Review Article
Heme oxygenase-1 in inflammation and cardiovascular disease

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Abstract: Cardiovascular disease accounts for 1 of every 2.9 deaths in the United States, thus the burden of the disease remains high. Given the high mortality and escalating healthcare cost for the disease, it is of urgent need to treat cardiovascular disease effectively. Heme oxygenase-1 (HO-1) catalyzes the oxidation of heme to generate carbon monoxide, biliverdin, and iron. These reaction products of HO-1 have potent anti-inflammatory and anti-oxidative functions. Although HO-1 is expressed at low levels in most tissues under normal basal conditions, it is highly inducible in response to various pathophysiological stresses. Numerous studies have indicated that HO-1 induction is an adaptive defense mechanism to protect cells and tissues against injury in many disease settings. This review highlights the role of HO-1 in inflammation and several cardiovascular diseases—atherosclerosis, myocardial infarction, graft survival after heart transplantation, and abdominal aortic aneurysm. Given that inflammation and oxidative stress are associated with development of cardiovascular disease and that HO-1 has anti-inflammatory and anti-oxidative properties, HO-1 is emerging as a great potential therapeutic target for treating cardiovascular disease.

Keywords: Heme oxygenase-1 (HO-1), inflammation, cardiovascular disease

Introduction

According to the 2011 update on heart disease and stroke statistics from American Heart Association, the 2007 overall death rate from cardiovascular disease was 251.2 per 100,000. Cardiovascular disease accounts for 33.6% of all 2,243,712 deaths in 2007, or 1 of every 2.9 deaths in the United States [1]. It is clear that the burden of cardiovascular disease remains high. Given the high mortality and the escalating healthcare cost, it is of urgent need to treat cardiovascular disease effectively. Numerous studies have shown that the development of cardiovascular disease is associated with inflammation and oxidative stress. Therefore, targeting inflammation and oxidative stress may be of great potential for treating cardiovascular disease.

Heme oxygenase (HO), the rate-limiting enzyme in heme degradation, catalyzes the oxidation of heme to generate several biologically active molecules—carbon monoxide (CO), biliverdin, and ferrous ion [2]. The endogenously produced CO can serve as a second messenger affecting several cellular functions, including inflammation, proliferation, and apoptosis [3, 4]. Biliverdin is subsequently reduced to bilirubin, both of which have antioxidant properties. Ferrous iron induces ferritin expression, which is important for iron sequestration. There are three isoforms in the HO family—HO-1, HO-2, and HO-3. They are products of different genes and are different in their regulation. HO-1 is normally expressed at low levels in most tissues/organs except for spleen; however, it is highly inducible in response to a variety of stimuli to protect cells against oxidative and inflammatory injury [4]. HO-2 is constitutively expressed in most tissues. HO-3 has similar protein structure to HO-2 but with lower enzymatic activity and is less well characterized. Through its induction in response to various pathophysiological stresses and the anti-inflammatory and anti-oxidative functions, numerous studies have indicated the therapeu-

tic potential of HO-1 in the prevention and treatment of cardiovascular disease.

The first genetic deficiency of HO-1 in human was reported in a young boy in 1999 [5] and a second case was reported recently in a young girl [6]. Both patients died in a young age, 6 and 15 years old, respectively [6, 7]. Both cases showed severe inflammatory phenotypes including elevated expression of inflammatory markers such as C-reactive protein, ferritin, and von Willebrand factor. They also had coagulopathy, nephritis, chronic inflammation, and increased susceptibility to atherosclerosis. The human HO-1 deficiency reveals a critical immunomodulatory role of HO-1 and highlights the essential function of HO-1 in human health and disease. This article reviews the roles of HO-1 in inflammation and cardiovascular disease such as atherosclerosis, myocardial infarction, graft survival after heart transplantation, and abdominal aortic aneurysm.

Inflammation and atherosclerosis

Coronary heart disease (CHD) remains a major health issue in the United States and developing countries. The CHD has a prevalence of 7.0 % in adults 20 years of age and it causes 1 of every 6 deaths with the mortality of 406,351 in the United States in 2007 [1]. CHD is a result of manifestations of atherosclerosis. Atherosclerotic lesions in coronary arteries can lead to blockage of blood flow and subsequent ischemia of the heart, which then results in myocardial infarction. The culprit for the initiation of atherosclerosis is thought to be circulating low-density lipoprotein (LDL). LDL particles can accumulate in the intima, become oxidized, and cause endothelial dysfunction. Monocytes and T cells are then attracted to these sites, adhere, and then migrate into the artery wall, forming the earliest atherosclerotic lesion—fatty streak [8]. As such, fatty streak is a pure inflammatory lesion with only monocyte-derived macrophages and T lymphocytes [9]. Further activation of immune cells in the early lesion propagates and amplifies the inflammatory response, attracting more immune cells and inducing medial smooth muscle cell (SMC) migration and proliferation into the lesion, leading to formation of advanced, complicated atherosclerotic lesions. In addition, the inflammatory mediators can cause apoptosis and necrosis of cells within the lesion, resulting in formation of necrotic core and a

potential for plaque rupture [8, 10]. It is clear from all the evidences that inflammation plays a key role in the pathogenesis of atherosclerosis, including initiation and progression, and thus atherosclerosis is recognized as a chronic inflammatory disease [8, 10-12]. Strategies targeting inflammation may be of therapeutic benefits in the prevention and treatment of atherosclerosis and cardiovascular disease.

HO-1 in inflammation and atherosclerosis

The role of HO-1 in inflammation was revealed in a report showing that upregulation of HO-1 results in suppression of immune effector functions [13]. Furthermore, HO-1 induction by cobalt protoporphyrin decreases the lymphoproliferative alloresponse and differentiation of cytotoxic T cells [13]. Given the anti-inflammatory function of HO-1, it may have a role in atherosclerosis. Interestingly, HO-1 expression is detected in atherosclerotic lesions, supporting a role for HO-1 in atherogenesis [14]. Indeed, HO-1 overexpression by pharmacological inducers or viral gene transfer inhibits atherogenesis in hypercholesterolemic animal models [15-17]. On the other hand, genetic ablation of both apoE and HO-1 in mice accelerates the development of atherosclerosis and exacerbates lesion formation (**Figure 1**), demonstrating unequivocally an essential role of HO-1 in protecting against atherogenesis [18]. The role of HO-1 in inflammatory cells and atherosclerosis is further emphasized in a study showing that HO-1 expression in macrophages increases antioxidant protection and decreases inflammatory components of atherosclerotic lesions [19]. Decreased and absent HO-1 expression in macrophages correlates with increased proinflammatory cytokines expression, such as monocyte chemotactic protein 1 and IL-6, scavenger receptor A expression, and foam cell formation [19]. It is particularly of interest that the anti-atherogenic effects of a number of mediators, including statins are mediated through HO-1 induction [20, 21]. Additionally, treatment of animals with Ginkgo biloba extract, known for its anti-atherogenic and vascular protective effects, enhances HO-1 expression in circulating monocytes and reduces leukocyte adherence to injured arteries [22]. Collectively, these studies establish that HO-1 is critical in anti-inflammation and protecting against vascular diseases that result from the manifestations of inflammation, such as atherosclerosis.
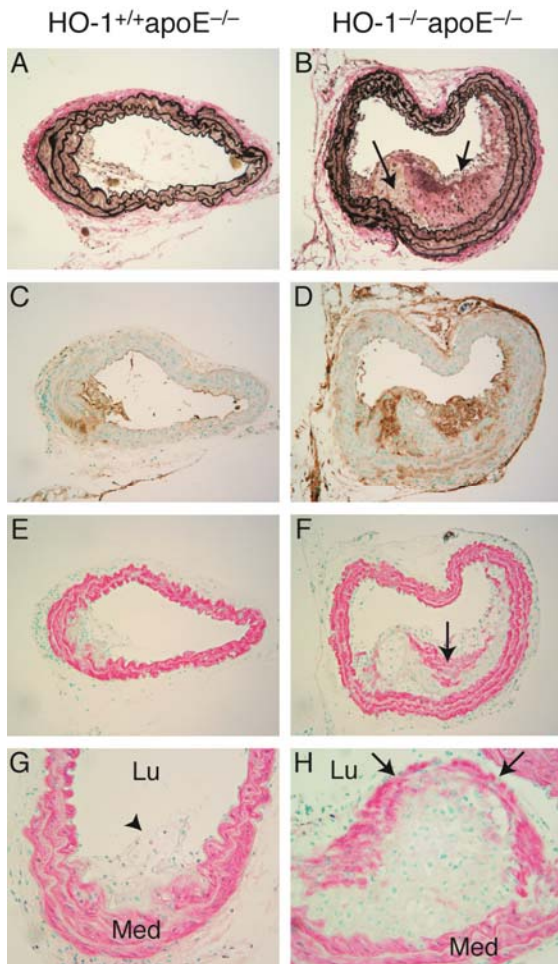
Figure 1. Increased atherosclerotic lesion formation in HO-1$^{-/-}$apoE$^{-/-}$ mice. Representative histological analysis of HO-1$^{+/+}$apoE$^{-/-}$ and HO‑1$^{-/-}$apoE$^{-/-}$ vessel sections after 8 weeks on Western diet. A and B, Verhoeff's staining for elastin (black) was performed on sections from the proximal brachiocephalic arteries from HO‑1$^{+/+}$apoE$^{-/-}$ (A) and HO‑1$^{-/-}$apoE$^{-/-}$ (B) mice. C and D, MOMA-2 staining for macrophages (brown) in proximal brachiocephalic arteries (x100) from HO-1$^{+/+}$apoE$^{-/-}$ (C) and HO‑1$^{-/-}$apoE$^{-/-}$ (D) mice. E through H, SM α-actin–stained (red) vessels from HO-1$^{+/+}$apoE$^{-/-}$ (E and G) and HO‑1$^{-/-}$apoE$^{-/-}$ (F and H) mice. In B, arrows indicate foam cells. In F, arrow indicates smooth muscle cells in the lesion. In G, arrowhead marks a small noncomplex lesion. In H, arrows mark the fibrous cap. Lu, lumen; Med, medial smooth muscle layers. Original magnification x100 (A through F), and x200 (G and H). Reproduced from Yet et al. [18] with permission.

HO-1 in myocardial infarction

A role for HO-1 in cardiac homeostasis was first

implicated in a study showing that HO-1 expression in the heart is increased in response to hyperthermia [23]. A follow-up study showed that ischemia/reperfusion substantially enhances HO-1 expression in the porcine heart, suggesting a potential role of HO-1 in the defense against pathophysiological stress [24]. In a genetic loss-of-function approach using HO-1 null (HO-1$^{-/-}$) mice, we demonstrate that in contrast to wild type mice, hypoxia induces severe right ventricular dilatation and infarction in HO-1$^{-/-}$ mice [25]. In addition, an absence of HO-1 exacerbates ischemia/reperfusion-induced myocardial damage [26]. Gain-of-function experiments using cardiac-specific HO-1 transgenic mice reveal that HO-1 overexpression reduces myocardial infarct size and inflammatory cell infiltration following ischemia/reperfusion injury (**Figure 2**), further demonstrating the protective role of HO-1 against myocardial infarction [27]. Studies using these cardiac-specific HO-1 transgenic mice in a heart failure model show that HO-1 overexpression significantly improved postinfarction survival and alleviated postinfarction pathological left ventricular remodeling [28]. In addition, HO-1 overexpression promotes neovascularization and ameliorates apoptosis in the heart failure model [28]. In light of the cardioprotective function of HO-1 and to avoid unwanted side effects of constitutively overexpressing HO-1, Tang et al. designed a hypoxia-regulated HO-1 gene therapy system, which can sense myocardial ischemia and turn on exogenous HO-1 expression [29, 30]. This vigilant plasmid-mediated HO-1 gene transfer improves contractile and diastolic performance after myocardial infarction [29, 30], indicating the therapeutic potential of HO‑1.

Many studies have demonstrated the promising role of adult bone marrow-derived stem cells in regenerating damaged myocardium [31-33]. However, the efficacy of cell therapy is limited by poor viability of graft cells, which might be due in part to ischemia and inflammatory response in the ischemic heart [34]. Interestingly, a hypoxia-regulated HO-1 vector modification of mesenchymal stem cells (MSCs) improves the survival of engrafted MSCs by protecting cells from apoptosis after implantation [35]. The ischemic hearts treated with HO-1-modified MSCs have reduced proinflammatory cytokine production, less inflammatory cell infiltration, and more importantly, have a better cardiac function [35]. In another gene transfer study,
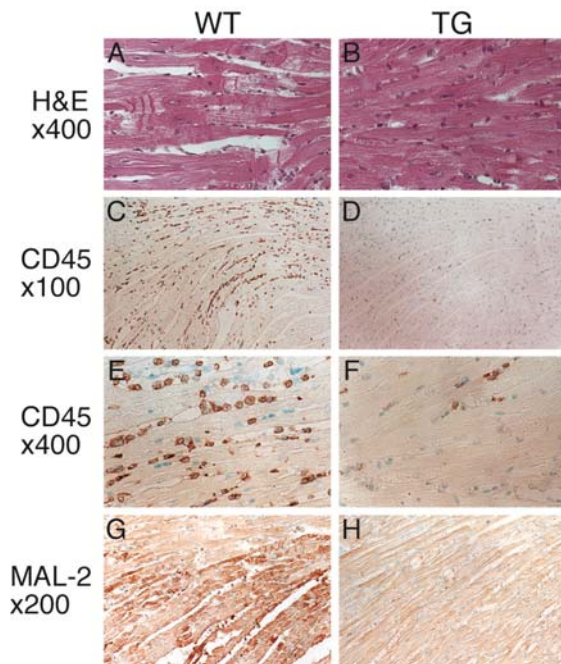
Figure 2. Reduced cardiomyocyte injury, inflammatory cell infiltration, and oxidative damage in transgenic mouse hearts after ischemia and reperfusion. Representative histological analysis of wild-type (WT, n = 4) and TG.H transgenic (TG, n = 4) heart sections after 1 h ischemia and 24 h reperfusion. A, Hematoxylin and eosin (H&E) stained left ventricles from the infarcted area of WT mice. B, H&E stained left ventricles from similar ischemic areas of TG mice. CD45 stained left ventricles from WT (C and E) and TG (D and F) mice. CD45 positive cells stained brown. MAL-2 immunostaining (brown) of the left ventricles from WT (G) and TG (H) mice. Original magnification: x400 (A and B), x100 (C and D), x400 (E and F), x200 (G and H). Reproduced from Yet et al. [27] with permission.

injection of adenoviral-HO-1-transduced MSCs into rat hearts 1 h after myocardial infarction reduces infarct size and significantly improves cardiac performance of HO-1-MSCs-treated hearts [36]. Furthermore, HO-1-MSCs-treated hearts have decreased proinflammatory cytokine but increased anti-inflammatory cytokine IL-10 expression [36]. Interestingly, HO-1-MSC increases TIMP2/3 expression and decreases MMP2/9 expression and thus normalizes the ratio of MMPs/TIMPs in transplanted hearts, suggesting modulating MMPs/TIMPs system might be another mechanism contributing to cardioprotective effect of HO-1 [37]. In a rat infarction model, transplantation of HO-1-overexpressing-MSCs results in increased resis-

tance to cell apoptosis and death and increased capillary density [38]. Taken together, combining stem cell therapy with HO-1 overexpression might provide a new therapeutic strategy for heart disease.

HO-1 in heart transplantation

Heart transplantation is the most effective therapy for end-stage heart failure. However, patients need lifelong immunosuppression remedy to prevent acute and chronic rejection by immune system for allograft survival and function. Current immunosuppressive drug treatments are effective for acute but not chronic rejection and are associated with significant side effects including renal toxicity, dyslipidemia, and diabetes [39]. Since HO-1 and its byproducts mediate many cytoprotective effects such as anti-inflammation, antioxidant, and anti-apoptosis, HO-1 may play a role in promoting graft survival after transplantation.

Induction of HO-1 has been shown to improve allograft/isograft survival for liver [40], thyroid [41], and kidney [42]. In a mouse cardiac allograft model, induction of HO-1 protects grafts against development of transplant arteriosclerosis, characteristic of chronic rejection [43]. In a mouse-to-rat cardiac xenograft model, the combination therapy of cobra venom factor (CVF) and cyclosporin A (CyA) in recipients effectively prolongs long-term graft survival [44]. Interestingly, this phenomenon is accompanied with HO-1 induction in endothelial cells, SMCs, and cardiac myocytes [45]. The importance of HO-1 in xenograft survival is further demonstrated in a genetic loss-of-function approach by using HO-1$^{-/-}$ mice. When HO-1$^{+/+}$ or HO-1$^{-/-}$ hearts are transplanted into rats treated with CVF plus CyA, HO-1$^{+/+}$ hearts survived long-term (more than 60 days) in rats whereas all HO-1$^{-/-}$ hearts are rejected ~3-4 days after transplant [45]. The rejected HO-1$^{-/-}$ hearts exhibit severe thrombosis in major coronary arteries, myocardial infarction, and activated host leukocyte infiltration [45]. On the other hand, hemin treatment induces HO-1 expression and increases the survival time of discordant cardiac xenograft by decreasing interstitial edema, lymphocyte infiltration, myocardial cell apoptosis, and thrombus formation in small vessels, and deposition of xenoantibodies IgM in the intima of vessels in transplanted heart [46]. These results indicate that HO-1 protects xenografts from injury and

thus preventing rejection. To explore the therapeutic potential of HO-1 overexpression by gene transfer, recombinant adeno-associated virus (rAAV) was used to increase HO-1 expression in heart grafts in a rat heart transplantation model [47]. rAAV-mediated HO-1 expression not only increases long-term graft survival but also reduces graft atherosclerosis. Furthermore, rAAV/ HO-1 treatment results in fewer infiltrating T cells and macrophages and lower expressions of macrophage migration inhibitory factor, TNF-α, and TGF-β_1 [47].

A study with 18 heart transplant patients shows an inverse correlation between HO-1 expression and the degree of cardiomyocyte apoptosis in biopsies from patients [48], suggesting HO-1 protects the graft. Supporting these findings, endomyocardial biopsies of transplanted hearts have higher HO-1 expression in the first two months [49], suggesting induction of HO-1 may protect transplanted hearts from injury [49]. In humans, a (GT)n dinucleotide repeat in the HO-1 gene promoter shows a length polymorphism that modulates the level of gene transcription [50]. Short (<25 GT) repeats are associated with an increased HO-1 upregulation in response to inflammatory stimuli than are longer repeats [51]. This ability to upregulate HO-1 serves as an endogenous protective mechanism in many human cardiovascular diseases [52]. Thus, one would predict that heart transplants with a short (GT)n HO-1 gene promoter polymorphism have a better outcome. However, a study with 152 heart allograft recipients with at least one-year survival reveals that HO-1 gene promoter polymorphism does not show an association with the development of cardiac allograft vasculopathy in heart transplants [53]. Another study also indicates no influence of HO-1 gene promoter polymorphism on the development of heart failure, graft survival, acute rejection, or transplant atherosclerosis [54]. Interestingly, Ohmann et al. [55] recently found that HO-1 polymorphisms with higher expression of HO-1 correlate with a reduced risk of late post-transplantation infection in pediatric heart. Although it is clear that HO-1 protects heart graft in animal models, the role of HO-1 in human heart transplants is somewhat controversial and thus warrants further study.

HO-1 in abdominal aortic aneurysm

Abdominal aortic aneurysm (AAA) is a relatively common and often fatal condition that primarily affects older patients [56, 57]. It is a leading cause of sudden death in men older than 55 years [58]. AAA is a localized dilatation of the abdominal aorta exceeding the normal diameter (~2 cm) by more than 50% [59], which is characterized by chronic aortic wall inflammation, loss of medial SMCs, and connective tissue degradation and remodeling. AAA is associated with old age, male gender, cigarette smoking, atherosclerosis, hypertension, and a genetic disposition [60, 61]. Screening studies in Europe show that ~5% of men 65 years of age have AAAs of 3 cm or more [62]. In the United States, the prevalence of AAAs 2.9 to 4.9 cm in diameter ranges from 1.3% in men 45 to 54 years of age to 12.5% in men 75 to 84 years of age [1]. With an aging population, the incidence and prevalence of AAA is certain to go up. Although the pathogenesis of AAA is not completely elucidated yet, it is well accepted that inflammation and oxidative stress are key factors inducing AAA formation. Inflammation-mediated proteolysis and disorganized extracellular remodeling within the aortic wall are critical pathophysiological events leading to progressive aortic enlargement and ultimate rupture [63]. In experimental animal models of AAA, genetic and pharmacological inhibition of reactive oxygen species (ROS) production and MMPs suppress aneurysm formation [64-66]. Thus, reducing ROS generation and inflammation and inhibiting MMP activity might be useful therapeutic strategies.

Interestingly, patients with AAA are less frequently carriers of short (<25 GT) repeat in the HO-1 gene promoter than healthy subjects. This suggests that short alleles, and thus, increased upregulation of HO-1, may be a protective anti-inflammatory factor against the development of AAA [67]. In animal models, flow loading significantly increases HO-1 expression and attenuates AAA formation [64]. Concomitant with increased HO-1 expression, ROS production is significantly reduced in flow-loaded AAAs, suggesting that flow loading and HO-1 induction may attenuate AAA enlargement via wall shear or strain-related reductions in oxidative stress [64]. Further supporting the notion that HO-1 may affect development and progression of AAA, HO-1 is shown to reduce levels of MMPs and inflammation. For example, HO-1 has been suggested to reduce MMP9 levels in human carotid enarterectomy tissues with intraplaque

hemorrhage [68] and in human atheromatous plaque [69]. The effects of HO-1 on MMPs may influence the stability of AAAs by attenuating elastin degradation and thereby preventing AAA rupture. The medial SMC apoptotic death contributes to the reduction of cellularity and subsequent impairment for the repair and maintenance of the arterial extracellular matrix in AAAs [70]. HO-1 also has anti-apoptotic activity [71], thus, HO-1 may participate in decreasing SMC apoptosis. Taken together, with the anti-oxidative, anti-inflammatory, and anti-apoptotic activities of HO-1, HO‑1 has a great potential to prevent/attenuate the development/progression of AAA. The role of HO-1 in the pathogenesis of AAA certainly warrants further investigation.

Therapeutic opportunities

As described above, through its enzymatic products HO-1 mediates many cellular functions in protecting cells and tissues against inflammation and oxidative stress. Numerous studies have indicated the protective function of HO-1 in many cardiovascular diseases. In light of the socioeconomic burden of cardiovascular disease, the therapeutic potential of HO-1 is of particular interest. Thus, induction of HO-1 pharmacologically or by other means might be a promising therapeutic intervention for preventing and treating these inflammatory cardiovascular diseases.

Acknowledgments

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