Hemeoxygenase-1 and Renal Ischaemia-Reperfusion Injury

David A. Ferenbach  David C. Kluth  Jeremy Hughes
MRC Centre for Inflammation Research, Queen’s Medical Research Institute, Edinburgh, UK

Keywords
Hemeoxygenase-1 · Acute kidney injury

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.

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.
Whilst the constitutively expressed enzymes hemeoxy-
genase-2 and -3 contribute a basal level of heme metabo-
lism, the predominant route of heme degradation is via
inducible hemeoxygenase-1 (HO-1). In addition to remov-
ing a source of oxidative stress, 2 products of heme me-
tabolism, biliverdin and carbon monoxide (CO), are rec-
ognized 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 availabil-
ity 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 im-
mune 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 ther-
apeutic manipulation are the subject of ongoing research.

**HO-1 and Renal IRI**

HO-1 is induced in a range of organs including the ro-
dent kidney in response to experimental IRI [8]. Further-
more, 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, lipopolysac-
charide administration and bile duct ligation, all of which
result in protection against a subsequent, more severe ex-
perimental 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 in-
flammatory cytokines. These diverse stimuli act via a
similarly broad range of signalling pathways. The pro-
moter 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 transcrip-
tion factor NF-E2-related factor-2 (Nrf2) is recognized as
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 protected 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

---

**Fig. 2.** Current working hypothesis of the putative actions of HO-1 in AKI.
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 converse-ly 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 chemotactrant 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 indispensable 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.

Acknowledgements

D.A.F. has received funding from Medical Research Scotland and Kidney Research UK. We apologize to the authors of the many noteworthy original papers we have been unable to quote due to restrictions in space.

References


