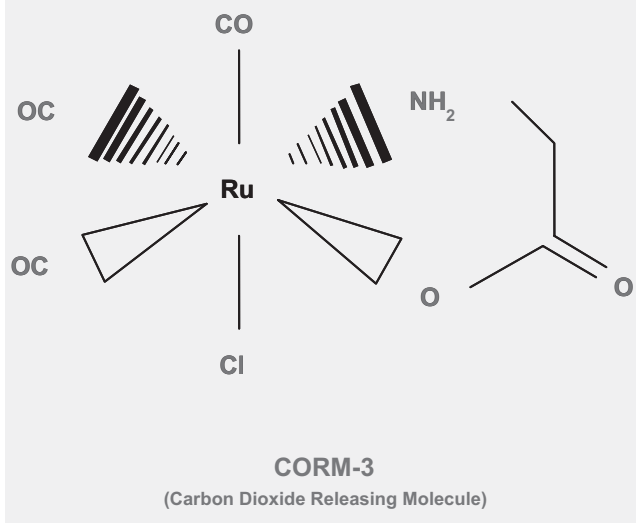

FIGURE 4. CORM-3, recently developed, supplies the CO molecule resulting in aortic vasodilatation and reduced blood pressure. This confirms the vasodilatory and cardiovascular benefits of CO.

tion enhanced the tolerance of engrafted mesenchymal stem cells to hypoxia-reoxygenation injury in vitro and improved their viability in an ischemic milieu. These findings may also make cell therapy more effective in infarct repair. However, it is important to remember that increased expression of *HO-1* protein alone is not a good indicator of increased HO activity; the latter must be measured.

CO RELEASING MOLECULES AND THEIR BENEFITS IN CARDIOVASCULAR DISEASE

The discovery that transitional metal carbonyls have the ability to bind and release CO has furthered the understanding of CO in vascular biology.[103] Metal carbonyls or CO-releasing molecules (CORMs) have contributed greatly to our understanding of the role each HO product might play in vascular cytoprotection.[103,104] Water-soluble CORM-3 [Ru(CO)3Cl(glycinate)] has recently been developed and has confirmed the vasodilatory and cardiovascular benefits of CO. CORM-3 (Fig. 4) has the capability of generating aortic vasodilation ex vivo and reduces blood pressure in vivo.[103–106] Administration of CORM-3 reduces infarct size in vivo when given at the time of reperfusion.[46] Infarct size was dramatically smaller in treated mice, suggesting that CORM-3 can be used to bypass *HO-1* induction and decrease injury during myocardial ischemia-reperfusion.[46] The cardioprotective effect of CORMs occurs through the release of CO. Pulmonary artery SMCs showed an elevation of *HO-1* in the presence of oxidants. Through the use of the CORM tricarbonyldichlororuthenium (II) dimer, the proliferation of pulmonary artery SMCs profoundly decreased.[82] It is apparent that the up-regulation of CO caused a profound opposing effect on hypertension. Rodella et al (personal communication) have shown that CORM-3 renders ECs resistant to oxidative stressors even when *HO-1*/*HO-2* are inhibited with stannous mesoporphyrin (tin mesoporphyrin, [SnMP]).

CO produced from heme metabolism in blood vessels is reported to elicit relaxation[32,107] through the elevation of cGMP levels.[108] Hemin, an inducer of HO, was administered and its effect on the pulmonary artery in young SHR studied.[109] Hemin decreased blood pressure by inducing the HO/CO-sGC/cGMP system. Impairment of this system in the pulmonary artery may be indicative of the pathogenesis and development of hypertension. Up-regulating the HO/CO system lowers blood pressure in young SHR but not in adults.[7,110] In young SHR, blood pressure rises and continues to increase with age, whereas adult SHR have an established hypertension.[7,109] It was hypothesized that if the HO/CO system is defective in young SHR, then *HO-1* inducers could enhance the activity of this system.

In assessing the effect of CORMs, such as CORM-3, it is essential to use a pharmacological dose. Motterlini's group has shown that a physiological dose of CO has a powerful vasodilator effect and attenuates the phenylephrine contracting effect.[106,111,112] However, nonphysiological levels of CORMs will result in less relaxation or contraction (R. Motterlini, personal communication).

This is essentially similar to the effect of acute and chronic *HO-1* inducers on cell survival and on heme-dependent enzymes.[113,114]

HO-1 AND ITS ROLE IN ATHEROSCLEROSIS AND VASCULAR INJURY

As atherosclerotic lesions progress, migration and proliferation of SMCs and deposition of fibrous tissue lead to an advanced, complicated lesion.[115] Various studies emphasize a distinct role of metabolic risk factors, like TNF, interleukin-1 (IL-1), IL-6, angiotensin, oLDL, obesity etc., as important contributors in the development of cardiovascular disease. This has triggered interest in investigating the role of HO in modulating the pathogenesis of cardiogenic disorders.

Considerable evidence suggests that the *HO-1*/CO system plays a beneficial role in atherosclerosis. *HO-1* is highly expressed in the endothelium and foam cells of atherosclerotic lesions in both humans and animals.[42] oLDL, a major determinant in the pathogenesis of atherosclerosis, is a potent inducer of *HO-1* in vascular cells.[116] *HO-1* in vascular ECs, VSMCs, and macrophages is markedly up-regulated by oLDL, whereas *HO-1* is not increased in vascular ECs or SMCs when exposed to native LDL.[41,117] The component in oLDL responsible for *HO-1* induction seems to be oxidized arachidonic acid-containing phospholipids, such as 1-palmitoyl-2-isoprostanoyl-sn-glycero-3-phosphorylcholine[41] and linoleyl hydroperoxide.[118] *HO-1* expression is observed throughout the development of the lesions, from an early fatty streak to an advanced complex atherosclerotic lesion[42] in human aortic endothelial and SMC, and its induction results in the attenuation of monocyte chemotaxis resulting from treatment with mildly oLDL in vitro and in vivo.[119] *HO-1* induction reduces atherosclerotic lesion size in Watanabe heritable hyperlipidemic rabbits[120] and LDL receptor knockout mice.[119] 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 with control animals, despite similar elevations in total plasma cholesterol levels.[121] Interdiction of the diabetic state in nonobese diabetic mice can be achieved by sustained induction of HO and subsequent effects produced by CO and bilirubin.[122] Pharmacological induction of *HO-1* and adenovirus-mediated gene transfer of *HO-1* decreases lesion formation in murine models of atherosclerosis, whereas the inhibition of HO activity promotes lesion development.[40]

Intravascular Dysfunction

ECs were long considered to be a passive monolayer of cells covering the inner part of vascular walls. These cells were regarded as a mechanical barrier between circulating blood and vascular structures. Now, after a series of biochemical and experimental studies, the endothelium is regarded as an organ, covering approximately 700 square meters in area. ECs produce NO as a result of either higher blood pressure or an increased demand for oxygen from the action of eNOS or the amino acid L-arginine. Prostacyclins are the other vasodilator produced by endothelium. Conversely, vasoconstrictors like endothelin 1, Ang II, and thromboxane A are produced in the vascular wall by ECs, acting counter to NO. These molecules impart the endothelium with a vasoregulatory function. Endothelial dysfunction is a well established response to cardiovascular risk factors, including diabetes, and precedes the development of atherosclerosis. Endothelial activation by oLDL and TNFα is central to the development of atherosclerotic lesions.[123–125] Endothelial dysfunction promotes both the early and late mechanisms of atherosclerosis, including up-regulation of adhesion molecules, increased chemokine secretion and leukocyte adherence, increased cell permeability, enhanced LDL oxidation, platelet activation, cytokine elaboration, and VSMC proliferation and migration.[126]

Endothelial *HO-1* overexpression attenuates production of inflammatory mediators and reverses the decrease in eNOS by oLDL and TNFα.[127] In addition, *HO-1* overexpression also improves oLDL and TNFα-impaired vasodilatory responses of aortic segments treated with oLDL.[127] Induction of *HO-1* and extracellular-superoxide dismutase by D-4F enhances the ability of high-density lipoprotein (HDL) to protect LDL against oxidation in atherosclerotic animals.[128] *HO-1* activation via D-4F (apolipoprotein A-1 mimetic peptide) enhances the ability of HDL to protect LDL against oxidation in atherosclerotic disease.[128,129] In fact, *HO-1* is expressed in atherosclerotic lesions[42,119,130] and stimulates cell cycle progression and proliferation in vascular endothelium.[131,132] Transduction of the *HO-1* gene into EC promotes their growth and the development of capillary-like tube structures (angiogenesis), while inhibition of *HO-1* activity blocks cell growth and tube formation.[133] However, additional studies are needed to determine whether HO influences angiogenesis and the underlying mechanism(s) of action. The ability of *HO-1* to stimulate EC regrowth at sites of arterial injury may prove a crucial mechanism in limiting lesion formation because the re-endothelialization of the vessel wall is thought to maintain the underlying SMC in a quiescent state. Thus, *HO-1* as an intrinsic antioxidant may have an important role in cellular protection against endothelial dysfunction and atherogenesis.

Inflammation

Oxidative stress and inflammation are accepted as major factors in the pathogenesis of atherosclerosis.[115] Increased levels of inflammation-related molecules like IL-1, the TNFα ligand, gamma interferon, platelet derived growth factor and fibroblast growth factor, plasma C reactive protein, fibrinogen, IL-6, complement, thrombin and heat shock proteins seen in atherosclerosis, further implies the pivotal role of inflammation in the pathogenesis of atherosclerosis.[134] Despite similar total plasma cholesterol levels in response to hypercholesterolemia, *HO-1*$^{-/-}$apoE$^{-/-}$ mice, in comparison with *HO-1*$^{+/+}$apoE$^{-/-}$ mice, had an accelerated and more advanced atherosclerotic lesion formation. In addition to greater lipid accumulation, advanced lesions from *HO-1*$^{-/-}$apoE$^{-/-}$ mice contained macrophages and smooth muscle α-actin-positive cells.[121] Moreover, decreased expression of *HO-1* in atherosclerotic plaques[135] and a decrease in experimental atherosclerosis after *HO-1* up-regulation[40,119] further establishes the protective role of *HO-1* against atherosclerosis.

CO inhibits the lipopolysaccharide-mediated expression of pro-inflammatory cytokines such as TNFα, IL-1β, and macrophage inflammatory protein-1β, while simultaneously increasing the expression of the anti-inflammatory cytokine IL-10 in both ECs and macrophages, suggesting a significant contribution of CO to the anti-inflammatory properties to increased levels of *HO-1* expression.[136] Furthermore, *HO-1*/CO activation can down-regulate the inflammatory response by blocking the release of NO from iNOS and the expression of granulocyte-macrophage colony stimulating factor from macrophages and SMC.[137,138] In addition, the activation of sGC and p38 mitogen-activated protein kinase (MAPK) have been implicated in suppression of inflammatory cytokines by *HO-1*/CO activation.[136,137]

Smooth Muscle Cell Proliferation

SMC proliferation and monocyte recruitment are essential steps for the development of atherosclerosis. *HO-1* was induced by hypoxia in VSMC, and SMC–derived CO inhibited EC production of endothelin-1, platelet-derived growth factor-B, and vascular endothelial growth factor.[139] The inhibition of these factors by CO led to a decrease in VSMC proliferation. In addition, SMC-derived CO

directly decreased VSMC growth by inhibiting E2F-1, a transcription factor that participates in the control of cell cycle progression from G1 to the S phase.[140] *HO-1* overexpression or the exogenous administration of CO arrests cultured SMC in the G0/G1 phase of the cell cycle.[89,141,142] Cell cycle progression inhibition was associated with a marked decrease in cyclin-dependent kinase 2 (cdk2) activity, a critical event required for S-phase entry and DNA synthesis.[142] 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.[89,142,143] The antiproliferative action of CO appears to be mediated by the sGC/cGMP pathway because inhibitors of GC and protein kinase C restored SMC growth.[89,141] Excessive SMC proliferation after endovascular injury is a major determinant of neointimal formation. The ability to inhibit SMC growth is related to the vasoprotective action of *HO-1* derived CO. *HO-1* directly stimulated vascular relaxation and inhibited vascular cell proliferation in a model of vascular injury in pigs using adenoviral *HO-1* gene transfer.[141] Moreover, prior induction of *HO-1* attenuates vascular neointimal formation after balloon injury of rat carotid arteries, while inhibition of HO activity exacerbates lesion formation.[88,144,145] Similar results were seen with adenovirus-mediated *HO-1* gene delivery after rat carotid artery injury,[92] indicating the pivotal role of increased levels of *HO-1* overexpression and HO activity in cellular protection.

Redox alterations promote neointimal growth in induced VSMC proliferation and the O_2^- generating enzyme NADPH oxidase plays a pivotal role in this scenario.[146] Bilirubin is a highly efficient radical scavenger and has the potential to influence cellular redox alterations, such as scavenging of peroxyl radicals,[22] which occur in response to vascular injury. Higher blood concentrations of bilirubin in Gunn rats suppress the development of vascular neointimal hyperplasia after balloon injury.[147] In this study, the SMCs from the injured vascular wall underwent substantial growth inhibition in response to injury. Transfection of rat carotid arteries before balloon injury with dominant-negative mutants of p38 MAPK inhibits neointimal formation.[137] Bilirubin/biliverdin arrest the cell cycle progression by interfering with the late G1 phase in growth-stimulated cultures of primary VSMCs.[147,148] Bilirubin exerts its growth suppressor functions in VSMCs, at least in part, by modulating the p38 MAPK signaling pathway.[147,149] More research is needed to establish the role of bilirubin/biliverdin in relation to VSMC proliferation and prevention. At present, the protective role of *HO-1* against VSMC proliferation in atherosclerosis is attributed to CO. However, this may be premature because HO and its byproducts (bilirubin and CO), by limiting VSMC growth, may act as key mediators in the compensatory response to vascular remodeling, and thus limit the damage caused by atherosclerosis.

Vasodilatation

CO and NO are both capable of inducing the relaxation of blood vessels through vasodilation and inhibiting the proliferation of VSMC.[82] Earlier studies have suggested that CO binds to and activates sGC, thereby increasing intracellular levels of cGMP, as has been demonstrated for NO.[39,150,151] Like NO, HO-derived CO influences the sGC and cGMP pathways, which serve to regulate both blood pressure and vascular contractility.[83] However, the physiological relevance of CO as a vasodilator is controversial. Indeed, overexpression of *HO-1* in arteries was reported to stimulate vascular relaxation, mediated by sGC and cGMP, independently of NO.[141] CO is more chemically stable than NO but is 1000-fold less potent than NO as a relaxing agent.[152] Thus, the biologic availability of NO and CO may differ.[150] CO likely acts via multiple mechanisms, including the direct modulation of cGMP levels and potassium channels in SMCs; and the indirect effects via modulation of endothelium-dependent vasoconstrictors and myogenic factors.[153] CO may also act as a physiological regulator of vascular tone through cGMP-mediated responses in large vessels (eg, aorta), or dilate the smaller renal arteries or plial arterioles via activation of calcium-activated potassium channels.[29,32] CO may play a role in blood pressure regulation in acute hypertension.[154,155] Recently, CO has been reported to exert cGMP-independent actions on calcium-dependent potassium channels[32,156] and on MAPK. CO directly activates calcium-dependent potassium channels, leading to vascular relaxation through calcium desensitization. Antiproliferative effects on VSMCs after balloon injury are mediated by the p38 MAPK pathway, including MAPK 3 and 6.[157] In addition, p38 MAPK pathway activation by CO has been observed to protect ECs from TNFα apoptosis.[158]

Regarding relevance of *HO-1* in atherosclerosis, it should be noted that *HO-1* is induced by most of the well-established cardiovascular risk factors in vascular cells and circulating macrophages. These *HO-1* responses seem to have a protective role in the vascular wall against atherogenesis through multiple pathways. Interventions aimed at modulating the levels of HO in the vascular wall, therefore, might be a novel target to treat or prevent atherosclerotic diseases.

HO-1 IN CORONARY SYNDROMES

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 arrhythmias after myocardial ischemia-reperfusion, whereas cardiac tissue damage is exacerbated by HO inhibitors.[62,159–161] Cardioselective overexpression of *HO-1* exerts a cardioprotective effect after myocardial ischemia/reperfusion in mice, and this effect is probably mediated via an antiapoptotic action of *HO-1* degradation products.[162] The hearts of transgenic mice expressing cardiac-specific *HO-1* showed an improved recovery of contractile performance during reperfusion after ischemia and protection against ischemia, in an *HO-1* dose-dependent manner as evident by decreased infarct size. 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.[163] These data demonstrate that *HO-1* gene transfer produces therapeutic, cardioprotective benefits by reducing oxidative stress and associated inflammation and cell death.

In addition, isolated hearts from heterozygote *HO-1* knockout mice demonstrate an increased susceptibility to ischemia-reperfusion injury relative to hearts from controls.[164] A maladaptive response consisting of enhanced ventricular dilatation, infarction, and thrombosis has also been reported in *HO-1* null mice during hypoxia.[165] Moreover, the preemptive delivery of *HO-1* inhibits post-myocardial infarct remodeling and restores ventricular function after ischemia-reperfusion.[166] A similar study demonstrated that rats having undergone successful *HO-1* gene administration had a dramatic reduction in left ventricular myocardial infarction after coronary artery ligation and release. In addition to the reduction in infarct size, there was a decrease in myocardial lipid peroxidation, proapoptotic Bcl-2-associated X protein (bax), and proinflammatory IL-1β protein abundance, as well as an increase in antiapoptotic Bcl-2 protein levels.[167] Induction of *HO-1* increases adult cardiomyocyte tolerance to ischemia after in vivo transplantation.[101] Autologous atrial cardiomyocytes are an available cell source for infarct repair; however, they undergo apoptosis, precluding their use as cellular repair grafts. Thus, preconditioning with *HO-1* acts to retain functional viability in vivo in adult cardiomyocyte cellular grafts after

implantation. This, in turn, may prove effective in repairing infarcted myocardium.[101]

The ultimate goal in the treatment of cardiovascular disease is the timely delivery of the best therapeutic agents to protect the heart from the deleterious effects of prolonged ischemia or the effects of repeated bouts of ischemia.[168] A preemptive strategy for tissue protection has been developed using an adeno-associated vector system, containing erythropoietin hypoxia response elements, for ischemia-regulated expression of the therapeutic human *HO-1* gene.[169] A single administration of this vector several weeks in advance of ischemia/reperfusion injury to the heart produced a rapid and timely induction of human *HO-1* during ischemia, which resulted in a dramatic reduction in tissue damage. In addition, overexpression of the therapeutic transgene prevented long-term pathologic tissue remodeling and normalized tissue function.[169] In the future, it may be possible, by a similar approach, to use *HO-1* as a therapy to repair tissue injury caused by ischemia and to protect against further ischemic episodes in individuals at risk for a second ischemic incident.

HO-1 AND THE EFFECTS OF ADIPONECTIN

Cardiovascular disease and obesity are associated with inflammation and endothelial dysfunction, which contribute to increasing the risk of renal dysfunction. The role of inflammation and Ang II in the development of vascular dysfunction, renal damage, and hypertension is well established.[170–173] Paradoxically, circulating adiponectin levels are diminished in obese individuals.[174] Adiponectin levels inversely correlate with the levels of Ang II, high blood pressure, and superoxide levels.[175] Low plasma adiponectin level (hypoadiponectinemia) is strongly associated with the increased prevalence of obesity.[174–176] Studies with adiponectin-deficient (adiponectin-knockout) mice suggest that low levels of adiponectin directly contribute to impairment in endothelial-dependent vasodilatation and increased intimal hyperplasia after acute vascular injury.[176,177] Recently, our group has shown that induction of *HO-1* was associated with a parallel increase in the serum levels of adiponectin.[178–180] Inhibition of HO activity decreases adiponectin levels.[178] Adiponectin, when elevated, decreases the levels of IL1, IL-6, and TNF[178–180] and has both anti-inflammatory and antioxidant properties.[159,181,182] Up-regulation of *HO-1* mediates secretion of adiponectin by adipocytes, which stimulates various anti-inflammatory molecules and activates AMPK-AKT pathways. Additionally, it seems that the antivascular dysfunction effect of increased HO activity may be due, in part, to blunting the vasoconstrictor action of 20-HETE ([20-hydroxyeicosatetraenoic acid] a hydroxy arachidonic acid, a cytochrome p450 metabolite),[183–186] that may also cause increased inflammation and decreased release of adiponectin. Adiponectin is an adipose tissue-specific protein that has proved to have both antiatherogenic and insulin-sensitizing properties.[187] Adiponectin exists in plasma as 3 different oligomeric forms (trimer, hexamer, and high molecular weight).[188] Recently it was reported that hypoadiponectinemia is a predictor of the development of hypertension.[189] However, the association between low adiponectin and the future occurrence of hypertension was independent of a homeostasis model assessment of insulin resistance,[189] thus suggesting that hypoadiponectinemia might also influence blood pressure through other mechanisms, including endothelial dysfunction and the activation of the inflammatory cascade.[190] Hypoadiponectinemia was shown to be a risk factor for hypertension after adjusting for age, body mass index, and total cholesterol levels.[191] An inverse relation exists between plasma adiponectin level and systolic blood pressure, which was significant in obese subjects.[192]

In addition, an increase in *HO-1* expression is also associated with an increase in adiponectin levels in heart eNOS, pAKT, and pAMPK levels. This is manifested by improved endothelial function and increased resistance to oxidants and apoptosis, thereby protecting the hearts of the chronic diabetic mice from ischemic injury (L'Abbate et al 2009, personal communication), supporting a report that adiponectin is critical for EC survival and function via the activation of eNOS, pAKT, and pAMPK.[193] Activation of AMPK is important in cellular energy homeostasis via stimulation of glucose transport, switching off energy consumption by decreasing lipogenesis, increasing fatty acid oxidation and adenosine triphosphate.[194–197] AMPK has been suggested as a therapeutic target for the amelioration of endothelial dysfunction, and as such, of vascular disease.[198–200] Activation of AMPK is known to reduce inflammation and improve insulin sensitivity and glucose tolerance.[201,202] Both pAMPK and pAKT use eNOS as a substrate and enhance the levels of peNOS,[48,203,204] suggesting that *HO-1*'s ability to prevent vascular dysfunction may be due to increases in pAMPK, pAKT, and NO availability.[48] Finally, an increase in *HO-1* decreases macrophages and adipocyte release of TNF, IL-1, and IL-6,[178–180,205,206] and restores vascular integrity by increasing endothelial progenitors homing.[207]

THERAPEUTIC APPLICATIONS OF *HO-1* IN VASCULAR DISEASE

HO has potential in the treatment of atherosclerosis, hypertension, and vascular injury in humans, by being delivered directly or by pharmacological activation of the *HO-1* gene. Most of the pharmacological inducers of *HO-1* used in experimental studies, such as hemin and heavy metals, show cellular and tissue toxicity, and the long-term adverse effects of *HO-1* gene delivery must be elucidated before its application in humans. However, recent studies revealed that some well-used drugs modulate *HO-1* expression in vascular cells.

Aspirin

Aspirin is known to reduce the incidence of thrombotic occlusive events such as myocardial infarction and stroke by inhibiting platelet COX-1 and COX-2 activity. Aspirin increased *HO-1* protein levels and activity in a dose-dependent manner in cultured ECs derived from human umbilical vein.[208] Pretreatment of cells with aspirin or bilirubin protected ECs from hydrogen peroxide-mediated toxicity. The authors concluded that aspirin targets *HO-1* via NO-dependent pathways similar to NOS blockers such as L-NAME, preventing aspirin-dependent *HO-1* induction.[208] *HO-1* is a cGMP-sensitive endothelial gene and a causal relationship exists between *HO-1* induction and endothelial protection by the cGMP/NO system.[209] Aspirin increases ferritin synthesis in ECs, presumably as a result of *HO-1* induction and iron release, suggesting a role in the prevention of endothelial injury during atherogenesis. Induction of *HO-1* activity is a novel mechanism by which aspirin prevents cellular injury under inflammatory conditions and in cardiovascular diseases.

Statins

Statins are widely used lipid-lowering agents that substantially decrease cardiovascular morbidity and mortality in patients with and without coronary disease. Simvastatin and lovastatin increase *HO-1* mRNA levels in cultured ECs derived from human umbilical vein.[208,210,211] Increased statin-mediated transcriptional expression of *HO-1* is associated with elevated *HO-1* protein levels and reduced free radical formation. Lee et al also reported that simvastatin activates *HO-1* in VSMCs in vitro and in vivo, and suggested the involvement of p38 and the p13K-AKT pathway in *HO-1*

induction.[212] These results may account for the pleiotropic antioxidant, anti-inflammatory, and antiatherogenic actions of statins.

Probucol

Probucol, a rarely used cholesterol lowering drug, is an antioxidant drug that reduces the risk of postangioplasty restenosis. The protective effect of probucol depends not only on its ability to inhibit lipid oxidation, but also on its ability to induce *HO-1*.[213] Probucol inhibits macrophage accumulation, stimulates reendothelialization, and inhibits VSMC proliferation; these processes are mediated via the induction of *HO-1*, an activity not shared by vitamin E. Vitamin E and other antioxidants have failed to protect against atherosclerotic diseases, but a striking exception is probucol, which retards atherosclerosis in carotid arteries and restenosis of coronary arteries after angioplasty.[214] These findings indicate the contribution of *HO-1* in the actions of probucol. The drug has recently lost popularity because it causes HDL reduction and QT prolongation.

Losartan

HO-1 is expressed in medial smooth muscle and adventitial cells in normotensive rat aortas, and this is markedly increased in adventitial and ECs in Ang II-induced hypertensive rat aortas. This results in the up-regulation of blood pressure. Losartan treatment reduced the levels of *HO-1* to that seen in control animals.[215] Losartan can markedly reduce pulmonary pressure and inhibit vascular remodeling in volume-overloaded left-to-right shunt rats, and this results in down-regulation of *HO-1* mRNA expression.[216] Unique renoprotective properties of angiotensin receptor blockers, independent of blood pressure lowering, can result in decreased oxidative stress, correction of chronic hypoxia, and inhibition of advanced glycation end product formation and of abnormal iron deposition.[217]

Resveratrol

Resveratrol is one of the major components of certain varieties of red grapes and may underlie the cardioprotective effects thought to be obtained from moderate red wine consumption. Recent studies have demonstrated that resveratrol can pharmacologically precondition the heart through an *HO-1*-dependent, NO-mediated mechanism. Resveratrol causes phosphorylation of p38 MAPKβ and AKT, as well as the inhibition of p38 MAPKα, which is completely reversed by tin protoporphyrin.[218] Pretreatment with resveratrol markedly reduces infarct size 24 hours after myocardial infarction and increases capillary density in the peri-infarct myocardium, along with better left ventricular function compared with control.[219] These results indicate that resveratrol generates cardioprotection by preconditioning the heart through an *HO-1*-mediated mechanism.[220] Currently, resveratrol, independent of red wine, is being evaluated as a drug to both prevent and treat cardiovascular disease.

Innovative Agents

There are other new therapeutic approaches directed at improving NO bioavailability, a defect in patients with coronary disease. One class includes direct stimulators of sGC, which enhance the sensitivity of the reduced enzyme to low levels of bioavailable NO (eg, riociquat 10).[221] Another class includes the sGC activators which activate the NO-unresponsive, heme-oxidized or heme-free enzyme. An agent in this class, ataciguat, stimulates the oxidized form of sGC.[222] Preclinical studies have shown that ataciguat can inhibit platelet activation in an animal model of acute thrombosis. The drug has also been shown to restore endothelial and vascular function in a diabetic animal model, to improve endothelial function and reduce plaque in an atherosclerosis model, and to improve ischemia-induced muscle fatigue in an arterial occlusive disease model.

Based on the above data, a randomized, double-blind, placebo-controlled trial (ACCELA) is underway in patients with peripheral arterial occlusive disease. Both of these new classes of drugs can reduce blood pressure and have the potential to prevent atherosclerosis, thrombosis, and inflammation.

CONCLUSIONS

The HO system plays a central role in many aspects of normal human physiology, as well as in the pathophysiology of myocardial ischemia/reperfusion, hypertension, cardiomyopathy, organ transplantation, endotoxemia, and pulmonary disorders. Active agents in these situations include not only the pro-oxidant heme, but also its metabolic products, CO and the bile pigments, biliverdin and bilirubin, as well as a wide array of genetic and metabolic processes, which respond when heme metabolism is perturbed.[223] The use of pharmacological and genetic interventions for regulating HO has provided important new insights into the interactions of the heme-HO system and biologic and pathologic events, and offers the potential for the development of new therapeutic strategies directed against recalcitrant disease processes. It is, for example, possible to envision the use of a single drug or gene intervention using site-specific expression to induce long-term prophylaxis against certain pathologic cardiovascular events or to promote and enhance repair processes in individuals who have experienced, or are at high risk for, cardiac injury. The use of stable gene integration may also lead to the development of more effective, perhaps long-lasting, therapies that can moderate other chronic diseases.

The HO system is on the one hand a seemingly simple biochemical axis, and on the other the hub of a complex of coupled processes whose actions and products exert a wide array of diverse and potent metabolic effects.[207] Down- regulating the HO system by pharmacological or genetic means has already led to new approaches for managing old clinical problems[15] and up-regulating the system offers promise for new approaches to experimental work in cell biology and new methods for moderating some of the consequences of clinical disorders. The use of pharmacological and genetic interventions for up-regulation of *HO-1* in the management of cardiovascular diseases appears to be especially promising. In the future, it may be possible to administer a drug or gene therapy using site specific expression of *HO-1* to promote long-term prophylaxis against secondary coronary events and to promote myocardial repair in patients who have experienced an infarct, as well as in those at high risk of myocardial injury. These pharmacologic or genetic strategies to regulate the heme-HO system could, as we have earlier suggested,[13] open up new therapeutic approaches to the effective management of a number of clinical disorders.

REFERENCES

1. Abraham NG, Drummond GS, Lutton JD, et al. The biological significance and physiological role of heme oxygenase. *Cell Physiol Biochem*. 1996;6: 129–168.
2. Cruse I, Maines MD. Evidence suggesting that the two forms of heme oxygenase are products of different genes. *J Biol Chem*. 1988;263:3348–3353.
3. Huang TJ, McCoubrey WK Jr, Maines MD. Heme oxygenase-2 interaction with metalloporphyrins: function of heme regulatory motifs. *Antioxid Redox Signal*. 2001;3:685–696.
4. Scapagnini G, D'Agata V, Calabrese V, et al. Gene expression profiles of heme oxygenase isoforms in the rat brain. *Brain Res*. 2002;954:51–59.
5. Ishikawa K, Takeuchi N, Takahashi S, et al. Heme oxygenase-2. Properties of the heme complex of the purified tryptic fragment of recombinant human heme oxygenase-2. *J Biol Chem*. 1995;270:6345–6350.
6. Abraham NG, da Silva JL, Lavrovsky Y, et al. Adenovirus-mediated heme oxygenase-1 gene transfer into rabbit ocular tissues. *Invest Ophthalmol Vis Sci*. 1995;36:2202–2210.
7. Abraham NG, Lavrovsky Y, Schwartzman ML, et al. Transfection of the

human heme oxygenase gene into rabbit coronary microvessel endothelial cells: protective effect against heme and hemoglobin toxicity. *Proc Natl Acad Sci USA*. 1995;92:6798–6802.

8. Abraham NG. Adenovirus-mediated heme oxygenase gene transfer into human hematopoietic $CD34^+$ stem cells. *Exp Hematol*. 1996;24:1053.
9. Mingone CJ, Gupte SA, Chow JL, et al. Protoporphyrin IX generation from delta-aminolevulinic acid elicits pulmonary artery relaxation and soluble guanylate cyclase activation. *Am J Physiol Lung Cell Mol Physiol*. 2006; 291:L337–L344.
10. Yang L, Quan S, Nasjletti A, et al. Heme oxygenase-1 gene expression modulates angiotensin II-induced increase in blood pressure. *Hypertension*. 2004;43:1221–1226.
11. Lavrovsky Y, Schwartzman ML, Levere RD, et al. Identification of binding sites for transcription factors NF-kappa B and AP-2 in the promoter region of the human heme oxygenase 1 gene. *Proc Natl Acad Sci USA*. 1994;91: 5987–5991.
12. Dennery PA, Spitz DR, Yang G, et al. Oxygen toxicity and iron accumulation in the lungs of mice lacking heme oxygenase-2. *J Clin Invest*. 1998;101:1001–1011.
13. Kappas A, Drummond G. Control of heme metabolism with synthetic metalloporphyrins. *J Clin Invest*. 1986;77:335–339.
14. Drummond GS, Kappas A. The cytochrome P-450-depleted animal: an experimental model for in vivo studies in chemical biology. *Proc Natl Acad Sci USA*. 1982;79:2384–2388.
15. Kappas A. A method for interdicting the development of severe jaundice in newborns by inhibiting the production of bilirubin. *Pediatrics*. 2004;113: 119–123.
16. Vile GF, Basu-Modak S, Wlatner C, et al. Heme oxygenase 1 mediates an adaptive response to oxidative stress in human skin fibroblasts. *Proc Natl Acad Sci USA*. 1994;91:2607–2610.
17. Wagener FA, da Silva JL, Farley T, et al. Differential effects of heme oxygenase isoforms on heme mediation of endothelial intracellular adhesion molecule 1 expression. *J Pharmacol Exp Ther*. 1999;291:416–423.
18. Balla G, Jacob HS, Balla J, et al. Ferritin: a cytoprotective antioxidant strategm of endothelium. *J Biol Chem*. 1992;267:18148–18153.
19. Hayashi S, Takamiya R, Yamaguchi T, et al. Induction of heme oxygenase-1 suppresses venular leukocyte adhesion elicited by oxidative stress: role of bilirubin generated by the enzyme. *Circ Res*. 1999;85:663–671.
20. Platt JL, Nath KA. Heme oxygenase: protective gene or Trojan horse. *Nat Med*. 1998;4:1364–1365.
21. Nath KA, Haggard JJ, Croatt AJ, et al. The indispensability of heme oxygenase-1 in protecting against acute heme protein-induced toxicity in vivo. *Am J Pathol*. 2000;156:1527–1535.
22. Stocker R, Yamamoto Y, McDonagh AF, et al. Bilirubin is an antioxidant of possible physiological importance. *Science*. 1987;235:1043–1046.
23. Stocker R, McDonagh AF, Glazer AN, et al. Antioxidant activities of bile pigments: biliverdin and bilirubin. *Methods Enzymol*. 1990;186: 301–309.
24. Wiesel P, Patel AP, DiFonzo N, et al. Endotoxin-induced mortality is related to increased oxidative stress and end-organ dysfunction, not refractory hypotension, in heme oxygenase-1-deficient mice. *Circulation*. 2000;102: 3015–3022.
25. Abraham NG, Kushida T, McClung J, et al. Heme oxygenase-1 attenuates glucose-mediated cell growth arrest and apoptosis in human microvessel endothelial cells. *Circ Res*. 2003;93:507–514.
26. Abraham NG, Rezzani R, Rodella L, et al. Overexpression of human heme oxygenase-1 attenuates endothelial cell sloughing in experimental diabetes. *Am J Physiol Heart Circ Physiol*. 2004;287:H2468–H2477.
27. Moqattash S, Lutton JD, bu-Hijleh MF, et al. Hemopoietic recovery from AZT toxicity with recombinant hemoglobin in a murine model of AIDS. *Acta Haematol*. 1994;92:182–186.
28. Otterbein LE, Kolls JK, Mantell LL, et al. Exogenous administration of heme oxygenase-1 by gene transfer provides protection against hyperoxia-induced lung injury. *J Clin Invest*. 1999;103:1047–1054.
29. Leffler CW, Nasjletti A, Yu C, et al. Carbon monoxide and cerebral microvascular tone in newborn pigs. *Am J Physiol*. 1999;276:H1641–H1646.
30. Leffler CW, Nasjletti A, Johnson RA, et al. Contributions of prostacyclin and nitric oxide to carbon monoxide-induced cerebrovascular dilation in piglets. *Am J Phyisiol Heart Circ Physiol*. 2001;280:H1490–H1495.
31. Adachi T, Ishikawa K, Hida W, et al. Hypoxemia and blunted hypoxic ventilatory responses in mice lacking heme oxygenase-2. *Biochem Biophys Res Commun*. 2004;320:514–522.
32. Kaide J-I, Zhang F, Wei Y, et al. Carbon monoxide of vascular origin attenuates the sensitivity of renal arterial vessels to vasoconstrictors. *J Clin Invest*. 2001;107:1163–1171.
33. Kaide J, Zhang F, Wei Y, et al. Vascular CO counterbalances the sensitizing influence of 20-HETE on agonist-induced vasoconstriction. *Hypertension*. 2004;44:210–216.
34. Kaide J, Wang MH, Wang JS, et al. Transfection of CYP4A1 cDNA increases vascular reactivity in renal interlobar arteries. *Am J Physiol Renal Physiol*. 2003;284:F51–F56.
35. Perrella MA, Yet SF. Role of heme oxygenase-1 in cardiovascular function. *Curr Pharm Des*. 2003;9:2479–2487.
36. Ferris CD, Jaffrey SR, Sawa A, et al. Haem oxygenase-1 prevents cell death by regulating cellular iron. *Nat Cell Biol*. 1999;1:152–157.
37. Conners MS, Schultz MC, Dunn MW, et al. Hypoxia-induced complications of soft contact lens wear are alleviated by heme oxygenase induction. *Invest Ophthalmol Vis Sci*. 1992;33:780–787.
38. Willis D, Moore AR, Frederick R, et al. Heme oxygenase: a novel target for the modulation of the inflammatory response. *Nat Med*. 1996;2:87–90.
39. Laniado-Schwartzman M, Abraham NG, Conners M, et al. Heme oxygenase induction with attenuation of experimentally induced corneal inflammation. *Biochem Pharmacol*. 1997;53:1069–1075.
40. Juan SH, Lee TS, Tseng KW, et al. Adenovirus-mediated heme oxygenase-1 gene transfer inhibits the development of atherosclerosis in apolipoprotein E-deficient mice. *Circulation*. 2001;104:1519–1525.
41. Ishikawa K, Navab M, Leitinger N, et al. Induction of heme oxygenase-1 inhibits the monocyte transmigration induced by mildly oxidized LDL. *J Clin Invest*. 1997;100:1209–1216.
42. Wang LJ, Lee TS, Lee FY, et al. Expression of heme oxygenase-1 in atherosclerotic lesions. *Am J Pathol*. 1998;152:711–720.
43. Pileggi A, Molano RD, Berney T, et al. Heme oxygenase-1 induction in islet cells results in protection from apoptosis and improved in vivo function after transplantation. *Diabetes*. 2001;50:1983–1991.
44. Hashiba T, Suzuki M, Nagashima Y, et al. Adenovirus-mediated transfer of heme oxygenase-1 cDNA attenuates severe lung injury induced by the influenza virus in mice. *Gene Ther*. 2001;8:1499–1507.
45. Amersi F, Buelow R, Kato H, et al. Upregulation of heme oxygenase-1 protects genetically fat Zucker rat livers from ischemia/reperfusion injury. *J Clin Invest*. 1999;104:1631–1639.
46. Guo Y, Stein AB, Wu WJ, et al. Administration of a CO-releasing molecule at the time of reperfusion reduces infarct size in vivo. *Am J Physiol Heart Circ Physiol*. 2004;286:H1649–H1653.
47. Yet SF, Tian R, Layne MD, et al. Cardiac-specific expression of heme oxygenase-1 protects against ischemia and reperfusion injury in transgenic mice. *Circ Res*. 2001;89:168–173.
48. Abraham NG, Kappas A. Pharmacological and clinical aspects of heme oxygenase. *Pharmacol Rev*. 2008;60:79–127.
49. Abraham NG, Friedland ML, Levere RD. Heme metabolism in hepatic and erythroid cells. In: Brown E, ed. *Progress in Hematology*. Vol XIII ed. New York, NY: Grune and Stratton; 1983:75–130.
50. Schacter BA. Heme catabolism by heme oxygenase: physiology, regulation, and mechanism of action. *Semin Hematol*. 1988;25:349–369.
51. Kushida T, LiVolti G, Goodman AI, et al. TNF-alpha-mediated cell death is attenuated by retrovirus delivery of human heme oxygenase-1 gene into human microvessel endothelial cells. *Transplant Proc*. 2002; 34:2973–2978.
52. Kushida T, Li Volti G, Quan S, et al. Role of human heme oxygenase-1 in attenuating TNF-alpha-mediated inflammation injury in endothelial cells. *J Cell Biochem*. 2002;87:377–385.
53. Stocker R, Glazer AN, Ames BN. Antioxidant activity of albumin-bound bilirubin. *Proc Natl Acad Sci USA*. 1987;84:5918–5922.
54. Frei B, Stocker R, Ames BN. Antioxidant defenses and lipid peroxidation in human blood plasma. *Proc Natl Acad Sci USA*. 1988;85:9748–9752.
55. Neuzil J, Stocker R. Free and albumin-bound bilirubin are efficient co-antioxidants for alpha-tocopherol, inhibiting plasma and low density lipoprotein lipid peroxidation. *J Biol Chem*. 1994;269:16712–16719.
56. Wu TW, Wu J, Li RK, et al. Albumin-bound bilirubins protect human ventricular myocytes against oxyradical damage. *Biochem Cell Biol*. 1991; 69:683–688.
57. Dore S, Takahashi M, Ferris CD, et al. Bilirubin, formed by activation of heme oxygenase-2, protects neurons against oxidative stress injury. *Proc Natl Acad Sci USA*. 1999;96:2445–2450.
58. Lleusy SF, Tomaro ML. Evidence of involvement of bilirubin as physio-

logical protector against oxidative damage. *Biochim Biophys Acta*. 1994; 1223:9–14.

59. Ossola JO, Tomaro ML. Heme oxygenase induction by cadmium chloride: evidence for oxidative stress involvement. *Toxicology*. 1995;104:141–147.
60. Ossola JO, Tomaro ML. Heme oxygenase induction by UVA radiation. A response to oxidative stress in rat liver. *Int J Biochem Cell Biol*. 1998;30: 285–292.
61. Clark JE, Foresti R, Green CJ, et al. Dynamics of haem oxygenase-1 expression and bilirubin production in cellular protection against oxidative stress. *Biochem J*. 2000;348(Pt 3):615–619.
62. Clark JE, Foresti R, Sarathchandra P, et al. Heme oxygenase-1-derived bilirubin ameliorates postischemic myocardial dysfunction. *Am J Physiol Heart Circ Physiol*. 2000;278:H643–H651.
63. Mazza F, Goodman A, Lombardo G, et al. Heme oxygenase-1 gene expression attenuates angiotensin II-mediated DNA damage in endothelial cells. *Exp Biol Med (Maywood)*. 2003;228:576–583.
64. Morita T, Imai T, Yamaguchi T, et al. Induction of heme oxygenase-1 in monocytes suppresses angiotensin II-elicited chemotactic activity through inhibition of CCR2: role of bilirubin and carbon monoxide generated by the enzyme. *Antioxid Redox Signal*. 2003;5:439–447.
65. Kwak JY, Takeshige K, Cheung BS, et al. Bilirubin inhibits the activation of superoxide-producing NADPH oxidase in a neutrophil cell-free system. *Biochim Biophys Acta*. 1991;1076:369–373.
66. Sano K, Nakamura H, Matsuo T. Mode of inhibitory action of bilirubin on protein kinase C. *Pediatr Res* 1985;19:587–590.
67. Rajagopalan S, Kurz S, Munzel T, et al. Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. *J Clin Invest*. 1996;97:1916–1923.
68. Ishizaka N, Aizawa T, Mori I, et al. Heme oxygenase-1 is upregulated in the rat heart in response to chronic administration of angiotensin II. *Am J Phyisiol Heart Circ Physiol*. 2000;279:H672–H678.
69. Sedlak TW, Snyder SH. Bilirubin benefits: cellular protection by a biliverdin reductase antioxidant cycle. *Pediatrics*. 2004;113:1776–1782.
70. Turkseven S, Kruger A, Mingone CJ, et al. Antioxidant mechanism of heme oxygenase-1 involves an increase in superoxide dismutase and catalase in experimental diabetes. *Am J Physiol Heart Circ Physiol*. 2005;289:H701–H707.
71. McClung JA, Morita T, Rodella L, et al. Heme oxygenase-1 prevents superoxide anion-associated vascular smooth muscle growth and decreases circulating endothelial cells in a rat model of balloon injury and restenosis in diabetes mellitus. *Circulation.* 2004;110:III-282.
72. Rezzani R, Quan S, Rodella L, et al. Heme oxygenase-1 upregulation attenuates glucose-mediated oxidative stress renal injury in *HO-2* knockout mice [abstract]. *Hypertension*. 2003;42:86.
73. Milstien S, Katusic Z. Oxidation of tetrahydrobiopterin by peroxynitrite: implications for vascular endothelial function. *Biochem Biophys Res Commun*. 1999;263:681–684.
74. Foresti R, Sarathchandra P, Clark JE, et al. Peroxynitrite induces haem oxygenase-1 in vascular endothelia to apoptosis. *Biochem J*. 1999;339:729–736.
75. Chang SH, Barbosa-Tessmann I, Chen C, et al. Glucose deprivation induces heme oxygenase-1 gene expression by a pathway independent of the unfolded protein response. *J Biol Chem*. 2002;277:1933–1940.
76. Mingone CJ, Turkseven S, Wolin MS, et al. Heme-oxygenase-1 modulates vascular responses in diabetic rats via guanylate cyclase activation: role of superoxide dismutase [abstract]. *FASEB.* 2005:8836.
77. Mansoor A, Mingone CJ, Turkseven S, et al. Down regulation of iNOS and in eNOS by overexpression of heme oxygenase-1 restore vascular response in diabetic rats. *FASEB.* 2005:7415.
78. Hill-Kapturczak N, Chang SH, Agarwal A. Heme oxygenase and the kidney. *DNA Cell Biol*. 2002;21:307–321.
79. Solari V, Piotrowska AP, Puri P. Expression of heme oxygenase-1 and endothelial nitric oxide synthase in the lung of newborns with congenital diaphragmatic hernia and persistent pulmonary hypertension. *J Pediatr Surg*. 2003;38:808–813.
80. Snyder SH, Baranano DE. Heme oxygenase: a font of multiple messengers. *Neuropsychopharmacology*. 2001;25:294–298.
81. Motterlini R, Foresti R, Intaglietta M, et al. NO-mediated activation of heme oxgenase: endogenous cytoprotection against oxidative stress to endothelium. *Am J Physiol*. 1996;270:H107–H114.
82. Stanford SJ, Walters MJ, Hislop AA, et al. Heme oxygenase is expressed in human pulmonary artery smooth muscle where carbon monoxide has an anti-proliferative role. *Eur J Pharmacol*. 2003;473:135–141.
83. Ndisang JF, Tabien HE, Wang R. Carbon monoxide and hypertension. *J Hypertens*. 2004;22:1057–1074.
84. Ndisang JF, Wu L, Zhao W, et al. Induction of heme oxygenase-1 and stimulation of cGMP production by hemin in aortic tissues from hypertensive rats. *Blood*. 2003;101:3893–3900.
85. Ou HS, Yang J, Dong LW, et al. Role of endogenous carbon monoxide in the pathogenesis of hypotension during septic shock. *Sheng Li Xue Bao*. 1999;51:1–6.
86. Beltowski J, Jamroz A, Borkowska E. Heme oxygenase and carbon monoxide in the physiology and pathology of the cardiovascular system [in polish]. *Postepy Hig Med Dosw (Online)*. 2004;58:83–99.
87. Ushiyama M, Morita T, Katayama S. Carbon monoxide regulates blood pressure cooperatively with nitric oxide in hypertensive rats. *Heart Vessels*. 2002;16:189–195.
88. Togane Y, Morita T, Suematsu M, et al. Protective roles of endogenous carbon monoxide in neointimal development elicited by arterial injury. *Am J Physiol Heart Circ Physiol*. 2000;278:H623–H632.
89. Otterbein LE, Zuckerbraun BS, Haga M, et al. Carbon monoxide suppresses arteriosclerotic lesions associated with chronic graft rejection and with balloon injury. *Nat Med*. 2003;9:183–190.
90. Kukoba TV, Moibenko OO, Kotsioruba AV. Cardioprotective effect of heme oxygenase-1 induction by hemin on the isolated rat heart during ischemia–reperfusion [in Ukrainian]. *Fiziol Zh*. 2003;49:14–21.
91. Yamada N, Yamaya M, Okinaga S, et al. Microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with susceptibility to emphysema. *Am J Hum Genet*. 2000;66:187–195.
92. Tulis DA, Durante W, Liu X, et al. Adenovirus-mediated heme oxygenase-1 gene delivery inhibits injury-induced vascular neointima formation. *Circulation*. 2001;104:2710–2715.
93. Li Volti G, Wang J, Traganos F, et al. Differential effect of heme oxygenase-1 in endothelial and smooth muscle cell cycle progression. *Biochem Biophys Res Commun*. 2002;296:1077–1082.
94. Kushida T, Quan S, Yang L, et al. A significant role for the heme oxygenase-1 gene in endothelial cell cycle progression. *Biochem Biophys Res Commun*. 2002;291:68–75.
95. Agarwal A, Balla J, Alam J, et al. Induction of heme oxygenase in toxic renal injury: a protective role in cisplatin nephrotoxicity in the rat. *Kidney Int*. 1995;48:1298–1307.
96. Lutton JD, da Silva J-L, Moqattash S, et al. Differential induction of heme oxygenase in the hepatocarcinoma cell line (Hep3b) by environmental agents. *J Cell Biochem*. 1992;49:259–265.
97. Kong D, Melo LG, Mangi AA, et al. Enhanced inhibition of neointimal hyperplasia by genetically engineered endothelial progenitor cells. *Circulation*. 2004;109:1769–1775.
98. Gu Y, Ortines R. Heme oxygenase-1 is cardioprotective in the remodeled, failing heart [abstract]. *Circulation.* 2004;110:III-201.
99. Seki T, Naruse M, Naruse K, et al. Induction of heme oxygenase produces load-independent cardioprotective effects in hypertensive rats. *Life Sci*. 1999;65:1077–1086.
100. Ooi H, Ito M, Pimental D. Heme oxygenase protects against β-adrenergic receptor-stimulated apoptosis in cardiac myocytes [abstract]. *Circulation.* 2004;110:III-5.
101. Kawamoto S, Flynn JP, Allen MD. Heme oxygenase-1 induction improves the viability of adult cardiomyocyte cellular grafts in vivo [abstract]. *Circulation.* 2004;110:III-302.
102. Tang YL, Tang Y, Zhang YC, et al. Mesenchymal stem cells protected with a hypoxia-regulated heme oxygenase-1 vector: a potential strategy to prevent graft cell death in the ischemic heart [abstract]. *Circulation.* 2004;110:III-3.
103. Motterlini R, Clark JE, Foresti R, et al. Carbon monoxide-releasing molecules: characterization of biochemical and vascular activities. *Circ Res*. 2002;90:E17–E24.
104. Chatterjee PK. Water-soluble carbon monoxide-releasing molecules: helping to elucidate the vascular activity of the "silent killer." *Br J Pharmacol*. 2004;142:391–393.
105. Clark JE, Naughton P, Shurey S, et al. Cardioprotective actions by a water-soluble carbon monoxide-releasing molecule. *Circ Res*. 2003;93:e2–e8.
106. Foresti R, Hammad J, Clark JE, et al. Vasoactive properties of CORM-3, a novel water-soluble carbon monoxide-releasing molecule. *Br J Pharmacol*. 2004;142:453–460.
107. Wang R, Wang Z, Wu L. Carbon monoxide-induced vasorelaxation and the underlying mechanisms. *Br J Pharmacol*. 1997;121:927–934.

108. Morita T, Perrella MA, Lee M, et al. Smooth muscle cell-derived carbon monoxide is a regulator of vascular cGMP. *Proc Natl Acad Sci USA*. 1995;92:1475–1479.
109. Ndisang JF, Wang R. Alterations in heme oxygenase/carbon monoxide system in pulmonary arteries in hypertension. *Exp Biol Med (Maywood)*. 2003;228:557–563.
110. Levere RD, Martasek P, Escalante B, et al. Effect of heme arginate administration on blood pressure in spontaneously hypertensive rats. *J Clin Invest*. 1990;86:213–219.
111. Sammut IA, Foresti R, Clark JE, et al. Carbon monoxide is a major contributor to the regulation of vascular tone in aortas expressing high levels of haeme oxygenase-1. *Br J Pharmacol*. 1998;125:1437–1444.
112. Motterlini R, Mann BE, Johnson TR, et al. Bioactivity and pharmacological actions of carbon monoxide-releasing molecules. *Curr Pharm Des*. 2003;9: 2525–2539.
113. Abraham NG, Mieyal PA, Quan S, et al. Modulation of cyclic GMP by retrovirus-mediated human heme oxgyenase-1 gene transfer in microvessel endothelial cells. *Am J Physiol*. 2002;283:L1117–L1124.
114. da Silva JL, Morishita T, Escalante B, et al. Dual role of heme oxygenase in epithelial cell injury: contrasting effects of short-term and long-term exposure to oxidant stress. *J Lab Clin Med*. 1996;128:290–296.
115. Ross R. Atherosclerosis–an inflammatory disease. *N Engl J Med*. 1999;340: 115–126.
116. Agarwal A, Balla J, Balla G, et al. Renal tubular epithelial cells mimic endothelial cells upon exposure to oxidized LDL. *Am J Physiol*. 1996;271: F814–F823.
117. Yamaguchi M, Sato H, Bannai S. Induction of stress proteins in mouse peritoneal macrophages by oxidized low-density lipoprotein. *Biochem Biophys Res Commun*. 1993;193:1198–1201.
118. Agarwal A, Shiraishi F, Visner GA, et al. Linoleyl hydroperoxide transcriptionally upregulates heme oxygenase-1 gene expression in human renal epithelial and aortic endothelial cells. *J Am Soc Nephrol*. 1998;9:1990–1997.
119. Ishikawa K, Sugawara D, Wang X, et al. Heme oxygenase-1 inhibits atherosclerotic lesion formation in ldl-receptor knockout mice. *Circ Res*. 2001;88:506–512.
120. Ishikawa K, Sugawara D, Goto J, et al. Heme oxygenase-1 inhibits atherogenesis in Watanabe heritable hyperlipidemic rabbits. *Circulation*. 2001; 104:1831–1836.
121. Yet SF, Layne MD, Liu X, et al. Absence of heme oxygenase-1 exacerbates atherosclerotic lesion formation and vascular remodeling. *FASEB J*. 2003; 17:1759–1761.
122. Li M, Peterson S, Husney D, et al. Interdiction of the diabetic state in NOD mice by sustained induction of heme oxygenase: possible role of carbon monoxide and bilirubin. *Antioxid Redox Signal*. 2007;9:855–863.
123. Gao X, Belmadani S, Picchi A, et al. Tumor necrosis factor-alpha induces endothelial dysfunction in Lepr(db) mice. *Circulation*. 2007; 115:245–254.
124. Korobowicz A. Biology of tumor necrosis factor type alpha (TNF-alpha) [in Polish]. *Pol Merkur Lekarski*. 2006;21:358–361.
125. Chen J, Liu Y, Liu H, et al. Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) transcriptional regulation by Oct-1 in human endothelial cells: implications for atherosclerosis. *Biochem J*. 2006;393:255–265.
126. Hadi HA, Carr CS, Al SJ. Endothelial dysfunction: cardiovascular risk factors, therapy, and outcome. *Vasc Health Risk Manag*. 2005;1:183–198.
127. Kawamura K, Ishikawa K, Wada Y, et al. Bilirubin from heme oxygenase-1 attenuates vascular endothelial activation and dysfunction. *Arterioscler Thromb Vasc Biol*. 2005;5:155–160.
128. Kruger AL, Peterson S, Turkseven S, et al. D-4F induces heme oxygenase-1 and extracellular superoxide dismutase, decreases endothelial cell sloughing, and improves vascular reactivity in rat model of diabetes. *Circulation*. 2005;111:3126–3134.
129. Peterson SJ, Husney D, Kruger AL, et al. Long-term treatment with the apolipoprotein A1 mimetic peptide increases antioxidants and vascular repair in type I diabetic rats. *J Pharmacol Exp Ther*. 2007;322:514–520.
130. Nakayama M, Takahashi K, Komaru T, et al. Increased expression of heme oxygenase-1 and bilirubin accumulation in foam cells of rabbit atherosclerotic lesions. *Arterioscler Thromb Vasc Biol*. 2001;21:1373–1377.
131. Dulak J, Loboda A, Zagorska A, et al. Complex role of heme oxygenase-1 in angiogenesis. *Antioxid Redox Signal*. 2004;6:858–866.
132. Bussolati B, Mason JC. Dual role of VEGF-induced heme-oxygenase-1 in angiogenesis. *Antioxid Redox Signal*. 2006;8:1153–1163.
133. Suzuki M, Iso-o N, Takeshita S, et al. Facilitated angiogenesis induced by heme oxygenase-1 gene transfer in a rat model of hindlimb ischemia. *Biochem Biophys Res Commun*. 2003;302:138–143.
134. Alvaro-Gonzalez LC, Freijo-Guerrero MM, Sadaba-Garay F. Inflammatory mechanisms, arteriosclerosis and ischemic stroke: clinical data and perspectives [in Spanish]. *Rev Neurol*. 2002;35:452–462.
135. Ameriso SF, Villamil AR, Zedda C, et al. Heme oxygenase-1 is expressed in carotid atherosclerotic plaques infected by *Helicobacter pylori* and is more prevalent in asymptomatic subjects. *Stroke*. 2005;36:1896–1900.
136. Otterbein LE, Bach FH, Alam J, et al. Carbon monoxide has anti-inflammatory effects involving the mitogen- activated protein kinase pathway. *Nat Med*. 2000;6:422–428.
137. Zhan Y, Kim S, Izumi Y, et al. Role of JNK, p38, and ERK in platelet-derived growth factor-induced vascular proliferation, migration, and gene expression. *Arterioscler Thromb Vasc Biol*. 2003;23:795–801.
138. Sawle P, Foresti R, Mann BE, et al. Carbon monoxide-releasing molecules (CO-RMs) attenuate the inflammatory response elicited by lipopolysaccharide in RAW264.7 murine macrophages. *Br J Pharmacol*. 2005;145:800–810.
139. Morita T, Kourembanas S. Endothelial cell expression of vasoconstrictors and growth factors is regulated by smooth muscle cell-derived carbon monoxide. *J Clin Invest*. 1995;96:2676–2682.
140. Morita T, Mitsialis SA, Hoike H, et al. Carbon monoxide controls the proliferation of hypoxic smooth muscle cells. *J Biol Chem*. 1997;272: 32804–32809.
141. Duckers HJ, Boehm M, True AL, et al. Heme oxygenase-1 protects against vascular constriction and proliferation. *Nat Med*. 2001;7:693–698.
142. Tulis DA, Keswani AN, Peyton KJ, et al. Local administration of carbon monoxide inhibits neointima formation in balloon injured rat carotid arteries. *Cell Mol Biol (Noisy-le-grand)*. 2005;51:441–446.
143. Taille C, El-Benna J, Lanone S, et al. Mitochondrial respiratory chain and NAD(P)H oxidase are targets for the antiproliferative effect of carbon monoxide in human airway smooth muscle. *J Biol Chem*. 2005;280:25350–25360.
144. Aizawa T, Ishizaka N, Taguchi J, et al. Balloon injury does not induce heme oxygenase-1 expression, but administration of hemin inhibits neointimal formation in balloon-injured rat carotid artery. *Biochem Biophys Res Commun*. 1999;261:302–307.
145. Tulis DA, Durante W, Peyton KJ, et al. Heme oxygenase-1 attenuates vascular remodeling following balloon injury in rat carotid arteries. *Atherosclerosis*. 2001;155:113–122.
146. Azevedo LC, Pedro MA, Souza LC, et al. Oxidative stress as a signaling mechanism of the vascular response to injury: the redox hypothesis of restenosis. *Cardiovasc Res*. 2000;47:436–445.
147. Ollinger R, Bilban M, Erat A, et al. Bilirubin: a natural inhibitor of vascular smooth muscle cell proliferation. *Circulation*. 2005;112:1030–1039.
148. Braun-Dullaeus RC, Mann MJ, Dzau VJ. Cell cycle progression: new therapeutic target for vascular proliferative disease. *Circulation*. 1998;98: 82–89.
149. Zhao M, Liu Y, Bao M, et al. Vascular smooth muscle cell proliferation requires both p38 and BMK1 MAP kinases. *Arch Biochem Biophys*. 2002; 400:199–207.
150. Maines MD. The heme oxygenase system: a regulator of second messenger gases. *Annu Rev Pharmacol Toxicol*. 1997;37:517–554.
151. Lozonschi L, Sunamura M, Kobari M, et al. Controlling tumor angiogenesis and metastasis of C26 murine colon adenocarcinoma by a new matrix metalloproteinase inhibitor, KB-R7785, in two tumor models. *Cancer Res*. 1999;59:1252–1258.
152. Furchgott RF, Jothianandan D. Endothelium-dependent and -independent vasodilation involving cyclic GMP: relaxation induced by nitric oxide, carbon monoxide and light. *Blood Vessels*. 1991;28:52–61.
153. Ryter SW, Alam J, Choi AM. Heme oxygenase-1/carbon monoxide: from basic science to therapeutic applications. *Physiol Rev*. 2006;86:583–650.
154. Motterlini R, Gonzales A, Foresti R, et al. Heme oxygenase-1 derived carbon monoxide contributes to the suppression of acute hypertensive responses in vivo. *Circ Res*. 1998;83:568–577.
155. Quan S, Feldman E, Yang L, et al. Distinct effect of retroviral-mediated IFN-alpha gene transfer on human erythroleukemic and CD34+ cell growth and differentiation. *J Hematother Stem Cell Res*. 1999;8:491–502.
156. Wang R, Wu L. The chemical modification of KC_a channels by carbon monoxide in vascular smooth muscle cells. *J Biol Chem*. 1997;272:8222–8226.

157. Amersi F, Shen XD, Anselmo D, et al. Ex vivo exposure to carbon monoxide prevents hepatic ischemia/reperfusion injury through p38 MAP kinase pathway. *Hepatology*. 2002;35:815–823.
158. Brouard S, Berberat PO, Tobiasch E, et al. Heme oxygenase-1-derived carbon monoxide requires the activation of transcription factor NF-kappa B to protect endothelial cells from tumor necrosis factor-alpha-mediated apoptosis. *J Biol Chem*. 2002;277:17950–17961.
159. L'Abbate A, Neglia D, Vecoli C, et al. Beneficial effect of heme oxygenase-1 expression on myocardial ischemia-reperfusion involves an increase in adiponectin in mildly diabetic rats. *Am J Physiol Heart Circ Physiol*. 2007;293:H3532–H3541.
160. Hangaishi M, Ishizaka N, Aizawa T, et al. Induction of heme oxygenase-1 can act protectively against cardiac ischemia/reperfusion in vivo. *Biochem Biophys Res Commun*. 2000;279:582–588.
161. Masini E, Vannacci A, Marzocca C, et al. Heme oxygenase-1 and the ischemia-reperfusion injury in the rat heart. *Exp Biol Med (Maywood)*. 2003;228:546–549.
162. Vulapalli SR, Chen Z, Chua BH, et al. Cardioselective overexpression of *HO-1* prevents I/R-induced cardiac dysfunction and apoptosis. *Am J Physiol Heart Circ Physiol*. 2002;283:H688–H694.
163. Melo LG, Agrawal R, Zhang L, et al. Gene therapy strategy for long-term myocardial protection using adeno-associated virus-mediated delivery of heme oxygenase gene. *Circulation*. 2002;105:602–607.
164. Yoshida T, Maulik N, Ho YS, et al. H(mox-1) constitutes an adaptive response to effect antioxidant cardioprotection: a study with transgenic mice heterozygous for targeted disruption of the Heme oxygenase-1 gene. *Circulation*. 2001;103:1695–1701.
165. Yet SF, Perrella MA, Layne MD, et al. Hypoxia induces severe right ventricular dilatation and infarction in heme oxygenase-1 null mice. *J Clin Invest*. 1999;103:R23–R29.
166. Liu X, Pachori AS, Ward CA, et al. Heme oxygenase-1 (*HO-1*) inhibits postmyocardial infarct remodeling and restores ventricular function. *FASEB J*. 2006;20:207–216.
167. Coito AJ, Buelow R, Shen XD, et al. Heme oxygenase-1 gene transfer inhibits inducible nitric oxide synthase expression and protects genetically fat Zucker rat livers from ischemia-reperfusion injury. *Transplantation*. 2002;74:96–102.
168. Stec DE. Smart gene therapy for the heart. *Hypertension*. 2004;43:720–721.
169. Pachori AS, Melo LG, Hart ML, et al. Hypoxia-regulated therapeutic gene as a preemptive treatment strategy against ischemia/reperfusion tissue injury. *Proc Natl Acad Sci USA*. 2004;101:12282–12287.
170. Liao TD, Yang XP, Liu YH, et al. Role of inflammation in the development of renal damage and dysfunction in angiotensin II-induced hypertension. *Hypertension*. 2008;52:256–263.
171. Letavernier E, Perez J, Bellocq A, et al. Targeting the calpain/calpastatin system as a new strategy to prevent cardiovascular remodeling in angiotensin II-induced hypertension. *Circ Res*. 2008;102:720–728.
172. Henke N, Schmidt-Ullrich R, Dechend R, et al. Vascular endothelial cell-specific NF-kappa B suppression attenuates hypertension-induced renal damage. *Circ Res*. 2007;101:268–276.
173. Gupta A, Rhodes GJ, Berg DT, et al. Activated protein C ameliorates LPS-induced acute kidney injury and downregulates renal INOS and angiotensin 2. *Am J Physiol Renal Physiol*. 2007;293:F245–F254.
174. Fontana L, Eagon JC, Trujillo ME, et al. Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. *Diabetes*. 2007;56:1010–1013.
175. Wang ZV, Scherer PE. Adiponectin, cardiovascular function, and hypertension. *Hypertension*. 2008;51:8–14.
176. Hopkins TA, Ouchi N, Shibata R, et al. Adiponectin actions in the cardiovascular system. *Cardiovasc Res*. 2007;74:11–18.
177. Ouchi N, Ohishi M, Kihara S, et al. Association of hypoadiponectinemia with impaired vasoreactivity. *Hypertension*. 2003;42:231–234.
178. Li M, Kim DH, Tsenovoy PL, et al. Treatment of obese diabetic mice with a heme oxygenase inducer reduces visceral and subcutaneous adiposity, increases adiponectin levels, and improves insulin sensitivity and glucose tolerance. *Diabetes*. 2008;57:1526–1535.
179. Kim DH, Burgess AP, Li M, et al. Heme oxygenase-mediated increases in adiponectin decrease fat content and inflammatory cytokines, tumor necrosis factor-alpha and interleukin-6 in Zucker rats and reduce adipogenesis in human mesenchymal stem cells. *J Pharmacol Exp Ther*. 2008;325:833–840.
180. Peterson SJ, Drummond G, Hyun KD, et al. L-4F treatment reduces adiposity, increases adiponectin levels and improves insulin sensitivity in obese mice. *J Lipid Res*. 2008;49:1658–1669.
181. Elbatarny HS, Netherton SJ, Ovens JD, et al. Adiponectin, ghrelin, and leptin differentially influence human platelet and human vascular endothelial cell functions: implication in obesity-associated cardiovascular diseases. *Eur J Pharmacol*. 2007;558:7–13.
182. Jung TW, Lee JY, Shim WS, et al. Adiponectin protects human neuroblastoma SH-SY5Y cells against acetaldehyde-induced cytotoxicity. *Biochem Pharmacol*. 2006;72:616–623.
183. Zou AP, Fleming JT, Falck JR, et al. 20-HETE is an endogenous inhibitor of the large-conductance Ca(2+)-activated K+ channel in renal arterioles. *Am J Physiol*. 1996;270:R228–R237.
184. Zou AP, Imig JD, Ortiz-de-Montellano PR, et al. Effect of P-450 omega-hydroxylase metabolites of arachidonic acid on tubuloglomerular feedback. *Am J Physiol*. 1994;266:F934–F941.
185. Zou AP, Ma YH, Sui ZH, et al. Effects of 17-octadecynoic acid, a suicide-substrate inhibitor of cytochrome P450 fatty acid omega-hydroxylase, on renal function in rats. *J Pharmacol Exp Ther*. 1994;268:474–481.
186. da Silva JL, Tiefenthaler M, Park E, et al. Tin-mediated heme oxygenase gene activation and cytochrome P450 arachidonate hydroxylase inhibition in spontaneously hypertensive rats. *Am J Med Sci*. 1994;307:173–181.
187. Patel JV, Lim HS, Hughes EA, et al. Adiponectin and hypertension: a putative link between adipocyte function and atherosclerotic risk? *J Hum Hypertens*. 2007;21:1–4.
188. Grana X, Reddy EP. Cell cycle control in mammalian cells: role of cyclins, cyclin dependent kinases (CDKs), growth suppressor genes and cyclin-dependent kinase inhibitors (CKIs). *Oncogene*. 1995;11:211–219.
189. Chow WS, Cheung BM, Tso AW, et al. Hypoadiponectinemia as a predictor for the development of hypertension: a 5-year prospective study. *Hypertension*. 2007;49:1455–1461.
190. Han SH, Quon MJ, Kim JA, et al. Adiponectin and cardiovascular disease: response to therapeutic interventions. *J Am Coll Cardiol*. 2007;49:531–538.
191. Iwashima Y, Katsuya T, Ishikawa K, et al. Hypoadiponectinemia is an independent risk factor for hypertension. *Hypertension*. 2004;43:1318–1323.
192. Li HY, Chiu YF, Hwu CM, et al. The negative correlation between plasma adiponectin and blood pressure depends on obesity: a family-based association study in SAPPHIRe. *Am J Hypertens*. 2008;21:471–476.
193. Ouchi N, Kobayashi H, Kihara S, et al. Adiponectin stimulates angiogenesis by promoting cross-talk between AMP-activated protein kinase and Akt signaling in endothelial cells. *J Biol Chem*. 2004;279:1304–1309.
194. Tomas E, Tsao TS, Saha AK, et al. Enhanced muscle fat oxidation and glucose transport by ACRP30 globular domain: acetyl-CoA carboxylase inhibition and AMP-activated protein kinase activation. *Proc Natl Acad Sci USA*. 2002;99:16309–16313.
195. Lihn AS, Pedersen SB, Lund S, et al. The anti-diabetic AMPK activator AICAR reduces IL-6 and IL-8 in human adipose tissue and skeletal muscle cells. *Mol Cell Endocrinol*. 2008;292:36–41.
196. Dyck JR, Lopaschuk GD. AMPK alterations in cardiac physiology and pathology: enemy or ally? *J Physiol*. 2006;574:95–112.
197. Laderoute KR, Amin K, Calaoagan JM, et al. 5′-AMP-activated protein kinase (AMPK) is induced by low-oxygen and glucose deprivation conditions found in solid-tumor microenvironments. *Mol Cell Biol*. 2006;26:5336–5347.
198. Schulz E, Dopheide J, Schuhmacher S, et al. Suppression of the JNK pathway by induction of a metabolic stress response prevents vascular injury and dysfunction. *Circulation*. 2008;118:1347–1357.
199. Chen K, Kirber MT, Xiao H, et al. Regulation of ROS signal transduction by NADPH oxidase 4 localization. *J Cell Biol*. 2008;181:1129–1139.
200. Murabito JM, Keyes MJ, Guo CY, et al. Cross-sectional relations of multiple inflammatory biomarkers to peripheral arterial disease: the Framingham Offspring Study. *Atherosclerosis.* In press.
201. Bandyopadhyay GK, Yu JG, Ofrecio J, et al. Increased malonyl-CoA levels in muscle from obese and type 2 diabetic subjects lead to decreased fatty acid oxidation and increased lipogenesis; thiazolidinedione treatment reverses these defects. *Diabetes*. 2006;55:2277–2285.
202. Yang X, Ongusaha PP, Miles PD, et al. Phosphoinositide signaling links O-GlcNAc transferase to insulin resistance. *Nature*. 2008;451:964–969.
203. Dimmeler S, Fleming I, Fisslthaler B, et al. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. *Nature*. 1999;399:601–605.
204. Chen ZP, Mitchelhill KI, Michell BJ, et al. AMP-activated protein kinase phosphorylation of endothelial NO synthase. *FEBS Lett*. 1999;443:285–289.
205. Wulster-Radcliffe MC, Ajuwon KM, Wang J, et al. Adiponectin differen-

tially regulates cytokines in porcine macrophages. *Biochem Biophys Res Commun*. 2004;316:924–929.

206. Rodella L, Lamon BD, Rezzani R, et al. Carbon monoxide and biliverdin prevent endothelial cell sloughing in rats with type I diabetes. *Free Radic Biol Med*. 2006;40:2198–2205.
207. Sambuceti G, Morbelli S, Vanella L, et al. Diabetes impairs the vascular recruitment of normal stem cells by oxidant damage; reversed by increases in pAMPK, heme oxygenase-1 and adiponectin. *Stem Cells.* In press.
208. Grosser N, Abate A, Oberle S, et al. Heme oxygenase-1 induction may explain the antioxidant profile of aspirin. *Biochem Biophys Res Commun*. 2003;308:956–960.
209. Polte T, Oberle S, Schroder H. The nitric oxide donor SIN-1 protects endothelial cells from tumor necrosis factor-alpha-mediated cytotoxicity: possible role for cyclic GMP and heme oxygenase. *J Mol Cell Cardiol*. 1997;29:3305–3310.
210. Grosser N, Hemmerle A, Berndt G, et al. The antioxidant defense protein heme oxygenase 1 is a novel target for statins in endothelial cells. *Free Radic Biol Med*. 2004;37:2064–2071.
211. Grosser N, Erdmann K, Hemmerle A, et al. Rosuvastatin upregulates the antioxidant defense protein heme oxygenase-1. *Biochem Biophys Res Commun*. 2004;325:871–876.
212. Lee TS, Chang CC, Zhu Y, et al. Simvastatin induces heme oxygenase-1: a novel mechanism of vessel protection. *Circulation*. 2004;110:1296–1302.
213. Heinecke JW. Lipoprotein oxidation in cardiovascular disease: chief culprit or innocent bystander? *J Exp Med*. 2006;203:813–816.
214. Wu BJ, Kathir K, Witting PK, et al. Antioxidants protect from atherosclerosis by a heme oxygenase-1 pathway that is independent of free radical scavenging. *J Exp Med*. 2006;203:1117–1127.
215. Ishizaka N, De Leon H, Laursen JB, et al. Angiotensin II-induced hypertension increases heme oxygenase-1 expression in rat aorta. *Circulation*. 1997;96:1923–1929.
216. Yuan LX, Liu HM, Li M, et al. Effects of Losartan on expression of heme oxygenases in volume-overloaded rats with left-to-right shunt [in Chinese]. *Sichuan Da Xue Xue Bao Yi Xue Ban*. 2005;36:668–671.
217. Izuhara Y, Nangaku M, Inagi R, et al. Renoprotective properties of angiotensin receptor blockers beyond blood pressure lowering. *J Am Soc Nephrol*. 2005;16:3631–3641.
218. Das S, Fraga CG, Das DK. Cardioprotective effect of resveratrol via *HO-1* expression involves p38 map kinase and PI-3-kinase signaling, but does not involve NFkappaB. *Free Radic Res*. 2006;40:1066–1075.
219. Kaga S, Zhan L, Matsumoto M, et al. Resveratrol enhances neovascularization in the infarcted rat myocardium through the induction of thioredoxin-1, heme oxygenase-1 and vascular endothelial growth factor. *J Mol Cell Cardiol*. 2005;39:813–822.
220. Huang HM, Liang YC, Cheng TH, et al. Potential mechanism of blood vessel protection by resveratrol, a component of red wine. *Ann NY Acad Sci*. 2005;1042:349–356.
221. Evgenov OV, Pacher P, Schmidt PM, et al. NO-independent stimulators and activators of soluble guanylate cyclase: discovery and therapeutic potential. *Nat Rev Drug Discov*. 2006;5:755–768.
222. Van Eickels M, Wassman S, Schäfer A, et al. Role of sGC activator ataciquat sodium (HMR1766) in cardiovascular disease. *BMC Pharmacol*. 2007;7(suppl 1):S4.
223. Abraham NG, Scapagnini G, Kappas A. Human heme oxygenase: cell cycle-dependent expression and DNA microarray identification of multiple gene responses after transduction of endothelial cells. *J Cell Biochem*. 2003;90:1098–1111.