ELSEVIER

Cardiovascular Research 41 (1999) 385–394

Cardiovascular Research

Review

Heme oxygenase–carbon monoxide signalling pathway in atherosclerosis: anti-atherogenic actions of bilirubin and carbon monoxide?

Richard C.M. Siow, Hideyo Sato[1], Giovanni E. Mann*

Vascular Biology Research Centre, School of Biomedical Sciences, King's College London, Campden Hill Road, London W8 7AH, UK

Received 4 June 1998; accepted 2 September 1998

Abstract

Atherosclerosis is a major contributor to cardiovascular disease, and genetic disorders of lipoprotein metabolism are recognized risk factors in atherogenesis. The gaseous monoxides nitric oxide (NO) and carbon monoxide (CO), generated within the blood vessel wall, have been identified as important cellular messengers involved in the regulation of vascular smooth muscle tone. Microsomal heme oxygenases degrade heme to biliverdin and CO, and the cytosolic enzyme biliverdin reductase then catalyzes reduction of biliverdin to bilirubin, both powerful chain-breaking antioxidants. Two principal isozymes of heme oxygenase have been identified, a constitutive isoform HO-2 (M_r~34 000) and an inducible isoform HO-1 (M_r~32 000), which is expressed at a low basal level in vascular endothelial and smooth muscle cells and is induced by heavy metals, oxidative stress, inflammatory mediators and oxidized low density lipoproteins. Although NO and CO modulate intracellular cGMP levels, platelet aggregation and smooth muscle relaxation, CO has a much lower affinity for soluble guanylyl cyclase than NO. Decreased production or sensitivity to NO in atherosclerosis may be compensated for by an induction of HO-1, with bilirubin acting as a cellular antioxidant and CO as a vasodilator. This review examines the evidence that oxidized low density lipoproteins (LDL), hypoxia and pro-inflammatory cytokines induce HO-1 expression and activity in vascular endothelial and smooth muscle cells, and evaluates the anti-atherogenic potential of the heme oxygenase signalling pathway.

Keywords: Atherosclerosis; Heme oxygenase; Carbon monoxide; Vascular smooth muscle; Endothelium; Oxidative stress protein; Antioxidant; Nitric oxide

1. Introduction

Over the past decade the field of cardiovascular research has increasingly embraced the hypothesis of 'free radical attack' to explain the pathogenesis of vascular disease and the 'cardioprotective' role of antioxidants [1]. The emphasis has been on the pathogenesis of atherosclerosis and vascular dysfunction associated with elevated plasma levels of low density lipoproteins (LDL) and their oxidative modification [2]. Oxidized LDL and reactive oxygen species influence vascular cell gene expression [3,4], promoting atherogenesis and endogenous antioxidant defences to counteract and prevent further oxidative damage [5,6]. Increased expression of the stress response protein heme oxygenase-1 (HO-1) in human atherosclerotic lesions [7] and vascular endothelial and smooth muscle cells exposed to oxidized LDL [8,9] may serve a multi-purpose role, via metabolism to the antioxidant biliverdin and the vasodilator carbon monoxide (CO) [10,11]. These adaptive responses may contribute to the maintenance of vascular tone and patency in atherosclerotic vessels and compensate for diminished nitric oxide (NO) generation and activity [12,13]. This review summarises the evidence that the heme oxygenase–CO signalling pathway plays a physiological role in the vasculature and evaluates its potential significance in the pathogenesis of atherosclerosis.

2. Heme metabolism and regulation of heme oxygenase induction

Heme plays a central role in eukaryotic metabolic

*Corresponding author. Tel.: +44-171-333-4450; fax: +44-171-333-4450; e-mail: giovanni.mann@kcl.ac.uk

[1]Present address: Department of Biochemistry, Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan.

Time for primary review 27 days.

0008-6363/99/$ – see front matter
PII: S0008-6363(98)00278-8

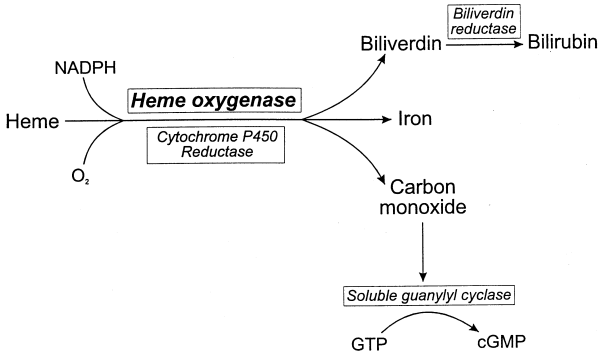

Fig. 1. Metabolism of heme by heme oxygenase. Heme is catabolised by heme oxygenases, acting in concert with NADPH-cytochrome P450 reductase, to form biliverdin, iron and carbon monoxide. Biliverdin is subsequently converted to bilirubin by biliverdin reductase. Carbon monoxide can activate soluble guanylyl cyclase in the cell in which CO is produced or in an adjacent cell by binding to its heme moiety. Activation of soluble guanylyl cyclase results in elevated cyclic guanosine monophosphate levels (cGMP) formed from guanosine triphosphate (GTP). Adapted from Marks et al. [10].

pathways, as the prosthetic moiety of hemoproteins is involved in cell respiration and oxidative biotransformations, including hemoglobin and myoglobin, cytochrome P450, nitric oxide synthase (NOS) and soluble guanylyl cyclase [11,14] (Fig. 1). Heme oxygenase plays an important biological role in the degradation of heme to regulate hemoprotein levels and protects cells from the deleterious effects of free heme [11]. Two isozymes of HO, expressed in the vasculature, have been characterized extensively, a 32 000 Da inducible isoform (HO-1) and a 36 000 Da constitutive isoform (HO-2) [11]. A third 33 000 Da constitutive isoform (HO-3) has been identified in brain, heart, kidney, liver, testis and spleen, yet to date there is no evidence that this isoform is expressed in the vasculature [15]. These isoforms are products of different genes, with HO-1 and HO-2 sharing only ~40% amino acid homology and HO-3 more closely related to HO-2 (~90%) (see Table 1). The catalytic activity of HO requires the concerted action of microsomal NADPH-cytochrome P450 reductase to transfer electrons to the heme–HO complex, which may account for their close cellular localisation [12].

Various regulatory elements in the 5′ flanking region of the human, rat and mouse HO-1 gene and associated transcription factors have been identified [16] and may be involved in HO-1 gene activation in response to hydrogen peroxide, heavy metals, heme and lipopolysaccharide. The inducer-responsive element, designated stress response element (StRE) [17] contains the sequence of the binding site for the transcription factor AP-1, and inhibition of its activation has recently been shown to decrease HO-1 mRNA expression in cytokine-treated human vascular endothelial cells [18]. Transcriptional activation of genes by electrophilic agents such as diethylmaleate (DEM), known to conjugate cellular glutathione, is mediated by an electrophile response element (EpRE), also designated antioxidant responsive element (ARE) [19]. Since DEM can induce HO-1 in vascular endothelial and smooth muscle cells [9,20], it is possible that EpRE-binding protein(s), in addition to AP-1, mediate HO-1 gene expression. In addition, NF-κB and AP-2 consensus binding sites have been identified in the regulatory sequences of the human HO-1 gene promoter region [21]. Thus, activation of AP-1, AP-2 and NF-κB may also be involved in the induction of HO-1 in vascular cells in atherogenesis [7,22]. Although oxidatively modified LDL and pro-inflammatory cytokines can activate these transcription factors in cells [3,4,23], NF-κB is only activated within cells of the atherosclerotic lesion but not in normal vessels [22,24]. HO-2 has been shown to be up-regulated by glucocor-

Table 1
Characteristics of heme oxygenase isoforms

Isoform	Molecular mass	HO-2 Amino acid homology	Physiological roles	Constitutive tissue expression	Modulators of expression
HO-1	~31 000–33 000	~40%	Heme catabolism Antioxidant defence Modulation of vascular tone and liver perfusion Neural signalling, Anti-inflammatory	Spleen Testis Liver	Oxidative stress oxidized LDL Cytokines, heavy metals, hypoxia nitric oxide, heat shock, endotoxin, heme, UV
HO-2	~34 000–36 000	100%	Heme catabolism Maintenance of vascular tone Neural signalling	Most tissues e.g. Brain, retina, liver, spleen, nervous system, testis, viscera, airways, kidney, vasculature	Expression enhanced by adrenal glucocorticoids
HO-3	~33 000	~90%	Heme binding Regulation of heme dependent genes	Most tissues	Not known

Information taken from Maines [11] and McCoubrey et al. [15].

ticoids in the brain [25], although its vascular expression appears to be constitutive [26].

3. Oxidative stress in atherogenesis

Vascular cells respond to an environment of 'oxidative stress', such as that found within the milieu of the developing atherosclerotic lesion, by inducing endogenous antioxidant defence mechanisms [6]. The key antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase have been studied extensively in view of their ability to attenuate oxidant injury [27]. The glutathione (GSH) redox cycle forms the primary defence against oxidative insult in many mammalian cell systems [28] and is induced in endothelial cells, smooth muscle cells and macrophages by oxidative stress agents including oxidized LDL [20,29]. Diminished production of NO by human endothelial cells depleted of GSH [30] may contribute to the impaired vascular reactivity associated with atherogenesis [12]. HO-1 is expressed in a variety of cells under conditions where GSH is overwhelmed by oxidant insult [30,31]. Moreover, it has recently been shown that a there is a diminished glutathione-related enzymatic antioxidant shield within human atherosclerotic lesions [32], and thus HO-1 induction may act as an alternative antioxidant defence mechanism. As summarized in Tables 2 and 3, induction of HO-1 in endothelial and smooth muscle cells by pro-atherogenic agents, such as heme [33], oxidized LDL [8,9], lipid metabolites [8,31], peroxynitrite [34], hypoxia [35–37], heavy metals [9,38] pro-inflammatory cytokines [18,39,40] and angiotensin II [41] could provide a secondary line of antioxidant defence and maintain vessel patency through the generation of CO. In patients with coronary heart disease, vasodilatation in response to NO donors may also involve the heme oxygenase pathway, since these compounds have been reported to induce HO-1 in vascular cells [34,42].

Levels of heme are elevated in atherosclerotic plaques due to the release of hemoproteins from necrotic and ruptured cells present within lesions leading to further LDL oxidation [43] and vascular cell toxicity [44]. Induction of HO-1 by heme serves to remove this pro-oxidant, while concomitant induction of ferritin expression [45] within vascular cells of atherosclerotic lesions [46] chelates free iron generated by increased HO activity, preventing further oxidative damage [47]. Other heme binding proteins are co-induced with HO-1 in vascular cells, macrophages and hepatocytes. These include a 23 000 Da macrophage stress protein (MSP23) [9,48] and its homologue heme binding protein (HBP23) [49], both of which are members of the peroxiredoxin family of antioxidant proteins [50].

4. Regulation of vascular tone by carbon monoxide

The mechanism by which CO activates soluble guanylyl cyclase (sGC) is similar to that of NO, and involves binding and dislocation of its heme-iron to induce a conformational change and activation of the catalytic site of guanylyl cyclase [10,11]. Endothelium-derived CO or NO diffuses to subjacent smooth muscle cells where activation of sGC results in elevated intracellular cGMP levels leading to smooth muscle relaxation [51]. As illustrated in Fig. 2, CO and NO can also be generated in smooth muscle cells in response to atherogenic stimuli or pro-inflammatory cytokines, respectively. The metabolic and functional links between CO and NO suggest that

Table 2
Heme oxygenase expression in vascular endothelium

Isoform	Species/vessel	Inducing agent	Literature
HO-1	Human and murine aorta in situ	Atherosclerotic lesion	Wang et al. (1998) [7]
HO-1	Human umbilical vein	Oxidized LDL	Agarwal et al. (1996) [104]
		BSO, Hyperoxia	Jornot & Junod (1993) [20]
		Heme, Tumour necrosis factor	Wagener et al. (1997) [102]
		Interleukin, Tumour necrosis factor	Terry et al. (1998) [18]
HO-1	Human aorta	Oxidized LDL, Hemin	Ishikawa et al. (1997) [8]
		Peroxynitrite, NO donors	Foresti et al. (1997) [34]
HO-1	Rat aorta in situ	Angiotensin II, Norepinephrine	Ishizaka et al. (1997) [41]
		Endotoxin	Yet et al. (1997) [40]
HO-1	Rat pulmonary microvessel	Hyperoxia	Visner et al. (1996) [101]
HO-1	Rat brain capillary	Hemin	Vigne et al. (1995) [33]
HO-1	Porcine aorta	NO donors, LPS, Interferon γ	Motterlini et al. (1996) [63]
		Haemoglobin, Hemin	Motterlini et al. (1995) [100]
HO-2	Rat vasculature	Basal	Grozdanovic (1996) [105]
	Rat aorta in situ	Angiotensin II	Ishizaka et al. (1997) [41]
HO-2	Porcine pulmonary artery in situ	Basal	Zakhary et al. (1997) [26]

Table 3
Heme oxygenase expression in vascular smooth muscle

Isoform	Species/vessel	Inducing agent	Literature
HO-1	Human and murine aorta in situ	Atherosclerotic lesion	Wang et al. (1998) [7]
HO-1	Human aorta	Oxidized LDL, Hemin	Ishikawa et al. (1997) [8]
HO-1	Human umbilical artery	Oxidized LDL, cadmium	Siow and Mann (1998) [38]
HO-1	Porcine aorta	Oxidized LDL, cadmium, glucose oxidase, diethylmaleate	Siow et al. (1995) [9]
HO-1	Rat aorta	Hypoxia	Morita et al. (1995) [37] Lee et al. (1997) [75]
		Hemin, Arsenite	Christodoulides et al. (1995) [106]
		Nitric oxide donors	Hartsfield et al. (1997) [42]
		NO donors and cytokines	Durante et al. (1997) [39]
		H_2O_2, hemin	Ishizaka and Griendling (1997) [103]
		Interleukin-1β	Yet et al. (1997) [40]
		Shear stress	Wagner et al. (1995) [69]
		Cyclic AMP	Durante et al. (1997) [79]
		Angiotensin II	Ishizaka and Griendling (1997) [103]
HO-1	Rat aorta in situ	Angiotensin II, LPS	Ishizaka et al. (1997) [41]
HO-2	Rat aorta	Interleukin-1β	Yet et al. (1997) [40]
		Hemin, Arsenite	Christodoulides et al.(1995) [106]
		Cyclic AMP	Durante et al. (1997) [79]
		Nitric oxide donors	Durante et al. (1997) [39]
HO-2	Rat aorta in situ	Basal expression	Grozdanovic and Gossrau (1996) [105]
		Angiotensin II	Ishizaka et al. (1997) [41]

vasodilator actions of CO may become important in atherogenesis, where endothelium-derived NO production is inhibited.

The effects of CO on vascular smooth muscle relaxation are blood vessel and species specific, with findings differing from one study to another. CO relaxes precontracted rabbit and dog aortic rings, with half maximal relaxation achieved between 20 and 40 μM [52]. In this study, the relative potency of CO to NO varied from 1:1000 for rabbit aorta to 1:1 for canine circumflex coronary artery. As the ability of NO to directly activate sGC is ~50-fold greater than CO [51], vasodilator actions of CO may be of particular importance in atherogenesis. The fact that heme [53], a potent inducer of HO-1, lowers blood pressure in spontaneously hypertensive rats suggests that endogenous CO formation may be sufficient to stimulate vascular smooth muscle relaxation in vivo. Further evidence for a physiological role of CO in regulating vascular tone is provided by the findings of Johnson et al. [54], who demonstrated a basal vasodepressor function of CO in normal rats. Inhibition of HO activity by administration of zinc deuteroporphyrin-2,4-bisglycol (a HO inhibitor) increases mean arterial blood pressure and total peripheral resistance [54]. This group subsequently reported that substrates for HO acutely lower blood pressure in genetically hypertensive but not in normotensive rats [55]. These findings are consistent with recent reports that vascular HO-1 expression is elevated in aortae of genetic [56] and angiotensin II induced hypertensive rats [41]. The most convincing evidence for regulation of vascular resistance by CO has been obtained in the isolated perfused rat liver [57], where all three HO isoforms are expressed constitutively [15,58]. In these elegant studies, Suematsu et al. [57] demonstrated that inhibition of HO activity, but not NOS activity, increased hepatic vascular resistance, which was reversed by exogenous CO.

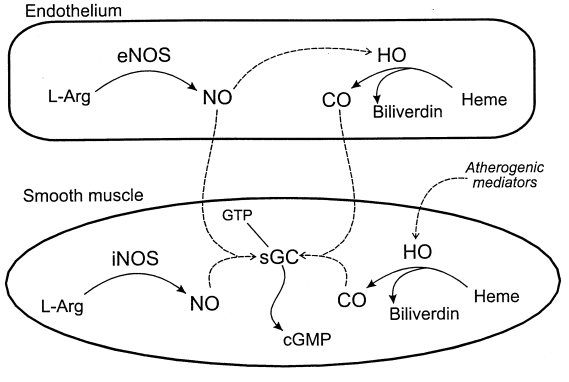

Fig. 2. Importance of the heme oxygenase–carbon monoxide and L-arginine–nitric oxide signalling pathways in vascular endothelial and smooth muscle cells in atherogenesis. Heme oxygenases (HO) metabolise heme to generate the antioxidant biliverdin and carbon monoxide (CO), which like nitric oxide (NO), stimulates soluble guanylyl cyclase (sGC) resulting in increased intracellular cGMP levels. Atherogenic and pro-inflammatory mediators such as oxidized LDL and cytokines decrease the expression and activity of endothelial nitric oxide synthase (eNOS) while inducing HO-1 and inducible nitric oxide synthase (iNOS) in smooth muscle cells. Diminished production or activity of NO by the endothelium in atherogenesis could be compensated for by induction of HO-1. Increased cGMP levels in vascular smooth muscle cells would sustain blood flow, while catabolism of heme and generation of biliverdin would attenuate cellular oxidative stress in atherogenesis.

Metalloporphyrin inhibitors of HO exert non-selective effects that may complicate the interpretation of experiments examining the vasodilator actions of CO. Zn and Sn protoporphyrin are widely used as inhibitors of HO, but also inhibit sGC activity and can modulate NOS activity [59]. Although interpretation of the reported actions of CO in the vasculature merit caution, there is now good evidence for a physiological role of heme oxygenase–CO pathway in vascular cells. In addition to activating sGC, vasodilator actions of CO appear to be mediated by direct modulation of high conductance Ca^{2+}-dependent potassium channels (K_{Ca}) in vascular smooth muscle cells [60]. CO induced increases in potassium efflux via K_{Ca} channels would hyperpolarise smooth muscle cells and in turn inactivate voltage-dependent Ca^{2+}-channels and reduce calcium sensitivity, leading to smooth muscle relaxation [61]. These changes in K_{Ca} channel activity have been attributed to a chemical modification of specific amino acid residues of the channel itself rather than to activation of cGMP-dependent kinases [60]. Modulation of the membrane potential by CO highlights another protective mechanism of the heme oxygenase pathway, since increased intracellular Ca^{2+} levels in vascular smooth muscle cells induced by oxLDL [62] would contribute to increased vascular contractility observed in atherogenesis.

5. Interactions between the NO and CO signalling pathways

Accumulating evidence indicates that NO donors and endogenously generated NO induce expression of HO-1 in vascular endothelial and smooth muscle cells [34,39,42]. This provides an endogenous adaptive defence mechanism against the oxidative stress associated with sustained production of NO [40,63]. As the heme moiety of NOS and sGC can serve as alternate substrates for HO [11], their activity may under certain conditions be downregulated. In addition, CO is able to bind to the heme moiety of NOS, and thereby inhibit L-arginine turnover and NO production [11]. Due to the close similarity of NOS and NADPH-cytochrome P450 reductase, electron transfer from NOS to HO can also occur, fuelling heme catabolism by HO [11]. By reducing intracellular heme levels in vascular cells, heme oxygenases may limit de novo synthesis of iNOS, whilst the iron generated by heme catabolism would further limit synthesis of iNOS through inhibition of nuclear transcription [64]. Expression of iNOS has been detected in animal and human atherosclerotic lesions and contributes to the formation of peroxynitrite [65–67]. Induction of HO-1 in atherogenesis in response to peroxynitrite [34] may attenuate vascular injury by inhibiting iNOS activity.

Shear stress induced vasodilatation is thought to be mediated by endothelium-derived NO [68], however, Wagner et al. [69] have recently reported that CO is also generated following induction of HO-1 in smooth muscle cells exposed to shear stress without iNOS induction. CO liberated from smooth muscle cells exposed to shear, increased intracellular cGMP levels in co-incubated platelets and inhibited agonist-induced aggregation. The heme oxygenase–CO pathway thus provides another line of defence in atherogenesis and hypertension, by maintaining blood flow and regulating platelet reactivity. In the context of atherosclerosis, impairment of the vascular NO signalling pathway could tip the balance in favour of HO as a salvage mechanism required to maintain vascular tone and function.

6. Regulation of heme oxygenase by hypoxia

Arterial wall hypoxia resulting from hypertension [70] and through the occlusion of the vasa vasorum [71], has been implicated in the pathogenesis of atherosclerosis. Several studies have reported that ischaemia/reperfusion upregulates HO-1 expression in porcine and rat hearts [72,73], and that hypoxia induces HO-1 levels in cultured vascular endothelial [74] and smooth muscle [35–37] cells. Hypoxia-dependent HO-1 gene activation in rat smooth muscle cells is mediated by a hypoxia-inducible factor-1 (HIF-1) [75], initially identified as a nuclear factor that binds to the enhancer sequences of the erythropoietin gene [76]. Hypoxia thus serves as an additional inducer of HO-1 in the vasculature, mediated by mechanisms different from those activated by oxidative stress. However, the oxidative stress responsive transcription factor NF-κB is also activated in hypoxia [77], and may contribute to the induction of HO-1. Under hypoxic conditions, cAMP can regulate HIF-1 binding to the HRE [78] and thereby also modulate HO-1 induction. In this context, Durante et al. [79] recently reported that elevation of cAMP or analogues of cAMP were able to induce HO-1 and generate CO in vascular smooth muscle cells. Induction of iNOS in vascular smooth muscle cells is also modulated by cAMP [80], suggesting that cAMP is an important regulator of both NO and CO signalling pathways under inflammatory conditions.

The significance for atherogenesis of HO-1 induction by hypoxia may lie in its ability to generate CO to modulate vascular resistance, since eNOS expression is suppressed by hypoxia [81]. Morita et al. [37] have reported that increased accumulation of cGMP in hypoxic smooth muscle cells was dependent on enhanced HO-1 expression and activity. Interestingly, hypoxia-induced release of CO from smooth muscle cells inhibits gene expression of endothelin-1 and platelet-derived growth factor in co-cultured endothelial cells [35]. Moreover, increased proliferation of hypoxic smooth muscle cells in response to serum or mitogens is inhibited by CO, acting via a cGMP-dependent pathway involving transcriptional regulation of cell cycle progression genes [36].

7. Heme oxygenase mediated antioxidant protection in atherogenesis

The role of HO-1 induction in cellular antioxidant defences in atherogenesis may be of greater importance than its involvement in vascular relaxation through CO generation. The clinical complications arising from atherosclerosis are directly related to elevated free radical generation and oxidation of lipids in the sub-endothelial space [2,82]. The induction of HO-1 by atherogenic and inflammatory mediators such as oxidized LDL and cytokines may ameliorate the insult to cells by restoring the balance of antioxidants and pro-oxidants in the vascular wall. In the context of this review, is of interest to note that there is increasing evidence that low serum concentrations of bilirubin are an independent risk factor for coronary artery disease [83–85]. Protection against atherosclerosis afforded by bilirubin may be due to its ability to protect LDL from oxidative modification by a variety of pro-oxidants and potentiate actions of vitamin C and E [86–88]. Moreover, bilirubin has been reported to inhibit the activity of protein kinase C (PKC) in human fibroblasts [89]. As many of the effects of atherogenic agents in vascular cells are mediated via activation of PKC [90], bilirubin could also exert cytoprotection independent of its known antioxidant properties. Although a cytoprotective role for bilirubin generated from HO in vivo in man remains to be established, bilirubin acts as an antioxidant in rats in vivo treated with lipopolysaccharide [106].

We have previously reported that mildly and highly oxidized LDL, but not native LDL, induce a time- (6–48 h) and concentration- (10–100 μg protein ml^{-1}) dependent expression of HO-1 in cultured vascular smooth muscle cells [9]. In these studies, the degree of LDL oxidation was carefully defined according to their electrophoretic mobilities and lipid hydroperoxide content [91]. In similar studies in rat aortic smooth muscle cells, we confirmed that mildly oxidized and highly oxidized LDL, but not native LDL, increased mRNA levels for HO-1 (see Fig. 3A). Ishikawa et al. [8] have recently reported that induction of HO-1 mRNA expression and activity in co-cultures of human aortic endothelial and smooth muscle cells by mildly oxidized LDL, oxidized lipid metabolites or hemin inhibits monocyte chemotaxis (see Fig. 3B), an early event in atherogenesis [82]. This finding was attributed to the generation of bilirubin and biliverdin by endothelial cells, since monocyte chemotaxis was attenuated by pretreatment with these products heme metabolism and reversed by inhibition of HO activity. Our recent studies in human umbilical artery smooth muscle cells have established that highly oxidized LDL induces HO-1 expression to a greater degree than mildly oxidized LDL (see Fig. 4A) and that pretreatment of cells with physiological concentrations of vitamin C (10–100 μM) attenuates the adaptive increase in HO-1 expression induced by highly oxidized LDL (see Fig. 4B) [38]. Together with our recent report that vitamin C also attenuates the adaptive increases in glutathione levels in human smooth muscle cells treated with oxidized LDL [29], these findings provide further evidence that antioxidant supplementation may be anti-atherogenic.

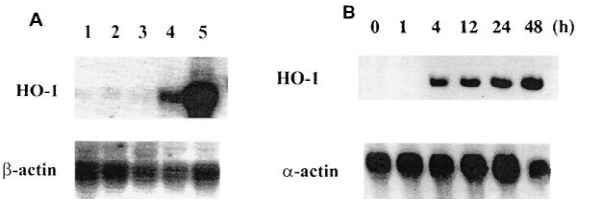

Fig. 3. Induction of heme oxygenase-1 (HO-1) in vascular cells by atherogenic oxidized low density lipoproteins (LDL). (A) induction of HO-1 mRNA in cultured rat aortic smooth muscle cells by oxidized LDL and cadmium. Cells were incubated for 8 h in the absence (lane 1) or presence of 100 (μg protein) ml^{-1} native (nLDL, lane 2), mildly oxidized (moxLDL, lane 3), or highly oxidized (oxLDL, lane 4) LDL or with 10 μM cadmium chloride (lane 5). LDL was oxidized for ~2 or 24 h with 5 μM $CuSO_4$ to obtain moxLDL and oxLDL, respectively [91]. HO-1 and β-actin mRNA levels were determined by Northern blot analysis [Siow & Mann, unpublished data]. (B) Time course of HO-1 mRNA induction in co-cultures of human aortic endothelial and smooth muscle cells by mildly oxidized LDL (taken from Ishikawa et al. [8]). Co-cultured cells were exposed for 0–48 h to 350 μg ml^{-1} co-culture modified LDL, prepared by prior incubation of native LDL in other co-cultures for 16 h. HO-1 and α-actin mRNA levels were determined by northern blot analysis. Data in all panels are representative of three independent experiments.

8. Future research — vascular gene transfer?

The potential for protecting vascular cells against oxidant injury in atherogenesis is highlighted by recent reports suggesting that HO-1 gene transfer confers vascular

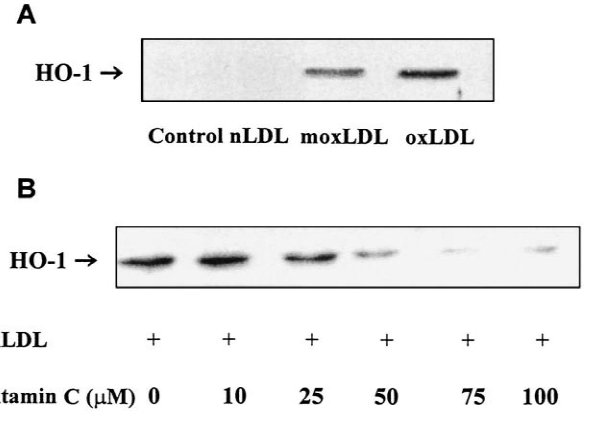

Fig. 4. Oxidized LDL induced expression of HO-1 in human umbilical artery smooth muscle cells and cytoprotective effects of vitamin C. (A) Smooth muscle cells were treated for 24 h with 100 (μg protein) ml^{-1} native (nLDL), mildly oxidized (moxLDL) or highly oxidized (oxLDL). (B) Cells were pretreated for 24 h with vitamin C (0–100 μM) and the culture medium then removed and cells incubated with 100 (μg protein) ml^{-1} oxLDL for a further 24 h. Expression of HO-1 protein was determined by Western blot analysis. Data are representative of three independent experiments (taken from [38]).

protection. The elegant studies by Abraham and co-workers [92,93] have shown that transfection of the human HO-1 gene into coronary endothelial cells attenuates the damaging effects of free heme and modulates the cell phenotype, inducing an angiogenic response to increase blood vessel formation. Transfection of HO-1 into human pulmonary epithelial cells [94] and hamster fibroblasts [95] has also been reported to increase their resistance to hyperoxic insult through the enhanced activity of HO-1. As levels of HO-1 are elevated in atherosclerotic lesions [7], enhanced angiogenesis within the atherosclerotic vessel wall may ameliorate ischaemia arising from occluded vasa vasorum [71]. Moreover, the recognition that HO-1 plays an important anti-inflammatory role [96] has important clinical implications for the treatment of a variety of inflammatory diseases.

Recent studies by Ishikawa et al. [97] have shown that induction of HO-1 expression and activity in LDL receptor knockout mice clearly inhibited the formation of atherosclerotic lesions, while Denery et al. [98] have reported that mice lacking HO-2 are more susceptible to oxygen toxicity. These reports highlight the potential of genetic manipulation of vascular genes to provide new insights into the physiological role(s) of heme oxygenases. Induction of the heme oxygenase–CO signalling pathway represents an adaptive response to oxidant injury, counteracting oxidant stress and perhaps impaired vascular relaxation through the generation of the chain-breaking antioxidants biliverdin and bilirubin and the gaseous vasodilator CO. Although the vasodilator potential of CO is significantly less than that of NO [11], the resistance of certain vascular beds, such as the hepatic circulation, is modulated by CO rather than NO [99]. As endothelium-derived NO synthesis and the sensitivity of target smooth muscle cells may be decreased in atherosclerosis, induction of the heme oxygenase–CO pathway could provide an important adaptive mechanism for moderating the severity of ischaemia, haemorrhage, thrombosis and atherosclerosis. Future studies using gene transfer approaches and transgenic animals would enable further elucidation of the protective role of heme oxygenase against atherosclerotic lesion formation.

9. Note added in proof

Since acceptance of this review, a number of important publications have further highlighted the potential anti-atherogenic properties of the vascular heme oxygenase pathway [107–110]. The most relevant has shown that expression of HO-1 is functionally associated with long term survival of cardiac xenografts [107]. In that study, the survival of rats receiving transplants from homozygous or heterozygous HO-1 deficient mice was markedly reduced. HO-1 was expressed in endothelium, smooth muscle and cardiac myocytes in transplanted hearts from wild type mice and was associated with no evidence of vascular cell injury or thrombosis. In contrast, rejected hearts from HO-1 deficient mice exhibited widespread apoptosis of vascular cells and myocytes, infiltration of host leukocytes, platelet aggregation and vascular thrombosis.

Acknowledgements

We gratefully acknowledge the financial support of the British Heart Foundation (PG/95148), Ministry of Agriculture, Fisheries and Food (ANO/413), British Council, Daiwa Anglo-Japanese Foundation and Japanese Ministry of Education, Science and Technology.

References

[1] Halliwell B. The role of oxygen radicals in human disease, with particular reference to the vascular system. Haemostasis 1993;23:118–126.
[2] Steinberg D. Low density lipoprotein oxidation and its pathobiological significance. J Biol Chem 1997;272:20963–20966.
[3] Ares M, Kallin B, Eriksson P, Nilsson J. Oxidized LDL induces transcription factor activator protein-1 but inhibits activation of nuclear factor-kappa-B in human vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 1995;15:1584–1590.
[4] Zhu Y, Lin JHC, Liao HL, et al. LDL induces transcription factor activator protein-1 in human endothelial cells. Arterioscler Thromb Vasc Biol 1998;18:473–480.
[5] Holvoet P, Collen D. Oxidized lipoproteins in atherosclerosis and thrombosis. FASEB J 1994;15:1279–1284.
[6] Sen C, Packer L. Antioxidant and redox regulation of gene-transcription. FASEB J 1996;10:709–720.
[7] Wang LJ, Lee TS, Lee FY, Pai RC, Chau LY. Expression of heme oxygenase-1 in atherosclerotic lesions. Am J Pathol 1998;152:711–720.
[8] Ishikawa K, Navab M, Leitinger N, Fogelman AM, Lusis AJ. Induction of heme oxygenase-1 inhibits the monocyte transmigration induced by mildly oxidized LDL. J Clin Invest 1997;100:1209–1216.
[9] Siow RC, Ishii T, Sato H, et al. Induction of the antioxidant stress proteins heme oxygenase-1 and MSP23 by stress agents and oxidised LDL in cultured vascular smooth muscle cells. FEBS Lett 1995;368:239–242.
[10] Marks G, Brien J, Nakatsu K, McLaughlin B. Does carbon monoxide have a physiological function? Trend Pharmacol Sci 1991;12:185–188.
[11] Maines MD. The heme oxygenase system: a regulator of second messenger gases. Annu Rev Pharmacol Toxicol 1997;37:517–554.
[12] Cox DA, Cohen ML. Effects of oxidized low-density lipoprotein on vascular contraction and relaxation: Clinical and pharmacological implications in atherosclerosis. Pharmacol Rev 1996;48:3–19.
[13] Jay MT, Chirico S, Siow RCM, et al. Modulation of vascular tone by low density lipoproteins: Effects on L-arginine transport and nitric oxide synthesis. Exp Physiol 1997;82:349–360.
[14] Padmanaban G, Venkateswar V, Rangarajan P. Haem as a multifunctional regulator. Trend Biol Sci 1989;14:492–496.
[15] McCoubrey WK, Huang TJ, Maines MD. Isolation and characterization of a cDNA from the rat brain that encodes hemoprotein heme oxygenase-3. Eur J Biochem 1997;247:725–732.
[16] Choi AMK, Alam J. Heme oxygenase-1: Function, regulation, and

implication of a novel stress-inducible protein in oxidant-induced lung injury. Am J Respir Cell Mol Biol 1996;15:9–19.
[17] Sun Y, Oberley L. Redox regulation of transcriptional activators. Free Rad Biol Med 1996;21:335–348.
[18] Terry CM, Clikeman JA, Hoidal JR, Callahan KS. Effect of tumor necrosis factor α and interleukin-1 α on heme oxygenase-1 expression in human endothelial cells. Am J Physiol 1998;274:H883–91.
[19] Prestera T, Talalay P, Alam J, Ahn YI, Lee PJ, Choi AM. Parallel induction of heme oxygenase-1 and chemoprotective phase 2 enzymes by electrophiles and antioxidants: regulation by upstream antioxidant-responsive elements (ARE). Mol Med 1995;1:827–837.
[20] Jornot L, Junod AF. Variable glutathione levels and expression of antioxidant enzymes in human endothelial cells. Am J Physiol 1993;264:L482–9.
[21] Lavrovsky Y, Schwartzman M, Levere R, Kappas A, Abraham N. Identification of binding-sites for transcription factors NF-κB and AP-2 in the promoter region of the human heme oxygenase-1 gene. Proc Natl Acad Sci USA 1994;91:5987–5991.
[22] Brand K, Page S, Walli AK, Neumeier D, Baeuerle PA. Role of nuclear factor-κB in atherogenesis. Exp Physiol 1997;82:297–304.
[23] Maziere C, DjavaheriMergny M, FreyFressart V, Delattre J, Maziere JC. Copper and cell- oxidized low-density lipoprotein induces activator protein 1 in fibroblasts, endothelial and smooth muscle cells. FEBS Lett 1997;409:351–356.
[24] Liao F, Andalibi A, Debeer F, Fogelman AM, Lusis AJ. Genetic control of inflammatory gene induction and NF-κB like transcription factor activation in response to an atherogenic diet in mice. J Clin Invest 1993;91:2572–2579.
[25] Raju VS, McCoubrey Jr. WK, Maines MD. Regulation of heme oxygenase-2 by glucocorticoids in neonatal rat brain: Characterization of a functional glucocorticoid response element. Biochim Biophys Acta 1997;1351:89–104.
[26] Zakhary R, Gaine SP, Dinerman JL, Ruat M, Flavahan NA, Snyder SH. Heme oxygenase 2: endothelial and neuronal localization and role in endothelium-dependent relaxation. Proc Natl Acad Sci USA 1996;93:795–798.
[27] Yu BP. Cellular defences against damage from reactive oxygen species. Physiol Rev 1994;74:139–162.
[28] Meister A. Glutathione-ascorbic acid antioxidant system in animals. J Biol Chem 1994;269:9397–9400.
[29] Siow RCM, Sato H, Bannai S, Leake DS, Pearson JD, Mann GE. Vitamin C protects human arterial smooth muscle cells against atherogenic lipoproteins: Effects of antioxidant vitamins on oxidized LDL-induced increases in cystine transport and glutathione. Arterioscler Thromb Vasc Biol 1998;18:1662–1670.
[30] Ghigo D, Alessio P, Foco A, et al. Nitric oxide synthesis is impaired in glutathione depleted human umbilical vein endothelial cells. Am J Physiol 1993;256:C728–C732.
[31] BasuModak S, Luscher P, Tyrrell RM. Lipid metabolite involvement in the activation of the human heme oxygenase-1 gene. Free Rad Biol Med 1996;20:887–897.
[32] Lapenna D, de Gioia S, Ciofani G, et al. Glutathione-related antioxidant defences in human atherosclerotic plaques. Circulation 1998;97:1930–1934.
[33] Vigne P, Feolde E, Ladoux A, Duval D, Frelin C. Contributions of NO synthase and heme oxygenase to cGMP formation by cytokine and hemin treated brain capillary endothelial cells. Biochem Biophys Res Commun 1995;214:1–5.
[34] Foresti R, Clark JE, Green CJ, Motterlini R. Thiol compounds interact with nitric oxide in regulating heme oxygenase-1 induction in endothelial cells. Involvement of superoxide and peroxynitrite anions. J Biol Chem 1997;272:18411–18417.
[35] Morita T, Kourembanas S. Endothelial cell expression of vasoconstrictors and growth factors is regulated by smooth muscle cell-derived carbon monoxide. J Clin Invest 1995;96:2676–2682.
[36] Morita T, Mitsialis SA, Koike H, Liu YX, Kourembanas S. Carbon monoxide controls the proliferation of hypoxic vascular smooth muscle cells. J Biol Chem 1997;272:32804–32809.
[37] Morita T, Perrella MA, Lee ME, Kourembanas S. Smooth muscle cell-derived carbon monoxide is a regulator of vascular cGMP. Proc Natl Acad Sci USA 1995;92:1475–1479.
[38] Siow RCM, Mann GE. Vitamin C attenuates induction of heme oxygenase-1 by oxidized LDL in human smooth muscle cells. J Physiol 1998;506P:37P.
[39] Durante W, Kroll MH, Christodoulides N, Peyton KJ, Schafer AI. Nitric oxide induces heme oxygenase-1 gene expression and carbon monoxide production in vascular smooth muscle cells. Circ Res 1997;80:557–564.
[40] Yet SF, Pellacani A, Patterson C, et al. Induction of heme oxygenase-1 expression in vascular smooth muscle cells. A link to endotoxic shock. J Biol Chem 1997;272:4295–4301.
[41] Ishizaka N, DeLeon H, Laursen JB, et al. Angiotensin II-induced hypertension increases heme oxygenase-1 expression in rat aorta. Circulation 1997;96:1923–1929.
[42] Hartsfield CL, Alam J, Cook JL, Choi AM. Regulation of heme oxygenase-1 gene expression in vascular smooth muscle cells by nitric oxide. Am J Physiol 1997;273:L980–L988.
[43] Paganga G, Riceevans C, Rule R, Leake D. The interaction between ruptured erythrocytes and low-density lipoproteins. FEBS Lett 1992;303:154–158.
[44] Balla G, Jacob HS, Eaton JW, Belcher JD, Vercellotti GM. Hemin: A possible physiological mediator of low density lipoprotein oxidation and endothelial injury. Arterioscler Thromb 1991;11:1700–1711.
[45] Balla J, Nath KA, Balla G, Juckett MB, Jacob HS, Vercellotti GM. Endothelial cell heme oxygenase and ferritin induction in rat lung by hemoglobin in vivo. Am J Physiol 1995;268:L321–L327.
[46] Pang J, Jiang M, Chen Y, et al. Increased ferritin gene-expression in atherosclerotic lesions. J Clin Invest 1996;97:2204–2212.
[47] Balla G, Jacob HS, Balla J, Rosenberg M, et al. Ferritin: a cytoprotective antioxidant strategem of endothelium. J Biol Chem 1992;267:18148–18153.
[48] Ishii T, Yamada M, Sato H, et al. Cloning and characterization of a 23 kDa stress induced mouse peritoneal macrophage protein. J Biol Chem 1993;268:18633–18636.
[49] Immenschuh S, Iwahara S, Satoh H, Nell C, Katz N, MullerEberhard U. Expression of the messenger RNA of heme-binding protein-23 is coordinated with that of heme oxygenase-1 by heme and heavy-metals in primary rat hepatocytes and hepatoma-cells. Biochem 1995;34:13407–13411.
[50] Chae HZ, Robison K, Poole LB, Church G, Storz G, Rhee SG. Cloning and sequencing of thiol-specific antioxidant from mammalian brain: Alkyl hydroperoxide reductase and thiol- specific antioxidant define a large family of antioxidant enzymes. Proc Natl Acad Sci USA 1994;91:7017–7021.
[51] Schmidt HHHW, Lohmann SM, Walter U. The nitric oxide and cGMP signal transduction system: Regulation and mechanism of action. Biochim Biophys Acta 1993;1178:153–175.
[52] Furchgott RF, Jothianahdan D. Endothelium-dependent and -independent vasodilation involving cyclic GMP: Relaxation induced by nitric oxide, carbon monoxide and light. Blood Vessels 1991;28:52–61.
[53] Levere RD, Martasek P, Escalante B, Schwartzman ML, Abraham NG. Effect of heme arginate administration on blood pressure in spontaneously hypertensive rats. J Clin Invest 1990;86:213–219.
[54] Johnson RA, Lavesa M, Askari B, Abraham NG, Nasjletti A. A heme oxygenase product, presumably carbon-monoxide, mediates a vasodepressor function in rats. Hypertension 1995;25:166–169.
[55] Johnson RA, Lavesa M, DeSeyn K, Scholer MJ, Nasjletti A. Heme oxygenase substrates acutely lower blood pressure in hypertensive rats. Am J Physiol 1996;271:H1132–H1138.
[56] Seki T, Naruse M, Naruse K, et al. Roles of heme oxygenase carbon monoxide system in genetically hypertensive rats. Biochem Biophys Res Commun 1997;241:574–578.
[57] Suematsu M, Goda N, Sano T, et al. Carbon monoxide: an endogenous modulator of sinusoidal tone in the perfused rat liver. J Clin Invest 1995;96:2431–2437.

[58] Goda N, Suzuki K, Naito M, et al. Distribution of heme oxygenase isoforms in rat liver. Topographic basis for carbon monoxide-mediated microvascular relaxation. J Clin Invest 1998;101:604–612.
[59] Grundemar L, Ny L. Pitfalls using metalloporphyrins in carbon monoxide research. Trend Pharmacol Sci 1997;18:193–195.
[60] Wang R, Wu LY. The chemical modification of K–Ca channels by carbon monoxide in vascular smooth muscle cells. J Biol Chem 1997;272:8222–8226.
[61] Quast U, Guillon J, Cavero I. Cellular pharmacology of potassium channel openers in vascular smooth-muscle. Cardiovasc Res 1994;28:805–810.
[62] Weisser B, Locher R, Mengden T, Vetter W. Oxidation of low density lipoprotein enhances its potential to increase intracellular calcium concentration in vascular smooth muscle cells. Arterioscler Thromb 1992;12:231–236.
[63] Motterlini R, Foresti R, Intaglietta M, Winslow RM. NO-mediated activation of heme oxygenase: endogenous cytoprotection against oxidative stress to endothelium. Am J Physiol 1996;270:H107–14.
[64] Weiss G, Wernerfelmayer G, Werner E, Grunewald K, Wachter H, Hentze M. Iron regulates nitric oxide synthase activity by controlling nuclear transcription. J Exp Med 1994;180:969–976.
[65] Darley-Usmar V, White R. Disruption of vascular signalling by the reaction of nitric oxide with superoxide: Implications for cardiovascular disease. Exp Physiol 1997;82:305–316.
[66] Buttery L, Springall D, Chester A, et al. Inducible nitric-oxide synthase is present within human atherosclerotic lesions and promotes the formation and activity of peroxynitrite. Lab Invest 1996;75:77–85.
[67] Luoma JS, Stralin P, Marklund SL, Hiltunen TP, Sarkioja T, Yla-Herttuala S. Expression of extracellular SOD and iNOS in macrophages and smooth muscle cells in human and rabbit atherosclerotic lesions. Colocalization with epitopes characteristic of oxidized LDL and peroxynitrite-modified proteins. Arterioscler Thromb Vasc Biol 1998;18:157–161.
[68] Awolesi M, Sessa W, Sumpio B. Cyclic strain up-regulates nitric-oxide synthase in cultured bovine aortic endothelial-cells. J Clin Invest 1995;96:1449–1454.
[69] Wagner C, Durante W, Christodoulides N, Hellums J, Schafer A. Hemodynamic forces induce the expression of heme oxygenase in cultured vascular smooth muscle cells. J Clin Invest 1997;100:589–596.
[70] Santilli SM, Fiegel VD, Knighton DR. Changes in the aortic wall oxygen tensions of hypertensive rabbits. Hypertension and aortic wall oxygen. Hypertension 1992;19:33–39.
[71] Barker S, Talbert A, Cottam S, Baskerville P, Martin J. Arterial intimal hyperplasia after occlusion of the adventitial vasa vasorum in the pig. Arterioscler Thromb 1993;13:70–77.
[72] Maulik N, Sharma HS, Das DK. Induction of the haem oxygenase gene expression during the reperfusion of ischemic rat myocardium. J Mol Cell Cardiol 1996;28:1261–1270.
[73] Sharma HS, Maulik N, Gho BC, Das DK, Verdouw PD. Coordinated expression of heme oxygenase-1 and ubiquitin in the porcine heart subjected to ischemia and reperfusion. Mol Cell Biochem 1996;157:111–116.
[74] Kourembanas S, Morita T, Liu Y, Christou H. Mechanisms by which oxygen regulates gene expression and cell–cell interaction in the vasculature. Kidney Int 1997;51:438–443.
[75] Lee PJ, Jiang BH, Chin BY, et al. Hypoxia-inducible factor-1 mediates transcriptional activation of the heme oxygenase-1 gene in response to hypoxia. J Biol Chem 1997;272:5375–5381.
[76] Wang GL, Semenza GL. Purification and characterization of hypoxia-inducible factor 1. J Biol Chem 1995;270:1230–1237.
[77] Koong A, Chen E, Giaccia A. Hypoxia causes the activation of nuclear factor-κB through the phosphorylation of i-κB-α on tyrosine residues. Cancer Res 1994;54:1425–1430.
[78] Kvietikova I, Wenger RH, Marti HH, Gassmann M. The hypoxia-inducible factor-1 DNA recognition site is cAMP-responsive. Kidney Int 1997;51:564–566.
[79] Durante W, Christodoulides N, Cheng K, Peyton KJ, Sunahara RK, Schafer AI. cAMP induces heme oxygenase-1 gene expression and carbon monoxide production in vascular smooth muscle. Am J Physiol 1997;273:H317–23.
[80] Imai T, Hirata Y, Kanno K, Marumo F. Induction of nitric oxide synthase by cyclic AMP in rat vascular smooth-muscle cells. J Clin Invest 1994;93:543–549.
[81] McQuillan L, Leung G, Marsden P, Kostyk S, Kourembanas S. Hypoxia inhibits expression of eNOS via transcriptional and post-transcriptional mechanisms. Am J Physiol 1994;36:H1921–H1927.
[82] Ross R. The pathogenesis of atherosclerosis: A perspective for the 1990s. Nature 1993;362:801–809.
[83] Madhavan M, Wattigney WA, Srinivasan SR, Berenson GS. Serum bilirubin distribution and its relation to cardiovascular risk in children and young adults. Atherosclerosis 1997;131:107–113.
[84] Schwertner HA, Jackson WG, Tolan G. Association of low serum concentration of bilirubin with increased risk of coronary artery disease. Clin Chem 1994;40:18–23.
[85] Hopkins PN, Wu LL, Hunt SC, James BC, Vincent GM, Williams RR. Higher serum bilirubin is associated with decreased risk for early familial coronary artery disease. Arterioscler Thromb Vasc Biol 1996;16:250–255.
[86] Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN. Bilirubin is an antioxidant of possible physiological importance. Science 1987;235:1043–1046.
[87] Neuzil J, Stocker R. Free and albumin-bound bilirubin are efficient co-antioxidants for α-tocopherol, inhibiting plasma and low density lipoprotein lipid peroxidation. J Biol Chem 1994;269:16712–16719.
[88] Yamaguchi T, Horio F, Hashizume T, et al. Bilirubin is oxidized in rats treated with endotoxin and acts as a physiological antioxidant synergistically with ascorbic acid in vivo. Biochem Biophys Res Commun 1995;214:11–19.
[89] Amit Y, Boneh A. Bilirubin inhibits protein kinase C activity and protein kinase C-mediated phosphorylation of endogenous substrates in human skin fibroblasts. Clin Chim Acta 1993;223:103–111.
[90] MietusSnyder M, Friera A, Glass CK, Pitas RE. Regulation of scavenger receptor expression in smooth muscle cells by protein kinase C-A role for oxidative stress. Arterioscler Thromb Vasc Biol 1997;17:969–978.
[91] Rice-Evans C, Leake D, Bruckdorfer R, Diplock AT. Practical approaches to low density lipoprotein oxidation: Whys, wherefores and pitfalls. Free Rad Res 1996;25:285–311.
[92] Abraham NG, Lavrovsky Y, Schwartzman ML, et al. Transfection of the human heme oxygenase gene into rabbit coronary microvessel endothelial cells: protective effect against heme and hemoglobin toxicity. Proc Natl Acad Sci USA 1995;92:6798–6802.
[93] Deramaudt BM, Braunstein S, Remy P, Abraham NG. Gene transfer of human heme oxygenase into coronary endothelial cells potentially promotes angiogenesis. J Cell Biochem 1998;68:121–127.
[94] Lee P, Alam J, Wiegand G, Choi A. Overexpression of heme oxygenase-1 in human pulmonary epithelial-cells results in cell-growth arrest and increased resistance to hyperoxia. Proc Natl Acad Sci USA 1996;93:10393–10398.
[95] Dennery P, Sridhar K, Lee C, et al. Heme oxygenase-mediated resistance to oxygen toxicity in hamster fibroblasts. J Biol Chem 1997;272:14937–14942.
[96] Willis D, Moore AR, Frederick R, Willoughby DA. Heme oxygenase: A novel target for the modulation of the inflammatory response. Nature Med 1996;2:87–90.
[97] Ishikawa K, Wang XP, Qiao JH, Navab M, Fogelman AM, Lusis AJ. Heme oxygenase-1 inhibits atherosclerotic lesion formation in LDL receptor knockout mice. Circulation 1997;8SS:619.
[98] Dennery PA, Spitz DR, Yang G, et al. Oxygen toxicity and iron accumulation in the lungs of mice lacking heme oxygenase-2. J Clin Invest 1998;101:1001–1011.
[99] Suematsu M, Wakabayashi Y, Ishimura Y. Gaseous monoxides: a new class of microvascular regulator in the liver. Cardiovasc Res 1996;32:679–686.

[100] Motterlini R, Foresti R, Vandegriff K, Intaglietta M, Winslow RM. Oxidative-stress response in vascular endothelial cells exposed to acellular hemoglobin solutions. Am J Physiol 1995;269:H648–55.
[101] Visner GA, Fogg S, Nick HS. Hyperoxia-responsive proteins in rat pulmonary microvascular endothelial cells. Am J Physiol 1996;270:L517–25.
[102] Wagener F, Feldman E, de Witte T, Abraham N. Heme induces the expression of adhesion molecules ICAM-1, VCAM-1, and E selectin in vascular endothelial cells. Proc Soc Exp Biol Med 1997;216:456–463.
[103] Ishizaka N, Griendling KK. Heme oxygenase-1 is regulated by angiotensin II in rat vascular smooth muscle cells. Hypertension 1997;29:790–795.
[104] Agarwal A, Balla J, Balla G, Croatt A, Vercellotti G, Nath K. Renal tubular epithelial cells mimic endothelial cells upon exposure to oxidized LDL. Am J Physiol 1996;271:F814–F823.
[105] Grozdanovic Z, Gossrau R. Expression of heme oxygenase-2 (HO-2)-like immunoreactivity in rat tissues. Acta Histochem 1996;98:203–214.
[106] Christodoulides N, Durante W, Kroll MH, Schafer AI. Vascular smooth muscle cell heme oxygenases generate guanylyl cyclase-stimulatory carbon monoxide. Circulation 1995;91:2306–2309.
[107] Soares MP, Lin Y, Anrather J, Csizmadia E, Takigami K, Sato K, Grey ST, Colvin RB, Choi AM, Poss KD, Bach FH. Expression of heme oxygenase-1 can determine cardiac xenograft survival. Nature Med 1998;4:1073–1077.
[108] Pannen BHJ, Kohler N, Hole B, Bauer M, Clemens MG, Geiger KK. Protective role of endogenous carbon monoxide in hepatic microcirculatory dysfunction after hemorrhagic shock in rats. J Clin Invest 1998;102:1220–1228.
[109] Motterlini R, Gonzales A, Foresti R, Clark JE, Green CJ, Winslow RM. Heme oxygenase-1 derived carbon monoxide contributes to the suppression of acute hypertensive responses in vivo. Circ Res 1998;83:568–577.
[110] Juckett M, Zheng Y, Yuan H, Pastor T, Antholine W, Weber M, Vercellotti G. Heme and the endothelium. Effects of nitric oxide on catalytic iron and heme degradation by heme oxygenase. J Biol Chem 1998;273:23388–23397.