Acta Biochimica Polonica

Vol. 52 No. 2/2005, 273–284

on line at www.actabp.pl

Review

Heme oxygenase-1 expression in disease states✪

Jessy Deshane, Marcienne Wright and Anupam Agarwal✉

Department of Medicine, Nephrology Research and Training Center, Division of Nephrology, University of Alabama at Birmingham, Birmingham, USA; ✉e-mail: agarwal@uab.edu

Received: 05 April, 2005; revised: 07 May, 2005; accepted: 11 May, 2005
available on line: 31 May, 2005

Heme oxygenase-1 (HO-1) is an enzyme which catalyzes the rate-limiting step in heme degradation resulting in the formation of iron, carbon monoxide and biliverdin, which is subsequently converted to bilirubin by biliverdin reductase. The biological effects exerted by the products of this enzymatic reaction have gained much attention. The anti-oxidant, anti-inflammatory and cytoprotective functions associated with HO-1 are attributable to one or more of its degradation products. Induction of HO-1 occurs as an adaptive and beneficial response to several injurious stimuli including heme and this inducible nature of HO-1 signifies its importance in several pathophysiological disease states. The beneficial role of HO-1 has been implicated in several clinically relevant disease states involving multiple organ systems as well as significant biological processes such as ischemia-reperfusion injury, inflammation/immune dysfunction and transplantation. HO-1 has thus emerged as a key target molecule with therapeutic implications.

Keywords: heme oxygenase-1, heme, cytoprotection, disease, polymorphisms

ENZYMATIC REACTION CATALYZED BY HEME OXYGENASE-1

Heme oxygenase is the rate limiting enzyme in the degradation of heme and results in the release of equimolar quantities of biliverdin, iron and carbon monoxide (CO) (Fig 1.) (Maines, 1997). Biliverdin reductase subsequently converts biliverdin to bilirubin. Amongst the two reported isoforms of heme oxygenase, HO-1 is the highly inducible enzyme by heme and various other stimuli including oxidative stress (Alam *et al.*, 1989; Camhi *et al.*, 1995; Durante *et al.*, 1997; Agarwal *et al.*, 1998; Camhi *et al.*, 1998; Alam *et al.*, 2000; Alcaraz *et al.*, 2001; Sikorski *et al.*, 2004). HO-2 is the constitutively expressed isoform. A third isoform HO-3 has also been described (McCoubrey *et al.*, 1997) but has recently been shown to be a pseudogene (Hayashi *et al.*, 2004). Although 45% amino-acid homology exists between HO-1 and HO-2, (Maines, 1997) they are differentially regulated and expressed in tissues. HO-1 is ubiquitously induced in mammalian tissues and is localized to the endoplasmic reticulum, while HO-2 is constitutively expressed in the brain, testes, endothelium, distal nephron segments, liver and myenteric plexus of the gut with subcellular localization in the mitochondria (reviewed in Agarwal & Nick, 2000). Recent studies have suggested that HO-1 is also present in caveoli (Jung *et al.*, 2003; Kim *et al.*, 2004). HO-1 plays a cytoprotective role in modulating tissue responses to injury in several pathophysiological states. HO-2, on the other hand, functions as a physiological regulator of cellular function (Wagener *et al.*, 1999).

The protective effects resulting from HO-1 activity are due to its inducibility by a variety of stimuli including heme, nitric oxide (NO), cadmium, growth factors, hyperoxia and others resulting in the liberation of its reaction products, which exert several biological effects including anti-oxidant, anti-inflammatory and anti-apoptotic properties (Choi & Alam, 1996; Platt & Nath, 1998; Nath, 1999; Dong *et al.*, 2000; Ryter & Choi, 2002; Otterbein *et al.*, 2003). The mechanisms underlying the beneficial effects of HO-1 and the role of the individual reaction products in mediating these cytoprotective properties

✪Presented at the XXXII Winter School, 3–7 March 2005, Zakopane, Poland.

Abbreviations: apoE, apolipoprotein E; CoPP, cobalt protoporphyrin; DOCA, deoxycorticosterone acetate; DS, Dahl salt sensitive; HO, heme oxygenase; HO-1, 2, 3, heme oxygenase-1, 2, 3; 13-HPODE, 13-hydroperoxy-9,11-octadecadienoic acid; ICAM-1, intercellular adhesion molecule-1; IL-1, 10, 13, interleukin-1, 10, 13; IUGR, intrauterine growth retardation; LPS, lipopolysaccharide; Nrf-2, NF-E2 related factor-2; Ox-LDL, oxidized low density lipoprotein; SHR, spontaneously hypertensive rat; SnPP, tin protoporphyrin; TNF-α, tumor necrosis factor-α; VCAM-1, vascular cell adhesion molecule-1; ZnPP, zinc protoporphyrin.

have been recently reviewed (Tomaro & Batlle, 2002; Kapitulnik, 2004; Ryter & Otterbein, 2004). The focus of this article is to provide a comprehensive review of the current literature on the functional role of HO-1 gene expression in different disease states. The molecular regulation of HO-1 gene expression has been reviewed elsewhere (Sikorski *et al*., 2004).

DISEASES ASSOCIATED WITH HO-1

The expression of HO-1 has been implicated in several disease states including atherosclerosis, hypertension, transplant rejection, acute renal injury hyperoxia and hypoxia-induced lung injury, cancer, as well as others (Table 1). More importantly, the expression of HO-1 modulates several critical biological processes such as ischemia-reperfusion injury, inflammation/immune dysfunction and transplantation in multiple organ systems.

HO-1 IN ISCHEMIA-REPERFUSION INJURY

Ischemia and reperfusion constitute a major mechanism of organ failure and tissue injury. HO-1 has been associated with a tissue protective role in ischemia-reperfusion injury in the heart, kidney, liver, brain and lung. One possible mechanism for this cytoprotection is perhaps by the modulation of the pro- and anti-apoptotic pathways by HO-1 (Tsuchihashi *et al*., 2004). Pachori and colleagues have shown that an adenoviral vector system containing the erythropoietin hypoxia response element for ischemia-regulated expression of the human HO-1 gene, conferred tissue protection in the heart, liver and skeletal muscle (Pachori *et al*., 2004). Both CO and bilirubin have been reported to mediate the protective effects of HO-1 expression in ischemia-reperfusion injury. Inhalation of CO is protective in

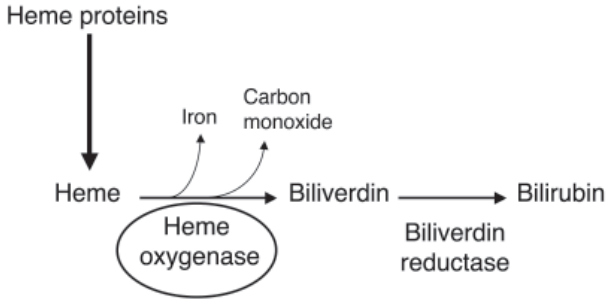

Figure 1. Schematic of the heme oxygenase catalyzed reaction.

Heme is cleaved by heme oxygenase to generate equimolar quantities of iron, carbon monoxide and biliverdin. Biliverdin is then converted by biliverdin reductase to bilirubin.

ischemia-reperfusion injury in the heart, lung, kidney and liver (Fujita *et al*., 2001; Nakao *et al*., 2003; Neto *et al*., 2004; Nakao *et al*., 2005). Studies using exogenous bilirubin have also shown that the protective effects of HO-1 activity in ischemia-reperfusion injury in the heart, liver and kidney are mediated through bilirubin (Clark *et al*., 2000; Kato *et al*., 2003; Adin *et al*., 2005). Thus, HO-1 expression serves as a protective response in ischemia-reperfusion, effects mediated *via* CO and/or bilirubin.

HO-1 IN INFLAMMATION

HO-1 plays an important role in the inflammatory response (Willis *et al*., 1996; Yet *et al*., 1997; Wang *et al*., 1998; Otterbein *et al*., 1999b; Ishikawa *et al*., 2001b; Kapturczak *et al*., 2004). The beneficial effects of HO-1 in inflammation were first reported by Willis and colleagues in a model of pleural inflammation (Willis *et al*., 1996). Inhibition of HO-1 using tin protoporphyrin (SnPP), significantly increased inflammatory infiltrate, while prior induction with hemin resulted in a significant reduction of inflammation suggesting that HO-1 activity modulates the inflammatory response. Similar findings have been reported in other models of inflammation as well (Vogt *et al*., 1996; Siow *et al*., 1999). Vogt and co-workers demonstrated a novel phenomenon of acquired resistance to renal tubular injury in glomerular inflammation that was dependent on the induction of HO-1 in renal tubules (Vogt *et al*., 1996). Induction of HO-1 by its inducer hemin has been shown to reduce inflammation of the gut and decreases mucosal injury in an animal model of small bowel ischemia (Attuwaybi *et al*., 2004).

The importance of HO-1 in inflammation is supported by findings in HO-1 knockout mice and the human HO-1 deficient child, both exhibiting a pro-inflammatory phenotype (Poss & Tonegawa, 1997a; 1997b; Yachie *et al*., 1999; Kapturczak *et al*., 2004). In addition, several pro-inflammatory mediators are activated in HO-1 deficiency (Kapturczak *et al*., 2004) and overexpression of HO-1 or its byproducts are anti-inflammatory. Furthermore, anti-inflammatory mediators such as IL-10 have been shown to confer protection through upregulation of HO-1 in a murine model of sepsis (Lee & Chau, 2002). IL-13, an immunoregulatory cytokine that is a key mediator in allergic inflammation, has also been shown to induce HO-1 (Ke *et al*., 2002). Similar to the effects of IL-10 in sepsis, HO-1 induction has been suggested to mediate the effects of IL-13 *in vivo* in rat cardiac allografts (Ke *et al*., 2002).

Although preinduction of HO-1 inhibits inflammation, pro-inflammatory mediators like TNF-α, IL-1, LPS and oxidized lipids are potent inducers of HO-1 expression in endothelial cells and macro-

phages (Camhi *et al.*, 1998; Terry *et al.*, 1999; Wagener *et al.*, 2003; Chen & Kunsch, 2004). In addition, several adhesion molecules that are key mediators of inflammation such as ICAM-1, VCAM-1 and selectins are activated by inducers of HO-1 (Wagener *et al.*, 1997; Soares *et al.*, 2004). In the context of vascular disorders and transplant rejection, activation, survival or apoptosis and differentiation of monocytes are crucial factors which determine fate of the disease. Recent studies by Lang and colleagues have demonstrated that the dose and time dependent induction of HO-1 by hemin inhibited apoptosis in monocytes despite the upregulation of caspase-3 pathways (Lang *et al.*, 2005). HO-1 induction has also been shown to inhibit microvascular endothelial cell leukocyte adhesion through the action of its metabolites, bilirubin and CO (Morisaki *et al.*, 2002; Zampetaki *et al.*, 2003; Keshavan *et al.*, 2005).

TRANSPLANTATION

Perhaps the most significant area that has generated research interest involving HO-1 is in the field of transplantation. HO-1 is induced in several models of acute transplant rejection and localizes predominantly to infiltrating cells (Agarwal *et al.*, 1996b; Avihingsanon *et al.*, 2002; Souza *et al.*, 2005). Such induction is functionally relevant, since the absence of HO-1 leads to accelerated graft rejection in cardiac allo- and xenotransplantation (Soares *et al.*, 1998; Holweg *et al.*, 2004). The functional significance of HO-1 in transplantation has been corroborated in other organ transplant models as well. In addition to transplant rejection, HO-1 induction also attenuates ischemia/reperfusion injury that affects donor organ quality and subsequent transplantation (Amersi *et al.*, 1999; Nath, 1999). Amersi and colleagues have demonstrated that overexpression of HO-1, using either cobalt protoporphyrin (CoPP) or adenoviral HO-1 gene transfer attenuated ischemia-reperfusion injury and prolonged survival, following cold ischemia/isotransplantation of livers (Amersi *et al.*, 1999). A recent study evaluating the effect of HO-1 upregulation showed that peritransplant upregulation of HO-1 by administration of CoPP significantly attenuated chronic rejection of renal allografts (Bedard *et al.*, 2005).

HO-1 and its byproduct CO prevent ischemia-reperfusion injury associated with heart transplantation (Sato *et al.*, 2001; Beltowski *et al.*, 2004; Braudeau *et al.*, 2004). Akamatsu and co-authors have shown that exposure of the donor and the graft to CO confers a protective effect in cardiac transplant associated ischemia-reperfusion injury. In addition CO (250 ppm) improves function of renal grafts and imparts significant protective effects against renal ischemia-reperfusion injury (Akamatsu *et al.*, 2004; Neto *et al.*, 2004a). RDP1258, a novel peptide derived from the HLA class I heavy chain, has been shown to possess immunoregulatory function *via* modulation of HO-1 enzyme activity (Cuturi *et al.*, 1999; Magee *et al.*, 1999). These recent developments provide new therapeutic approaches in the overall success of organ transplantation and prolongation of graft survival.

ATHEROSCLEROSIS

The expression of HO-1 in atherosclerosis is a protective response. This is supported by the following findings. First, an abundance of HO-1 (mRNA and protein) has been identified in human atherosclerotic plaques, providing *in vivo* relevance to this enzyme in atherosclerosis (Wang *et al.*, 1998). Increased HO-1 expression is also present in advanced lesions in animal models of atherosclerosis (Wang *et al.*, 1998). Secondly, overexpression of HO-1 in the vasculature in apolipoprotein E (apoE)-deficient mice attenuates the development of atherosclerosis (Juan *et al.*, 2001). Thirdly, inhibition of HO enzyme

Table 1. Disease states associated with heme oxygenase-1

General	
	Ischemia reperfusion
	Inflammation
	Immune dysfunction
	Transplantation
Specific diseases	
Cardiovascular	
	Myocardial infarction
	Atherosclerosis
	Hypertension
	Vascular restenosis
Kidney	
	Acute renal failure
	Glomerulonephritis
	Diabetic kidney disease
	Polycystic kidney disease
	Sickle cell renal disease
Lung	
	Hypoxia and hyperoxia induced lung injury
	Emphysema
	Pleuritis
	Asthma
Liver	
	Sepsis
	Cirrhosis
Nervous system	
	Spinal cord injury
	Cerebrovascular accident
	Alzheimer's disease
Pancreas	
	Acute pancreatitis
Others	
	Pre-eclampsia and intrauterine growth retardation
	Cancer
	Iron-related disorders
	Keratitis
	Retinopathy of prematurity
	Acquired immunodeficiency syndrome

activity in Watanabe heritable hyperlipidemic rabbits leads to accelerated atherosclerosis (Ishikawa *et al.*, 2001a). Hoekstra and coworkers have also reported that differences in susceptibility to atherosclerosis between resistant and susceptible strains of Japanese quail may be due to differences in endothelial HO and anti-oxidant components (Hoekstra *et al.*, 2003). Fourth, transgenic mice deficient in HO-1 in an apoE knockout background develop significantly more atherosclerosis compared to wild-type mice (Yet *et al.*, 2003). Finally, atherogenic lipoproteins like oxidized LDL that have been implicated in the pathogenesis of atherosclerosis (Shi *et al.*, 2000; Furnkranz *et al.*, 2005) are potent inducers of HO-1 in vascular cells and renal tubular epithelial cells (Agarwal *et al.*, 1996a). More importantly, oxidized LDL-mediated HO-1 induction inhibits monocyte chemotaxis (Ishikawa *et al.*, 1997), a key inflammatory event in the pathogenesis of atherosclerosis.

The major stimulus for the induction of HO-1 in atherosclerotic plaques is oxidized LDL (Agarwal *et al.*, 1996a; Ishikawa *et al.*, 1997) and more specifically, its fatty acid component, linoleyl hydroperoxide (Agarwal *et al.*, 1998). 13-HPODE, one of the major components of oxidized LDL induces HO-1 *via* transcriptional mechanisms (Agarwal *et al.*, 1998). Our laboratory has identified a distal *cis*-acting region in the human HO-1 promoter that regulates this response in human aortic endothelial cells (Hill-Kapturczak *et al.*, 2003). Studies to further delineate this region are in progress. In murine macrophages, OxLDL causes nuclear accumulation of Nrf2, which in turn activates HO-1 (Ishii *et al.*, 2004). Bach-1 has recently been identified as a potential transcriptional repressor for HO-1. Although HO-1 has been implicated in the protective response against atherosclerosis, the functional role of Bach-1 in modulating this response is not well understood. In a recent study involving cuff injury in Bach-1 deficient mice, Bach-1 was shown to play a critical role in the regulation of HO-1 expression, macrophage function, smooth muscle cell proliferation and neointima formation (Omura *et al.*, 2005). In smooth muscle cells derived from Bach-1 deficient mice, HO-1 expression was increased and associated with decreased proliferation compared with wild type cells (Omura *et al.*, 2005). Thus during inflammation or atherogenesis, Bach-1 may regulate HO-1 gene expression and this hypothesis requires further investigation.

VASCULAR RESTENOSIS AND OTHER CARDIOVASCULAR DISEASES

Several lines of evidence suggest that upregulation of HO-1 may be an important protective factor after balloon angioplasty in cardiovascular diseases such as vascular restenosis (Ishikawa, 2003; Schillinger *et al.*, 2004). Prior induction of HO-1 by chemical and genetic manipulation attenuates vascular neointimal proliferation following balloon injury, while inhibition of HO enzyme activity, leads to worsening of the lesion (Aizawa *et al.*, 1999; Tulis *et al.*, 2001a; 2001b). HO-1 knockout mice demonstrate exaggerated vascular neointimal proliferation following wire-induced injury (Duckers *et al.*, 2001). In recent work, Visner and coworkers have suggested that the antiproliferative effects of rapamycin in vascular smooth muscle cells are mediated through the induction of HO-1 (Visner *et al.*, 2003). Rapamycin-coated stents have been used to prevent restenosis following angioplasty and these findings implicate HO-1 as the underlying mechanism for the beneficial effects of rapamycin in vascular injury.

Recent studies focusing on a (GT)n repeat region in the proximal human HO-1 promoter have yielded interesting results in vascular restenosis. A study investigating the association of length polymorphisms of the human HO-1 promoter and peripheral vascular restenosis showed significantly reduced level of inflammation following balloon angioplasty in patients with short (GT)n repeats (<25) when compared to longer (GT)n repeats (reviewed in Exner *et al.*, 2004; Schillinger *et al.*, 2004). These findings were confirmed in coronary artery restenosis wherein the carriers of longer (GT)n repeats had a 3.74 fold higher risk for restenosis compared with those with shorter (GT)n repeats. Significant association was also observed between HO-1 (GT)n polymorphisms and abdominal aortic aneurysms (Schillinger *et al.*, 2002). On the other hand, no association has been found between HO-1 (GT)n repeat polymorphism and Kawasaki disease and systemic vasculitis in Japanese children (Kanai *et al.*, 2003).

RENAL DISEASES

Studies utilizing chemical inducers and inhibitors as well as HO-1 knockout mice have shown that the expression of HO-1 is cytoprotective in heme and non-heme mediated models of renal injury (Nath *et al.*, 1992; Agarwal *et al.*, 1995; Shiraishi *et al.*, 2000). A detailed review of this area is summarized in a recent article from our group (Hill-Kapturczak *et al.*, 2002).

HYPOXIA/HYPEROXIA LUNG INJURY AND EMPHYSEMA

HO-1 is protective in both hyperoxia as well as hypoxia-induced lung injury (Choi & Alam, 1996; Taylor *et al.*, 1998; Otterbein *et al.*, 1999a; Christou *et al.*, 2000; Zampetaki *et al.*, 2003). The generation of CO appears to be the mechanism involved in these

models since exogenous administration of CO protects against lung injury (Otterbein *et al.*, 1999b), results that are similar to HO-1 gene delivery studies (Otterbein *et al.*, 1999a). In mouse lung ischemia-reperfusion injury models as well as primary rat pulmonary artery endothelial cells, overexpression of HO-1 attenuates apoptosis and knockdown of HO-1 by siRNA in endothelial cells increases anoxia-reoxygenation induced apoptosis (Zhang *et al.*, 2004).

Studies carried out in patients with emphysema (Yamada *et al.*, 2000) suggests that long (GT)n repeats reduces HO-1 inducibility in response to smoking and thus perhaps a much higher risk for development of chronic obstructive pulmonary disease. On the contrary, a study constituting 621 smokers found no link between HO-1 promoter genotype and loss of lung function (He *et al.*, 2002).

PRE-ECLAMPSIA AND INTRA-UTERINE GROWTH RETARDATION

Endothelial oxidative stress plays a significant role in the pathophysiology of preeclampsia, a hypertensive disorder in pregnancy (Lum & Roebuck, 2001). Critical inflammatory processes like increased leukocyte-endothelial interaction/endothelial dysfunction, associated upregulation of cellular adhesion molecules and endothelial permeability by reactive oxygen species are involved in the development of this condition. HO-1 has been proposed to be involved in these processes. In a study investigating the effect of HO-1 activation on TNF-α induced placental damage and feto-placental circulation, induction of HO-1 significantly attenuated the inflammatory response mediated cellular damage in placental villous explants (Ahmed *et al.*, 2000). Recent studies have also shown that large amounts of peroxynitrite are generated in the maternal vasculature (Zhao *et al.*, 2004) suggesting a possible role for peroxynitrite in the pathogenesis in preeclampsia. Endothelial oxidative stress induced by peroxynitrite upregulated adhesion molecule expression and induced HO-1. Treatment of endothelial cells with either peroxynitrite scavenger or HO-1 inhibitor abolished the increased expression of adhesion molecules. Therefore, the modulation of expression of adhesion molecules may be mediated by HO-1 regulation (Zhao *et al.*, 2004).

Damage to the endothelium and impaired microvascularization are commonly linked with recurrent miscarriages. HO-1 protein levels were significantly lower in placentae from cases with preeclampsia, compared with gestationally matched normal pregnancies (Lash *et al.*, 2003). A study investigating 162 women with recurrent miscarriages compared to a group of postmenopausal healthy women showed a significant association between HO-1 (GT)n repeat polymorphisms and incidence of miscarriages (Denschlag *et al.*, 2004). In pregnant women who had a fetus with IUGR, levels of HO-1 expression in placental trophoblasts were significantly reduced when compared to a group of normal pregnant women (Wang & Yu, 2002). On the contrary, an earlier study trying to correlate expression of HO-1 and HO-2 to preeclampsia and fetal growth restriction, showed that reduced expression of HO-2 in endothelial cells under these abnormal conditions may be responsible for reduced placental blood flow (Barber *et al.*, 2001). However, no significant difference in HO-1 expression levels was noted in endothelial cells and in the placental bed in preeclampsia or fetal growth restriction. McLaughlin and coauthors on the other hand, have found increased HO-1 expression in chorionic villi and fetal membranes from preeclamptic pregnancies compared to normotensive controls (McLaughlin *et al.*, 2003).

CO, one of the products of heme degradation by HO-1, has been considered as a vascular relaxant (McFaul & McGrath, 1987). Studies of inhibition of HO-1 in isolated perfused placentae showed increase in placental perfusion pressure suggesting that CO levels are perhaps crucial for maintenance of blood flow in the placenta which is of vital importance for a healthy pregnancy (Lyall *et al.*, 2000). In preconstricted placental arteries, hemin reduced vascular tension significantly and hemin induced vascular relaxation as well as production of CO, was inhibited by SnPP (Ahmed *et al.*, 2000) suggesting a role for HO-1 as an endogenous placental factor conferring cytoprotection and placental blood vessel relaxation.

HYPERTENSION

Johnson and coworkers demonstrated in Dahl salt sensitive (DS) rats, that coronary arterial HO-1 expression was increased with salt induced hypertension, and cardioprotection was provided by promoting coronary vasodilation (Johnson *et al.*, 2004b). On the other hand, endothelium dependent vasodilator responses were attenuated in arterioles from another severely salt sensitive model of hypertension, deoxycorticosterone acetate (DOCA) rats and not in the spontaneously hypertensive (SHR) rat model (Johnson *et al.*, 2004a). Using an inhibitor, which abolishes endogenous CO production, they show data which suggests that DOCA-salt hypertension is associated with increased generation of endogenous CO which may play a role in endothelial dysfunction. Yang and coauthors have demonstrated that overexpression of HO-1 leads to a reduction in pressor responsiveness to angiotensin II (Yang *et al.*, 2004). This is most likely due to the increased generation of one of the HO-1 metabolites, presumably CO, which has the ability to inhibit vascular reactivity to constrictor stimuli. Several studies have

documented the induction of vascular, cardiac and renal HO-1 in response to angiotensin II both *in vitro* and *in vivo* (Aizawa *et al.*, 2000; Haugen *et al.*, 2000; Das *et al.*, 2004). Motterlini and coworkers have also shown previously that HO-1 derived CO plays a role in the suppression of an acute hypertensive response *in vivo* (Motterlini *et al.*, 1998).

DIABETES

Oxidative stress and generation of reactive oxygen species, specifically superoxide anion has been implicated in the cardiovascular complications seen in patients with diabetes (Giugliano *et al.*, 1995; Mohamed *et al.*, 1999). Hyperglycemia has been shown to mediate endothelial dysfunction, delayed cell replication and enhanced apoptosis (Lorenzi *et al.*, 1987; Baumgartner-Parzer *et al.*, 1995; Zou *et al.*, 2002). These events seem to be reversible by increased expression of anti-oxidant enzymes such as HO-1 (Lorenzi *et al.*, 1985; Curcio & Ceriello, 1992). Cosso and coauthors have shown that diabetes induces an increase in oxidative stress and results in upregulation of HO-1 in liver (Cosso *et al.*, 2001). Increased HO-1 expression has also been observed in glomerular cells of diabetic rats (Agarwal & Nick, 2000; Hayashi *et al.*, 2001). Quan and coworkers have reported a decrease in HO activity in the early stages of diabetes and an increase in number of circulating endothelial cells in streptozotocin-induced diabetic rats (Quan *et al.*, 2004). Overexpression HO-1 in diabetic rats resulted in increased serum bilirubin, reduced production of reactive oxygen species and attenuated sloughing of endothelial cells (Abraham *et al.*, 2004; Quan *et al.*, 2004). Interestingly, hyperglycemia *per se* represses HO-1 gene expression (Abraham *et al.*, 2003) while low glucose induces HO-1 gene expression (Chang *et al.*, 2003).

In rodent models of islet transplantation induction of HO-1 in islet cells resulted in a protective response from pro-apoptotic stimuli and improved islet function (Pileggi *et al.*, 2001; Tobiasch *et al.*, 2001). Studies conducted in diabetic and non diabetic HO-1–/– and +/+ mice have shown that animals lacking HO-1 are more susceptible to damage from myocardial ischemia-reperfusion injury and the presence of diabetes worsens the injury (Liu *et al.*, 2005). Myocardial infarct size was significantly higher in HO-1 deficient mice, whereas, overexpression of HO-1 conferred protection against myocardial injury in diabetic rats (Liu *et al.*, 2005).

CANCER

It is well known that HO-1 is expressed in a variety of tumors (Goodman *et al.*, 1997; Doi *et al.*, 1999; Tsuji *et al.*, 1999; Deininger *et al.*, 2000; Fang *et al.*, 2003) and that HO-1 directly contributes to rapid tumor growth *via* its anti-oxidative and anti-apoptotic effects (Doi *et al.*, 1999; Tanaka *et al.*, 2003). The anti-apoptotic action of HO-1 is believed to be mediated by multiple mechanisms including decreased levels of intracellular pro-oxidants and increased bilirubin and CO levels. CO exerts its anti-apoptotic effect by inhibiting expression of the tumor suppressor protein, p53, and release of mitochondrial cytochrome *c* (Liu *et al.*, 2002). In a study investigating the relationship between expression levels of HO-1 and cervical lymph node metastasis of tongue squamous cell carcinoma, low HO-1 expression was associated with lymph node metastasis (Yanagawa *et al.*, 2004) and hence suggested to be a possible clinical marker for the disease. Fang and coworkers have shown in human colon carcinoma cells that treatment with a HO inhibitor, ZnPP, enhanced the chemotherapeutic response of tumor cells and reduced tumor growth suggesting that HO-1 may be an attractive target for chemotherapeutic intervention (Fang *et al.*, 2003). Chen and coauthors have demonstrated in papillary thyroid carcinoma cells that induction of HO-1 markedly reduces the sensitivity of the cells to apoptotic stimuli (Chen *et al.*, 2004). Thus HO-1 may be an effective target for anti-cancer therapy.

However, HO-1 has also been shown to have a protective effect in cancer which is contradictory to its tumorigenic properties. Results from a study conducted to establish an association between incidence of lung adenocarcinoma and HO-1 polymorphisms among Japanese patients compared to controls showed that large (GT)n repeats in the HO-1 gene promoter may be directly correlated with the development of the disease (Kikuchi *et al.*, 2005). Recent studies also demonstrate an association between risk of oral squamous cell carcinoma amongst areca chewers and longer (GT)n repeat alleles in the HO-1 promoter and suggests that shorter (GT)n repeats may in fact confer protection against oral carcinogenesis (Chang *et al.*, 2004). Further studies will delineate the dual role played by HO-1 in cancer and the underlying mechanisms.

CEREBROVASCULAR ACCIDENT

Studies on focal cerebral ischemia in rats showed that treatment with an HO inhibitor, ZnPP before ischemia significantly reduced the infarct size and edema following the event (Kadoya *et al.*, 1995). Recent evidence indicates that prolonged expression of HO-1 in glial cells in human brains following focal cerebral infarctions or traumatic brain injury helps in the recovery of neuronal tissue following these insults (Beschorner *et al.*, 2000). In a study involving 399 patients with ischemic cerebrovascular

events, and 398 healthy control subjects, short <25 (GT)n repeats in the HO-1 promoter conferred a reduced risk for cerebrovascular events in people with normal plasma lipid levels (Funk *et al.*, 2004). These studies also show a contradictory role for HO-1 in this disease context. Since specificity of HO inhibitors is questionable, studies using genetic manipulation of HO-1 would provide more insight into the underlying mechanisms.

DRUGS

Several important therapeutic agents have been shown to induce HO-1 expression and mediate their beneficial effects, at least in part, through the induction of HO-1. For example, rapamycin, an immunosuppressive drug which has significant antiproliferative actions is a potent inducer of HO-1 expression in vascular cells (Visner *et al.*, 2003). Such induction is functionally important since HO inhibition with tin protoporphyrin leads to a loss of the antiproliferative effect of rapamycin in smooth muscle cells. Several studies have shown the beneficial effects of statins in reducing the mortality rate in patients with coronary heart disease (LaRosa, 2000; Vaughan *et al.*, 2000). Mechanisms beyond the lipid-lowering effects *per se* significantly contribute to the antiatherogenic and tissue protective properties of statins. Recent studies have shown that statins, albeit at relatively high concentrations, are potent inducers of HO-1 *in vitro* and *in vivo* (Grosser *et al.*, 2004a; 2004b; Lee *et al.*, 2004). It has also been suggested that the anti-inflammatory as well as the antiproliferative actions of statins are mediated through the induction of HO-1.

Probucol, a cholesterol lowering drug which inhibits atherosclerosis and vascular restenosis has been shown to protect against smooth muscle cell proliferation by inducing expression of HO-1 (Deng *et al.*, 2004). On the other hand, treatment with antioxidants such as probucol, completely normalized the HO-1 induction observed in diabetic glomeruli (Gorogawa *et al.*, 2002; Koya *et al.*, 2003). Other therapeutic agents such as aspirin and dopamine have also been shown to induce HO-1 (Berger *et al.*, 2000; Grosser *et al.*, 2003).

SUMMARY

In summary, induction of HO-1 plays an important role in the pathophysiology of several diseases such as atherosclerosis, hypertension, acute renal injury, lung injury, cancer as well as others involving multiple organ systems. Upregulation of HO-1 by various stimuli also modulates key biological processes including inflammation, ischemic injury and transplant rejection. Evaluation of the role played by products of the HO-1 catalyzed reaction in mediating the protective response will provide further insight into the underlying mechanisms of the cytoprotective effect elicited by HO-1.

REFERENCES

Abraham NG, Kushida T, Mcclung J, Weiss M, Quan S, Lafaro R, Darzynkiewicz Z, Wolin M (2003) Heme oxygenase-1 attenuates glucose-mediated cell growth arrest and apoptosis in human microvessel endothelial cells. *Circ Res* **93**: 507–514.

Abraham NG, Rezzani R, Rodella L, Kruger A, Taller D, Li Volti G, Goodman AI, Kappas A (2004) Overexpression of human heme oxygenase-1 attenuates endothelial cell sloughing in experimental diabetes. *Am J Physiol Heart Circ Physiol* **287**: H2468–2477.

Adin CA, Croker BP, Agarwal A (2005) Protective effects of exogenous bilirubin on ischemia-reperfusion injury in the isolated, perfused rat kidney. *Am J Physiol Renal Physiol* **288**: F778–784.

Agarwal A, Nick HS (2000) Renal response to tissue injury: lessons from heme oxygenase-1 gene ablation and expression. J *Am Soc Nephrol* **11**: 965–973.

Agarwal A, Balla J, Alam J, Croatt AJ, Nath KA (1995) Induction of heme oxygenase in toxic renal injury: a protective role in cisplatin nephrotoxicity in the rat. *Kidney Int* **48**: 1298–1307.

Agarwal A, Balla J, Balla G, Croatt AJ, Vercellotti GM, Nath KA (1996a) Renal tubular epithelial cells mimic endothelial cells upon exposure to oxidized LDL. *Am J Physiol* **271**: F814–823.

Agarwal A, Kim Y, Matas AJ, Alam J, Nath KA (1996b) Gas-generating systems in acute renal allograft rejection in the rat. Co-induction of heme oxygenase and nitric oxide synthase. *Transplantation* **61**: 93–98.

Agarwal A, Shiraishi F, Visner GA, Nick HS (1998) Linoleyl hydroperoxide transcriptionally upregulates heme oxygenase-1 gene expression in human renal epithelial and aortic endothelial cells. *J Am Soc Nephrol* **9**: 1990–1997.

Ahmed A, Rahman M, Zhang X, Acevedo CH, Nijjar S, Rushton I, Bussolati B, St John J (2000) Induction of placental heme oxygenase-1 is protective against TNF alpha-induced cytotoxicity and promotes vessel relaxation. *Mol Med* **6**: 391–409.

Aizawa T, Ishizaka N, Taguchi J-I, Kimura S, Kurokawa K, Ohno M (1999) Balloon injury does not induce heme oxygenase-1 expression, but administration of hemin inhibits neointimal formation in balloon-injured rat carotid artery. *Biochem Biophys Res Commun* **261**: 302–307.

Aizawa T, Ishizaka N, Taguchi J, Nagai R, Mori I, Tang SS, Ingelfinger JR, Ohno M (2000) Heme oxygenase-1 is upregulated in the kidney of angiotensin II-induced hypertensive rats: possible role in renoprotection. *Hypertension* **35**: 800–806.

Akamatsu Y, Haga M, Tyagi S, Yamashita K, Graca-Souza AV, Ollinger R, Czismadia E, May GA, Ifedigbo E, Otterbein LE, Bach FH, Soares MP (2004) Heme oxygenase-1-derived carbon monoxide protects hearts from transplant-associated ischemia reperfusion injury. *FASEB J* **18**: 771–772.

Alam J, Shibahara S, Smith A (1989) Transcriptional activation of the heme oxygenase gene by heme and cadmium in mouse hepatoma cells. *J Biol Chem* **264**: 6371–6375.

Alam J, Wicks C, Stewart D, Gong P, Touchard C, Otterbein S, Choi AM, Burow ME, Tou J (2000) Mechanism of heme oxygenase-1 gene activation by cadmium in MCF-7 mammary epithelial cells. Role of p38 kinase and Nrf2 transcription factor. *J Biol Chem* **275**: 27694–27702.

Alcaraz MJ, Habib A, Creminon C, Vicente AM, Lebret M, Levy-Toledano S, Maclouf J (2001) Heme oxygenase-1 induction by nitric oxide in raw 264.7 macrophages is upregulated by a cyclo-oxygenase-2 inhibitor. *Biochim Biophys Acta* **1526**: 13–16.

Amersi F, Buelow R, Kato H, Ke B, Coito AJ, Shen XD, Zhao D, Zaky J, Melinek J, Lassman CR, Kolls JK, Alam J, Ritter T, Volk HD, Farmer DG, Ghobrial RM, Busuttil RW, Kupiec-Weglinski JW (1999) Upregulation of heme oxygenase-1 protects genetically fat Zucker rat livers from ischemia/reperfusion injury. *J Clin Invest* **104**: 1631–1639.

Attuwaybi BO, Kozar RA, Moore-Olufemi SD, Sato N, Hassoun HT, Weisbrodt NW, Moore FA (2004) Heme oxygenase-1 induction by hemin protects against gut ischemia/reperfusion injury. *J Surg Res* **118**: 53–57.

Avihingsanon Y, Ma N, Csizmadia E, Wang C, Pavlakis M, Giraldo M, Strom TB, Soares MP, Ferran C (2002) Expression of protective genes in human renal allografts: a regulatory response to injury associated with graft rejection. *Transplantation* **73**: 1079–1085.

Barber A, Robson SC, Myatt L, Bulmer JN, Lyall F (2001) Heme oxygenase expression in human placenta and placental bed: reduced expression of placenta endothelial HO-2 in preeclampsia and fetal growth restriction. *FASEB J* **15**: 1158–1168.

Baumgartner-Parzer SM, Wagner L, Pettermann M, Grillari J, Gessl A, Waldhausl W (1995) High-glucose-triggered apoptosis in cultured endothelial cells. *Diabetes* **44**: 1323–1327.

Bedard EL, Jiang J, Parry N, Wang H, Liu W, Garcia B, Kim P, Chakrabarti S, Buelow R, Zhong R (2005) Peritransplant treatment with cobalt protoporphyrin attenuates chronic renal allograft rejection. *Transpl Int* **18**: 341–349.

Beltowski J, Jamroz A, Borkowska E (2004) Heme oxygenase and carbon monoxide in the physiology and pathology of the cardiovascular system]. *Postepy Hig Med Dosw* (Online) **58**: 83–99 (in Polish).

Berger SP, Hunger M, Yard BA, Schnuelle P, Van Der Woude FJ (2000) Dopamine induces the expression of heme oxygenase-1 by human endothelial cells *in vitro*. *Kidney Int* **58**: 2314–2319.

Beschorner R, Adjodah D, Schwab JM, Mittelbronn M, Pedal I, Mattern R, Schluesener HJ, Meyermann R (2000) Long-term expression of heme oxygenase-1 (Ho-1, HSP-32) following focal cerebral infarctions and traumatic brain injury in humans. *Acta Neuropathol (Berl)* **100**: 377–384.

Braudeau C, Bouchet D, Tesson L, Iyer S, Remy S, Buelow R, Anegon I, Chauveau C (2004) Induction of long-term cardiac allograft survival by heme oxygenase-1 gene transfer. *Gene Ther* **11**: 701–710.

Camhi SL, Alam J, Otterbein L, Sylvester SL, Choi AM (1995) Induction of heme oxygenase-1 gene expression by lipopolysaccharide is mediated by AP-1 activation. *Am J Respir Cell Mol Biol* **13**: 387–398.

Camhi SL, Alam J, Wiegand GW, Chin BY, Choi AM (1998) Transcriptional activation of the HO-1 gene by lipopolysaccharide is mediated by 5′ distal enhancers: role of reactive oxygen intermediates and AP-1. *Am J Respir Cell Mol Biol* **18**: 226–234.

Chang SH, Garcia J, Melendez JA, Kilberg MS, Agarwal A (2003) Haem oxygenase 1 gene induction by glucose deprivation is mediated by reactive oxygen species via the mitochondrial electron-transport chain. *Biochem J* **371**: 877–885.

Chang KW, Lee TC, Yeh WI, Chung MY, Liu CJ, Chi LY, Lin SC (2004) Polymorphism in heme oxygenase-1 (HO-1) promoter is related to the risk of oral squamous cell carcinoma occurring on male areca chewers. *Br J Cancer* **91**: 1551–1555.

Chen XL, Kunsch C (2004) Induction of cytoprotective genes through nrf2/antioxidant response element pathway: a new therapeutic approach for the treatment of inflammatory diseases. *Curr Pharm Des* **10**: 879–891.

Chen GG, Liu ZM, Vlantis AC, Tse GM, Leung BC, Van Hasselt CA (2004) Heme Oxygenase-1 protects against apoptosis induced by tumor necrosis factor-alpha and cycloheximide in papillary thyroid carcinoma cells. *J Cell Biochem* **92**: 1246–1256.

Choi AM, Alam J (1996) Heme Oxygenase-1: function, regulation, and implication of a novel stress-inducible protein in oxidant-induced lung injury. *Am J Respir Cell Mol Biol* **15**: 9–19.

Christou H, Morita T, Hsieh CM, Koike H, Arkonac B, Perrella MA, Kourembanas S (2000) Prevention of hypoxia-induced pulmonary hypertension by enhancement of endogenous heme oxygenase-1 in the rat. *Circ Res* **86**: 1224–1229.

Clark JE, Foresti R, Sarathchandra P, Kaur H, Green CJ, Motterlini R (2000) Heme oxygenase-1-derived bilirubin ameliorates postischemic myocardial dysfunction. *Am J Physiol Heart Circ Physiol* **278**: H643–651.

Cosso L, Maineri EP, Traverso N, Rosatto N, Pronzato MA, Cottalasso D, Marinari UM, Odetti P (2001) Induction of heme oxygenase 1 in liver of spontaneously diabetic rats. *Free Radic Res* **34**: 189–191.

Curcio F, Ceriello A (1992) Decreased cultured endothelial cell proliferation in high glucose medium is reversed by antioxidants: new insights on the pathophysiological mechanisms of diabetic vascular complications. *In vitro Cell Dev Biol* **28a**: 787–790.

Cuturi MC, Christoph FA, Woo J, Iyer S, Brouard S, Heslan JM, Pignon P, Soulillou JP, Buelow R (1999) Rdp1258, a new rationally designed immunosuppressive peptide, prolongs allograft survival in rats: analysis of its mechanism of action. *Mol Med* **5**: 820–832.

Das DK, Maulik N, Engelman RM (2004) Redox regulation of angiotensin II signaling in the heart. *J Cell Mol Med* **8**: 144–152.

Deininger MH, Meyermann R, Trautmann K, Duffner F, Grote EH, Wickboldt J, Schluesener HJ (2000) Heme oxygenase (HO)-1 expressing macrophages/microglial cells accumulate during oligodendroglioma progression. *Brain Res* **882**: 1–8.

Deng YM, Wu BJ, Witting PK, Stocker R (2004) Probucol protects against smooth muscle cell proliferation by upregulating heme oxygenase-1. *Circulation* **110**: 1855–1860.

Denschlag D, Marculescu R, Unfried G, Hefler LA, Exner M, Hashemi A, Riener E-K, Keck C, Tempfer CB, Wagner O (2004). The size of a microsatellite polymorphism of the haem oxygenase 1 gene is associated with idiopathic recurrent miscarriage. *Mol Hum Reprod* **10**: 211–214.

Doi K, Akaike T, Fujii S, Tanaka S, Ikebe N, Beppu T, Shibahara S, Ogawa M, Maeda H (1999) Induction of haem oxygenase-1 nitric oxide and ischaemia in experimental solid tumours and implications for tumour growth. *Br J Cancer* **80**: 1945–1954.

Dong Z, Lavrovsky Y, Venkatachalam MA, Roy AK (2000) Heme oxygenase-1 in tissue pathology: the yin and yang. *Am J Pathol* **156**: 1485–1488.

Duckers HJ, Boehm M, True AL, Yet SF, San H, Park JL, Clinton Webb R, Lee ME, Nabel GJ, Nabel EG (2001) Heme oxygenase-1 protects against vascular constriction and proliferation. *Nat Med* **7**: 693–698.

Durante W, Kroll MH, Christodoulides N, Peyton KJ, Schafer AI (1997) Nitric oxide induces heme oxygenase-1 gene expression and carbon monoxide production in vascular smooth muscle cells. *Circ Res* **80**: 557–564.

Exner M, Minar E, Wagner O, Schillinger M (2004) The role of heme oxygenase-1 promoter polymorphisms in human disease. *Free Radic Biol Med* **37**: 1097–1104.

Fang J, Sawa T, Akaike T, Akuta T, Sahoo SK, Khaled G, Hamada A, Maeda H (2003) *In vivo* antitumor activity of pegylated zinc protoporphyrin: targeted inhibition of heme oxygenase in solid tumor. *Cancer Res* **63**: 3567–3574.

Fujita T, Toda K, Karimova A, Yan SF, Naka Y, Yet SF, Pinsky DJ (2001) Paradoxical rescue from ischemic lung injury by inhaled carbon monoxide driven by derepression of fibrinolysis. *Nat Med* **7**: 598–604.

Funk M, Endler G, Schillinger M, Mustafa S, Hsieh K, Exner M, Lalouschek W, Mannhalter C, Wagner O (2004) The effect of a promoter polymorphism in the heme oxygenase-1 gene on the risk of ischaemic cerebrovascular events: the influence of other vascular risk factors. *Thromb Res* **113**: 217–223.

Furnkranz A, Schober A, Bochkov VN, Bashtrykov P, Kronke G, Kadl A, Binder BR, Weber C, Leitinger N (2005) Oxidized phospholipids trigger atherogenic inflammation in murine arteries. *Arterioscler Thromb Vasc Biol* **25**: 633–638.

Giugliano D, Ceriello A, Paolisso G (1995) Diabetes mellitus, hypertension, and cardiovascular disease: which role for oxidative stress? *Metabolism* **44**: 363–368.

Goodman AI, Choudhury M, Da Silva JL, Schwartzman ML, Abraham NG (1997) Overexpression of the heme oxygenase gene in renal cell carcinoma. *Proc Soc Exp Biol Med* **214**: 54–61.

Gorogawa S, Kajimoto Y, Umayahara Y, Kaneto H, Watada H, Kuroda A, Kawamori D, Yasuda T, Matsuhisa M, Yamasaki Y, Hori M (2002) Probucol preserves pancreatic beta-cell function through reduction of oxidative stress in type 2 diabetes. *Diabetes Res Clin Pract* **57**: 1–10.

Grosser N, Abate A, Oberle S, Vreman HJ, Dennery PA, Becker JC, Pohle T, Seidman DS, Schroder H (2003) Heme oxygenase-1 induction may explain the antioxidant profile of aspirin. *Biochem Biophys Res Commun* **308**: 956–960.

Grosser N, Erdmann K, Hemmerle A, Berndt G, Hinkelmann U, Smith G, Schroder H (2004a) Rosuvastatin upregulates the antioxidant defense protein heme oxygenase-1. *Biochem Biophys Res Commun* **325**: 871–876.

Grosser N, Hemmerle A, Berndt G, Erdmann K, Hinkelmann U, Schurgerc S, Wijayanti N, Immenschuh S, Schroder H (2004b) The antioxidant defense protein heme oxygenase 1 is a novel target for statins in endothelial cells. *Free Radic Biol Med* **37**: 2064–2071.

Haugen EN, Croatt AJ, Nath KA (2000) Angiotensin II induces renal oxidant stress *in vivo* and heme oxygenase-1 *in vivo* and *in vitro*. *Kidney Int* **58**: 144–152.

Hayashi K, Haneda M, Koya D, Maeda S, Isshiki K, Kikkawa R (2001) Enhancement of glomerular heme oxygenase-1 expression in diabetic rats. *Diabetes Res Clin Pract* **52**: 85–96.

Hayashi S, Omata Y, Sakamoto H, Higashimoto Y, Hara T, Sagara Y, Noguchi M (2004) Characterization of rat heme oxygenase-3 gene. Implication of processed pseudogenes derived from heme oxygenase-2 gene. *Gene* **336**: 241–250.

He JQ, Ruan J, Connett JE, Anthonisen NR, Pare PD, Sandford AJ (2002) Antioxidant gene polymorphisms and susceptibility to a rapid decline in lung function in smokers. *Am J Respir Crit Care Med* **166**: 323–328.

Hill-Kapturczak N, Chang SH, Agarwal A (2002) Heme oxygenase and the kidney. *DNA Cell Biol* **2:** 307–321.

Hill-Kapturczak N, Voakes C, Garcia J, Visner G, Nick HS, Agarwal A (2003) A cis-acting region regulates oxidized lipid-mediated induction of the human heme oxygenase-1 gene in endothelial cells. *Arterioscler Thromb Vasc Biol* **23**: 1416–1422.

Hoekstra KA, Godin DV, Kurtu J, Cheng KM (2003) Heme Oxygenase and antioxidant status in cultured aortic endothelial cells isolated from atherosclerosis-susceptible and -resistant Japanese quail. *Mol Cell Biochem* **252**: 253–262.

Holweg CT, Balk AH, Snaathorst J, Van Den Engel S, Niesters HG, Maat AW, Zondervan PE, Weimar W, Baan CC (2004) Intragraft heme oxygenase-1 and coronary artery disease after heart transplantation. *Transpl Immunol* **13**: 265–272.

Ishii T, Itoh K, Ruiz E, Leake DS, Unoki H, Yamamoto M, Mann GE (2004) Role of Nrf2 in the regulation of CD36 and stress protein expression in murine macrophages: activation by oxidatively modified LDL and 4-hydroxynonenal. *Circ Res* **94**: 609–616.

Ishikawa K (2003) Heme oxygenase-1 against vascular insufficiency: roles of atherosclerotic disorders. *Curr Pharm Des* **9**: 2489–2497.

Ishikawa K, Navab M, Leitinger N, Fogelman AM, Lusis AJ (1997) Induction of heme oxygenase-1 inhibits the monocyte transmigration induced by mildly oxidized ldl. *J Clin Invest* **100**: 1209–1216.

Ishikawa K, Sugawara D, Goto J, Watanabe Y, Kawamura K, Shiomi M, Itabe H, Maruyama Y (2001a) Heme oxygenase-1 inhibits atherogenesis in Watanabe heritable hyperlipidemic rabbits. *Circulation* **104**: 1831–1836.

Ishikawa K, Sugawara D, Wang X, Suzuki K, Itabe, H, Maruyama Y, Lusis AJ (2001b). Heme oxygenase-1 inhibits atherosclerotic lesion formation in LDL-receptor knockout mice. *Circ Res* **88**: 506–512.

Johnson FK, Durante W, Peyton KJ, Johnson RA (2004a) Heme oxygenase-mediated endothelial dysfunction in DOCA-salt, but not in spontaneously hypertensive, rat arterioles. *Am J Physiol Heart Circ Physiol* **286**: H1681–1687.

Johnson RA, Teran FJ, Durante W, Peyton KJ, Johnson FK (2004b) Enhanced heme oxygenase-mediated coronary vasodilation in Dahl salt-sensitive hypertension. *Am J Hypertens* **17**: 25–30.

Juan SH, Lee TS, Tseng KW, Liou JY, Shyue SK, Wu KK, Chau LY (2001) Adenovirus-mediated heme oxygenase-1 gene transfer inhibits the development of atherosclerosis in apolipoprotein E-deficient mice. *Circulation* **104**: 1519–1525.

Jung NH, Kim HP, Kim BR, Cha SH, Kim GA, Ha H, Na YE, Cha YN (2003) Evidence for heme oxygenase-1 association with caveolin-1 and -2 in mouse mesangial cells. *IUBMB Life* **55**: 525–532.

Kadoya C, Domino EF, Yang GY, Stern JD, Betz AL (1995) Preischemic but not postischemic zinc protoporphyrin treatment reduces infarct size and edema accumulation after temporary focal cerebral ischemia in rats. *Stroke* **26**: 1035–1038.

Kanai M, Tanabe S, Okada M, Suzuki H, Niki T, Katsuura M, Akiba T, Hayasaka K (2003) Polymorphisms of heme oxygenase-1 and bilirubin UDP-glucuronosyl transferase genes are not associated with kawasaki disease susceptibility. *Tohoku J Exp Med* **200**: 155–159.

Kapitulnik J (2004) Bilirubin: an endogenous product of heme degradation with both cytotoxic and cytoprotective properties. *Mol Pharmacol* **66**: 773–779.

Kapturczak MH, Wasserfall C, Brusko T, Campbell-Thompson M, Ellis TM, Atkinson MA, Agarwal A (2004) Heme oxygenase-1 modulates early inflammatory responses: evidence from the heme oxygenase-1-deficient mouse. *Am J Pathol* **165**: 1045–1053.

Kato Y, Shimazu M, Kondo M, Uchida K, Kumamoto Y, Wakabayashi G, Kitajima M, Suematsu M (2003) Bilirubin rinse: A simple protectant against the rat liver graft injury mimicking heme oxygenase-1 preconditioning. *Hepatology* **38**: 364–373.

Ke B, Shen XD, Zhai Y, Gao F, Busuttil RW, Volk HD, Kupiec-Weglinski JW (2002) Heme oxygenase 1 mediates the immunomodulatory and antiapoptotic effects of interleukin 13 gene therapy *in vivo* and *in vitro*. *Hum Gene Ther* **13**: 1845–1857.

Keshavan P, Deem TL, Schwemberger SJ, Babcock GF, Cook-Mills JM, Zucker SD (2005) Unconjugated bilirubin inhibits vcam-1-mediated transendothelial leukocyte migration. *J Immunol* **174**: 3709–3718.

Kikuchi A, Yamaya M, Suzuki S, Yasuda H, Kubo H, Nakayama K, Handa M, Sasaki T, Shibahara S, Sekizawa K, Sasaki H (2005) Association of susceptibility to the development of lung adenocarcinoma with the heme oxygenase-1 gene promoter polymorphism. *Hum Genet* 1–14.

Kim HP, Wang X, Galbiati F, Ryter SW, Choi AM (2004) Caveolae compartmentalization of heme oxygenase-1 in endothelial cells. *FASEB J* **18**: 1080–1089.

Koya D, Hayashi K, Kitada M, Kashiwagi A, Kikkawa R, Haneda M (2003) Effects of antioxidants in diabetes-induced oxidative stress in the glomeruli of diabetic rats. *J Am Soc Nephrol* **14**: S250–253.

Lang D, Reuter S, Buzescu T, August C, Heidenreich S (2005) Heme-induced heme oxygenase-1 (HO-1) in human monocytes inhibits apoptosis despite caspase-3 up-regulation. *Int Immunol* **17**: 155–165.

LaRosa JC (2000) Statins and risk of coronary heart disease. *JAMA* **283**: 2935–2936.

Lash GE, Mclaughlin BE, Macdonald-Goodfellow SK, Smith GN, Brien JF, Marks GS, Nakatsu K, Graham CH (2003) Relationship between tissue damage and heme oxygenase expression in chorionic villi of term human placenta. *Am J Physiol Heart Circ Physiol* **284**: H160–167.

Lee TS, Chau LY (2002) Heme Oxygenase-1 mediates the anti-inflammatory effect of interleukin-10 in mice. *Nat Med* **8**: 240–246.

Lee TS, Chang CC, Zhu Y, Shyy JY (2004) Simvastatin induces heme oxygenase-1: a novel mechanism of vessel protection. *Circulation* **110**: 1296–1302.

Liu X, Wei J, Peng DH, Layne MD, Yet SF (2005) Absence of heme oxygenase-1 exacerbates myocardial ischemia/reperfusion injury in diabetic mice. *Diabetes* **54**: 778–784.

Liu XM, Chapman GB, Peyton KJ, Schafer AI, Durante W (2002) Carbon monoxide inhibits apoptosis in vascular smooth muscle cells. *Cardiovasc Res* **55**: 396–405.

Lorenzi M, Cagliero E, Toledo S (1985) Glucose toxicity for human endothelial cells in culture. Delayed replication, disturbed cell cycle, and accelerated death. *Diabetes* **34**: 621–627.

Lorenzi M, Nordberg JA, Toledo S (1987) High glucose prolongs cell-cycle traversal of cultured human endothelial cells. *Diabetes* **36**: 1261–1267.

Lum H, Roebuck KA (2001) Oxidant stress and endothelial cell dysfunction. *Am J Physiol Cell Physiol* **280**: C719–741.

Lyall F, Barber A, Myatt L, Bulmer JN, Robson SC (2000) Heme oxygenase expression in human placenta and placental bed implies a role in regulation of trophoblast invasion and placental function. *FASEB J* **14**: 208–219.

Magee CC, Azuma H, Knoflach A, Denton MD, Chandraker A, Iyer S, Buelow R, Sayegh M (1999) *In vitro* and *in vivo* immunomodulatory effects of RDP1258, a novel synthetic peptide. *J Am Soc Nephrol* **10**: 1997–2005.

Maines MD (1997) The heme oxygenase system: a regulator of second messenger gases. *Ann Rev Pharmacol And Toxicol* **37:** 517–554.

McCoubrey WK Jr, Huang TJ, Maines MD (1997) Isolation and characterization of a cDNA from the rat brain that encodes hemoprotein heme oxygenase-3. *Eur J Biochem* **247**: 725–732.

Mcfaul SJ, Mcgrath JJ (1987) Studies on the mechanism of carbon monoxide-induced vasodilation in the isolated perfused rat heart. *Toxicol Appl Pharmacol* **87**: 464–473.

McLaughlin BE, Lash GE, Smith GN, Marks GS, Nakatsu K, Graham CH, Brien JF (2003) Heme oxygenase expression in selected regions of term human placenta. *Exp Biol Med (Maywood)* **228**: 564–567.

Mohamed AK, Bierhaus A, Schiekofer S, Tritschler H, Ziegler R, Nawroth PP (1999) The role of oxidative stress and NF-κB activation in late diabetic complications. *Biofactors* **10**: 157–167.

Morisaki H, Katayama T, Kotake Y, Ito M, Handa M, Ikeda Y, Takeda J, Suematsu M (2002) Carbon monoxide modulates endotoxin-induced microvascular leukocyte adhesion through platelet-dependent mechanisms. *Anesthesiology* **97**: 701–709.

Motterlini R, Gonzales A, Foresti R, Clark JE, Green CJ, Winslow RM (1998) Heme oxygenase-1–derived carbon monoxide contributes to the suppression of acute hypertensive responses *in vivo*. *Circ Res* **83**: 568–577.

Nakao A, Kimizuka K, Stolz DB, Neto JS, Kaizu T, Choi AM, Uchiyama T, Zuckerbraun BS, Nalesnik MA, Otterbein LE, Murase N (2003) Carbon monoxide inhalation protects rat intestinal grafts from ischemia/reperfusion injury. *Am J Pathol* **163**: 1587–1598.

Nakao A, Neto JS, Kanno S, Stolz DB, Kimizuka K, Liu F, Bach FH, Billiar TR, Choi AM, Otterbein LE, Murase N (2005) Protection against ischemia/reperfusion injury in cardiac and renal transplantation with carbon monoxide, biliverdin and both. *Am J Transplant* **5**: 282–291.

Nath KA (1999) Heme oxygenase-1: a redoubtable response that limits reperfusion injury in the transplanted adipose liver. *J Clin Invest* **104**: 1485–1486.

Nath KA, Balla G, Vercellotti GM, Balla J, Jacob HS, Levitt MD, Rosenberg ME (1992) Induction of heme oxygenase is a rapid, protective response in rhabdomyolysis in the rat. *J Clin Invest* **90**: 267–270.

Neto JS, Nakao A, Kimizuka K, Romanosky AJ, Stolz DB, Uchiyama T, Nalesnik MA, Otterbein LE, Murase N (2004) Protection of transplant-induced renal ischemia-reperfusion injury with carbon monoxide. *Am J Physiol Renal Physiol* **287**: F979–989.

Omura S, Suzuki H, Toyofuku M, Ozono R, Kohno N, Igarashi K (2005) Effects of genetic ablation of bach1 upon smooth muscle cell proliferation and atherosclerosis after cuff injury. *Genes Cells* **10**: 277–285.

Otterbein LE, Kolls JK, Mantell LL, Cook JL, Alam J, Choi AM (1999a) Exogenous administration of heme oxygenase-1 by gene transfer provides protection against hyperoxia-induced lung injury. *J Clin Invest* **103**: 1047–1054.

Otterbein LE, Mantell LL, Choi AMK (1999b) Carbon monoxide provides protection against hyperoxic lung injury. *Am J Physiol Lung Cell Mol Physiol* **276**: L688–694.

Otterbein LE, Soares MP, Yamashita K, Bach FH (2003) Heme oxygenase-1: unleashing the protective properties of heme. *Trends Immunol* **24**: 449–455.

Pachori AS, Melo LG, Hart ML, Noiseux N, Zhang L, Morello F, Solomon SD, Stahl GL, Pratt RE, Dzau VJ (2004) Hypoxia-regulated therapeutic gene as a preemptive treatment strategy against ischemia/reperfusion tissue injury. *Proc Natl Acad Sci USA* **101**: 12282–12287.

Pileggi A, Molano RD, Berney T, Cattan P, Vizzardelli C, Oliver R, Fraker C, Ricordi C, Pastori RL, Bach FH, Inverardi L (2001) Heme oxygenase-1 induction in islet cells results in protection from apoptosis and improved *in vivo* function after transplantation. *Diabetes* **50**: 1983–1991.

Platt JL, Nath KA (1998) Heme oxygenase: protective gene or trojan horse. *Nat Med* **4**: 1364–1365.

Poss KD, Tonegawa S (1997a) Heme oxygenase-1 is required for mammalian iron reutilization. *Proc Natl Acad Sci USA* **94**: 10919–10924.

Poss KD, Tonegawa S (1997b) Reduced stress defense in heme oxygenase-1-deficient cells. *Proc Natl Acad Sci USA* **94**: 10925–10930.

Quan S, Kaminski PM, Yang L, Morita T, Inaba M, Ikehara S, Goodman AI, Wolin MS, Abraham NG (2004) Heme oxygenase-1 prevents superoxide anion-associated endothelial cell sloughing in diabetic rats. *Biochem Biophys Res Commun* **315**: 509–516.

Ryter SW, Choi AM (2002) Heme oxygenase-1: molecular mechanisms of gene expression in oxygen-related stress. *Antioxid Redox Signal* **4**: 625–632.

Ryter SW, Otterbein LE (2004) Carbon monoxide in biology and medicine. *Bioessays* **26**: 270–280.

Sato K, Balla J, Otterbein L, Smith RN, Brouard S, Lin Y, Csizmadia E, Sevigny J, Robson SC, Vercellotti G, Choi AM, Bach FH, Soares MP (2001) Carbon monoxide generated by heme oxygenase-1 suppresses the rejection of mouse-to-rat cardiac transplants. *J Immunol* **166**: 4185–4194.

Schillinger M, Exner M, Mlekusch W, Domanovits H, Huber K, Mannhalter C, Wagner O, Minar E (2002) Heme oxygenase-1 gene promoter polymorphism is associated with abdominal aortic aneurysm. *Thromb Res* **106**: 131–136.

Schillinger M, Exner M, Minar E, Mlekusch W, Mullner M, Mannhalter C, Bach FH, Wagner O (2004) Heme oxygenase-1 genotype and restenosis after balloon angioplasty: a novel vascular protective factor. *J Am Coll Cardiol* **43**: 950–957.

Shi W, Haberland ME, Jien ML, Shih DM, Lusis AJ (2000) Endothelial responses to oxidized lipoproteins determine genetic susceptibility to atherosclerosis in mice. *Circulation* **102**: 75–81.

Shiraishi F, Curtis LM, Truong L, Poss K, Visner GA, Madsen K, Nick HS, Agarwal A (2000) Heme oxygenase-1 gene ablation or expression modulates cisplatin-induced renal tubular apoptosis. *Am J Physiol Renal Physiol* **278**: F726–736.

Sikorski EM, Hock T, Hill-Kapturczak N, Agarwal A (2004) The story so far: molecular regulation of the heme oxygenase-1 gene in renal injury. *Am J Physiol Renal Physiol* **286**: F425–441.

Siow RC, Sato H, Mann GE (1999) Heme oxygenase-carbon monoxide signalling pathway in atherosclerosis: anti-atherogenic actions of bilirubin and carbon monoxide? *Cardiovasc Res* **41**: 385–394.

Soares MP, Lin Y, Anrather J, Csizmadia E, Takigami K, Sato K, Grey ST, Colvin RB, Choi AM, Poss KD, Bach FH (1998) Expression of heme oxygenase-1 can determine cardiac xenograft survival. *Nat Med* **4**: 1073–1077.

Soares MP, Seldon MP, Gregoire IP, Vassilevskaia T, Berberat PO, Yu J, Tsui TY, Bach FH (2004) Heme oxygenase-1 modulates the expression of adhesion molecules associated with endothelial cell activation. *J Immunol* **172**: 3553–3563.

Souza AI, Felkin LE, Mccormack AM, Holder A, Barton PJ, Banner NR, Rose ML (2005) Sequential expression of three known protective genes in cardiac biopsies after transplantation. *Transplantation* **79**: 584–590.

Tanaka S, Akaike T, Fang J, Beppu T, Ogawa M, Tamura F, Miyamoto Y, Maeda H (2003) Antiapoptotic effect of haem oxygenase-1 induced by nitric oxide in experimental solid tumour. *Br J Cancer* **88**: 902–909.

Taylor JL, Carraway MS, Piantadosi CA (1998) Lung-specific induction of heme oxygenase-1 and hyperoxic lung injury. *Am J Physiol Lung Cell Mol Physiol* **274**: L582–590.

Terry CM, Clikeman JA, Hoidal JR, Callahan KS (1999) TNF-alpha and IL-1alpha induce heme oxygenase-1 via protein kinase C, Ca^{2+}, and phospholipase A2 in endothelial cells. *Am J Physiol* **276**: H1493–1501.

Tobiasch E, Gunther L, Bach FH (2001) Heme oxygenase-1 protects pancreatic beta cells from apoptosis caused by various stimuli. *J Invest Med* **49**: 566–571.

Tomaro ML, Batlle AM (2002) Bilirubin: its role in cytoprotection against oxidative stress. *Int J Biochem Cell Biol* **34**: 216–220.

Tsuchihashi S, Fondevila C, Kupiec-Weglinski JW (2004) Heme oxygenase system in ischemia and reperfusion injury. *Ann Transplant* **9**: 84–87.

Tsuji MH, Yanagawa T, Iwasa S, Tabuchi K, Onizawa K, Bannai S, Toyooka H, Yoshida H (1999) Heme oxygenase-1 expression in oral squamous cell carcinoma as involved in lymph node metastasis. *Cancer Lett* **138**: 53–59.

Tulis DA, Durante W, Liu X, Evans AJ, Peyton KJ, Schafer AI (2001a) Adenovirus-mediated heme oxygenase-1 gene delivery inhibits injury-induced vascular neointima formation. *Circulation* **104:** 2710–2715.

Tulis DA, Durante W, Peyton KJ, Evans AJ, Schafer AI (2001b) Heme oxygenase-1 attenuates vascular remodeling following balloon injury in rat carotid arteries. *Atherosclerosis* **155**: 113–122.

Vaughan CJ, Gotto AM Jr, Basson CT (2000) The evolving role of statins in the management of atherosclerosis. *J Am Coll Cardiol* **35**: 1–10.

Visner GA, Lu F, Zhou H, Liu J, Kazemfar K, Agarwal A (2003) Rapamycin induces heme oxygenase-1 in human pulmonary vascular cells: implications in the antiproliferative response to rapamycin. *Circulation* **107**: 911–916.

Vogt BA, Shanley TP, Croatt A, Alam J, Johnson KJ, Nath KA (1996) Glomerular inflammation induces resistance to tubular injury in the rat. A novel form of acquired, heme oxygenase-dependent resistance to renal injury. *J Clin Invest* **98**: 2139–2145.

Wagener Feldman E, de Witte T, Abraham NG (1997) Heme induces the expression of adhesion molecules ICAM-1, VCAM-1, and E selectin in vascular endothelial cells. *Proc Soc Exp Biol Med* **216**: 456–463.

Wagener FA, Da Silva JL, Farley T, De Witte T, Kappas A, Abraham NG (1999) Differential effects of heme oxygenase isoforms on heme mediation of endothelial intracellular adhesion molecule 1 expression. *J Pharmacol Exp Ther* **291**: 416–423.

Wagener FA, Volk HD, Willis D, Abraham NG, Soares MP, Adema GJ, Figdor CG (2003) Different faces of the heme-heme oxygenase system in inflammation. *Pharmacol Rev* **55**: 551–571.

Wang YP, Yu YH (2002) Expression of endogenous heme oxygenase on surface of placental trophoblasts of pregnant women with intrauterine growth retardation of the fetus. *Di Yi Jun Yi Da Xue Xue Bao* **22**: 637–639.

Wang LJ, Lee TS, Lee FY, Pai RC, Chau LY (1998) Expression of heme oxygenase-1 in atherosclerotic lesions. *Am J Pathol* **152**: 711–720.

Willis D, Moore AR, Frederick R, Willoughby DA (1996) Heme oxygenase: a novel target for the modulation of the inflammatory response. *Nat Med* **2**: 87–90.

Yachie A, Niida Y, Wada T, Igarashi N, Kaneda H, Toma T, Ohta K, Kasahara Y, Koizumi S (1999) Oxidative stress causes enhanced endothelial cell injury in human heme oxygenase-1 deficiency. *J Clin Invest* **103**: 129–135.

Yamada N, Yamaya M, Okinaga S, Nakayama K, Sekizawa K, Shibahara S, Sasaki H (2000) Microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with susceptibility to emphysema. *Am J Hum Genet* **66**: 187–195.

Yanagawa T, Omura K, Harada H, Nakaso K, Iwasa S, Koyama Y, Onizawa K, Yusa H, Yoshida H (2004) Heme oxygenase-1 expression predicts cervical lymph node metastasis of tongue squamous cell carcinomas. *Oral Oncol* **40**: 21–27.

Yang L, Quan S, Nasjletti A, Laniado-Schwartzman M, Abraham NG (2004) Heme oxygenase-1 gene expression modulates angiotensin II-induced increase in blood pressure. *Hypertension* **43**: 1221–1226.

Yet SF, Pellacani A, Patterson C, Tan L, Folta SC, Foster L, Lee WS, Hsieh CM, Perrella MA (1997) Induction of heme oxygenase-1 expression in vascular smooth muscle cells. A link to endotoxic shock. *J Biol Chem* **272**: 4295–4301.

Yet SF, Layne MD, Liu X, Chen YH, Ith B, Sibinga NE, Perrella MA (2003) Absence of heme oxygenase-1 exacerbates atherosclerotic lesion formation and vascular remodeling. *FASEB J* **17**: 1759–1761.

Zampetaki A, Minamino T, Mitsialis SA, Kourembanas S (2003) Effect of heme oxygenase-1 overexpression in two models of lung inflammation. *Exp Biol Med* **228**: 442–446.

Zhang X, Shan P, Jiang D, Noble PW, Abraham NG, Kappas A, Lee PJ (2004) Small interfering RNA targeting heme oxygenase-1 enhances ischemia-reperfusion-induced lung apoptosis. *J Biol Chem* **279**: 10677–10684.

Zhao S, Zhang Y, Gu Y, Lewis DF, Wang Y (2004) Heme Oxygenase-1 mediates up-regulation of adhesion molecule expression induced by peroxynitrite in endothelial cells. *J Soc Gynecol Investig* **11**: 465–471.

Zou MH, Shi C, Cohen RA (2002) High glucose *via* peroxynitrite causes tyrosine nitration and inactivation of prostacyclin synthase that is associated with thromboxane/prostaglandin H_2 receptor-mediated apoptosis and adhesion molecule expression in cultured human aortic endothelial cells. *Diabetes* **51**: 198–203.