Review

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

Richard C.M. Siow, Hideyo Sato¹, 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 (Mw ~34 000) and an inducible isoform HO-1 (Mw ~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. © 1999 Elsevier Science B.V. All rights reserved.

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

---

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

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

0008-6363/99/$ – see front matter © 1999 Elsevier Science B.V. All rights reserved.

PII: S0008-6363(98)00278-8

Time for primary review 27 days.
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 (SiRE) [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 expres-
sion. 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

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

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 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 |
|---|---|---|
| **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] |
|          |                    | 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 | Vugler et al. (1996) [101] |
| **HO-1** | Rat brain capillary | Hemin | Vugler 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] |
| **HO-2** | 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

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Species/vessel</th>
<th>Inducing agent</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>HO-1</td>
<td>Human and marine aorta in situ</td>
<td>Atherosclerotic lesion</td>
<td>Wang et al. (1998) [7]</td>
</tr>
<tr>
<td>HO-1</td>
<td>Human aorta</td>
<td>Oxidized LDL, Hemin</td>
<td>Ishikawa et al. (1997) [8]</td>
</tr>
<tr>
<td>HO-1</td>
<td>Human umbilical artery</td>
<td>Oxidized LDL, cadmium</td>
<td>Siow and Mann (1998) [38]</td>
</tr>
<tr>
<td>HO-1</td>
<td>Porcine aorta</td>
<td>Oxidized LDL, cadmium, glucose oxidase, diethylmaleate</td>
<td>Siow et al. (1995) [9]</td>
</tr>
<tr>
<td>HO-1</td>
<td>Rat aorta</td>
<td>Hypoxia</td>
<td>Morita et al. (1995) [37]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hemin, Arsenite</td>
<td>Christodoulides et al. (1995) [106]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nitric oxide donors</td>
<td>Hartfield et al. (1997) [42]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NO donors and cytokines</td>
<td>Durante et al. (1997) [39]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H$_2$O$_2$, hemin</td>
<td>Ishizaka and Griendling (1997) [103]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Interleukin-1β</td>
<td>Yet et al. (1997) [40]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Shear stress</td>
<td>Wagner et al. (1995) [69]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cyclic AMP</td>
<td>Durante et al. (1997) [79]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Angiotensin II</td>
<td>Ishizaka and Griendling (1997) [103]</td>
</tr>
<tr>
<td>HO-1</td>
<td>Rat aorta in situ</td>
<td>Angiotensin II, LPS</td>
<td>Ishizaka et al. (1997) [41]</td>
</tr>
<tr>
<td>HO-2</td>
<td>Rat aorta</td>
<td>Interleukin-1β</td>
<td>Yet et al. (1997) [40]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hemin, Arsenite</td>
<td>Christodoulides et al. (1995) [106]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cyclic AMP</td>
<td>Durante et al. (1997) [79]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nitric oxide donors</td>
<td>Durante et al. (1997) [39]</td>
</tr>
<tr>
<td>HO-2</td>
<td>Rat aorta in situ</td>
<td>Basal expression</td>
<td>Grozdanovic and Gossrau (1996) [105]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Angiotensin II</td>
<td>Ishizaka et al. (1997) [41]</td>
</tr>
</tbody>
</table>

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 NO activity, increased hepatic vascular resistance, which was reversed by exogenous CO.
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 1-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 hyperpolarise smooth muscle cells. 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.

6. Regulation of heme oxygenase by hypoxia

Arterial wall hypoxia resulting from hypertension [70] and through the occlusion of the vas 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-\(\kappa\)B 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 cytoprotective 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.

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...
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 hypoxic 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 antiatherogenic 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

[16] Choi AMK, Alam J. Heme oxygenase-1: Function, regulation, and


