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Hemeoxygenase-1 and Renal Ischaemia-Reperfusion Injury

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Abstract

Degradation by the inducible enzyme hemeoxygenase-1 (HO-1) is the principal route of mammalian heme metabolism. The resultant generation of free iron, carbon monoxide and biliverdin results in myriad actions including promoting cell survival, circulatory integrity and immunomodulation. This review examines the evidence from both human studies and work performed in experimental models implicating the intrinsic heme-HO-1 pathway as important in determining both the susceptibility and severity of acute kidney injury. Additional work using chemical inducers of HO-1 has demonstrated the efficacy of strategies to upregulate enzyme activity in ameliorating the severity of experimental ischaemia-reperfusion injury whilst genetic ablation of HO-1 or pharmacological inhibition of HO-1 activity results in an augmented injury phenotype. There remain a multitude of candidate pathways to account for the therapeutic efficacy of HO-1 induction. Although this may reflect a truly multifactorial mechanism of action, the identification of the relative contribution of key components such as carbon monoxide generation remains critical to allow the rational design of agents for translational application in human disease.

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Introduction

Acute kidney injury (AKI) is a devastating clinical condition associated with high levels of morbidity and mortality despite best supportive care and lacks any specific interventions to improve outcome. The majority of cases of AKI do not arise from primary renal disease but rather as a result of systemic insults such as dehydration, surgery or sepsis leading to renal hypoperfusion. If renal perfusion is not immediately restored, then ischaemia-reperfusion injury (IRI) can occur in the kidney leading to acute tubular necrosis (ATN) and functional derangement in the form of AKI. Research using experimental animal models has led to an increasing appreciation that the eventual injury phenotype is determined by a balance between intrinsic and extrinsic factors promoting cellular function and survival, and the deleterious effects of circulatory dysfunction and immune activation within the kidney.

The Biological Role of Hemeoxygenase-1

Hemeoxygenases are the enzymes responsible for the catalysis of heme molecules in mammalian cells. They play a vital biological role in the response to cellular injury, which can result in the denaturing of heme-containing compounds. These free heme molecules act as a potent source of secondary oxidative stress if not promptly metabolized.
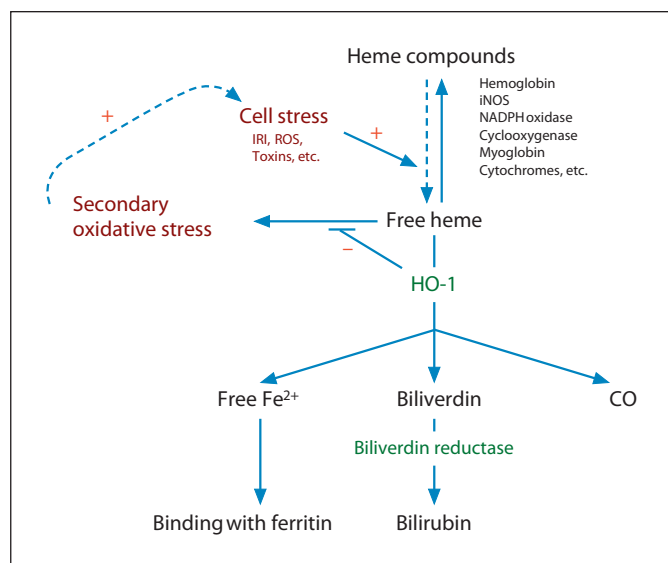
Fig. 1. The heme-HO-1 pathway. ROS = Reactive oxygen species; iNOS = inducible nitric oxide synthase; NADPH = nicotinamide-adenine dinucleotide phosphate reduced form.

Whilst the constitutively expressed enzymes hemeoxygenase-2 and -3 contribute a basal level of heme metabolism, the predominant route of heme degradation is via inducible hemeoxygenase-1 (HO-1). In addition to removing a source of oxidative stress, 2 products of heme metabolism, biliverdin and carbon monoxide (CO), are recognized to possess immunomodulatory, anti-apoptotic and in the case of CO, vasoactive properties [1] (fig. 1). HO-1 induction is also coupled to the increased availability of ferritin, leading to prompt conjugation and removal of free iron – another source of potential oxidative stress.

As such, HO-1 may modulate the behaviour of the immune system and the function of the microcirculation, all of which may ameliorate IRI. Accordingly, both the role of HO-1 in the renal response to IRI and its potential therapeutic manipulation are the subject of ongoing research.

HO-1 and the Kidney

Our current knowledge of the role of HO-1 in renal disease is largely based on experience of animal models of kidney disease, with additional insights from limited studies performed in human renal biopsy material. Heme molecules are generated ubiquitously by all nucleated cells in the body including those of the kidney, resulting in the need for a similarly widespread availability of HO-1 for heme metabolism. Both experimental models and human biopsy material have demonstrated that HO-1 is expressed at low levels within the healthy kidney. Also, human data suggest that HO-1 is a component of the tubulointerstitial response to injury, with tubular induction of HO-1 correlating with reduced markers of oxidative stress [2]. These findings are consistent with the single reported case of human HO-1 deficiency, which was characterized by systemic inflammation, haemolysis and a nephropathy with progressive tubulointerstitial inflammation [3].

The kidney can be subject to additional heme loads after tissue injury via the circulation – with experimental models demonstrating the key role for HO-1 in the successful metabolism of otherwise cytotoxic quantities of myoglobin after induction of rhabdomyolysis [4]. Consistent with this, the HO-1$^{-/-}$ mouse demonstrates exaggerated iron accumulation in the kidney under physiologic conditions with heightened susceptibility to AKI following models of IRI, rhabdomyolysis and cisplatin-induced AKI [5–7].

HO-1 and Renal IRI

HO-1 is induced in a range of organs including the rodent kidney in response to experimental IRI [8]. Furthermore, data from pre- and post-implantation biopsies of human renal allografts demonstrate HO-1 induction in post-implantation specimens, with the maximal HO-1 induction evident in allografts exhibiting delayed graft function [9]. Renal HO-1 expression can be induced by diverse noxious stimuli including hypoxia, lipopolysaccharide administration and bile duct ligation, all of which result in protection against a subsequent, more severe experimental IRI insult; this implicates HO-1 induction as a potential mediator of the ischaemic pre-conditioning phenomenon (reviewed in [10]).

HO-1 Upregulation as Therapy in Renal IRI

HO-1 gene transcription is influenced by pleiotropic signals including heme itself, heat shock, hypoxia (in part via stabilization of hypoxia-inducible factor-1α), and inflammatory cytokines. These diverse stimuli act via a similarly broad range of signalling pathways. The promoter region of HO-1 contains binding sites for nuclear factor (NF)-κB and activator protein-1 and 2, amongst others (comprehensively reviewed in [11]). The transcription factor NF-E2-related factor-2 (Nrf2) is recognized as
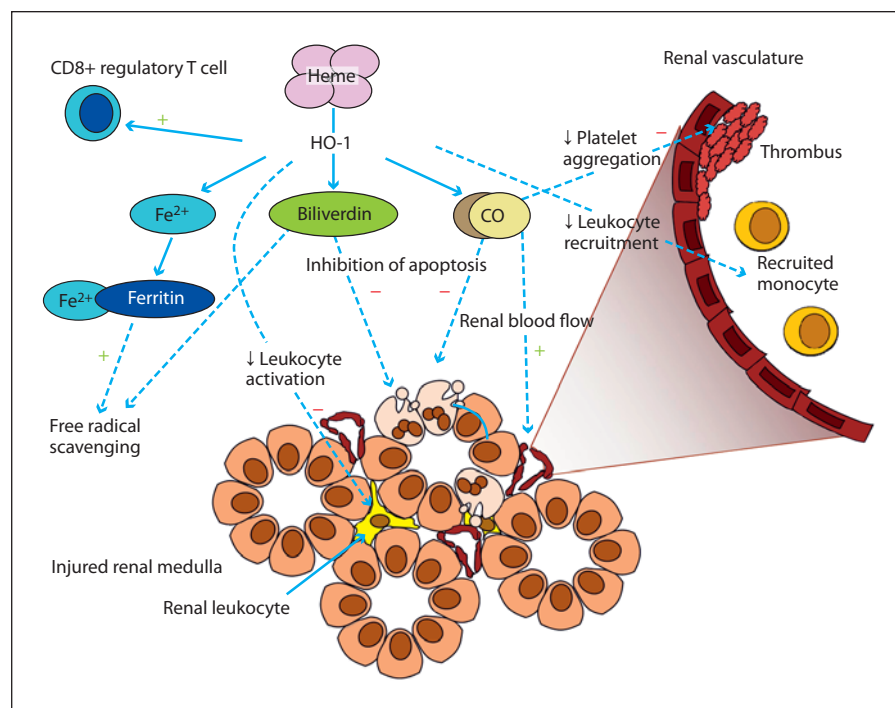
Fig. 2. Current working hypothesis of the putative actions of HO-1 in AKI.

the key mediator of HO-1 induction and protection seen after experimental IRI both in vivo and in vitro [11, 12], and is a focus of ongoing research.

Much of our understanding of the potential of HO-1 as a therapeutic target in renal IRI came from studies examining whether the administration of pharmacological agents to either induce or inhibit HO-1 modulates the outcome of experimental renal IRI (reviewed in [10]). In general, chemically induced upregulation of HO-1 prior to the induction of IRI results in functional and structural protection, whilst the inhibition of HO-1 activity typically abolishes the protected phenotype and often augments injury. Such approaches are not without caveats, as the widely used HO-1 inducer hemin is itself a pro-oxidant whilst HO-1-inhibiting protoporphyrin compounds almost ubiquitously inhibit the activity of other physiologically relevant enzymes, such as inducible nitric oxide synthase [13].

Putative Mechanisms Mediating HO-1 Protection in IRI

Given that HO-1 activity results in the removal of a potent cell stressor and the production of biologically active metabolites, the mechanisms underlying the protect-

ed phenotypes reproducibly seen following HO-1 induction are complex and likely multifactorial (fig. 2). The development of novel carbon monoxide-releasing molecules (CORMs) that act as CO donors in vivo will allow the relative contribution of the downstream products of HO activity (including HO-1) to be probed. Indeed, studies with pre-administration of CORMs have demonstrated functional protection comparable to HO-1 induction in IRI, implicating CO as a key component of the protected renal phenotype associated with HO-1 induction [14].

Effects on Renal Blood Flow and Microcirculation

Chemical inhibition of HO-2 and HO-1 activity in the healthy kidney results in reduced medullary blood flow, thus supporting a role for HO-1 in the maintenance of medullary perfusion under physiological conditions [15]. The potential importance of induced HO-1 in maintaining renal perfusion has been demonstrated by studies involving the pre-treatment of donor rats with hemin to induce HO-1 expression. The subsequent transplants exhibited preserved renal function, with an increase in capillary blood flow and the diameter of intra-renal vessels by intravital microscopy [16]. It is now recognized that CO exerts important effects on the circulation via its potent vasodilatory effects and potentially via the inhibition of platelet aggregation [17]. In explanted kidneys in an

isolated organ-perfusion circuit, CORM-derived CO had beneficial effects on renal blood flow and creatinine clearance. This is clearly independent of any effects on systemic haemodynamics, demonstrating the importance of the intra-renal actions of CO [18]. Micropuncture studies have demonstrated that HO-1 induction by stannous mesoporphyrin inhibits tubuloglomerular feedback-induced afferent arteriolar vasoconstriction [19]. This effect is reproduced by the administration of either a CORM or exogenous biliverdin, thus implicating both heme metabolites in the mediation of this effect. Studies combining inhaled CO with infused bilirubin in rat renal transplantation demonstrated synergistic effects on both graft survival and on glomerular filtration rate and blood flow rates [20].

Effects on Cell Apoptosis and Survival

In keeping with the cytoprotective role of HO-1, there is a consistent pattern of reduced cell death present throughout studies of HO-1 upregulation whilst conversely HO-1 inhibition or constitutive absence results in increased levels of apoptosis and necrosis. The pleiotropic effects of HO-1 make dissection of the exact mechanism of this pro-survival effect problematic. Although it remains possible that it is a secondary phenomenon due to the improved tissue perfusion and reduced immune cell activation, there is considerable evidence from other organs implicating CO as an anti-apoptotic signal when present in physiologic concentrations [21]. Additional in vitro evidence from studies of cultured tubular epithelial cells has implicated HO-1 as promoting cell survival by induction of the cyclin-dependent kinase inhibitor p21 [22].

Modulation of Local or Systemic Immune Phenotype

The role of HO-1 in the modulation of immune responses is a rapidly evolving field in immunology, with an increasing recognition that HO-1 may act as a 'molecular brake' on the activation, recruitment and amplification of immune responses (reviewed in [1]). Overexpression of HO-1 results in reduced expression of endothelial leukocyte adhesion molecules and reduced activity of the NF-κB pathway whilst constitutively HO-1-deficient animals exhibit increased levels of monocyte chemoattractant protein-1 [5]. Constitutive HO-1 expression by the Kupffer cells of the liver has been demonstrated to be of benefit in hepatic IRI [23], and HO-1 is induced within renal macrophages by statin therapy, an intervention associated with a protected phenotype in IRI [24]. Recently published work has implicated HO-1 as a target antigen for CD8+ regulatory T cells, resulting in profound immunomodulation of cellular immune responses [25]. This finding reinforces results from HO-1-null animals showing expanded regulatory T cell numbers, which fail to adopt a regulatory phenotype in vivo due to the absence of HO-1 within the antigen-presenting cell [26]. Given the recognized role of lymphocyte populations in determining susceptibility to IRI, this adds an additional 'extra-enzymatic' arm to the immunomodulatory properties of HO-1.

HO-1 – a Target for Translatable Therapies?

Understanding the mechanisms mediating the initiation and propagation of renal IRI remains the focus of intense research worldwide. Despite this, the challenge remains to generate therapies that can be translated successfully 'from bench to bedside' to improve outcome in what remains a devastating clinical problem. As with many agents showing therapeutic promise in animal studies, the utility of HO-1-based interventions are currently limited by the apparent need to administer therapy prior to the onset of the renal insult.

There are, however, a number of clinical settings such as major cardiac or vascular surgery and admissions into critical care environments where AKI may be probable, or in the case of renal transplantation, inevitable. Additionally, the advent of better identification of individuals at high risk of developing AKI or incipient AKI using criteria such as RIFLE may allow windows of therapeutic opportunity to be identified to allow timely intervention. There is clearly a need for HO-1-inducing agents licensed for use in humans, or other novel interventions such as CORM administration or the inhalation of low-concentration CO during 'high-risk' surgery. The documented HO-1-inducing effects of statins coupled to their established safety profile make them intriguing candidates, although it is of note that the multi-centre ALERT study looking at statin administration in transplantation showed no positive impact on acute or chronic rejection or graft function over 5+ years [27], in contrast to their promising immunomodulatory effects in experimental disease.

Conclusions

The heme-HO-1 system appears of intrinsic importance in the evolution of AKI. Further elucidation of which aspects of the HO-1 pathway are indispensible to

renal protection will be necessary, potentially coupled to the generation of novel therapeutic molecules to harness such mechanisms and allow successful translation to the clinic.

References

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

2 Morimoto K, Ohta K, Yachie A, Yang Y, Shimizu M, Goto C, Toma T, Kasahara Y, Yokoyama H, Miyata T, Seki H, Koizumi S: Cytoprotective role of heme oxygenase (HO)-1 in human kidney with various renal diseases. Kidney Int 2001;60:1858–1866.

3 Koizumi S: Human heme oxygenase-1 deficiency: a lesson on serendipity in the discovery of the novel disease. Pediatr Int 2007;49:125–132.

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

5 Pittock ST, Norby SM, Grande JP, Croatt AJ, Bren GD, Badley AD, Caplice NM, Griffin MD, Nath KA: MCP-1 is up-regulated in unstressed and stressed HO-1 knockout mice: pathophysiologic correlates. Kidney Int 2005;68:611–622.

6 Shiraishi F, Curtis LM, Truong L, Poss K, Visner GA, Madsen K, Nick HS, Agarwal A: Heme oxygenase-1 gene ablation or expression modulates cisplatin-induced renal tubular apoptosis. Am J Physiol Renal Physiol 2000;278:F726–F736.

7 Nath KA, Haggard JJ, Croatt AJ, Grande JP, Poss KD, Alam J: The indispensability of heme oxygenase-1 in protecting against acute heme protein-induced toxicity in vivo. Am J Pathol 2000;156:1527–1535.

8 Maines MD, Mayer RD, Ewing JF, McCoubrey WK Jr: Induction of kidney heme oxygenase-1 (HSP32) mRNA and protein by ischemia/reperfusion: possible role of heme as both promotor of tissue damage and regulator of HSP32. J Pharmacol Exp Ther 1993; 264:457–462.

9 Ollinger R, Kogler P, Biebl M, Sieb M, Sucher R, Bösmüller C, Troppmair J, Mark W, Weiss H, Margreiter R: Protein levels of heme oxygenase-1 during reperfusion in human kidney transplants with delayed graft function. Clin Transplant 2008;22:418–423.

10 Nath KA: Heme oxygenase-1: a provenance for cytoprotective pathways in the kidney and other tissues. Kidney Int 2006;70:432–443.

11 Sikorski EM, Hock T, Hill-Kapturczak N, Agarwal A: The story so far: molecular regulation of the heme oxygenase-1 gene in renal injury. Am J Physiol Renal Physiol 2004; 286:F425–F441.

12 Leonard MO, Kieran NE, Howell K, Burne MJ, Varadarajan R, Dhakshinamoorthy S, Porter AG, O'Farrelly C, Rabb H, Taylor CT: Reoxygenation-specific activation of the antioxidant transcription factor Nrf2 mediates cytoprotective gene expression in ischemia-reperfusion injury. FASEB J 2006;20:2624–2626.

13 Appleton SD, Chretien ML, McLaughlin BE, Vreman HJ, Stevenson DK, Brien JF, Nakatsu K, Maurice DH, Marks GS: Selective inhibition of heme oxygenase, without inhibition of nitric oxide synthase or soluble guanylyl cyclase, by metalloporphyrins at low concentrations. Drug Metab Dispos 1999;27:1214–1219.

14 Vera T, Henegar JR, Drummond HA, Rimoldi JM, Stec DE: Protective effect of carbon monoxide-releasing compounds in ischemia-induced acute renal failure. J Am Soc Nephrol 2005;16:950–958.

15 Zou AP, Billington H, Su N, Cowley AW Jr: Expression and actions of heme oxygenase in the renal medulla of rats. Hypertension 2000;35:342–347.

16 Hölzen JP, August C, Bahde R, Minin E, Lang D, Heidenreich S, Dietl KH, Spiegel HU: Influence of heme oxygenase-1 on microcirculation after kidney transplantation. J Surg Res 2008;148:126–135.

17 Chlopicki S, Olszanecki R, Marcinkiewicz E, Lomnicka M, Motterlini R: Carbon monoxide released by CORM-3 inhibits human platelets by a mechanism independent of soluble guanylate cyclase. Cardiovasc Res 2006; 71:393–401.

18 Bagul A, Hosgood SA, Kaushik M, Nicholson ML: Carbon monoxide protects against ischemia-reperfusion injury in an experimental model of controlled nonheartbeating donor kidney. Transplantation 2008;85:576–581.

19 Ren Y, D'Ambrosio MA, Wang H, Liu R, Garvin JL, Carretero OA: Heme oxygenase metabolites inhibit tubuloglomerular feedback (TGF). Am J Physiol Renal Physiol 2008;295:F1207–F1212.

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

21 Brouard S, Otterbein LE, Anrather J, Tobiasch E, Bach FH, Choi AM, Soares MP: Carbon monoxide generated by heme oxygenase 1 suppresses endothelial cell apoptosis. J Exp Med 2000;192:1015–1026.

22 Inguaggiato P, Gonzalez-Michaca L, Croatt AJ, Haggard JJ, Alam J, Nath KA: Cellular overexpression of heme oxygenase-1 up-regulates p21 and confers resistance to apoptosis. Kidney Int 2001;60:2181–2191.

23 Devey L, Ferenbach D, Mohr E, Sangster K, Bellamy CO, Hughes J, Wigmore SJ: Tissue-resident macrophages protect the liver from ischemia-reperfusion injury via a heme oxygenase-1-dependent mechanism. Mol Ther 2009;17:65–72.

24 Gueler F, Park JK, Rong S, Kirsch T, Lindschau C, Zheng W, Elger M, Fiebeler A, Fliser D, Luft FC, Haller H: Statins attenuate ischemia-reperfusion injury by inducing heme oxygenase-1 in infiltrating macrophages. Am J Pathol 2007;170:1192–1199.

25 Andersen MH: Identification of heme oxygenase-1' specific regulatory CD8+ T cells in cancer patients. J Clin Invest 2009;119:2245–2256.

26 George JF, Braun A, Brusko TM, Joseph R, Bolisetty S, Wasserfall CH, Atkinson MA, Agarwal A, Kapturczak MH: Suppression by CD4+CD25+ regulatory T cells is dependent on expression of heme oxygenase-1 in antigen-presenting cells. Am J Pathol 2008;173:154–160.

27 Fellstrom B, Holdaas H, Jardine AG, Holme I, Nyberg G, Fauchald P, Gronhagen-Riska C, Madsen S, Neumayer HH, Cole E, Maes B, Ambuhl P, Olsson AG, Hartmann A, Logan JO, Pedersen TR: Effect of fluvastatin on renal end points in the Assessment of Lescol in Renal Transplant (ALERT) trial. Kidney Int 2004;66:1549–1555.