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Role Of Hemoglobin/Heme Scavenger Protein Hemopexin In Atherosclerosis And Inflammatory Diseases

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Abstract

Purpose of the review—Hemoglobin (Hb) and its scavenger proteins haptoglobin (Hp) and hemopexin (Hx) associate with HDL and influence the inflammatory properties of HDL. Moreover, HDL from Hx-null mice is proinflammatory. In addition, Hx deficiency is implicated in a number of other inflammatory diseases such as septic shock and experimental autoimmune encephalomyelitis. This review highlights studies that demonstrate novel insights into the physiological protective role of hemopexin in inflammatory diseases.

Recent findings—Recent studies demonstrate that Hx-dependent uptake of extracellular heme leads to the de-activation of Bach1 repression leading to the transcriptional activation of anti-oxidant HO-1 gene. Levels of circulating Hx have been implicated in the prognosis for patients with septic shock. In addition, Hx therapy has been shown to be beneficial in cardiovascular disease, cerebral ischemic injury and experimental autoimmune encephalomyelitis.

Summary—These studies suggest that heme scavenging is a major mechanism by which Hx defends against oxidative stress and related inflammatory disorders. Hx therapy may provide a novel protective role against heme and oxidative stress mediated inflammatory conditions including atherosclerosis.

Keywords

Heme; Oxidative stress; Inflammation; Hemopexin therapy

INTRODUCTION

When red blood cells are damaged within the vascular compartment, hemoglobin (Hb) escapes into the plasma, dimerizes, and is rapidly bound by Hp. Scavenging of Hb by Hp is critical for preventing Hb mediated oxidative damage. Haptoglobin (Hp) is an acute phase,

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Conflicts of interest:

None

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plasma-born glycoprotein that is produced mainly by hepatocytes, but also by other cell types including adipocytes and lung cells, and is well known for its ability to tightly bind free Hb following its release from erythrocytes (4). Hp plasma concentrations are high, ranging from 0.3 mg/mL to 3.0 mg/mL, yielding an Hp/Hb molar ratio of 400:1 and allowing effective Hb scavenging. This activity of Hp is important for the prevention of iron loss via Hb filtration by the glomeruli and renal damage. Following its formation, Hb/Hp complex is taken up by various scavenger receptors in the liver and other tissues. One such receptor is the CD163 receptor, present on macrophages and Kupffer cells. The CD163 receptor strongly binds the Hb/Hp complex and facilitates its endocytosis and degradation (5). Since haptoglobins are not recycled, severe hemolytic diseases result in rapid haptoglobin depletion and rapid accumulation of free heme. Hemopexin (Hx) is an acute phase glycoprotein that is able to bind an equimolar amount of heme with high affinity (6). Many types of cells synthesize Hx including hepatic parenchymal cells, ganglionic and photoreceptor cells of the retina, kidney mesangial cells, and cells in the central and peripheral nervous system (7–9). In adults, the serum Hx concentration ranges from 0.40 to 1.5 mg/mL, and the level of Hx increases in response to high concentrations of free heme. Hx scavenges free heme, and the resultant heme–Hx complex is taken up by the liver through receptor-mediated endocytosis (10). CD91/LRP1 is the scavenger receptor identified for scavenging the heme-Hx complex from circulation to prevent heme mediated tissue damage (10).

Watanabe *et al.* (2) reported that Hb is associated with HDL in mouse models of atherosclerosis. Watanabe *et al.* (2) demonstrated that Hb when associated with HDL is predominantly in the oxyHb form and can effectively consume nitric oxide and contract arterial vessels *ex vivo* (2). In a subsequent study, Watanabe et al. (3) reported that Hb scavenger proteins, Hp and Hx are significantly increased in the apoA-I-containing particles of HDL both in mouse models of atherosclerosis and in coronary heart disease (CHD) patients, when compared with their wild type counterparts, respectively. Atherosclerosis is the leading cause of morbidity and mortality in Western society. Studies in both mice and humans suggest that the anti- or proinflammatory nature of HDL may be a more sensitive predictor of the risk for CHD events than HDL-cholesterol levels alone. Watanabe *et al.* (3) further demonstrated that the association of Hb, Hp, and Hx proteins with HDL positively correlates with inflammatory properties of HDL and systemic inflammation in CHD patients. Watanabe *et al.* (3) showed that HDL from $Hp^{-/-}$ mice under atherogenic conditions does not accumulate Hb and is anti-inflammatory whereas HDL from $Hx^{-/-}$ mice was pro-inflammatory, indicating a novel protective role of Hx in the inflammatory properties of HDL in atherogenic mice. These studies suggested for the first time that i) the Hb/Hx/Hp pathway, in part, modulates the inflammatory properties of HDL in both mice and humans and ii) the dissociation of Hb/Hp complex from apoA-I containing particles of HDL may be a novel target for the treatment of CHD and other chronic inflammatory diseases.

The role of Hx and Hp in atherosclerosis has not been directly evaluated to date. In this review, we will discuss some recent publications that underscore the importance of Hx therapy in several inflammatory conditions including atherosclerosis.

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Hx is required for heme-dependent activation of heme oxygenase 1

During intravascular or extravascular hemolysis, Hx binds and transports cytotoxic free heme, to hepatocytes and macrophages where it can be efficiently catabolized. The heme that is transported from the blood stream to the liver and macrophages by Hx is catabolized by anti-inflammatory gene, heme oxygenase 1 (HO-1) (11, 12). Heme has been shown to induce the expression of HO-1 by inactivating the transcription repressor Bach1 through direct binding (13). However, the source of heme for the regulation of the Bach1-HO-1 axis had been unclear. Since extracellular heme exists as a complex with Hx in serum under the physiological conditions, Hada *et al.* (13) examined the effects of (recombinant) rHx-bound heme on HO-1 expression and Bach1 in Hepa-1c1c7 liver cells as well as THP-1 macrophage cells. Hada *et al.* demonstrated that rHx-bound heme was internalized into the cells via endocytosis, resulting in HO-1 expression and inactivation of Bach1, suggesting that i) induction of HO-1 expression is Hx-dependent and ii) Hx plays an antiatherogenic role. It should be noted that Hx has also been shown to exert antiatherogenc activity in a non-HO-1 dependent mechanism (14). Liang X, et al. (14) reported that Hx limits TLR4 and TLR2 agonist-induced macrophage cytokine production directly through a mechanism distinct from HO-1 that does not involve binding and clearing of free heme (14).

Hx levels predict Hemolytic diseases and Septic shock

Heme is an essential molecule for living aerobic organisms and is involved in a remarkable array of diverse biological processes and disease conditions. Hemolytic diseases such as sickle cell disease and B-thalassemia are characterized by enhanced intravascular hemolysis resulting in heme-catalyzed reactive oxygen species (ROS) generation, which leads to endothelial dysfunction and oxidative damage (15). In addition, low serum Hx levels are related to sepsis severity and can indicate poor prognosis for septic shock patients (16). Jung JY et al. (16) measured the concentrations of Hx in a rat model featuring distinct grades of severity of endotoxemia and in patients who survived and did not survive septic shock. Jung JY et al. (16) showed that the levels of Hx are substantially lower during severe endotoxemia than during mild endotoxemia in the rat model. In addition, serum Hx levels are dissimilar in patients under septic conditions of distinct severity and, the initial serum Hx concentration in non-survivors is significantly lower than in survivors. Jung JY et al. (16) concluded that serum Hx concentration can help predict the 28-day mortality among septic shock patients, and suggested that a diminished level of Hx might be associated with poor prognosis in severe sepsis validating previous reports by Larsen R, et al. (17). Moreover, previous studies also reported that Hx can potently suppress the synergistic effect exerted by heme and hemoglobin on sterile and septic inflammatory stimuli such as HMGB1 and LPS (18–20).

Hx administration protects cerebral ischemic injury

Free heme is released from methemoglobin following cerebral ischemia-reperfusion and represents a toxic component in the peripheral blood. Hx, due to its high binding affinity to heme and an efficient scavenger of heme in overloaded peripheral blood was suggested to be neuroprotective. Studies by Li RC et al. (21) reported that Hx-null mice sustain greater infarct volumes and behavioral deficits than their wild-type counterparts in a model of transient middle cerebral artery occlusion, indicating its neuroprotective role against stroke-

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related damage (21). However, it has not been clear how Hx exerted its neuropotective role until a recent study by Dong B, et al. (22) who investigated the expression of Hx in the normal rat brain by immunofluorescent staining. Dong B, et al. (22) showed that Hx is mainly expressed in the neurons and a small portion of astrocytes after a short-term middle cerebral artery occlusion followed by 24-hour reperfusion in the rat brain. Dong B, et al. (22) further demonstrated that administration of exogenous Hx at the onset of reperfusion dose-dependently reduced the infarct volumes and improved the outcome of cerebral ischemia-reperfusion injury. These studies (22) confirmed the neuroprotective effects of Hx and provide new insights into the potential therapeutic role of Hx in cerebral ischemia.

Hx administration improves cardiovascular function

Circulating ferrous heme (on Hb) is considered as one of the important pathogenic factors in cardiovascular disease (CVD). Increased circulating ferrous heme containing Hb i) scavenges nitric oxide (NO), a potent vasodilator (23) and ii) promotes the generation of ROS, which leads to endothelial activation (24), and iii) induces smooth muscle proliferation (25), contributing further to cardiovascular pathology (26). Using a model of heme overload in Hx-null mice, Vinchi *et al.* (15) demonstrated that when excess heme is bound to Hx, ROS production and adhesion molecule expression are reduced and NO availability is increased. Using B-thalassemia and sickle cell disease mouse models of hemolytic diseases Vinchi *et al.* (15) discovered that Hx therapy preserves cardiovascular function and normalization of blood pressure by promoting heme recovery and hepatic detoxification of heme mainly through the induction of HO-1 activity. The authors suggested that Hx treatment is a promising novel therapy to protect against heme-induced cardiovascular dysfunction in hemolytic diseases (15).

Hx and heme-iron recycling

Hemopexin limits iron access to microorganisms mediating heme-iron recycling. Fiorito *et al.* (27) recently demonstrated increased iron levels in the duodenum of Hx-null mice due to increased iron uptake by enterocytes and storage in ferritins. The study reported that Hx deficiency resulted in enhanced heme catabolism in the duodenum by inducing HO-1 activity. The transfer of iron from the enterocytes to the liver was unaffected indicative of abnormal levels of iron deposits in the duodenal mucosa. In addition, they showed that absence of Hx did not affect the expression of iron transporters in the duodenum cells despite increased iron accumulation. This provides new insights to the understanding of body iron homeostasis and possibly at identifying strategies to increase/reduce iron absorption in the therapy of metabolic disorders of iron deficiency and overload respectively (27).

Hx in autoimmune disorders

Hx levels increases and remains high in experimental autoimmune encephalomyelitis (EAE) mice, the mouse model of multiple sclerosis. EAE induced in Hx knockout mice, developed EAE clinically earlier and exacerbated compared with mice having Hx due to a large amount of CD4+-infiltrating T cells. Hx-null mice developed sever EAE due to a greater number of infiltrating and circulating Th17 cells. Hx therapy in Hx-null mice before EAE induction reduced the Th17 expansion, as well as disease severity, and was comparable with those of

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wild-type mice. Taken together, these data indicate that Hx has a negative regulatory effect in Th17-mediated inflammation and propose hemopexin's therapy to limit the expansion of this cell subset in inflammatory and autoimmune diseases (28).

CONCLUSION

Hx is implicated in a number of inflammatory diseases. Heme scavenging appears to be the primary mechanism by which Hx protects against oxidative stress and related inflammatory disorders. Hx has been shown to modulate the expression of the anti-inflammatory protein HO-1 *via* mechanisms that include the removal and/or inactivation of transcription suppressors. Many inflammatory conditions including cerebral ischemic function, septic shock, and cardiovascular function are exacerbated in Hx deficiency. Recent studies in animal models underscore the importance of Hx therapy against heme and oxidative stress mediated inflammatory conditions. Future studies should be directed to test Hx therapy in humans.

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KEY POINTS

1. Hb/Hx/Hp pathway, in part, modulates the inflammatory properties of HDL in both atherosclerotic mice and humans
2. HDL from Hx-null mice is proinflammatory indicating a novel protective role of Hx in the inflammatory properties of HDL.
3. Hx has been shown to modulate the expression of the anti-oxidant enzyme HO-1.
4. Acute or chronic inflammatory conditions such as cerebral ischemia, septic shock, and cardiovascular disease are aggravated in the absence of Hx.
5. Hx therapy against heme mediated oxidative stress can improve inflammatory conditions.