Haem oxygenase-1 and cardiovascular disease: mechanisms and therapeutic potential

Kim H. CHAN*†, Martin K. C. NG*† and Roland STOCKER‡
*Department of Cardiology, Royal Prince Alfred Hospital, Camperdown, NSW 2050, Australia, †The Heart Research Institute, Newtown, NSW 2042, Australia, and §School of Medical Sciences (Pathology) and Bosch Institute, University of Sydney, Sydney, NSW 2006, Australia

ABSTRACT
Cardiovascular disease remains the leading cause of death worldwide. Despite progress in management, there remain a significant number of patients who are not eligible for current treatment options. Traditionally, HO-1 (haem oxygenase-1), one of two isoenzymes that initiate haem catabolism, was thought to only play a metabolic role. However, HO-1 is now recognized to have additional protective activities in states of heightened noxious stimuli or stress such as acute coronary syndromes. The present review article provides an overview of the mode of action of HO-1 in vascular protection, with particular emphasis on its atheroprotective, anti-inflammatory and antioxidative properties, as well as its role in vascular repair. Furthermore, we present evidence for the protective effects of HO-1 in CVD (cardiovascular disease) in both animal and human studies. Given its potential in vascular protection and repair, strategies aimed at inducing HO-1 emerge as a novel and alternative therapeutic target in the management of CVD.

INTRODUCTION
HO (haem oxygenase) was first discovered in the late 1960s to catalyse the rate-limiting step of haem degradation into CO (carbon monoxide), Fe²⁺ (ferrous iron) and biliverdin [1]. Although known initially for its role in haem catabolism and erythrocyte turnover, it has become increasingly recognized that HO-1, the inducible isoenzyme of HO, plays an important role in vascular biology [2–4]. The protective biological activities conferred by HO-1 include its antioxidant, anti-inflammatory, anti-apoptotic and pro-angiogenic properties [5]. This cardioprotective role of HO-1 is confirmed further by experimental models involving HO-1-knockout animals, as well as the discovery of the only known case of human HO-1 deficiency [6,7]. In the present review, we will explore our current understanding of the protective mechanisms of HO-1 in vascular disease, provide an overview of the role of HO-1 in CVD (cardiovascular disease) in humans and present some emerging therapeutic options for HO-1 induction in treating CVD.

EVIDENCE FOR THE PROTECTIVE ROLE OF HO-1 AGAINST CVD
The relationship of HO with atherosclerotic vascular disease was suggested initially in 1994 by an observational study...
Evidence supporting the vascular protective role of HO-1 in humans

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study reporting that low serum concentrations of bilirubin (converted from biliverdin) were associated with an increased risk of CAD (coronary artery disease) [8]. As a result, over the last decade, the interest in HO has shifted greatly from a metabolic to a protective function of the enzyme in a variety of conditions associated with cellular stress and pathologies [4].

The vascular protective effects of HO-1 have been demonstrated in several animal models involving HO-1 modulation and HO-1-knockout mice, and in human studies on HO-1 expression and CVD outcomes (Table 1). In addition, the only known case of human HO-1 deficiency provides insight into the protective mechanism of HO-1 in CVD [6]. Furthermore, HO-1 polymorphism in humans, resulting in variation in HO-1 activity between individuals, has been shown to correlate with vascular disease and outcomes. Finally, elevated blood bilirubin concentrations commonly are associated with protection from CVD, although hepatic UDP-glucoronyltransferase 1–1 (which transforms bilirubin into a water-soluble excretable metabolite) rather than HO-1 is thought to be the main factor controlling serum bilirubin [9].

Protective role of HO-1 in animal models of CVD

There have been numerous in vivo models involving chemical modulation and gene transfer of HO-1, showing the protective properties of HO-1 in vascular disease. However, the use of chemical inducers and inhibitors (e.g. tin or zinc protoporphyrin) may have many effects beyond altering HO-1 [10]. Therefore HO-1 gene knockout or overexpression animals and the human HO-1 deficiency are valuable additional models in evaluating the importance of HO-1 in protecting against vascular disease. The HO-1-knockout mice demonstrate features of the human iron overload syndrome, including splenomegaly, tissue iron deposition, hepatomegaly and hepatic fibrosis, growth retardation and premature death [11]. However, HO-1-knockout mice do not demonstrate any visible phenotype suggestive of defective vascular morphogenesis [2]. Yet et al. [12] reported that mice deficient in both HO-1 and ApoE (apolipoprotein E) have increased atherosclerosis and vein graft stenosis compared with mice not deficient in HO-1, therefore suggesting a protective role of HO-1 in atherosclerosis. In addition, Deshane et al. [13] have demonstrated that HO-1-deficient ECs (endothelial cells) in vitro and HO-1-knockout mice in vivo show impaired angiogenesis in an SDF-1 (stromal cell-derived factor-1)-dependent pathway. In addition, it has been shown that, compared with their wild-type counterparts, HO-1-knockout mice have impaired neovascularization during healing in a cutaneous wound injury model, whereas skin-specific HO-1 overexpression in transgenic mice was associated with enhanced neovascularization during wound healing [13,14]. These in vivo findings suggest that HO-1 plays a critical role in angiogenesis, assuming that the differences observed in HO-1-knockout mice are merely due to an absence of HO-1.

Human HO-1 deficiency

The first and only known case of human HO-1 deficiency was described in 1999 by Yachie et al. [6]. They described a case of a 6-year-old boy with severe growth retardation characterized by marked intravascular haemolysis, hyperlipidaemia and endothelial injury. Aortic fatty streaks and fibrous plaques were present on autopsy, and marked endothelial injury was evident on electron microscopy of the renal glomeruli, revealing endothelial swelling, detachment and subendothelial deposition. The extensive endothelial injury may be a result of impaired protection from haem-induced oxidative cell injury. Bilirubin is a well known antioxidant [15], and its production was impaired in this patient. Bilirubin also attenuates the oxidation of LDL (low-density lipoprotein)-cholesterol [16]. Therefore the decreased bilirubin production, coupled with increased haem concentrations, may have promoted oxidative modification of LDL, leading to further oxidative vascular endothelial injury in this patient [17]. To summarize, the human case of HO-1 deficiency exhibited phenotypical changes that are similar to those of HO-1-knockout mice, although the degree of oxidative damage was much more severe.
**HO-1 polymorphism and expression in humans**

Although human HO-1 deficiency is exceedingly rare, it appears that the length of the GT repeat in the HO-1 promoter is a much more common determinant of the variation of HO-1 activity in humans. It is thought that shorter GT repeats have higher HO-1 transcriptional activity and expression compared with longer GT repeats. A protective role of HO-1 in CVD is supported by several human studies reporting that shorter GT repeats were associated with reduced risk of CAD and coronary events [18,19]. Longer GT repeats were also associated with a significantly higher risk of developing restenosis after coronary stenting [20]. However, other studies have failed to find an association between GT repeat length and CAD [21] or coronary restenosis [22].

These studies may be explained by the fact that the GT repeat promoter polymorphism significantly modulates a cytoprotective, pro-angiogenic and anti-inflammatory function of HO-1 in human ECs [23]. Cells carrying the short GT repeat had better survival under oxidative stress, enhanced VEGF (vascular endothelial growth factor)-induced proliferation and decreased production of pro-inflammatory mediators [23]. More recently, Cheng et al. [24] have reported that HO-1 expression closely correlates with plaque vulnerability in human carotid endarterectomy specimens and that it is associated with plaque-destabilizing factors such as MMP-9 (matrix metalloproteinase-9), IL (interleukin)-6 and IL-8. In the same study, pharmaceutical and genetic modulation of HO-1 expression in a murine model of vulnerable plaque determined lesion outcome by affecting plaque stability. Taken together, these studies therefore suggest that HO-1 may play a protective and therapeutic role against CVD. However, other studies have reported conflicting findings, where higher HO-1 protein expression in leucocytes was associated with more severe CAD [25]. A possible explanation for these apparently disparate findings is that HO-1 may be induced by the CAD and that the higher HO-1 expression is a consequence, rather than a cause, of the disease process.

**Relationship between bilirubin levels and vascular disease**

Bilirubin is a by-product of haem degradation initiated by HO-1. The well-known antioxidant properties of bilirubin [15] may confer the cardioprotective effects of HO-1. Several human studies reported a protective role of bilirubin against CAD [8,9]. More recently, Kimm et al. [26] also reported an inverse relationship between bilirubin level and the incidence of ischaemic stroke. These findings are supported by studies showing an inverse relationship between bilirubin concentrations and surrogate markers of atherosclerosis, including endothelial dysfunction (assessed by flow-mediated dilatation) and carotid intima-media thickness [27]. In addition, longer GT repeats in the HO-1 gene promoter were associated with lower bilirubin concentrations and a higher risk of CAD [28]. However, after adjusted analyses, the effect of the HO-1 promoter polymorphism on a risk of CAD was no longer present, suggesting that the effect of HO-1 promoter polymorphism might be conveyed through its influence on bilirubin [28].

**POTENTIAL MODE OF ACTION OF HO-1**

HO-1 exerts anti-atherogenic actions and promotes vascular repair, in part via its products CO and biliverdin/bilirubin.

**Protection from atherosclerosis**

The atherosclerotic plaque is characterized by a state of heightened inflammation, oxidative stress and endothelial dysfunction [29]. The increased expression of HO-1 in atherosclerotic lesions [30] suggested a possible role for HO-1 in vascular protection. The atheroprotective properties of HO-1 include its anti-inflammatory, antioxidant and vasodilatory actions, and its inhibitory actions on smooth muscle cell proliferation (Figure 1).

**Anti-inflammatory activities**

There is compelling evidence that HO-1 exerts an anti-inflammatory action and is important in regulating inflammation in vivo. Compared with wild-type mice, HO-1-knockout mice exhibit hallmarks of a progressive chronic inflammatory state as evidenced by the higher peripheral blood leucocyte count, splenomegaly (due to extramedullary haematopoeisis and follicular hyperplasia), high splenic and lymph node CD4+/CD8+ T-cell ratios with numerous activated CD4+ T-cells, hepatic vessel wall inflammatory cell infiltrates, and adherence of monocytes to the vessel wall [11]. More recently, Orozco et al. [31] found that, compared with wild-type mice, peritoneal macrophages from HO-1-knockout mice exhibit increased pro-inflammatory cytokines such as MCP-1 (monocyte chemoattractant protein-1) and IL-6. Similarly, and as mentioned above, the only known case of human HO-1 deficiency also exhibited hallmarks of a pro-inflammatory state, characterized by marked leucocytosis and lymphadenopathy [6]. Several studies have shown that HO-1 induction or administration of CO inhibits the production of LPS (lipopolysaccharide)-induced pro-inflammatory cytokines such as TNF (tumour necrosis factor)-α, IL-1 and MIP-1β (macrophage inflammatory protein-1β) [32,33], and increases LPS-induced expression of the anti-inflammatory cytokine IL-10 [32]. Furthermore, HO-1 attenuates endothelial dysfunction by decreasing pro-inflammatory cytokines...
HO-1 and its products exert anti-inflammatory, antioxidant and vasodilatory activities, and inhibit VSMC proliferation. These activities confer protection against atherosclerosis.

Figure 1  Atheroprotective properties of HO-1
HO-1 and its products exert anti-inflammatory, antioxidant and vasodilatory activities, and inhibit VSMC proliferation. These activities confer protection against atherosclerosis.

such as MCP-1 and M-CSF (macrophage colony-stimulating factor) [34]. HO-1 induction also attenuates the microvascular EC-leucocyte adhesion interaction, and this is possibly mediated through the action of bilirubin [35]. More recently, studies reported that HO-1 overexpression inhibits TNF-α-mediated E-selectin and VCAM-1 (vascular cell adhesion molecule-1) production [34,36]. These studies suggest that products of HO-1-catalysed haem degradation mediate the anti-inflammatory activities of HO-1.

Antioxidant activities
The antioxidant protective properties of HO-1 have been published extensively. Compared with wild-type mice, the liver from HO-1-knockout mice show higher levels of oxidized proteins and lipid peroxidation [11]. Furthermore, peritoneal macrophages from HO-1-knockout mice, compared with wild-type controls, exhibit increased ROS (reactive oxygen species) [31]. Likewise, cells from the human case of HO-1 deficiency showed increased sensitivity to haemin-induced oxidative injury [6].

The substrate of HO-1, haem, is highly cytotoxic and the endothelium may be at greatest risk of exposure to these cytotoxic effects [37]. Furthermore, haem promotes the oxidation of LDL, and oxidized LDL is cytotoxic to ECs [38] and has potential pro-atherogenic activities [39]. HO-1 therefore plays a homoeostatic role to counteract oxidative-induced cell injury, being up-regulated during stress and degrading haem to CO, Fe2⁺ and biliverdin/bilirubin. Addition of bilirubin to the culture medium was reported to markedly reduce the cytotoxicity produced by oxidants [40]. Similarly, haem pre-treatment also resulted in increased resistance against oxidative cell injury; however, this protective effect occurred only in cells that were actively producing bilirubin as a consequence of increased haem availability and utilization by HO-1 [40]. These observations suggest that product(s) of HO-1-mediated haem degradation are cytoprotective. CO generated by HO-1 has also been shown to possess anti-apoptotic properties, and it has been suggested that this gaseous molecule offers cytoprotection against oxidative stress [41]. However, there may be differential effects of CO depending on the concentration of CO administered, as higher doses can be pro-apoptotic [42]. It is also important to note that up-regulation of HO-1 is often associated with increased ferritin [43], which sequesters redox-active iron, a toxic by-product of haem degradation [17].
prevent atherogenesis, in part through the antioxidant action of its metabolites.

Despite the abundant findings on the role of HO-1 as an antioxidant, fundamental problems remain with implicating oxidative stress as a cause of atherosclerosis [44]. These include the poor performance of antioxidant strategies in limiting either atherosclerosis or cardiovascular events, and observations in animals that suggest dissociation between atherosclerosis and lipoprotein oxidation [44,45]. It remains to be established that oxidative damage is a cause rather than bystander in atherosclerosis.

**Smooth muscle cell proliferation**

The inhibitory effects of HO-1 on the proliferative response to vascular injury were reported by several in vivo experiments. Expression of HO-1 in pig arteries inhibits cell proliferation and lesion formation after arterial injury [46]. Similarly, HO-1-knockout mice have hyperplastic arteries with increased cell proliferation and intima-media ratio compared with controls after intravascular injury [46].

Several animal studies have reported that HO-1 induction inhibits VSMC (vascular smooth muscle cell) proliferation, highlighting HO-1 as a potential therapeutic target against occlusive vascular disease. In a rat carotid artery balloon injury model, induction of HO-1 with haem or HO-1 adenoviral gene delivery attenuates vascular remodelling and neointima formation following balloon injury compared with controls [47,48]. This beneficial effect was abolished by concomitant administration of the HO-1 inhibitor SnPP (tin protoporphyrin IX chloride). More recently, probucol, a drug that induces HO-1, has been shown to inhibit VSMC proliferation and decrease the intima-media ratio in balloon-injured rabbit aortas [49–51].

Inhibition of HO-1 with SnPP abolished the ability of probucol to inhibit VSMC proliferation and decrease the intima-media ratio, suggesting that the vascular protective effects of probucol were mediated via the HO-1 pathway [49–51]. Apart from inhibiting VSMC proliferation, HO-1 induction exerts an opposite effect on ECs by promoting proliferation and re-endothelialization at the site of vascular injury, thereby conferring further protection from atherosclerosis [49]. An earlier study also reported that probucol promotes re-endothelialization and decreases luminal stenosis in the stented segment of the rabbit iliac artery [52]. The findings from these studies may help explain the inverse relationship between HO-1 expression and rates of restenosis after angioplasty.

The inhibitory effects of VSMC proliferation via the HO-1 pathway may be due to the products of haem catabolism. Morita and Kourembanas [53] reported that CO inhibits VSMC proliferation by decreasing the production of endothelial mitogens ET-1 (endothelin-1) and PDGF-β (platelet-derived growth factor-β). More recently, CO derived from HO-1 has been reported to inhibit in vitro and in vivo VSMC proliferation via the transcription factor Yin Yang [54]. Likewise, bilirubin and biliverdin inhibit serum-derived VSMC-cycle progression in vitro via inhibition of the MAPK (mitogen-activated protein kinase) signalling pathways [55].

**Vasodilatory activities**

The vasodilatory properties of HO-1 are thought to be mediated largely by CO. Early studies have suggested that the mechanism of this action arises from the binding of CO to the haem iron of cytochrome P450 [56]. Subsequently, it became evident that the vasodilatory properties of CO may be mediated by activation of sGC (soluble guanylate cyclase), resulting in increased concentrations of cGMP [57], an effect independent of NO [46]. However, CO is a much weaker activator of sGC than NO, with the purified enzyme being activated 100–200-fold by 0.5 % NO, but only about 4-fold by approx. 100 % CO [58]. Consistent with this, CO was reported to be approx. 1000 times less potent than NO as a vasodilator [57]. However, endogenous CO (produced as a result of HO-1 induction) has been reported to elevate cGMP levels in VSMCs [59]. Another study also demonstrated that inhibition of sGC completely abolishes the vasodilation induced by CO in rabbit aortic rings, consistent with the hypothesis that CO-induced vascular relaxation is mediated by sGC [60]. However, sGC inhibition resulted in only partial attenuation of NO-induced relaxation [60], suggesting that a component of NO-induced relaxation is independent of sGC/cGMP.

Although sGC and cGMP appear to play a role in CO-induced vasodilation, mechanisms involving the K⁺ channel have also been reported. Wang et al. [61] reported that CO induces endothelium-independent vasodilation in pre-contracted rat tail arteries, and blockade of either the cGMP pathway or calcium-activated K⁺ channels results in partial inhibition of vasodilation. However, when both cGMP and K⁺ channels were inhibited, the CO-induced vasodilation was completely abolished [61]. Furthermore, HO-1 inhibition results in abolition of haem-inhibited vasodilation, suggesting a role for endogenous CO from vascular tissues in vasodilation. Foresti et al. [62] also observed that vasodilation induced by CORM (CO-releasing molecule) involves both elevation of cGMP and activation of ATP-dependent K⁺ channels.

HO-1 may also regulate arterial dilation through the eNOS (endothelial NO synthase)/NO pathway. Kawamura et al. [34] demonstrated that HO-1 overexpression alleviates dysfunction in vascular ECs exposed to oxidized LDL and TNF-α by restoring eNOS expression. Similar effects were observed by pretreatment with bilirubin, but not CO [34]. However, others reported increased HO-1 activity to decrease eNOS expression and activity in human ECs [63],
HO-1 and its products protect against atherosclerotic vascular disease by promoting vascular repair. HO-1 enhances restoration of endothelial lining at sites of injury and protects against ischaemia by promoting angiogenesis. Both of these actions are mediated through EPC recruitment and local EC proliferation.

In summary, the findings from the studies described above suggest that CO-mediated vasodilatation probably involves multiple mechanisms, and that both the cGMP pathway and K⁺ channels may play a role in this. The complex interaction between CO and NO may result in divergent effects on vascular tone, depending on the concentration of CO. In addition, HO-1 may alter eNOS expression and activity. Finally, it remains to be established how much CO is produced endogenously, as CO is much less potent than NO in its vasodilatory properties [57], and whether HO-1 induction actually plays an important role in vasodilation in vivo [4].

Most recently, Jones et al. [66] reported that, compared with wild-type mice, superior mesenteric arteries from HO-1-knockout mice had impaired relaxation ex vivo. Compared with arteries from wild-type mice, much of the sGC in arteries from HO-1-knockout mice was in the oxidized state [66]. These findings suggest that HO-1 plays a significant role in the maintenance of sGC in a reduced state, making it resistant to degradation and thereby conferring its vasodilatory properties [66].

**Vascular repair**

Apart from the anti-inflammatory, antioxidative and vasodilatory actions, HO-1 may also attenuate progression of atherosclerosis by promoting vascular repair (Figure 2). There is evidence that HO-1 enhances restoration of the endothelial lining and increases blood flow to ischaemic sites through angiogenesis.

**Pro-angiogenic activities**

The pro-angiogenic effects of HO-1 have been demonstrated in vivo in a number of animal models. In a rat hindlimb ischaemia model, blood flow recovery and capillary density are greater in ischaemic limbs injected with HO-1 adenovirus, whereas co-administration of HO-1 inhibitor SnPP attenuates blood flow recovery [67]. Likewise, in a murine myocardial infarction model, transfection with HO-1 resulted in increased vascularization and functional recovery [68]. Lastly, modulation of HO-1 expression has also been shown to have an impact on tumour growth and angiogenesis [2]. The pro-angiogenic effects of HO-1 are probably mediated by key angiogenic factors, including VEGF and SDF-1, as well as EPC (endothelial progenitor cell) recruitment to sites of vascular injury.

HO-1 plays an important role in VEGF production. In rat VSMCs, inhibition of HO-1 by SnPP results
in decreased VEGF production in both normoxic and hypoxic conditions [69]. Stimulation of HO-1 activity by haemin increases VEGF production and this effect is abolished by HO-1 inhibition. Likewise, cells overexpressing HO-1 have enhanced production of VEGF protein. In addition, exposure of VSMCs to CO dramatically increases VEGF production, whereas biliverdin and bilirubin have no effect, and iron is inhibitory [69]. However, another study suggested that, in human keratinocytes, biliverdin can act as a potent inducer of pro-angiogenic factors such as VEGF and IL-8 [70]. A potential serious problem with in vitro studies employing the addition of bile pigments and iron is the low solubility of these agents. Overexpression of HO-1 or incubation of ECs with CORM also led to an increase in capillary sprouting [71]. Together, these studies suggest that the increases in VEGF production and pro-angiogenic effects of HO-1 may, in part, be mediated through CO.

There is evidence that HO-1 is also a downstream mediator of VEGF-induced angiogenesis. The HO-1 inhibitor SnPP has been reported to decrease the VEGF-mediated pro-angiogenic effects of ECs by decreasing their proliferation, migration, tubulogenesis on Matrigel matrix and capillary sprouting [71]. HO-1 overexpression, on the other hand, enhances capillary sprouting in response to VEGF exposure [71]. ECs from HO-1-knockout mice also fail to proliferate in the presence of VEGF [2]. Bussolati et al. [72] have also demonstrated that VEGF induced HO-1 expression and activity in human ECs and HO-1 inhibition abolished VEGF-mediated angiogenesis.

Apart from its effect and interaction with angiogenic factors, it is also possible that HO-1 promotes angiogenesis by decreasing the production of anti-angiogenic mediators. sFlt-1 (soluble Flt-1; also known as soluble VEGF receptor-1) and sEng (soluble endoglin) are inhibitors of angiogenesis that are elevated in preeclampsia [73]. A study has shown that overexpression of HO-1 in ECs inhibits VEGF-mediated sFlt-1 release and IFNγ (interferon γ)- and TNF-α-induced sEng release, whereas HO-1 inhibition potentiates sFlt-1 and sEng production from ECs and placental villous explants [74]. In addition, HO-1-knockout mice produce higher levels of sFlt-1 and sEng compared with wild-type mice.

A study has reported a link between HO-1 and SDF-1, a key angiogenic chemokine involved in EPC recruitment, ischaemia-mediated neovascularization and vascular repair [13,75]. Deshane et al. [13] demonstrated that the addition of SDF-1 to human aortic ECs induces HO-1 mRNA and HO-1 protein expression and enzyme activity independent of VEGF. SDF-1-induced angiogenesis (assessed with Matrigel tubulogenesis and migration assays) is impaired in HO-1-deficient ECs, suggesting that the pro-angiogenic effects of SDF-1 are mediated via HO-1. Likewise, SDF-1 induced capillary sprouting in an aortic ring angiogenic assay is impaired in HO-1-knockout compared with wild-type mice, and CO reverses this effect [13]. Furthermore, the pro-angiogenic effects of SDF-1 are impaired in HO-1-knockout mice in both the Matrigel plug and wound healing models, and HO-1-knockout EPCs show defective homing and re-endothelialization of the retinal vasculature in a murine model of retinal ischaemia-induced vascular repair [13]. These findings suggest a mechanistic role for HO-1 in SDF-1-mediated angiogenesis.

Apart from the link between HO-1 and key angiogenic factors, the pro-angiogenic effects of HO-1 may be mediated via its effect on ECs and recruitment of EPCs from the bone marrow. It has been proposed that putative EPCs mobilize from the bone marrow into the circulation and participate in neovascularization at sites of ischaemia [76]. Tongers et al. [77] have reported that blood flow recovery after hindlimb ischaemia surgery was impaired in mice receiving the HO-1 inhibitor SnPP. This may be explained by a decrease in Sca-1+/Kdr+ progenitor cells after HO-1 inhibition, as such inhibition caused fewer LacZ+ cells to be detected in the ischaemic hindlimb muscle of lethally irradiated wild-type mice receiving Tie2-lacZ transgenic bone marrow cells [77]. Another study also reported that, in a murine hindlimb ischaemia model, EPC levels are higher in mice receiving the HO-1 inducer probucol compared with placebo [78]. An earlier study showed, that compared with wild-type, bone marrow cells from HO-1-knockout mice have a decreased ability to form endothelial colony forming units [79]. In that study, treatment with the HO-1 inducer succinobucol increased the number of bone-marrow-derived endothelial colony forming units in wild-type but not HO-1-knockout mice [79], suggesting a role for HO-1 in the formation of progenitor cells. Likewise, treatment of wild-type mice with succinobucol was associated with an increase in the number of both circulating and bone-marrow-derived progenitor cells, including non-haematopoietic (CD34+/Flk1+/CD45−) and haematopoietic (CD34+/Flk1−/CD45+) cells [79]. Furthermore, HO-1 induction with probucol promotes endothelial regeneration in injured rabbit aortas [49]. These findings suggest that HO-1 contributes to vascular repair by local EC proliferation and by increasing recruitment of progenitor cells from the bone marrow.

It appears that HO-1 may have different roles in angiogenesis depending on the in vivo environment. In an elegant study, Bussolati et al. [72] assessed the role of HO-1 in two models of angiogenesis. First, in a non-inflammatory angiogenesis model, HO-1 inhibition enhances leucocyte infiltration, leading to increased VEGF-induced angiogenesis. However, when leucocyte migration was blocked, HO-1 inhibitors significantly decrease VEGF-induced angiogenesis. In contrast, in the LPS-induced model of inflammatory angiogenesis, induction of HO-1 inhibits leucocyte migration [72].
The results from that study suggest a dual role of HO-1 in chronic inflammation: first, an anti-inflammatory action inhibiting leucocyte infiltration, and secondly, the promotion of VEGF-mediated non-inflammatory angiogenesis that facilitates tissue repair [72].

Re-endothelialization

The vascular protective actions of HO-1 may also result from its ability to promote re-endothelialization, a key reparative process in response to vascular injury. Previous studies have shown that HO-1 is induced at sites of vascular injury, suggesting a key role of HO-1 in the reparative process and control of intimal hyperplasia [46]. The HO-1 inducer probucol promotes re-endothelialization in balloon-injured rabbit aortas, whereas inhibition of HO-1 abolishes the ability of probucol to promote re-endothelialization, suggesting that the vascular reparative properties of probucol are mediated via HO-1 [49–51]. It has also been reported that HO-1 contributes to vascular repair by both promoting local EC proliferation at the border zone of the injured artery [49] and by increasing circulating EPCs derived from the bone marrow [79]. In a rabbit model of aortic balloon injury, Sca-1+/Flk1+ EPCs were shown to adhere to the luminal surface of the injured vessel via a HO-1 pathway [79]. More recently, Weigel et al. [80] reported that, in a rodent carotid injury model, CO exposure accelerates EC proliferation and enhances recruitment of bone-marrow-derived EPCs and vessel repair in an NO-dependent manner. It is therefore possible that the beneficial effects of HO-1 on re-endothelialization may be mediated at least in part by CO.

**HO-1 AS A THERAPEUTIC TARGET IN CVD**

CVD remains the leading cause of death worldwide. Despite considerable progress in the management of CAD over the past three decades, there remains a significant number of patients who are not adequately served by current treatment approaches such as angioplasty or CABG (coronary artery bypass grafting) [81]. The need to better serve such ‘no option’ patients represents a major unmet clinical need within cardiovascular medicine.

Experimental models have shown that HO-1 possesses atheroprotective, pro-angiogenic, anti-inflammatory and antioxidative properties. Given its potential in vascular protection and repair, modulation of HO-1 activity promises to be an exciting new and alternative option in the armamentarium of cardiovascular therapies.

**HO-1 induction using drug therapy**

Probucol, a rarely used lipid-lowering drug, up-regulates HO-1 mRNA, protein and activity in vitro and in vivo [50], reduces restenosis after coronary intervention, and promotes regression of atherosclerosis when assessed by carotid ultrasonography [82,83]. Succinobucol is a derivative of probucol that also induces HO-1 [79], but has lower QT-interval-prolonging effects compared with probucol [83]. Therefore the ARISE (Aggressive Reduction of Inflammation Stops Events) trial assessed the effectiveness of succinobucol in preventing cardiovascular events [84]. The study randomized over 6000 patients who suffered from a recent acute coronary event to either succinobucol or placebo. Contrary to its anticipated benefit, the study reported that succinobucol had no beneficial effect on the primary end point of time to the first occurrence of cardiovascular death, resuscitated cardiac arrest, non-fatal myocardial infarction, non-fatal stroke, hospitalization for unstable angina or coronary revascularization [84]. However, the study did show benefits with succinobucol on the composite secondary end point of cardiovascular death, cardiac arrest, myocardial infarction or stroke, as well as the tertiary end point of prevention of new-onset diabetes [84]. The potential anti-diabetic effect is possibly consistent with succinobucol’s antioxidant and anti-inflammatory actions on the pancreas [85,86]. The study [84] also revealed that succinobucol led to the development of several unfavourable cardiovascular risk factor profiles, including hypertension, raised LDL levels, decreased HDL (high-density lipoprotein) levels and increased expression of CRP (C-reactive protein) (a marker of inflammation). It is possible that these adverse effects may have offset any potential benefit from succinobucol with regards to the primary cardiovascular end point [87]. Therefore the development of an HO-1-inducing drug without these ‘off-target’ adverse effects may result in improved CVD outcomes.

Apart from probucol and its analogues, several other drugs have also been implicated to modulate HO-1 expression. Statins (also known as 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors) are a class of lipid-lowering agents that have been shown to reduce cardiovascular events [88]. In addition to potential antioxidant and anti-inflammatory actions [89], these drugs have been reported to modulate HO-1 expression. Grosser et al. [90] demonstrated that high statin concentrations resulted in increased HO-1 mRNA expression in ECs. However, the concentration of statin used in these experiments was much higher than that achieved in clinical situations. In addition, the effect of statins on HO-1 expression may depend on the cell type [91]. It therefore remains to be established whether the dose of statin used clinically has any effect on HO-1 expression.

More recently, attention has been drawn to another lipid-lowering drug, fenofibrate, and its effects in reducing cardiovascular events in patients with Type 2 diabetes [92]. It is known that fenofibrate mainly exerts its actions via the activation of specific nuclear receptors called
HO-1 induction using gene therapy

To date, there have been numerous studies demonstrating enhanced blood flow recovery after hindlimb ischaemia and improved functional recovery after myocardial infarction in animal experiments after transfection with the HO-1 gene [67,95]. Gene transfer therapy allows us not only to reconstitute missing or dysfunctional genes, but also to modify the expression of genes that are already present [3]. Indeed, to date, there have been several human pilot studies involving the use of gene therapy in the treatment of critical limb ischaemia in peripheral arterial disease [96]. However, the long-term outcomes and safety data are still unknown and one must be wary of potential adverse effects, including the potential for angiogenesis-triggered malignancies and the impact of angiogenesis on physiological or pathological processes, as well as the inflammatory response exerted by viral vectors [3,96]. HO-1 gene transfer therapy offers an exciting and novel therapeutic option for ameliorating ischaemic symptoms and treating CVD, and may therefore form the basis for subsequent human pilot studies.

HO-1 induction and cell therapy

Cell therapy using stem and progenitor cells for therapeutic angiogenesis offers great potential in the treatment CVD, particularly for patients ineligible for current treatment options [81]. Given their role in development, maintenance and repair of senescent or diseased adult tissues, the description of EPCs by Asahara et al. [76] over a decade ago represents a novel alternative treatment modality for regenerative cardiovascular medicine. In view of the findings in experimental models suggesting a role of HO-1 in EPC mobilization to sites of vascular injury [13,79], modulation of HO-1 activity by gene therapy offers an exciting new therapeutic avenue in the treatment of CVD.

Apart from EPCs, bone-marrow-derived MSCs (mesenchymal stem cells) show promise in myocardial regeneration and repair in animal models of myocardial infarction [97]. This may be due to up-regulation of HO-1 expression in infarcted hearts after MSC transplantation [98]. The role of HO-1 in promoting myocardial repair by MSCs is suggested further by a study showing that, compared with control cells, MSCs transfected with HO-1 have greater survival and regenerative functional capacity in a murine myocardial infarction model [99]. More recently, Lin et al. [68] reported that, in a murine myocardial infarction model, transfection with HO-1 provides protection at least in part by promoting neovascularization through inducing angiogenic factors, such as VEGF and SDF-1, and by enhancing recruitment of circulating progenitor/stem cells to the infarcted region. However, the role of HO-1 expression in bone-marrow-derived progenitor/stem cell recruitment in humans remains unknown currently.

CONCLUSIONS

There is compelling evidence from pre-clinical studies for a vascular protective role of HO-1 via its anti-atherogenic, anti-inflammatory, antioxidant and pro-angiogenic activities. This has generated immense interest in HO-1 as a vascular therapeutic target. However, the only large-scale randomized clinical trial of an HO-1 inducer (succinobucol) has yielded disappointing and neutral results [84]. It is possible that a number of adverse ‘off-target’ effects of succinobucol may have offset its potential benefits. The development of a HO-1 therapy without these ‘off-target’ effects may result in improved cardiovascular outcomes. Furthermore, the use of HO-1 gene therapy may allow for local administration at sites of ischaemia, without the potential for systemic adverse side effects. Ultimately, the development of novel HO-1 drug and gene therapies may provide us with an alternative and exciting therapeutic option in the treatment of CVD by promoting angiogenesis and reducing atherosclerosis and restenosis after angioplasty.

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