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CLINICAL REVIEW

Prevention

Haeme oxygenase signalling pathway: implications for cardiovascular disease

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Evidence now points to the haeme oxygenase (HO) pathway as a possible actor in modulating risk for cardiovascular disease (CVD). In particular, the HO pathway may represent a key endogenous modulator of oxidative, inflammatory, and cytotoxic stress while also exhibiting vasoregulatory properties. In this review, we summarize the accumulating experimental and emerging clinical data indicating how activity of the HO pathway and its products may play a role in mechanisms underlying the development of CVD. We also identify gaps in the literature to date and suggest future directions for investigation. Because HO pathway activity can be influenced not only by genetic traits and environmental stimuli but also by a variety of existing pharmacologic interventions, the pathway could serve as a prime target for reducing the overall burden of CVD. Further work is needed to determine the role of HO pathway products as possible prognostic markers of risk for clinical CVD events and the extent to which therapeutic augmentation or inhibition of HO pathway activity could serve to modify CVD risk.

Keywords Heme oxygenase pathway • Carbon monoxide • Bilirubin • Biliverdin • Cardiovascular disease

Introduction

Considerable evidence now points to the haeme oxygenase (HO) pathway as a possible central actor in modulating risk for cardiovascular disease (CVD). The ameliorative properties of the HO pathway were first shown in an animal model of haeme protein-induced kidney injury,[1] with subsequent work demonstrating that HO induction protects endothelial cells (ECs) *in vitro*.[2] Intriguing early data in humans included the autopsy report of hyperlipidaemia, fatty streaks, and fibrous plaques in the aorta of an HO-1 deficient 6-year-old boy.[3] Supporting the concept that intact HO pathway activity is upregulated in response to vascular stress, another study found that HO-1 expression in adults with atherosclerosis was higher in association with worse lesion type and grade of stenosis.[4] Research to date now suggests that the HO pathway may represent one of the most important endogenous modulators of oxidative, inflammatory, and cytotoxic stress while also exhibiting vasoregulatory properties. Herein, we review the accumulating experimental and emerging clinical data indicating how activity of the HO pathway and its products may play a key role in mechanisms underlying the development of CVD.

Haeme metabolism and the haeme oxygenase pathway

Haeme forms the prosthetic moiety within haemoproteins [i.e. haemoglobin, myoglobin, cytochrome *c*, cytochrome P450, catalase, myeloperoxidase, nitric oxide synthase (NOS), and guanylate cyclase] and is involved in numerous biological processes including oxygen transport, cellular respiration, oxidative biotransformations, host defence, and regulation of vascular tone. While haeme is essential for life, free haeme within cells (i.e. cytosolic 'uncommitted' haeme that is not a part of haeme proteins) can be pro-inflammatory and cytotoxic, particularly in ECs,[5] via generation of reactive oxygen species (ROS) and lipid peroxidation. Efficient degradation of excess haeme is needed to avert such toxicity and, thus, intracellular levels of free haeme are tightly regulated by the HO family of proteins.[6]

The HO proteins catalyse the oxidative degradation of haeme, producing equimolar amounts of carbon monoxide (CO), iron (Fe^{2+}), and biliverdin-IXα (*Figure 1*). Biliverdin-IXα is converted to bilirubin-IXα by cytosolic biliverdin reductase, and bilirubin-IXα is a potent endogenous antioxidant[7] with recently recognized

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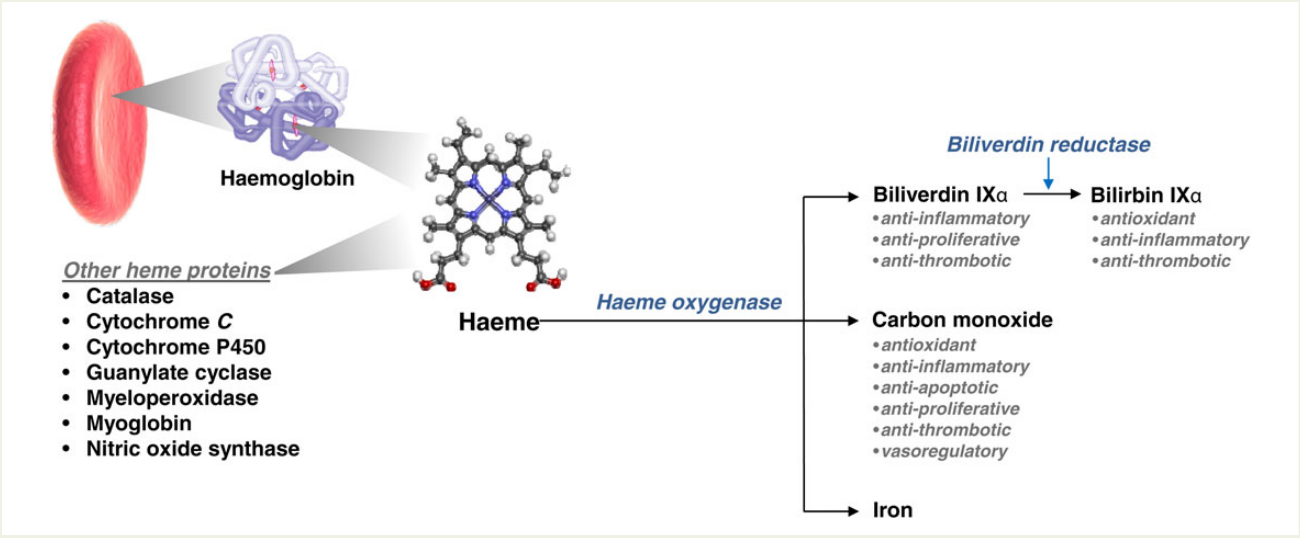

Figure 1 Overview of haeme metabolism and the central role of haeme-oxygenase activity.

anti-inflammatory properties.[8] Iron induces expression of ferritin, which sequesters iron and also exerts antioxidant[2] and anti-apoptotic[9] effects. Of the three direct products of haeme metabolism, however, CO has been most extensively studied. Carbon monoxide is a diatomic gas with numerous biological functions including protection against oxidative injury,[10–12] inflammation,[13] and cell death.[12,14,15] Furthermore, CO has been shown to inhibit cellular proliferation,[16] suppress matrix production,[17] and increase fibrinolysis.[18] Notably, CO shares many similarities with nitric oxide (NO), such as the ability to inhibit smooth muscle cell (SMC) proliferation[19] and platelet aggregation,[20] as well as modulate vascular tone by increasing cyclic guanosine monophosphate (cGMP) levels.[6,21]

Regulation of haeme oxygenase expression

The HO-1 and HO-2 isoforms are encoded by the *HMOX1* and *HMOX2* genes, respectively. HO-2 is constitutively expressed in multiple tissues, including the vasculature, but its expression is not generally inducible.[22,23] HO-1 is also ubiquitous and expressed most strongly in tissues involved in haemoglobin metabolism. Importantly, in other tissues such as the vascular endothelium and SMC, HO-1 is expressed at low levels basally, but is induced in response to diverse stimuli (*Figure 2*) such as haeme, endotoxin, ROS, NO, cytokines, growth factors, hypoxia, and hyperoxia.[23] In particular, HO-1 expression in the vasculature is upregulated in response to oxidized lipids and phospholipids,[24] vascular injury,[25] and laminar flow.[26]

Although regulation of HO-1 expression is predominantly at the transcriptional level, extracellular stimuli activate kinase signalling cascades that regulate transcription factor binding to the HO-1 promoter. All three mitogen-activated protein kinase (MAPK) pathways (i.e. extracellular signal-regulated kinases 1/2, c-Jun-N-terminal kinase, and p38 MAPK) have been implicated in regulating HO-1 expression.[23] In vascular EC, the selective COX-2 inhibitor, celecoxib, has been shown to induce HO-1 expression via PI3K activation and

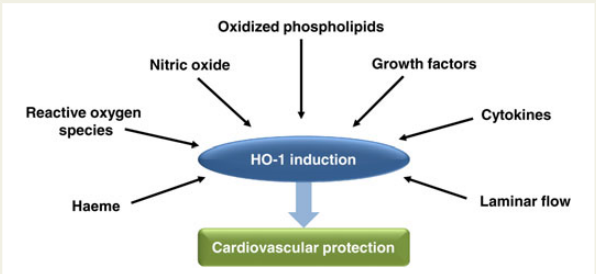

Figure 2 Several stimuli have been shown to induce HO-1 activity and, in turn, upregulated HO-1 expression may provide cardiovascular protection.

translocation of nuclear factor erythroid 2-related factor 2 (Nrf2).[27] Additionally, HO-1 is induced in EC by TNF-α and IL-1α in a PKC-dependent fashion via activation of arachidonic acid.[28]

One of the main regulators of HO-1 transcription is Nrf2, an oxidant responsive transcription factor. Nuclear factor erythroid 2-related factor 2 transactivates the HO-1 promoter, while the haeme-binding protein Bach1 represses HO-1 transcription.[29] Both Nrf2 and Bach1 have been shown to play key roles in cardiometabolic disease by regulating HO-1 expression.[30–37] Defective Nrf2 signalling has been implicated in the pathophysiology of diabetes[35,38,39] and coronary artery disease (CAD).[37,40,41] Additionally, Nrf2 has been shown to protect against glucose-induced apoptosis in cardiomyocytes.[36] Deficiency of Bach1 is also protective in animal models of atherosclerosis,[31] myocardial ischaemia-reperfusion injury,[34] and vascular injury (*Figure 3*).[30]

Haeme oxygenase gene expression in humans

Variation in the HO-1 gene has been related to cardiovascular risk in humans. The most extensively studied HO-1 gene variant in humans

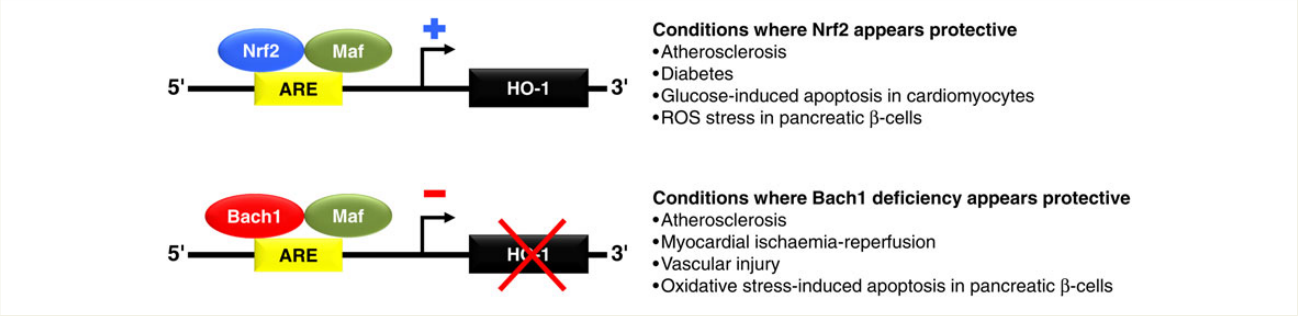

Figure 3 Certain transcription factors, Nrf2 and Bach1, appear to play an important role in regulating HO-1 expression in cardiovascular conditions. Specifically, Nrf2 and Bach1 form heterodimers with Maf proteins and bind to consensus antioxidant response element (ARE) sequences in the HO-1 promoter. Nrf2 transactivates the HO-1 promoter and may provide protection against diabetes and cardiovascular disease. Bach-1 competes with Nrf2/Maf dimers and represses HO-1 transcription. Accordingly, deficiency of Bach1 has been shown to be protective in animal models of atherosclerosis, myocardial ischaemia-reperfusion injury, and vascular injury.

is the dinucleotide repeat polymorphism, [GT]*n*.[6,42] This variant is the most frequent dinucleotide repeat scattered throughout human and animal genomes, and many repeat regions are length polymorphic. With respect to the HO-1 promoter region, length of the (GT) repeat region in the HO-1 promoter has been related inversely to HO-1 expression.[43] Importantly, this finding appears associated with CVD risk in humans. In the presence of pre-existing risk factors such as hypertension, metabolic syndrome, and diabetes, a larger number of (GT) repeats is generally related to increased risk of CVD (*Table 1*). Conversely, under similar circumstances, a smaller number of (GT) repeats has generally been related to less CVD (*Table 1*). Interestingly, these promoter polymorphisms have not been associated with CVD risk in the general population, supporting the concept that oxidative and metabolic stress may be required to induce HO-1 expression.

Although not as thoroughly investigated as the [GT]*n* polymorphism, there is at least one SNP in the proximal HO-1 promoter, T(-413)A, that has been associated with susceptibility to CVD.[6,44,45] The AA genotype of the T(-413)A polymorphism has been correlated with a lower incidence of CAD,[44,45] although the recent meta-analysis called this association into question given inconsistencies in the Hardy–Weinberg equilibrium in some studies.[46]

Haeme oxygenase and mechanisms related to cardiovascular disease

Multiple experimental studies have demonstrated a role for HO-1 and its products in the setting of hypertension, diabetes, vascular injury, atherosclerosis, and ischaemia reperfusion.[25,30,47–65] There are numerous mechanisms by which HO-1 activity may impact cardiovascular risk including a variety of antioxidant, anti-inflammatory, anti-apoptotic, anti-proliferative, anti-thrombotic, and vasoregulatory effects.

Antioxidant protection

HO-1 has well-described antioxidant cytoprotective effects in many cell types and disease models.[6,10–12] In atherosclerotic lesions, HO-1 is upregulated in EC and SMC with particularly high expression in macrophages and foam cells, where oxidized phospholipids co-localize with HO-1.[58] Inhibition of HO-1 enhances atherosclerosis and increases plasma lipid hydroperoxide levels in LDL-receptor knockout mice, suggesting that HO-1 may protect against lipid peroxidation in atherosclerosis.[58] In turn, HO-1 overexpression in cardiomyocytes appears to protect against reperfusion injury as well as attenuate cardiac inflammation and oxidative damage to cardiomyocytes.[63] Additionally, HO-1 expression correlates with plaque destabilizing factors such as matrix metalloproteinase-9, and overexpression of HO-1 has been shown to prevent progression of atherosclerotic lesions to vulnerable plaques.[66]

Products of the HO pathway can also exert antioxidant properties. Carbon monoxide can bind to haeme proteins (e.g. NADPH oxidase and cytochrome *c* oxidase) to inhibit electron transport and ROS generation. Conversely, CO can also bind and inhibit the catalytic function of other haeme proteins, which may lead to pro-oxidant effects under certain conditions.[67] Bilirubin is a potent ROS scavenger that can prevent oxidation of LDL and other lipids.[68,69] In addition, bilirubin can decrease ROS in EC,[70] protect against oxidative damage in ventricular myocytes,[71] and reduce infarct size and mitochondrial damage following myocardial ischaemia reperfusion.[61] In cerebrovascular EC, both CO and bilirubin appear to attenuate TNF-α-induced apoptosis and inhibit superoxide anion production.[72]

Interestingly, HO-1 and CO may also crosstalk with NOS enzymes and modulate levels of NO in the vasculature. Although NO-induced HO-1 expression is cytoprotective in EC,[73] excess NO can react with ROS to generate peroxynitrite that can promote lipid peroxidation and cell death within the vasculature.[74] In turn, HO-1 and CO can bind to the haeme moiety of NOS and may down-regulate NOS expression to reduce NO production[75] in the vasculature, which may be protective in certain circumstances. Accordingly, in a rabbit model of atherosclerosis, induction of HO-1 inhibited progression of atherosclerosis and was associated with reduced expression of inducible NOS and NO production, while inhibition of HO-1 had opposite effects.[76]

Anti-inflammatory activity

HO-1 is a well-recognized modulator of inflammation. Complete absence of HO-1 in mice results in a chronic multi-systemic

Table 1 Studies relating haeme oxygenase pathway activity to clinical phenotypes and outcomes in humans

Phenotype	No. of studies	No. of subjects per study	Study sampling characteristics	Main findings
HO-1 promoter polymorphism				
Hypertension	5	152–1998	Community sample[148,150]; Hypertension vs. controls[149]; MetS patients vs. controls;[151] Arsenic-exposed individuals[152]	• AA SNP is associated with increased risk of hypertension in women[148] and in individuals with MetS[151] • Shorter GT repeats/greater HO-1 expression is associated with lower diastolic blood pressure,[151] lower systolic blood pressure and less hypertension[152] • HO-1 promoter SNPs associated with hypertension[149] • Longer GT repeats/lower HO-1 expression is associated with lower risk of essential hypertension[150]
Metabolic syndrome	2	152–468	MetS and controls;[153] T2DM, MetS, and controls[154]	• No difference in promoter length or allelic frequency between MetS and controls,[153] or between T2DM, MetS, and controls[154] but S/M genotype was increased in T2DM and MetS patients compared with controls[154]
Diabetes	4	189–3089	T2DM and controls,[155,156] T2DM (no controls),[157] T2DM, MetS, and controls[154]	• L allele/longer GT repeats/lower HO-1 expression is associated with increased odds of T2DM[155] • S allele/shorter GT repeats/greater HO-1 expression is increased in T2DM and MetS patients compared with controls[154] • Allelic frequencies did not differ between T2DM and control groups,[156,157] and T2DM, MetS, and control groups[154]
Cardiovascular disease	16	70–4596	CAD vs. controls,[43,44,160,162,164] CAD,[159,161,163] T2DM[158] post-ischaemic stroke vs. controls,[165,166] haemodialysis patients vs. controls,[167] peripheral arterial disease,[168] chronic stable angina[169] and arsenic-exposed individuals[170,171]	• Longer GT repeats/lower HO-1 expression is associated with increased CAD,[43,158] worse coronary scores,[161] increased CVD events and all-cause mortality,[167] and increased odds of stroke[165] • Shorter GT repeats/greater HO-1 expression is associated with more favorable lipid profiles,[162] lower severity of CAD,[163] lower CAD risk under high oxidative stress,[164] decreased stroke risk in the absence of hyperlipidemia,[166] lower adjusted hazard ratio for coronary events,[168] less carotid atherosclerosis,[170] and reduced cardiovascular mortality[171] • AA SNP is associated with less ischaemic heart disease[158] • No relationships observed between AA SNP or GT repeats and CAD[160]
Restenosis after intervention	7	96–1357	Coronary stenting,[172–174,178] balloon angioplasty or stenting[175–177]	• Long allele carriers had increased risk of restenosis[172,173] and adverse cardiac events[172] • Short allele carriers had decreased risk of restenosis[175,177] • No difference in restenosis,[174,178] but short allele carriers had lower IL-6[178]
Indirect Measures of HO-1 Activity				
Metabolic syndrome	8	1423–12 342	Community sample,[95,145,179–182] adult women,[94] children and adolescents[183]	• Higher exhaled CO more likely to develop MetS[145] • Serum total bilirubin inversely related to prevalence of hyperinsulinaemia, and systemic inflammation,[95] MetS and insulin resistance,[94,95,179,180,182,183] but not in multivariable models[181]
Diabetes	5	417–5960	T2DM patients vs. controls,[132] T2DM patients,[184] community sample[186] children and adolescents[183]	• Plasma HO-1 concentration increased in T2DM cases compared with controls[132] • Total serum bilirubin inversely related to HbA1c levels in T2DM patients,[184] prevalence of MetS and insulin resistance in adults,[179] and insulin resistance in children and adolescents[183] • Higher serum bilirubin associated with increased risk of developing T2DM[186]
Cardiovascular disease	10	53–130 052	T2DM vs. controls,[4] community sample,[144,145,187,188,190] statin-treated cohort,[189] men,[191,192] overweight/obese high cardiovascular risk patients[193]	• HO-1 expression increased with increasing stenotic grade, and was higher in diabetic subjects[4] • Higher exhaled CO associated with incident CVD[145] and with developing overt CVD in the presence of subclinical CVD[144] • Higher blood COHb levels associated with higher incidence of cardiac events and deaths[191] • Higher serum bilirubin associated with lower Framingham risk score,[187] and lower risk of MI, CAD, and CVD events in men, less clearly in women[188] • Lower serum bilirubin associated with increased risk of CAD,[190,192] all CVD events, MI, and all-cause mortality,[189] and stroke[189,190] • Bilirubin was not a risk factor independent from traditional cardiovascular risk factors[193]

CAD, coronary artery disease; MI, myocardial infarction; CVD, cardiovascular disease; MetS, metabolic syndrome; T2DM, type 2 diabetes mellitus.

inflammatory disorder with evidence of vascular and perivascular involvement.[77] When exposed to lipopolysaccharide (LPS), HO-1 deficient (HO-1$^{-/-}$) mice have greater end-organ damage and reduced survival.[78] In turn, HO-1 induction by haemoglobin[79,80] as well as biliverdin[8] can attenuate lung inflammation, decrease pro-inflammatory cytokine expression, and improve survival following LPS exposure.

Notably, CO has been shown to mediate many of the anti-inflammatory effects of HO-1. Administration of CO to LPS-stimulated macrophages inhibits NF-κB activation and secretion of granulocyte macrophage-colony-stimulating factor.[81] Carbon monoxide has also been shown to decrease expression of TNF-α, IL-1β, and macrophage inflammatory protein-1β, while increasing expression of the anti-inflammatory cytokine IL-10 in macrophages and in mice.[13] Additionally, recent studies suggest that HO-1 may play a role in alternative activation of macrophages towards an M2 anti-inflammatory phenotype.[82,83]

HO-1 has been shown to exhibit anti-inflammatory effects in the vasculature, as well as globally. In EC, overexpression of HO-1 and bilirubin attenuate TNF-α-induced upregulation of VCAM-1 and E-selectin by inhibiting NF-κB activation.[84] Furthermore, overexpression of HO-1 or its products *in vivo* have led to protective anti-inflammatory as well as anti-proliferative effects in models of vascular injury, in-stent restenosis, and transplant arteriosclerosis.[85–87] Overexpression or induction of HO-1, as well as CO administration, have been shown to reduce leukocyte infiltration, pro-inflammatory cytokine expression, NF-κB activation, and apoptosis, in addition to attenuating intimal proliferation in rat aortic allografts and stented arteries.[85–87]

The anti-inflammatory effects of bilirubin and biliverdin may also protect against CVD risk.[88–95] Biliverdin decreases IL-6 secretion *in vitro* in both macrophages and LPS-stimulated EC.[8] In the vasculature, HO-1 induction down-regulates oxidant-induced leukocyte rolling and adhesion, and this finding appears mediated by bilirubin and biliverdin.[96] Bilirubin has also been shown to attenuate upregulation of E-selectin, VCAM-1, and ICAM-1, as well as to inhibit neutrophil adhesion in TNF-α-stimulated EC.[97]

Effects on apoptosis

Whereas oxidative stress and inflammation can lead to apoptosis within the vasculature, HO-1 and its products may counter this process. In EC, CO protects against apoptosis following TNF-α and anoxia-reoxygenation via activation of the p38 MAPK pathway.[14,98,99] In vascular smooth muscle cells (VSMCs), absence of HO-1 has been shown to increase susceptibility to oxidant stress and cell death in a vein graft stenosis model.[100] Paradoxically, overexpression of HO-1 and bilirubin in VSMC have also been shown to stimulate apoptosis.[101] Notably, CO did not have an effect on apoptosis in this study and, in a separate study, was shown to inhibit VSMC apoptosis via soluble guanylate cyclase (sGC) activation and suppression of p53 expression.[102] In addition, HO-1 induction by haemin decreases SMC apoptosis and prevents atherosclerotic plaque progression *in vivo*.[103] Furthermore, HO-1 overexpression in the myocardium decreases lipid peroxidation, IL-1β expression, pro-apoptotic signalling, and myocardial infarct size.[64] Taken together, HO-1 and the products of haeme metabolism may have differential effects on apoptosis depending on the cell type and mechanism of cellular injury, although most studies suggest that the HO-1-CO pathway confers anti-apoptotic properties in the setting of vascular injury.

Effects on cellular proliferation

HO-1 has potent anti-proliferative effects in the vasculature. HO-1 overexpression in a femoral artery injury model inhibited arterial remodelling by reducing VSMC proliferation and inducing expression of the cell cycle inhibitor p21[25]; in contrast, absence of HO-1 exaggerated cellular proliferation and enhanced vascular lesion formation.[25] Overexpression of HO-1 also decreased VSMC proliferation in models of transplant atherosclerosis and in-stent restenosis.[86,87] Carbon monoxide has been shown to mediate these protective effects of HO-1 on VSMC proliferation. In aortic transplant and carotid artery injury models, CO inhibited VSMC proliferation and attenuated intimal hyperplasia in injured vessels and aortic transplant allografts.[85,104] In addition to VSMC proliferation, migration of VSMC may contribute to intimal thickening following vascular injury, and the HO-1/CO pathway has recently been shown to attenuate VSMC migration.[105] Notably, overexpression of HO-1, CO gas, or treatment with a CO-releasing molecule (CORM) each decreased migration of VSMC via NOX1 inhibition.[105]

Although CO is best known for modulating the anti-proliferative effects of HO-1, emerging data suggest that biliverdin may have anti-proliferative properties as well.[88–90] Biliverdin has been shown to attenuate intimal hyperplasia and decreased EC apoptosis in vein grafting and balloon angioplasty models.[90] Biliverdin was also found to decrease SMC migration *in vitro*.[90] Additionally, hyperbilirubinaemic Gunn rats develop minimal intimal hyperplasia following balloon injury.[88] In mechanistic *in vitro* studies, bilirubin attenuates VSMC proliferation and arrests the cell cycle by inhibiting phosphorylation of the retinoblastoma tumour suppressor protein (Rb).[88]

HO-1 and CO may also play a role in regulating proliferation of EC and angiogenesis. HO-1 overexpression increased proliferation and capillary tube formation in coronary EC,[106] while inhibition of HO-1 inhibited VEGF-induced angiogenesis.[107] In addition, HO-1 deficient EC have been shown to have reduced angiogenesis that was rescued by CORM.[108] HO-1 has also been shown to influence the mobilization of endothelial progenitor cells (EPCs) following vascular injury.[109,110] Overexpression of HO-1 or CO inhalation accelerated re-endothelialization of denuded vessels and enhanced EPC mobilization after carotid artery injury.[109] In contrast, HO-1$^{-/-}$ animals generated fewer endothelial colony forming cells[110] and had reduced EPC mobilization and decreased re-endothelialization following vascular injury.[109] Thus, HO-1 may promote EC repair, yet inhibit proliferation and migration of VSMC, thereby preventing the development of intimal lesions at multiple cellular levels. Taken together, the beneficial effects of the HO-1/CO pathway may provide dual vascular protection to promote repair in the setting of vascular injury, further highlighting the central role of HO-1 in cardioprotection.

Anti-thrombotic activity

Induction of HO-1 enzymatic activity and CO have demonstrated beneficial effects on platelet aggregation and thrombus formation.[18,20,98,111–116] CO has well-described inhibitory effects on platelet aggregation via activation of sGC and increased platelet cGMP levels.[20] In addition, induction of HO-1 and bilirubin have been shown to delay thrombus formation, suggesting that bilirubin has anti-thrombotic properties as well.[111] Absence of HO-1 leads to

accelerated arterial thrombus formation and EC apoptosis following vascular injury that could be rescued by CO and biliverdin.[116] HO-1$^{-/-}$ mice also have increased mortality following aortic allograft transplantation due to graft thrombosis that was attenuated by CORM or adoptive transfer of wild-type platelets.[114] Similarly, HO-1 inhibition in rats led to graft rejection following heart transplantation with coronary artery thrombosis, leukocyte infiltration, and myocardial infarction which could be attenuated by CO.[113] HO-1$^{-/-}$ mice also have exaggerated venous thrombosis following inferior vena cava ligation, with increased expression of tissue factor, selectins, and pro-inflammatory signaling.[115] Furthermore, HO-1 gene transfer into injured carotid arteries of apolipoprotein E null mice leads to earlier thrombolysis, with reduced fibrin deposition and decreased expression of plasminogen activator inhibitor-1.[113]

Vasoregulation

Although HO-1 has been shown to modulate vascular tone in experimental studies, the physiologic significance of HO-1 on vascular reactivity *in vivo* remains unknown. In studies where induction of HO-1 decreased blood pressure in hypertensive animals, the vasodilatory effects have been attributed to CO.[117,118] Overexpression of HO-1 decreased vasoreactivity of pig arteries *ex vivo*, in a manner that appeared related to a cGMP-dependent mechanism independent of NO.[25] Additional studies have demonstrated that exogenously administered CO relaxes isolated aortas in an endothelium- and NO-independent fashion.[119,120] Endogenous CO release has also been shown to dilate blood vessels in the liver, skeletal muscle, and brain.[121–123] The mechanism by which CO mediates vasodilation has largely been attributed to sGC activation and increases in cGMP but, compared with NO, CO is a weak activator of sGC.[119,124] Additional mechanisms of CO-induced vasorelaxation include stimulation of calcium-activated potassium channels (BK_{Ca}) in VSMC,[125] as well as modulation of endothelial-derived vasoconstrictors.[126] Furthermore, in some vascular tissues under certain conditions, CO has been shown to have vasoconstrictive effects by inhibiting endothelial NO synthase (eNOS) expression and diminishing NO production.[127,128]

The HO pathway and cardiovascular risk factors

Extending from the experimental data focused on the mechanistic contributors to CVD, HO-1 has been shown to be upregulated in the setting of cardiovascular risk factors such as cigarette smoking,[129] hyperglycaemia,[130] and hypertension.[131] Although increased plasma and monocyte HO-1 levels have been observed in persons with type 2 diabetes,[132,133] the role of the HO-1–CO pathway in diabetes and metabolic disease is incompletely understood. Multiple experimental studies have demonstrated a protective role for HO-1 and its products in relation to insulin resistance and diabetes.[50–53,91,92,134–138] In rodent obesity models, HO-1 induction decreases weight gain, reduces adiposity, and improves insulin sensitivity and glucose tolerance.[134–138] Furthermore, HO-1 induction leads to increased levels of adiponectin and PPAR-γ in adipocytes, reduced adipocyte size,[138] and decreased adipogenesis in obese mice.[139] However, a recent study suggests that HO-1 activity is paradoxically a maladaptive contributor to obesity-related insulin resistance and diabetes.[140] These seemingly contradictory bodies of data could be related to a differential effect of HO-1 metabolism products or a dose-dependent effect of HO pathway activity.

Translating an old paradox into a new paradigm

The dual effects of the HO pathway, having been demonstrated in multiple settings, warrant special attention. Depending on the experimental conditions, HO-1 and its products have been observed to exert differential effects. For instance, HO-1 and CO exert antiproliferative effects in VSMCs but pro-proliferative effects in ECs.[106,107] Most studies suggest that HO-1 and its products confer anti-apoptotic properties in the face of vascular injury, but overexpression of HO-1 and bilirubin has also been shown to stimulate apoptosis in VSMC.[101] Similarly, whereas CO administration demonstrates vasodilatory effects in most studies, CO has also been shown to have vasoconstrictive effects under different experimental conditions.[127,128]

Just as experimental studies have demonstrated variable HO-1 and CO activity in the setting of different experimental conditions, clinical studies have also produced apparently conflicting results (*Table 1*). On the one hand, genetic polymorphisms leading to increased HO-1 expression have been associated with lower risk for hypertension, diabetes, and CVD in both referral and general population samples. On the other hand, indirect measures of HO-1 activity have been variably associated with increased risk for metabolic traits and CVD in selected and unselected community cohorts. There are several possible reasons for discrepant findings including differences in study design, potential confounders, and limitations of the various indirect measures of HO-1 activity used. It is also likely that while physiologic levels of HO-1 pathway activity are essential for health, measures of increased HO-1 activity reflect a compensatory—and, in some situations, an excessive—response to pathologic stress.

Overall, the apparent paradox of differential effects of HO-1 and its products in experimental models and the both very low and very high levels of HO-1 activity observed in association with adverse clinical outcomes may, in fact, reflect the central biological role of the HO-1 pathway in maintaining cellular and tissue homeostasis (*Figure 4*). This phenomenon has been demonstrated for well-established markers of cardiovascular stress, including conventional inflammatory markers (i.e. c-reactive protein, interleukins) and natriuretic peptides, for which genetic deficiencies predispose to adverse disease phenotypes even while excess circulating levels are also consistently associated with adverse clinical events.[141–143]

Future directions

Taken together, prior investigations of the HO pathway underscore its potential role in modulating risk for CVD and, in turn, to serve as a therapeutic target with wide ranging implications. To this end, there is more work to be done. For instance, the extent to which measures of HO-1 activity may serve as reliable prognostic markers of clinical cardiovascular risk has yet to be established. Most genetic studies of HO-1 variants have been performed in Asian cohorts and, thus, require validation in other populations. The largest studies of CO and clinical outcomes have relied predominantly on measures of

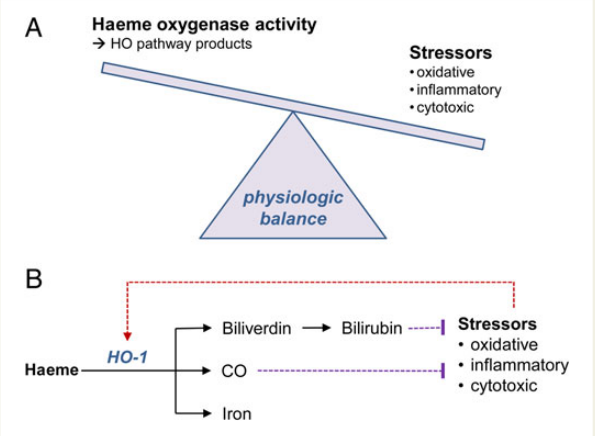

Figure 4 Schematic displaying the possible relationship between HO activity and cardiometabolic stressors, where maintenance of physiologic balance (*A*) involves HO pathway products countering stressors that activate HO activity (*B*).

CO in exhaled breath[144,145]; even though associations with cardiovascular and metabolic endpoints in these studies were significant after accounting for potential confounders (e.g. smoking status and lung disease), additional investigations using more direct measures of endogenous CO are needed. Measures of endogenous CO are preferred in part because circulating levels of the other HO pathway products, biliverdin and iron, are more prone to variation due to the activity of other metabolic pathways. In addition, gaseous or water-soluble tablet delivery of CO (i.e. in the form of CORM) has shown promise as agents for inducing HO-1 activity. Interestingly, HO-1 is also induced by many existing therapeutic agents including statins, rapamycin, paclitaxel, NO, aspirin, and probucol.[146,147] Of course, the extent to which certain pre-clinical or clinical disease states could benefit from induction of deficient HO-1 activity, as opposed to inhibition of excess HO-1 activity, remains unknown.

Overall, a large body of accumulating and emerging evidence highlights the need for more research of the HO pathway and its products, particularly endogenous CO, with respect to the development of CVD in humans. Ongoing investigations in the field promise to improve our understanding of how activity of the HO pathway may be harnessed to optimize human health and reduce the global burden of CVD. Accordingly, further discoveries regarding the therapeutic potential of interventions targeting the HO pathway appear to be on the horizon.

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