STATE-OF-THE-ART PAPER

Hemoxygenase-1 in Cardiovascular Disease

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Hemoxygenase (HO)-1 is an inducible isoform of the first and rate-controlling enzyme of the degradation of heme into iron, carbon monoxide, and biliverdin, the latter being subsequently converted into bilirubin. Several positive biological effects exerted by this enzyme have gained attention, as anti-inflammatory, anti-apoptotic, angiogenic, and cytoprotective functions are attributable to carbon monoxide and/or bilirubin. Thus, the physiological induction of HO-1 may be an adaptive and beneficial response to several possibly noxious stimuli, including heme itself, suggesting a potentially autoprotective and autodefensive role in several pathophysiological states including acute coronary syndromes and stroke. This review article provides a comprehensive overview of the biochemistry, physiology, and pathophysiology of HO-1 in relation to cardiovascular disease (CVD). Furthermore, we present some of the emerging evidence in support of the view that the induction of the HO-1 gene may be a new opportunity to target the pathophysiology of CVD, with therapeutic implications for management. (J Am Coll Cardiol 2008;52:971–8) © 2008 by the American College of Cardiology Foundation

Hemoxygenase (HO)-1, which is inducible by factors that include heavy metals and reactive oxygen species, catalyzes the first and rate-controlling step of the degradation of heme into ferrous (Fe^{2+}) iron, carbon monoxide (CO), and biliverdin, the latter being subsequently converted into bilirubin (1). These products may have physiological and pathological functions, such as in protection from oxidative stress, a process that may be important in the pathophysiology of several cardiovascular diseases (2,3). The growing interest in HO-1 is now such that the induction of the HO-1 gene has been proposed as a new therapy (4). In this review article, we provide a comprehensive overview of enzymatic and biological aspects of HO-1, as well as its reaction products (chiefly, CO and bilirubin) that illustrate the significance of this molecule in the pathophysiology of cardiovascular disease (CVD). In addition, we present some of the salient lines of evidence in support of the therapeutic and clinical implications of the modification of the activity of this enzyme.

Search Strategy

We performed a literature search (1966 to May 2008) on "haemoxygenase-1" and "heme-oxygenase-1" using Medline, EMBASE, PubMed, and Cochrane electronic bibliographic databases, as well as scanned relevant reference lists from included articles.

Biochemistry and Genetics of HO-1

Hemoxygenase, originally identified by Tenhunen et al. (5), has 3 isoforms. The first, HO-1, is a 32-kDa protein, inducible by numerous stimuli, that catalyzes the first and rate-limiting step in the degradation of the protoporphyrin ring of tetrapyrrole heme from effete red blood cells, yielding equimolar quantities of biliverdin IXa, CO, and iron (5,6). Biliverdin (through the action of biliverdin reductase) is converted to bilirubin, and iron is sequestered into ferritin. Interestingly, HO-1 utilizes heme as both a prosthetic group and a substrate (1). The second isoform of hemoxygenase, HO-2, a constitutively synthesized 36-kDa protein, is generally unresponsive to any of the inducers of HO-1. The third isoform, HO-3, also catalyzes heme degradation, but much less so when compared with HO-2 (7,8). Although heme is the typical HO-1 inducer, others include endotoxin, heavy metals, oxidants, and hypoxia (Table 1). A common feature of several of these inducers is their ability to generate reactive oxygen species, suggesting that HO-1 provides potent cytoprotective effects (9–16) (Table 1).

Bilirubin. Several lines of evidence suggest that biliverdin and bilirubin may be part of a cell defense strategy in response to oxidative stress. Both molecules are natural antioxidants, and high-normal serum levels of bilirubin are inversely related to the atherogenic risk, possibly by inhibitory effects against low-density lipoprotein oxidation and the scavenging of oxygen radicals (17,18). Additionally, an

inhibitory effect on protein kinase C and protein phosphorylation activity has been shown, both of which lead to inhibition of proatherogenic factors (19, 20). Bilirubin also provides cardioprotection against reperfusion injury, such as by the suppression of the oxidation of lipid membranes (21,22). The hypothesis that the protection provided by bilirubin in the ischemic myocardium could be clinically significant is supported by an inverse correlation between plasma bilirubin and the risk of coronary artery disease (CAD) and decreased antioxidant activity of bilirubin in atherosclerotic lesions (23–25). The increased intracellular bilirubin as a consequence of HO-1 induction also implies that CO production may be enhanced. Indeed, Morita et al. (17) have suggested that stimuli that amplify the generation of bilirubin also act in an adaptive reaction against oxidative insults, so that the action of the HO-1 pathway in raising endogenous bilirubin levels may represent an additional option in frustrating oxidative stress that may, eventually, have relevant clinical impact.

Carbon monoxide. Morita et al. (17) also summarized the evidence that CO has a physiological role in the regulation of vascular tone similar to that of nitric oxide (NO), one mechanism for which may be increased intracellular cyclic guanine monophosphate (cGMP) (26). However, the precise physiological significance of CO in relation to NO as a vasodilator is contentious. For example, the increase in cGMP is induced in vitro by perhaps 130-fold by NO, whereas it is only induced 4-fold by CO. Rodent models suggest that overproduction of CO might impair NO-elicited generation of soluble guanylate cyclase, resulting in inhibition of the cGMP increase in the aortas of transgenic mice that over-expressed HO-1, suggesting that CO may

result in protection from acute hypertension (27,28). Carbon monoxide may also limit the development of vascular diseases because of an effect on smooth muscle cell proliferation and death, whereas lack of HO-1 in (−/−) KO mice leads to pulmonary hypertension (29–31).

Iron. Ferrous iron, a possible electron donor with cytotoxic potential in generating reactive oxygen species is an additional regulator of HO-1 (32). Thus ferritin, primarily a liver-derived, iron-binding protein, may act as an indirect antioxidant by sequestering iron. The augmentation of intracellular ferritin through HO-1 reduces the cytotoxic effects of heme and hydrogen peroxide in vascular endothelial cells (33), and may protect against ischemia-reperfusion injury through its cytoprotective effects on the endothelium and its ability to keep labile iron pools low and thereby reduce oxidant-induced lipid peroxidation (34). However, one hesitates to leap to the conclusion that these relationships are causal. Nevertheless, the weight of published data suggests that, of the 3 products of HO-1, the potentially protective effects of bilirubin and/or CO seem likely to exceed the potentially harmful effects of iron.

Pathophysiological Processes of HO-1

The previous section provided evidence to suggest that HO-1 can (through its products CO and bilirubin) function as a potentially important cytoprotective molecule. Many workers assume that the up-regulation of HO-1 by stress-causing agents could mediate cytoprotection against subsequent noxious stimuli and that this can be an important physiological process. However, although physiologically low concentrations of heme are cytoprotective as they induce the rapid up-regulation of HO-1, excess pathological amounts of heme out-strip the ability of HO-1 to metabolize it so that residual heme (librating free iron) may act deleteriously on tissue by pro-oxidative and proinflammatory effects (35–37). Mechanisms by which HO-1 provides protection against cardiopathology include antioxidant activity of bilirubin (22–25), sequestration of iron by ferritin (33), and an antifibrinolytic and vasodilative effect of CO (26–31,38) (Fig. 1).

Inflammation and antioxidant function. The mechanisms by which HO-1 is anti-inflammatory generate considerable research activity but remain unclear, although animal model clues exist, such as a relationship between HO-1 and cytokines (39–41). A rat model of hepatic ischemic and reperfusion injury (which activates toll-like receptor-4 signaling) has been used as evidence of a novel mechanism by which HO-1 exerts adaptive cytoprotective and anti-inflammatory functions (41,42). In the latter, cobalt-protoporphyrin–induced HO-1 over-expression reduced liver damage and down-regulated activation of signal transducers and activator of transcription 1 by the type-1

Table 1	Inducers/Stimulators of HO-1 Activity
Cytokines (IL-10, IL-13, IL-18)	
Endotoxin*	
Growth factors (TGF-beta, PDGF, VEGF)	
Heme	
Heavy metals (e.g., sodium arsenite, cadmium, tin, lead)	
Hypoxia* and hyperoxia*	
Nitric oxide	
Oxidants (e.g., hydrogen peroxide,* peroxynitrite*)	
Oxidized LDL	
Thiol scavengers	
Ultraviolet radiation*	

Pooled from references 9–17, 70, 71, and elsewhere. *Also promotors of oxidative stress.
HO = hemoxygenase; IL = interleukin; LDL = low-density lipoprotein; PDGF = platelet-derived
growth factor; TGF = transforming growth factor; VEGF = vascular endothelial growth factor.
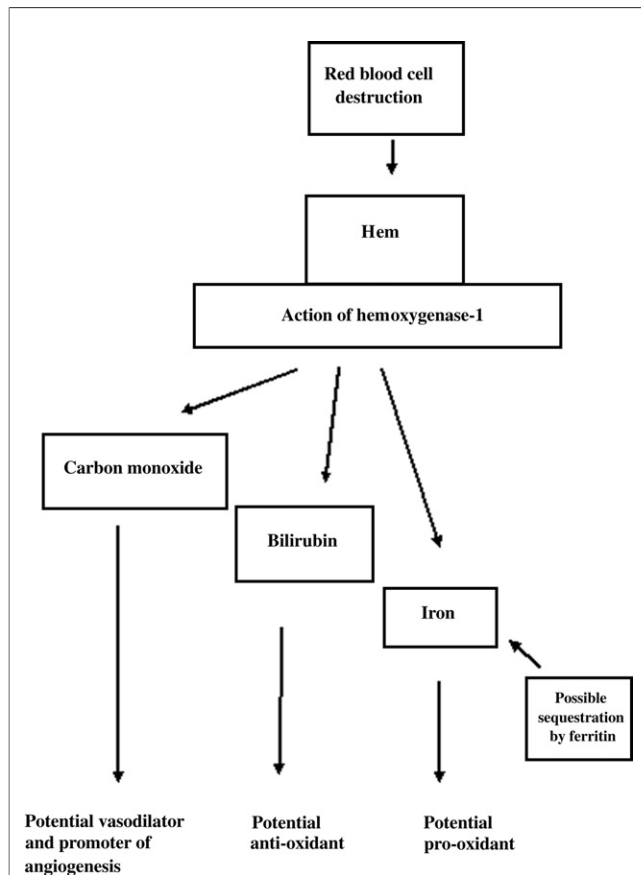
Figure 1	**Schematic of Hemoxygenase-1–Catalyzed Biochemical Reactions**

Hem released from red blood cells is enzymatically converted into carbon monoxide, bilirubin, and iron. The former is a vasodilator and promoter of angiogenesis. Bilirubin has potential antioxidant activity. Free ferrous iron has potential pro-oxidant activity, although this may be limited by its sequestration by ferritin.

interferon pathway downstream of toll-like receptor-4, which in turn decreased CXCL-10 production. The antioxidant activity of bilirubin may feed through to antiatherogenic properties—possibly by protecting low density lipoprotein cholesterol from oxidation (22,43,44).

Apoptosis. Hemoxygenase-1 appears to have a role in reducing the proapoptotic effects of tumor necrosis factor (TNF), hyperglycemia, and iron, some of which could involve CO (45–48). At the intracellular level, this may involve expression of p38 mitogen-activated protein kinase enzymes and possibly the activation of nuclear factor kappa B (49,50). Adenovirus-mediated transfection of the HO-1 gene into rat hearts resulted in a reduction in infarct size that was accompanied by decreases in lipid peroxidation and in proapoptotic Bax and proinflammatory interleukin-1-beta protein abundance, with a parallel increase in antiapoptotic Bcl-2 protein level (51). Various models of transplantation, hyperglycemia, and tissue culture suggest HO-1 inhibits apoptosis by suppressing cytotoxic, inflammatory, and signaling cytokines (52–55).

Hypoxia and ischemia/reperfusion injury. Hypoxia is thought to be a key determinant in clinical pathology, and thus several lines of research have linked it (albeit indirectly) with HO-1. For example, the HO-1–bilirubin pathway can defend cells from reoxygenation injury, and restricting vascular smooth muscle cell growth by increasing the release of CO may represent a route to limiting pulmonary hypertension (56,57). Interestingly, in a rat model, monotherapy with either CO or biliverdin did not alter the survival of heart grafts, and dual treatment increased survival from 0% to 80%, with a significant decrease of myocardial injury and improved cardiac function (58). This provides tantalizing data that may conceivably translate into a human therapy, as is implied by various reviewers, for example, Chen et al. (59) and Immenschuh and Schroder (60).

Angiogenesis. A link between HO-1 and angiogenesis is relatively recent. Transfection of rabbit cells with the human HO-1 gene resulted in a 2-fold increase in blood vessel formation (61); other investigators used a transfection model to show increased blood flow and, crucially, a relationship with the angiogenic stimulant vascular endothelial growth factor (VEGF) (62,63). Jozkowicz et al. (64) showed that CO could drive VEGF expression, and Bussolati et al. (65) linked inflammation with angiogenesis by proposing a dual action of HO-1 in an anti-inflammatory action and in the promotion of VEGF-driven angiogenesis. The role of HO-1 in angiogenesis has been recently reviewed (66,67).

Thus, interest in HO-1 in cardiology may be justified by aspects such as inflammation, antioxidant functions, apoptosis, hypoxia, and ischemia/reperfusion injury, and angiogenesis (Fig. 1).

Table 2	**Studies Reporting the Association Between HO-1 and CAD in Humans**			
Author (Ref. #)	Year	n	Disease	Key Findings
Chen et al. (80)	2005	135	AMI, UAP, SAP	HO-1 expression in patients with CAD significantly higher than in patients without CAD.
Gulesserian et al. (76)	2005	199	CAD	Low HO-1 level inducibility, may represent an independent prognostic marker for restenosis after angioplasty.
Kaneda et al. (75)	2002	577	CAD	Patients with shorter GT (<25 repeats) less likely to have CAD than patients with long GT (>29 repeats).
Li et al. (79)	2006	110	CAD	Significantly higher HO-1 protein leukocyte expression in patients with CAD than in patients without CAD.

AMI = acute myocardial infarction; CAD = coronary artery disease; GT = glutathione thymidine dinucleotide; HO = hemoxygenase; SAP = stable angina pectoris; UAP = unstable angina pectoris.

Table 3	Studies Reporting the Association of HO-1 With PVDs and DM			
Author (Ref. #)	Year	n	Disease	Key Findings
Chen et al. (74)	2002	474	DM	Greater expression of long GT repeats (≥32), thus might have higher oxidative stress and increased risk for CAD.
Schillinger et al. (70)	2002	271	CAD, AAA, PAD	Significant differences of HO-1 expression among 3 groups of patients: group with AAA had lower risk than other groups; thus up-regulation of HO-1 may be a protective anti-inflammatory factor against development of AAA.
Schillinger et al. (69)	2004	381	PVD	Significant short (<25 GT) repeats in HO-1 gene expression confer a reduced risk for restenosis after balloon angioplasty.
Dick et al. (73)	2005	472	PVD	Significant short (<25 GT) repeats in HO-1 gene expression confer a reduced risk for MI.

AAA = abdominal aortic aneurysms; DM = diabetes mellitus; GT = glutathione thymidine dinucleotide; MI = myocardial infarction; PAD = peripheral artery disease; PVD = peripheral vascular disease; other abbreviations as in Table 2.

Clinical Aspects of HO-1 in CVDs

The evidence for the protective role of HO-1 in clinical CVD is not only supported by experimental findings in cell culture and animal models (as discussed) but also by clinical studies in humans (Tables 2 and 3).

Genetics of HO-1. Exner et al. (68) reported that the number of glutathione thymidine dinucleotide (GT) repeats in the promoter region of the HO-1 gene modulates the level of gene transcription. The presence/absence of short/long GT repeats had a bearing on 6-month restenosis after femoropopliteal balloon dilatation, possibly associated with differences in levels of inflammatory marker C-reactive protein (69). They also reported that this polymorphism may be important in abdominal aortic aneurysm and renal (but not cardiac) allografting (70–72). A 21-month follow-up of 472 patients with peripheral artery disease indicated that the HO-1 genotype is potentially protective against adverse coronary events (73). Others (74,75) have also looked at this polymorphism in CAD and/or diabetes mellitus, speculating that diabetic persons carrying longer (GT) repeats might have higher oxidative stress and increased susceptibility to the development of CAD (i.e., the patients with fewer GT repeats were less likely to have CAD). The long (>29 repeats) polymorphic allele of the HO-1 gene promoter, which leads to low HO-1 inducibility, may be an independent prognostic marker for restenosis after percutaneous coronary intervention and stent implantation (76). Another polymorphism [the T(−413)A (AA/TA+TT) variant] of the HO-1 gene is associated with an increased incidence of hypertension in women (77). Other polymorphisms and a microsatellite marker seem to have no significant role on outcome of kidney transplantation (78).

CAD. Considerable animal data justify interest in HO-1 in human CAD, where the clear implication is that increased activity of the HO-1 gene (and therefore its products) is beneficial. Expression of the HO-1 protein was assessed in monocytes and lymphocytes from patients with acute myocardial infarction, patients with unstable angina pectoris, and patients with stable angina pectoris (79,80). There were significant differences of HO-1 expression—highest for the group with acute myocardial infarction, followed by the group with unstable angina pectoris, and finally by the group with stable angina pectoris. Within the patients with angiographically-defined CAD, HO-1 was highest in those with a greater disease burden. One interpretation of these data is that higher HO-1 expression is a consequence of the disease process and so may be a defense (self-limiting) mechanism. Morsi et al. (81) provided insightful data by obtaining endothelial cells from patients with advanced or early lesions and from coronary arteries free of disease. The HO-1 expression and (crucially) its biological activity (in terms of bilirubin release per mg of protein) were only present in cells from advanced atherosclerotic lesions. Interestingly, there also is a strong correlation between HO-1 and VEGF, although one hesitates before leaping to the conclusion that this raised VEGF may have been driven by HO-1 and/or its products.

Diabetes mellitus. In humans, the (GT)n HO-1 gene promoter polymorphism may influence clinical outcome, a putative mechanism being resistance/susceptibility to oxidative stress (74–76). De Silva et al. (82), examining retinal pigment epithelium, found significantly decreased levels of HO-1 messenger ribonucleic acid (mRNA), namely, 340 to 450 HO-1 mRNA copies/ng of total ribonucleic acid, in tissue from diabetic patients as compared with 425 to 8,000 HO-1 mRNA copies/ng of total ribonucleic acid in retinal pigment epithelium from normal donors and 460 to 7,605 copies/ng in hypertensive donor eyes. Increased monocyte HO-1 gene expression in diabetic patients falls upon metabolic improvement, possibly related to oxidative stress, although others found lower HO-1 skeletal muscle cell expression (83,84). Leukocyte HO-1 gene expression is significantly lower in patients with and without diabetic microangiopathy compared with control subjects, correlates negatively with a marker of oxidative stress, glycosylated hemoglobin, and diabetes duration, and normalization of blood glucose results in a reduction in HO-1 antigen in the cytoplasm of mononuclear leukocytes (85,86). Arredondo et al. (87) assessed the length of (GT)n repeats in the HO-1 gene promoter and also HO-1 enzymatic activity in mononuclear cells from diabetic patients. Although patients had significantly greater iron stores and HO activity than did control subjects, with a positive association between serum iron and HO activity in the diabetic patients, allelic frequency did not differ significantly between diabetic patients and control subjects.

Cerebrovascular disease. Beschorner et al. (88) demonstrated increased accumulation of HO-1+ microglia/macrophages at hemorrhagic lesions as early as 6 h after traumatic brain injury trauma that was still pronounced after 6 months. In contrast, after focal cerebral infarctions, HO-1+ microglia/macrophages accumulated within focal hemorrhages only and were absent in nonhemorrhagic regions. They speculated that prolonged expression of HO-1 in glial cells in human brains after traumatic brain injury and cerebral infarction helps in the recovery of neuronal tissue after these insults. Morgan et al. (89) assessed the role of GT repeats in the HO-1 promotor in 69 patients with cerebral aneurysms and 230 age-matched control subjects, and found that patients were more likely to have more than 36 repeats than were control subjects. The authors speculate that facilitated up-regulation of HO-1 may be protective against the development of intracranial aneurysms. They wisely point out, however, that because of the relatively small sample size and modest statistical significance, the data must be interpreted with caution and the association needs to be confirmed in large studies. For example, follow-up of 472 patients with advanced peripheral artery disease found that the status of the short/long GT genotype failed to identify 40 patients who had a cerebrovascular event but did instead associate with 48 patients who had a coronary event (73).

A Role for HO-1 in CVD?

Naturally, a caveat for the association(s) between greater activity of the HO-1 gene, its enzyme product, and the products of the enzyme relate to "cause or effect" phenomenon. It could be argued that raised levels and activity simply reflect more serious disease and an attempt by the body to limit the disease. Although data point to the likelihood that the activity of HO-1 leads to an active protection against the disease process, that may be one speculation too far. Perhaps the disease process effectively swamps the ability of HO-1 to limit cell damage that could lead to clinical disease (Tables 2 and 3). Possible mechanisms by which HO-1 contributes to pathogenesis are summarized in Figure 1.

Unsurprisingly, the combination of tissue protective and smooth muscle relaxing properties makes HO-1 an interesting objective for the drug treatment of CVD (4). Immenschuh and Ramadori (90) speculated that the therapeutic approaches intended at moderately increasing HO-1 expression in tissue might be beneficial in a number of disease states that probably relate to vascular disorders. Leaving aside the potential of gene therapy as being distant from the clinic (91), some current pharmacological agents act to induce HO-1. Some statins seem able to increase endothelial HO-1 mRNA levels in a concentration- and time-dependent fashion, although, whereas atorvastatin enhances the expression of endothelial nitric oxide synthase, HO-1 is not significantly affected (92–94). However, other

studies show that simvastatin activates HO-1 in vascular smooth muscle cells in vitro and in vivo (95). Aspirin (30 to 300 μM) increased human umbilical vein endothelial cell HO-1 protein levels in a concentration-dependent fashion up to 5-fold over basal levels (96), and more recent evidence points to a possible role for a peroxisome proliferators-activated receptors system (97).

Conclusions

Several of the numerous pathophysiological processes in atherosclerosis are, in theory, amenable to the action of 2 of the products of HO-1 (i.e., CO and bilirubin), whereas the third (i.e., iron) may be toxic. However, iron may be sequestered by ferritin, and the vast weight of published reports focuses on possible benefits of CO and bilirubin. For example, oxidative injury (such as to low-density lipoprotein cholesterol), which is thought to be a common feature of many pathophysiological processes, may be attenuated by HO-1. Thus, CO and bilirubin may play an important beneficial role in conditions such as hypertension, acute renal injury, and lung injury (17,37), and may well operate through the up-regulation of HO-1 in endothelial cells by various stimuli (such as hypoxia).

In addition, HO-1 is induced by some of the well-established cardiovascular risk factors, and appears to have a protective role in the vascular wall against atherogenesis through several pathways. However, in contrast to the implication of intracellular and pericellular activity of HO-1, little is known about plasma levels of this enzyme and, in particular, whether raised or lowered levels are present in CVD, and if such levels correlate with bilirubin and other plasma biomarkers. For example, if there is raised plasma HO-1 in CVD, is this indicative of potential or present protection (possibly driven by the pathology) or would it simply reflect leakage from damaged cells?

A number of therapeutic agents that are able to manipulate HO-1 gene expression have been recognized, suggesting that manipulation of the HO-1 gene might be a new avenue in the prevention and/or treatment of CVD (5,90). However, the more direct gene therapy approach, proven in animals, remains an intriguing opportunity to treat cardiovascular (and other) diseases. Nevertheless, whether these preliminary but promising reports come to fruition in the clinical setting is unknown, and a continuing weakness in the study of HO-1 is the lack of good clinical data. One example is a fascinating case report of a 6-year-old boy with severe HO-1 deficiency (98). Consistent with the cell biology and animal models described above (Fig. 1), he exhibited hematuria, proteinuria, a microcytic hemolytic anemia, increased iron-binding capacity, ferritin, and iron deposition alongside raised von Willebrand factor (marking endothelial cell damage). Crucially, serum bilirubin was constantly low whereas serum heme was extremely high. Undoubtedly,

more attention to HO-1 biology may provide novel insights into the pathophysiology of CVD.

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Key Words: cardiovascular disease ∎ hemoxygenase-1 ∎ heme ∎ bilirubin ∎ carbon monoxide.