Heme oxygenase-1: A provenance for cytoprotective pathways in the kidney and other tissues

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Heme oxygenase (HO) is the rate-limiting enzyme in the degradation of heme, converting heme to biliverdin, during which iron is released and carbon monoxide (CO) is emitted; biliverdin is subsequently converted to bilirubin by biliverdin reductase. At least two isozymes possess HO activity: HO-1 represents the isozyme induced by diverse stressors, including ischemia, nephrotoxins, cytokines, endotoxin, oxidants, and vasoactive substances; HO-2 is the constitutive, glucocorticoid-inducible isozyme. HO-1 is upregulated in the kidney in assorted conditions and diseases. Interest in HO is driven by the capacity of this system to protect the kidney against injury, a capacity likely reflecting, at least in part, the cytoprotective properties of its products: in relatively low concentrations, CO exerts vasorelaxant, antiapoptotic, and anti-inflammatory effects while bile pigments are antioxidant and anti-inflammatory metabolites. This article reviews the HO system and the extent to which it influences the function of the healthy kidney; it summarizes conditions and stimuli that elicit HO-1 in the kidney; and it explores the significance of renal expression of HO-1 as induced by ischemia, nephrotoxins, nephritides, transplantation, angiotensin II, and experimental diabetes. This review also points out the tissue specificity of the HO system, and the capacity of HO-1 to induce renal injury in certain settings. Studies of HO in other tissues are discussed insofar as they aid in elucidating the physiologic and pathophysiologic significance of the HO system in the kidney.

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Le Chatelier's famous principle recognized that chemical systems in equilibria, subjected to an external constraint, adjust themselves to oppose the effect of the external constraint. This capacity to resist the perturbing influence of imposed stress is equally true for biologic systems, and broadly underlies fundamental and diverse biologic phenomena such as homeostasis as a requirement for health, maintenance of the constancy of the internal environment, adaptive alterations in nephrons surviving after renal injury, and cytoprotective responses in injured tissues.

A widely and readily recruited response in injured tissue centers on the induction of heme oxygenase-1 (HO-1),[1–6] and evidence that the elicitation of HO-1 can reduce tissue injury was first derived in 1992 by studies involving the kidney.[7] A substantial and rapidly growing literature indicates that HO-1 provides the provenance for pathways that can interrupt virtually all major mechanisms of tissue injury, including those that impose vasoconstriction and vascular injury, ischemia, inflammation, immune injury, oxidative stress, cell cycle dysregulation, and sublethal and lethal cell damage, and that such pathways of protection, emanating from HO-1, may be proffered in virtually all tissues.[8–25] Ironically, these cytoprotective properties of HO are derived, at least in part, from products, such as carbon monoxide (CO) and bile pigments, which were once regarded as invariantly injurious in nature. Notably, while the clinical toxicity of CO is clearly recognized, much smaller quantities of CO are remarkably cytoprotective, antiapoptotic, vasorelaxant, and anti-inflammatory.[26–29] Bile pigments, long regarded as contributors to renal disease and other adverse consequences of hyperbilirubinemic states, are now recognized as anti-inflammatory and antioxidant when present in low concentrations.[30–34] Interest in HO is also driven by the similarity and interaction that exist between the HO/CO and nitric oxide synthase/nitric oxide systems,[35,36] and the provision by HO of CO, a gaseous product which, like nitric oxide, is involved in cell signaling.[28,29] Finally, the clinical relevance of the study of HO-1 is substantiated by the occurrence of renal and other diseases in patients genetically unable to express HO-1,[37–39] and by the predisposition towards assorted diseases,[40] including dysfunction of hemodialysis arteriovenous fistulae,[41] in individuals expressing polymorphisms in the HO-1 gene, which lead to lesser amounts of HO activity.

This review discusses the functional significance of the HO system in the kidney in health and disease; comprehensively covered elsewhere are areas such as regulation of expression of HO-1,[20,42] the significance of the HO/CO system in systemic hypertension,[22,43–45] and gene therapy based on HO-1.[22] Findings from other organ systems, where relevant to the kidney, are discussed in the present review.

OVERVIEW OF HEME METABOLISM, HEME TOXICITY, AND THE HO SYSTEM

As erythrocytes age, hemoglobin is progressively oxidized and destabilized, and it was in the course of studies seeking to determine the metabolic fate of the heme prosthetic group in senescent erythrocytes that HO was discovered in 1968.[46] In a reaction that requires oxygen and nicotinamide adenine dinucleotide phosphate (reduced form), HO facilitates the opening of the heme ring and its conversion to biliverdin, the release of iron from the heme ring, and the attendant emission of CO; biliverdin is subsequently converted to bilirubin by the enzyme biliverdin reductase (Figure 1).

By degrading heme, HO critically regulates the prevailing cellular levels of heme, the latter arising from the synthesis of heme or the release of heme from destabilized heme proteins (Figure 1). Heme is employed as a prosthetic group in diverse proteins which comprehensively affect cellular function (Table 1). However, when cells are injured, heme proteins may be denatured and destabilized, thereby incurring the liberation of heme,[47,48] the latter inflicting cell injury when present in relatively large amounts.[49–53] The nephrotoxic potential of heme is supported by clinical observations: the use of substantial amounts of heme (as hematin) to induce remission in patients with acute intermittent porphyria can precipitate acute tubular necrosis.[54]

The capacity to degrade heme is possessed by two and possibly three members of the HO family. HO-1 is the inducible isozyme that largely accounts for increased HO activity in stressed organs and tissues, and is, arguably, the most widely inducible protein ever described.[4,6] HO-2, the product of a different gene, is the constitutive isozyme that has as its major stimulus, corticosteroids;[55] HO-3 may be a pseudogene.[56]

The possibility that induction of HO-1 was a cytoprotective response was raised by studies in 1989 which identified HO as the 32 kDa protein commonly induced in injured cells.[57] To account for such induction of HO-1, it was hypothesized that HO-1 exerted a protective antioxidant

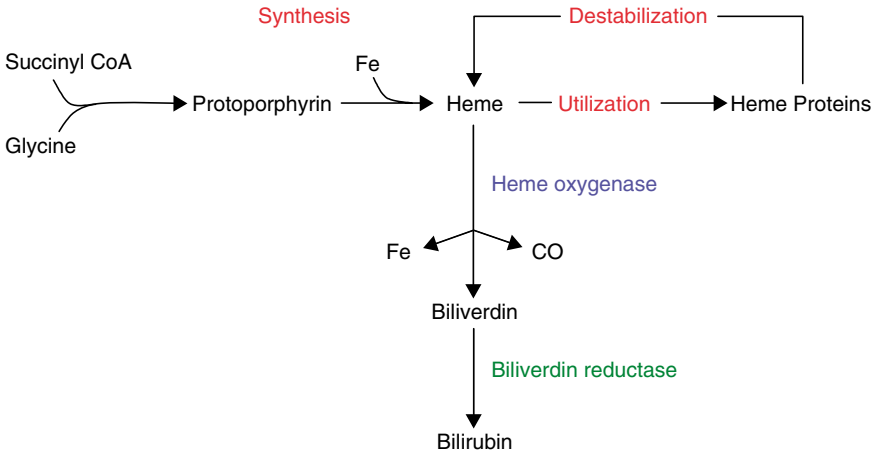

Figure 1 | Overview of cellular metabolism of heme depicting synthesis and utilization of heme, and destabilization of heme proteins. HO catalyzes the conversion of heme to biliverdin, releasing iron (Fe) and CO; biliverdin reductase catalyzes the conversion of biliverdin to bilirubin.

Table 1 | Some properties of heme

A Prosthetic group in diverse proteins	Heme in relatively larger quantities can be cytotoxic by impairing the following targets:[49–53]	Heme in relatively smaller quantities can exert cytoprotection by inducing HO-1 (see text)
Hemoglobin	Plasma membrane	
Myoglobin	Cytoskeleton	
Mitochondrial cytochromes	Mitochondria	
Microsomal cytochromes	Cytosolic enzymes	
NADPH oxidase	DNA	
Nitric oxide synthase		
Guanylate cyclase		
Glutathione peroxidase		
Cyclo-oxygenase		
Catalase		

HO, heme oxygenase; NADPH, nicotinamide adenine dinucleotide phosphate (reduced form).

response which enabled cells not only to remove heme, a pro-oxidant, but also to replace it by bilirubin, a potent antioxidant.[58] This hypothesis was tested in the glycerol model of acute renal failure, the latter exhibiting myolysis, hemolysis, and heme protein-induced renal injury.[7] In this model, HO-1 mRNA and HO activity were rapidly induced, and the competitive inhibitor of HO, tin protoporphyrin, significantly worsened the course of acute renal insufficiency; conversely, the prior induction of HO-1 by small, non-toxic doses of hemoglobin strikingly protected against acute renal failure.[7] Renal injury was reduced in this model, even when HO-1 was induced by endotoxin[59] and nephrotoxic serum,[60] and by hyperbilirubinemic states incurred by bile duct ligation.[61] Finally, HO-$1^{-/-}$ mice, when challenged by heme proteins, exhibit increased renal accumulation of heme, worse renal injury, and increased mortality.[62] Studies in this model also demonstrated that the induction of HO-1 was coupled to the synthesis of the iron-sequestering protein, ferritin.[7] Ferritin avidly binds iron and interrupts redox cycling of iron, thereby preventing iron from serving as a catalyst for oxidant stress.[63] Subsequent studies demonstrated that the induction of HO-1 is also coupled to the synthesis of iron-exporting proteins[64] and the critical role of HO-1 in maintaining iron homeostasis *in vivo*.[65] The linkage of HO-1 to mechanisms that safely sequester and/or export iron thus mitigates the risk of cytotoxicity arising from iron released from the heme ring.

Heme induces HO-1 by binding to and inactivating the repressor protein, Bach 1, thereby unfettering HO-1 gene transcription from inhibition imposed by Bach 1;[20,42] additionally, in cells such as renal tubular epithelial cells, heme facilitates the activation of HO-1 gene transcription by Nrf2.[20,42,66] The extent to which such mechanisms apply to other inducers of HO-1 is currently unresolved, as is the extent to which the release of heme from intracellular heme proteins underlies the induction of HO-1 by various stimuli and insults. Heme may also induce HO-1 via redox-sensitive, signal transduction pathways such as nuclear factor-kappa B (NF-κB); heme activates these pathways by its direct pro-oxidant effects or by iron released from heme.[67] While the upregulation of HO-1 by its myriad stimuli commonly involves oxidative stress and redox-sensitive pathways, the underpininings of such induction are quite complex, and depend on the applied stimulus, the tissue and cell type involved, and the species from which cells were derived; notably, the regulation of the HO-1 gene may differ significantly in humans as compared to other species.[20,42]

EXPRESSION AND FUNCTION OF HO IN THE HEALTHY KIDNEY

HO activity in the healthy kidney largely reflects HO-2, which is expressed in the pre-glomerular vasculature, the thick ascending limb, distal convoluted and connecting tubules, and the collecting duct; HO-1 is quite weakly expressed in proximal and distal tubules, in the loop of Henle, and in medullary collecting tubules.[68–70] Heme and heavy metals increase expression of HO-1 in arterioles as well as tubules.[69]

Table 2 | Mechanisms accounting for the vasorelaxant effects of the HO/CO system

CO-dependent vasorelaxation
Stimulation of guanylate cyclase
Release of nitric oxide stored within cells
Increased activity of calcium-activated potassium channels (K_{ca})
Impaired generation of vasoconstricting cytokines (e.g., endothelin-1)
Decreased synthesis of cytochrome *P*450-dependent vasoconstrictors (e.g., 20-HETE) since CO inhibits cytochrome *P*450 activity
Reduced supply of heme impairs cytochrome *P*450 activity and the attendant generation of cytochrome *P*450-dependent vasoconstrictors
Scavenging of superoxide anion by bile pigments

CO, carbon monoxide; HETE, hydroxyeicosatetraenoic acid; HO, heme oxygenase.

HO activity contributes to the regulation of renal hemodynamics. For example, inhibitors of HO activity infused in the renal medulla reduce medullary blood flow,[71] and when infused systemically, these inhibitors reduce total renal blood flow (RBF), the latter more effectively reduced when nitric oxide synthase is concomitantly inhibited.[72] Administration of inhibitors of HO activity directly into the renal artery significantly reduces glomerular filtration rate (GFR), RBF, and renal production of nitric oxide, and all of these effects can be reversed by CO-releasing molecules.[73] Additionally, CO derived from the renal vasculature can mitigate the vasoconstricting effects of various agonists,[74] and renal generation of CO is increased, presumably as a compensatory mechanism, when renal production of nitric oxide is inhibited.[75] Thus, GFR and RBF of the healthy kidney are maintained, at least in part, by basal HO activity and the vasorelaxant effects of CO, the latter likely dependent on renal generation of nitric oxide. Table 2 lists mechanisms which contribute to the vasorelaxant effects of HO/CO.[22,43–45,76–78] In addition to its hemodynamic effects, HO activity may promote sodium and fluid absorption in the loop of Henle.[79,80]

EXPRESSION OF HO-1 IN THE DISEASED KIDNEY

Table 3 lists conditions and stimuli that upregulate HO-1 in the kidney. In human nephropathies and kidney transplants, and in animal models of renal disease, stimulated expression of HO-1 is largely observed in the renal tubular epithelium. In proteinuric human kidney disease, HO-1 protein is induced in tubular epithelial cells, more prominently in distal tubules rather than proximal tubules, but is not expressed in resident glomerular cells; expression of HO-1 protein in proximal tubules, but not in distal tubules, correlates with proteinuria, hematuria, and tubulointerstitial disease.[117,118] The propensity for upregulation of HO-1 protein to occur in renal tubules but not in glomerular cells in kidney disease may relate to the differential sensitivity and response to oxidant stress exhibited by these cells.[119] For example, upon exposure to heme, proximal tubular epithelial cells as compared to mesangial cells more vigorously express

Table 3 | Conditions and stimuli that may be accompanied by increased expression of HO-1 in the kidney (inducers that are not referenced are discussed elsewhere in the text)

Assorted human nephropathies	Vasoactive substances
Kidney transplant[81]	Angiotensin II
Kidney disease models	Nitric oxide[96]
Renal ischemia	Dopamine[97]
Glomerulonephritides	Atrial natriuretic peptide[98]
Diabetic nephropathy	Carbon monoxide[99]
Heme protein-induced	Therapeutic/dietary substances
Toxic nephropathy	Gentamicin
Urinary tract obstruction[82]	Cyclosporine[100]
Acute rejection[83]	Parenteral iron preparations[101]
Liver disease	Morphine[102]
Endotoxin-induced	Curcumin[103]
Polycystic kidney disease[84]	Endogenous proteins or other stimuli
Aging[85]	Ngal
Radiation nephropathy[86]	CD40[104]
Cytokines/growth factors	Stra13[105]
Interleukin–1β[87]	SSAT[106]
TGF-β1[88]	15dPGJ2[107]
HGF[89]	Assorted stressors/toxins
BMP-7[90]	Heavy metals[108]
VEGF[91]	Puromycin[109]
Oxidant stress	Phenylhydrazine[110]
Hydrogen peroxide	Bromobenzene[111]
Heme	Osmotic stress[112]
Iron	Increased temperature
Glutathione depletion[92]	Hemodynamic stress[113]
Oxidized LDL[93]	Sickle cell nephropathy[114]
Linoleyl hydroperoxide[94]	Models of systemic hypertension[115]
Hypochlorite-modified LDL[95]	Renal carcinoma[116]

BMP, bone morphogenetic protein; HO, heme oxygenase; CD, cluster of differentiation; HGF, hepatocyte growth factor; LDL, low-density lipoprotein; Ngal, neutrophil gelatinase-associated lipocalin; SSAT, spermidine/spermine *N*-1-acetyl-transferase; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.

HO-1, and HO-1 so induced, efficiently protects against oxidant injury in tubular epithelial cells.[119]

The induction of HO-1 in the tubular epithelium in proteinuric states cannot be simply ascribed to increased trafficking of albumin *per se* across the proximal tubule;[120] such expression more likely reflects concomitant injury to tubular epithelial cells occurring *pari passu* with glomerular disease, and/or the proinflammatory, pro-oxidant, or other perturbing effect of specific proteins or other species appearing in the urinary space.[120]

ACUTE RENAL INJURY

Tables 4 and 5 summarize the salient findings from studies that examine the significance of induction of HO-1 in the kidney following toxic and ischemic insults, respectively.

In acute ischemic injury, approaches based on inhibition of HO activity or induction of HO-1 have yielded mixed results (Table 5). The reasons for these divergent findings may reside in the lack of specificity of agents employed, or differences in the models of ischemia. The former consideration is obviated in the HO-$1^{-/-}$ mouse.[135] Following relatively mild renal ischemia that exerts little effect in HO-$1^{+/+}$ mice, HO-$1^{-/-}$ mice exhibit marked deterioration in renal function and heightened renal upregulation of caspase-3.[135] Renal ischemia caused increased mortality in HO-$1^{-/-}$ mice

Table 4 | Summary of studies examining the functional significance of induction of HO-1 in toxic nephropathy

Model	Reference
Cisplatin	
Inhibition of HO activity worsens renal injury	48
HO-$1^{-/-}$ mice exhibit worse renal injury and more severe apoptosis	121
Upregulation of HO-1 protects against cell injury *in vitro*	121
Inhibition of HO activity worsens cell injury *in vitro*	122
Gentamicin	
No apparent effect on inhibiting HO activity	48
Cyclosporine	
Induction of HO-1 reduces renal injury	123
Mercuric chloride	
Prior induction of HO-1 does not protect against, nor does inhibition of HO activity worsen, renal injury induced by a higher dose	124
Prior induction of HO-1 protects against renal injury induced by a lower dose	125
Maleate nephropathy	
Inhibition of HO activity worsens proteinuria, histologic injury, and apoptosis	120
Potassium dichromate	
Induction of HO-1 protects against renal injury	126

HO, heme oxygenase.

Table 5 | Effect of modulating HO activity in renal ischemia–reperfusion injury

Inhibition of HO activity by metalloporphyrins	References
Worsening of renal injury	127
No apparent effect	48,128
Reduction of renal injury with high doses and no apparent effect with low doses	129
Non-specific induction of HO activity	
Protective effects provided by various inducers	92,130–134
Exacerbatory effect provided by hemin	129

HO, heme oxygenase.

but no mortality in HO-$1^{+/+}$ mice, and in surviving HO-$1^{-/-}$ mice, marked renal histologic injury occurred. This exacerbation in renal injury in HO-$1^{-/-}$ mice was accompanied by a heightened inflammatory response, as reflected by increased activation of NF-κB and NF-κB-dependent genes such as monocyte chemoattractant protein-1.[135] Increased sensitivity to renal ischemia is also acutely observed in studies of HO-$1^{-/-}$ mice subjected to the one-kidney, one-clip model; in these studies, expression of endothelin-1 is increased, and an ET_A receptor antagonist prevents such sensitivity to renal ischemia.[136]

Products of HO may protect against renal ischemic injury as shown by studies utilizing CO-releasing compounds, the latter attenuating the rise in serum creatinine and histologic injury when administered before the ischemic insult.[128] These

compounds also induce HO-1 in the ischemic kidney, but such induction is not necessary for the protective effects of CO-releasing molecules.[128] Bilirubin may also mitigate renal ischemic injury, as demonstrated in studies using the isolated perfused kidney.[137]

Studies of renal transplantation in syngeneic rats provide additional evidence for the protective effects of HO-1 in the ischemic kidney. For example, the induction of HO-1 by cobalt protoporphyrin,[138] heat pre-conditioning,[138] or adenoviral gene transfer[139] improves graft function and reduces structural injury. In such models, exposure to CO is also protective: recipients of a kidney transplant from syngeneic rats maintained in a CO-containing atmosphere (250 parts per million) exhibit less renal dysfunction, histologic injury, and mortality, as compared to similarly transplanted rats maintained in room air.[140] In this model, a combination of CO and biliverdin confers beneficial effects greater than either product alone.[141]

The protective effects of HO-1 and its products in acute renal injury likely reflect the vasorelaxant, anti-inflammatory, and antiapoptotic effects of the HO system. The antiapoptotic effects of HO-1 in the kidney were first described in cisplatin nephropathy.[121] While the basis for the antiapoptotic effects of HO-1 in the kidney is unresolved, it may involve the induction of p21, an antiapoptotic cell cycle inhibitor that is inducible by iron and CO.[142–144] Studies in other tissues demonstrate that the antiapoptotic effects of HO-1 are largely mediated by CO, and can interrupt either the mitochondrial or death receptor apoptotic pathways via mechanisms that depend on the model system employed.[145–151] For example, in assorted models, the antiapoptotic effects of HO-1/CO are ascribed to the following: guanylate cyclase;[145] activation of p38 mitogen-activated protein kinase;[146] p38β mitogen-activated protein kinase-dependent, Hsp70-effected mechanisms;[149] signaling pathways involving PI3K/Akt, p38 mitogen-activated protein kinase, and STAT3;[150] and interruption of GADD153-dependent mechanisms.[151] Induction of HO-1 may also prevent cell death by facilitating extracellular transport of iron, thereby suppressing iron-driven oxidant stress.[152]

RENAL INFLAMMATION

Nephrotoxic serum nephritis provides a model of anti-glomerular basement membrane nephritis. In the accelerated and heterologous subtypes of nephrotoxic serum nephritis, HO-1 is induced in glomerular intracapillary mononuclear cells;[153] HO-1 is also upregulated in renal tubules in the heterologous subtype of this model.[60,153] Prior induction of HO-1 by hemin reduces proteinuria and glomerular infiltration of leukocytes in either subtype, and in accelerated nephrotoxic serum nephritis, hemin also reduces formation of glomerular microthrombi.[153] The protective effect of hemin is accompanied by HO-mediated reduction in glomerular expression of inducible nitric oxide synthase (iNOS), the latter considered a mediator of glomerular injury in nephrotoxic serum nephritis.[154,155] Similar approaches demonstrate a protective role for HO-1 in murine lupus nephritis wherein hemin attenuated proteinuria and decreased, in glomeruli, expression of iNOS, presence of immune reactants, and histologic injury; hemin also reduced systemic levels of anti-DNA antibodies.[156] CO may mediate the suppressive effect of HO-1 on iNOS expression since, as shown in studies in a model of obliterative bronchiolitis, CO inhibits NF-κB-driven iNOS transcription.[157] A protective role for HO-1 is also incriminated in the anti-Thy 1 model of glomerulonephritis.[158]

HO-1 also suppresses inflammation in models of tubulointerstitial disease.[159] For example, when repetitively exposed to heme proteins, HO-$1^{-/-}$ mice, as compared with HO-$1^{+/+}$ mice, exhibit an amplified inflammatory response, intense upregulation of monocyte chemoattractant protein-1, and increased activation of NF-κB.[159] These findings raise the possibility that HO-1, by controlling cellular redox, can inhibit activation of NF-κB and NF-κB-driven cytokine expression. Indeed, even in the basal, unstressed state, HO-$1^{-/-}$ mice exhibit increased systemic levels of monocyte chemoattractant protein-1 and expression of monocyte chemoattractant protein-1 mRNA in circulating leukocytes.[135]

Other studies utilizing HO-$1^{-/-}$ mice underscore the proclivity towards a proinflammatory state fostered by the inability to express HO-1. For example, HO-$1^{-/-}$ mice exhibit striking mortality following the administration of lipopolysaccharide,[160] whereas splenocytes from HO-$1^{-/-}$ mice, when exposed to lipopolysaccharide, exhibit increased production of proinflammatory cytokines with a preponderating Th1 profile.[161]

Products of HO-1, such as CO, exert anti-inflammatory effects. In lipopolysaccharide-stimulated macrophages, CO, at low concentrations, attenuates generation of proinflammatory cytokines, while stimulating production of anti-inflammatory cytokines such as IL-10;[26] these anti-inflammatory effects involve activation of p38 mitogen-activated protein kinase.[26] The upregulation of IL-10 is of particular interest since IL-10 is a recognized protectant against renal injury;[162] additionally, IL-10 exerts anti-inflammatory effects via HO-dependent mechanisms,[163] thereby providing a positive feedback loop between HO/CO and IL-10. CO also suppresses expression of proinflammatory cytokines such as endothelin-1 and PDGF.[76]

HO/CO can inhibit T cells.[164,165] In this regard, the suppressive effects of regulatory T cells (Treg) on cellular proliferation and cytokine production involve the induction of HO-1 and increased HO activity in Treg.[164] Such effects may be mediated by CO since CO inhibits T-cell proliferation by impairing the synthesis of IL-2[166] and by suppressing caspase activity,[167] the latter contributing to T-cell proliferation. Induction of HO-1 can also suppress the maturation of dendritic cells and their capacity to promote inflammation and T-cell proliferation.[168]

Bile pigments possess anti-inflammatory properties, and can recapitulate the inhibitory effects of HO-1 on the

following proinflammatory changes: endothelial adhesion of leukocytes on oxidant-exposed mesenteric vessels;[33] tumor necrosis factor α-induced endothelial activation;[169] and upregulation of selectins in endotoxin-treated kidneys.[170] These effects of bile pigments may involve oxidant-scavenging properties,[78] their capacity to inhibit extracellular signal-regulated protein kinase 1/2 phosphorylation,[171] and their inhibition of nicotinamide adenine dinucleotide phosphate (reduced form) oxidase activity.[172]

KIDNEY TRANSPLANTATION

Studies in the late 1990s demonstrated that HO-1 reduced vascular rejection in murine cardiac allografts,[173] and prolonged the survival of cardiac xenografts.[174] Upregulation of HO-1 also protects renal allografts. For example, in acute kidney rejection in the rat, immune-modulatory peptides that induce HO-1 *in vivo*, when administered with low doses of cyclosporine A, reduced allograft injury and improved graft function.[175] In chronic renal allograft rejection in the rat, the upregulation of HO-1 in the donor kidney by cobalt protoporphyrin, administered prior to transplantation, reduced proteinuria, decreased allograft histologic injury, and improved allograft survival.[176] Upregulation of HO-1 by cobalt protoporphyrin in brain-dead donors similarly promotes survival of the kidney graft.[177]

Overexpression of HO-1 in the recipient can also reduce chronic kidney allograft injury.[178] Relevant to this finding are the observations that preservation of cardiac allografts and the prevention of aortic allograft rejection may be more effectively determined by systemic as compared with regional overexpression of HO-1.[179,180] In this regard, evidence has appeared indicating that HO-1 can engender activation-induced cell death of alloreactive T cells (AICD), an effect of HO-1 which would facilitate graft tolerance.[181] Thus, upregulation of HO-1 may confer complementary beneficial effects to the transplanted kidney: induction of HO-1 in the renal allograft may render the kidney resistant to ischemic injury and the adverse effects of the rejection process, while systemic upregulation of HO-1 may mitigate the immune processes that drive rejection.[181,182]

The clinical importance of HO-1 in maintaining kidney graft survival is underscored by several observations: polymorphisms in the HO-1 gene associated with increased HO activity predict improved graft function and survival, when such polymorphisms are expressed by the donor;[183,184] improved early function of renal transplants occurs in recipients treated with bioflavonoids, agents which induce HO-1;[185] and rapamycin, an effective immune suppressive agent, relies on HO-1 for some of its biologic effects.[186]

ANGIOTENSIN II-INDUCED RENAL INJURY

Chronic administration of angiotensin II induces systemic hypertension, proteinuria, oxidative stress, and HO-1.[187,188] In the kidney such upregulation is mainly in the renal tubular epithelium, whereas in the vasculature it occurs largely in endothelial and adventitial cells.[187–189] This upregulation of HO-1 is functionally significant: inhibition of HO activity worsens proteinuria and GFR, whereas the administration of hemin attenuates hypertension, proteinuria, the reduction in GFR, and apoptosis induced by angiotensin II;[188,190] additionally, overexpression of HO-1 by a retroviral approach reduces the pressor response to angiotensin II.[191]

The vasorelaxant effects of HO are likely mediated through CO and bilirubin. In the isolated perfused kidney, angiotensin II exerts pressor effects and generates CO via HO-1; inhibiting HO blocks the generation of CO and exaggerates the pressor effect of angiotensin II.[192] Bilirubin also attenuates the pressor effects of angiotensin II, as demonstrated in studies utilizing the hyperbilirubinemic Gunn rat.[78] This model exhibits resistance to the pressor effect of angiotensin II, less impairment in endothelium-dependent vascular relaxation, and greater preservation of vascular content of tetrahydrobiopterin, the latter representing an essential co-factor for endothelial nitric oxide synthase activity.[78] By scavenging oxidants, bilirubin preserves the vascular content of tetrahydrobiopterin, thereby optimizing endothelial nitric oxide synthase activity and, in turn, vascular relaxation.[78]

HO-1 also attenuates the cytotoxic effects of angiotensin II, as shown in studies utilizing tubular epithelial cells derived from the proximal tubule[193] or the thick ascending limb.[194]

EXPERIMENTAL DIABETES

In streptozotocin-induced diabetes, HO-1 is upregulated in mesangial and glomerular epithelial cells.[195,196] While such expression may be attenuated by insulin or antioxidants, its functional significance with regard to diabetic glomerular injury is currently unaddressed.[195,196] Streptozotocin-induced diabetes, imposed in states characterized by decreased HO activity as occurs in HO-$2^{-/-}$ mice, stimulates superoxide anion production and provokes prominent tubulointerstitial injury; remarkably, these adverse effects of streptozotocin-induced diabetes in HO-$2^{-/-}$ mice are attenuated when HO-1 is induced in these mice.[197] Upregulation of HO-1 also protects against oxidative stress, endothelial cell loss, and vascular dysfunction that occur in streptozotocin-induced diabetes,[198–201] and endothelial overexpression of HO-1 reduces glucose-induced apoptosis of endothelial cells.[202] In aggregate, these findings indicate that, in the diabetic milieu, upregulation of HO-1 can confer salutary effects in the kidney and vasculature.

SPECIFICITY AND DUALITY OF EFFECTS OF THE HO SYSTEM

The effects of induced HO-1 are often tissue-specific and target-dependent. For example, while inhibiting the growth of renal tubular epithelial cells,[142] HO-1 promotes the growth of endothelial cells.[203] This duality of effects of HO-1 is reflected by the actions of its products: in endotoxin-treated rats, CO inhibits iNOS expression in the injured lung but promotes iNOS expression in the injured liver;[204] CO inhibits oxidant generation by inhibiting nicotinamide adenine dinucleotide phosphate (reduced form) oxidase, but may

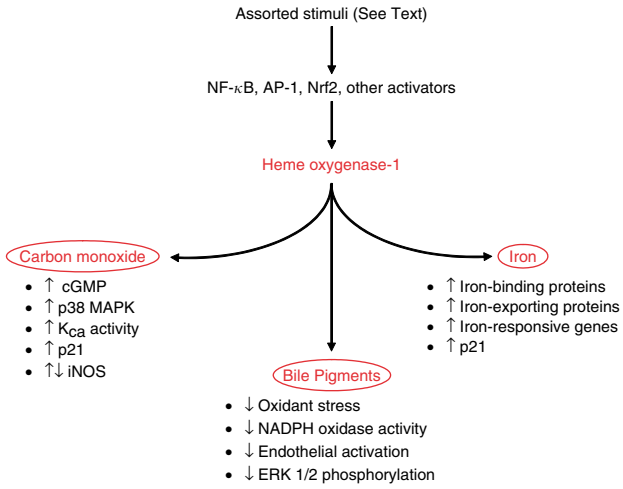

Figure 2 | Cellular effects of products of the HO-1 system.

promote mitochondrial generation of oxidants by inhibiting mitochondrial cytochromes.[205] While CO is clearly vasorelaxant, evidence has emerged that, under certain conditions, CO may inhibit vasorelaxant responses by inhibiting endothelial nitric oxide synthase.[77,206] Thus, the actions of HO and its products often depend on the specific tissue, cell type, and cellular target that are involved, and the pathophysiologic setting in which HO-1 is induced.

With these caveats in mind, Figure 2 schematizes some of the distal effects of products of HO activity.

ADVERSE EFFECTS OF INDUCED HO-1

Induction of HO-1 may lead to renal injury in certain settings.[207,208] Renal injury is reduced, for instance, when HO activity is inhibited by metalloporphyrins in proximal tubules harvested from rats subjected to heme protein-induced renal injury,[209] and in oxidant-exposed, tubular epithelial cells.[210] Such toxicity may reflect the fact that in sufficient amounts, the products of HO can all be harmful. Indeed, as pointed out by Paracelsus some 500 years ago, 'Poison is in everything, and no thing is without poison. The dosage makes it a poison or a remedy'.[211] While non-specific effects are a consideration in studies utilizing metalloporphyrin inhibitors,[212,213] inhibition of HO activity may confer protection in the following manner. HO activity releases iron from heme and, if the induction of HO activity is inadequately coupled to processes that sequester or export iron, the attendant elevation in cellular iron may drive iron-dependent oxidative stress. In this regard, in fibroblasts with varying levels of genetically induced HO-1 overexpression, relatively lower levels of overexpression protect against oxidant injury while higher levels of HO-1 expression exacerbate such injury, the latter associated with increased cellular levels of iron.[214,215] Excessive amounts of CO and bile pigments can also prove damaging. For example, the vasorelaxant effects of HO-1, induced in the vasculature, presumably acting through CO, may adversely affect systemic and renal hemodynamics following endotoxemia,[216] and promote systemic vasodilatation in cirrhosis.[217] Finally, it is possible that the protective effects observed in some studies utilizing metalloporphyrin inhibitors may reflect not the inhibition of HO activity but the beneficial effects of HO-1 protein, the latter reciprocally induced when HO activity is inhibited.[129,218] Through protein–protein interactions or other effects, HO-1 protein, independent of HO activity, may confer cytoprotective properties.

CONCLUSION

The protective effects conferred by HO-1 and its products in models of renal injury offer exciting therapeutic prospects for human kidney disease. The challenges, however, are many and include the following: the delivery of requisite and optimal amounts of product such that protection is achieved and toxicity is avoided; the determination of which specific HO product, or combination of products, is required for renal protection in a given setting; and methods that effectively deliver such products specifically to the kidney. Moreover, strategies that rely on the upregulation of HO-1 are faced with issues such as the cell and target specificity of the effects of HO-1, and the possibility that sustained induction of HO-1 may be damaging.

Additional therapeutic approaches may be based on biliverdin reductase: this biliverdin-inducible enzyme not only converts biliverdin to bilirubin but also is now recognized as a serine/threonine kinase[219] and as a protectant, independent of HO-1, in the kidney.[220] Another avenue may seek to discover non-toxic endogenous substances that induce HO-1, and utilize such substances in protecting against tissue injury. In this regard, induction of HO-1 turns out to be the mechanism underlying the protection against renal ischemic injury conferred by neutrophil gelatinase-associated lipocalin, a protein which can induce renal tubules to develop from mesenchyme,[221] and is strongly expressed after renal ischemia.[134] Remarkably, the induction of HO-1 and attendant renal protection by neutrophil gelatinase-associated lipocalin involve the intracellular delivery of iron by neutrophil gelatinase-associated lipocalin.[134,221] These seminal findings not only point the way for a novel therapeutic approach but also may alter existing paradigms regarding the toxicity of cellular iron: by eliciting HO-1 and other iron-responsive genes, increments in cellular levels of iron may recruit protective pathways, which, in aggregate and ultimately, more than override the intrinsic cytotoxicity of iron.[134]

In addition to its therapeutic implications, the facile recruitment of HO-1 in injured tissue affords insights into the nature of cellular resistance to stress. As defined and discussed by Barabasi in his masterful analysis of networks, the *robustness* of biologic systems – their capacity to survive stress – is dependent on the *interconnectivity* of nodes that comprise their underlying networks, particularly so on those uncommon nodes in the network that are *highly connected* and are termed *hubs*; the importance of hubs is underscored by the fact that the integrity and existence of a network are

imperiled when hubs are incapacitated.[222,223] In this regard, HO-1 is an outstanding example of a hub in a network, receiving as it does afferent signals from manifold stimuli, and engendering, in turn, nuanced efferent responses via CO, bile pigments, and iron, and its linkage to biliverdin reductase (Figure 2). The significance of HO-1 as a cytoprotective strategy thus draws upon two essential properties: the vigilance of HO-1 in sensing cellular stress and its ready recourse to diverse intracellular networks, thereby enabling a swift and often salutary response to such stress.

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