REVIEW —Current Perspective—

Fundamental Role of Heme Oxygenase in the Protection Against Ischemic Acute Renal Failure

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ABSTRACT—Oxidative stress conditions such as oxidant stimuli, inflammation, exposure to xenobiotics and ionizing irradiation provoke cellular responses, principally involving transcriptional activation of genes encoding proteins that participate in the defense against oxidative tissue injuries. Excess of free heme, which is released from hemeproteins under these conditions, may constitute a major threat because it catalyzes the formation of reactive oxygen species. Exposure of mammalian cells to oxidative stimuli induces heme oxygenase-1 (HO-1), the rate-limiting enzyme in heme degradation, as well as the 32-kDa heat shock protein. In various tissue injury systems, HO-1 induction has been shown to confer protection, while its abrogation has been shown to accelerate cellular injuries. In this review, recent findings concerning the role of HO-1 as a protective response against oxidative stress conditions are summarized, with a particular emphasis on its protective role in ischemic acute renal failure.

Keywords: Ischemic acute renal failure, Heme oxygenase, Tin, Heme, Oxidative stress

1. Introduction

Heme oxygenase (HO)-1 is the rate-limiting enzyme in the degradation of heme, which is catalyzed by a sequence of three enzymatic reactions; i.e., NADPH-cytochrome P450 reductase, HO-1 and biliverdin reductase (1). HO-1 converts heme to biliverdin IX α , carbon monoxide and iron. Among the three HO isoforms known, HO-1 is highly inducible by a vast array of stimuli, including oxidative stress, heat shock, UV radiation, ischemia-reperfusion, heavy metals, bacterial lipopolysaccharide (LPS), cytokines, and nitric oxide, and its substrate, heme (1). HO-2 and HO-3 are largely expressed in a constitutive fashion and probably function as heme binding molecules in normal cells (2, 3). Until recently, the heme-derived metabolites were thought to be useless waste or toxic products, but recent data suggest that they may have significant biological properties, such as anti-oxidative, anti-inflammatory, anti-apoptotic, signaling, and immune modulatory properties, and suppressing activities on adhesion molecule expression (4). The strong adaptive response of HO-1 to

various stimuli suggests an entirely new paradigm that HO-1 may play a significant role in the protection against inflammatory processes and oxidative tissue injuries.

Acute renal failure (ARF) is a frequent complication in critically ill patients in the intensive care unit (5). It may occur either as a part of the multiple organ dysfunction syndrome or as an isolated event. Accumulating data over the last two decades have indicated that ARF is characterized by a decrease in glomerular capillary permeability, back-leaks of glomerular filtrates, tubular obstruction and hemodynamic abnormalities (6). Reactive oxygen species (ROS) is thought to play an important role in the pathogenesis of ARF, and its formation can be greatly accelerated by the presence of iron. In certain models of ARF, the iron released from hemeproteins in the kidney is thought to be involved in ROS formation, thereby in the disease progression.

2. HO-1 induction in the experimental model of ARF

There are several experimental models of ARF, each with a unique feature. Each model has been used for assessing the role of HO-1 induction in the protection of the kidney from oxidative tissue injuries.

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Glycerol-induced ARF

The glycerol-induced ARF is most commonly used and is prepared by subcutaneous or intramuscular injection of hypertonic glycerol to rats (7). In this model, there are skeletal muscle injuries, termed "rhabdomyolysis", resulting in the release of myoglobin into plasma. It has been estimated that approximately one third of the patients with rhabdomyolysis develop ARF and that rhabdomyolysis may account for approximately 10% of all cases of ARF. It has been postulated that a large amount of heme released from myoglobin may be directly responsible for attendant lipid peroxidation (8).

In the kidney of rats treated with glycerol for 6 h, HO-1 mRNA was found to increase more than 50-fold, compared with that in untreated animals (9). The blockade of the increased HO activity by tin protoporphyrin (Sn-PP), a competitive inhibitor of HO, significantly aggravated the renal injury in this model. Namely, serum creatinine concentrations increased more than twofold in Sn-PP treated animals compared with untreated controls, suggesting that renal injury was aggravated by the inhibition of HO activity. In contrast, induction of HO-1 by pretreatment of animals with hemoglobin prior to glycerol injection showed significant protection against the development of ARF (9). Thus, in this model, the exposure of the kidney to a single pretreatment of hemoglobin elicited induction of adaptive cellular responses that facilitated the clearance of inordinate amounts of cytotoxic free heme. These findings thus indicate that induction of HO-1, which itself is a free heme-mediated process, also serves to clear an excess amount of free heme, ultimately resulting in a beneficial adaptive response.

Cisplatin-induced toxic renal injury

Cisplatin is a commonly used anticancer drug, but its use has to be often curtailed by its significant side effects, particularly nephrotoxicity. Cisplatin preferentially accumulates in the kidney and results in injuries in the proximal tubules (10). This unique effect of cisplatin was utilized to prepare an experimental model of toxic ARF. Both necrosis and apoptosis of renal tubular cells were observed in the cisplatin-induced ARF (11). Unlike in the glycerol-induced ARF, the oxidative stress in cisplatin-induced ARF appears to be unrelated to myoglobin, but rather may be due to the iron that is derived from microsomal cytochrome P450 in the kidney (12). It has been shown that HO-1 is induced in a time and dose-dependent fashion in the kidney after the administration of cisplatin, and administration of Sn-PP, which inhibits HO activity, aggravated the renal injury (13). HO-1 knockout mice have been shown to be highly sensitive to the toxic effect of cisplatin, and they developed more severe renal failure and have a higher degree of renal tubular apoptosis compared with the wild-type mice treated

with cisplatin (11). In contrast, both pre-induction of HO-1 by hemin treatment and over-expression of the *ho-1* gene by gene targeting resulted in a significant amelioration of the cisplatin-induced renal injury (11).

Ischemic ARF

Ischemic ARF (IARF), which is due to the reperfusion injury of the kidney, accompanies acute tubular epithelial cell injury, and it is the major form of ARF of all ARF episodes in intensive care units. The IARF injury is thought to be due to ROS generated by reperfusion, which has been suggested to be a result of the rapid release of heme from microsomal cytochrome P450 (14). The reversibility of renal function in IARF critically depends on the length of the ischemic treatment prior to reperfusion, e.g., longer than 60-min ischemia, resulting in an irreversible renal damage (15). Rats following a unilateral nephrectomy, rats with the ligation of a contralateral renal artery, or rats with bilateral ligation followed by reperfusion, have been used as the experimental model of IARF. We found that both HO-1 mRNA and its enzyme activity were significantly increased in the reversible IARF model (16). Inhibition of HO activity by Sn-mesoporphyrin (Sn-MP) resulted both in a marked increase in intracellular heme content and in the aggravation of renal function in this model. HO-1 induction thus plays an important role in the protection against renal dysfunction due to oxidative damages caused by heme.

3. HO inhibition worsens, while its induction alleviates IARF

Certain metalloporphyrins have been shown to act as competitive inhibitors of HO activity (17). For example, Sn-PP or Sn-MP has been shown to be a clinically useful inhibitor of HO activity and is used for the treatment of neonatal jaundice (17). Sn-MP is a stronger inhibitor of HO activity than Sn-PP, presumably due to its better water solubility (17). In contrast to Sn-PP or Sn-MP, tin chloride (SnCl₂) treatment has been shown to potently induce HO-1, which is almost entirely restricted to the kidney (18). We examined the effect of SnCl₂ or Sn-MP in the kidney in rats with IARF as well as their effects on renal function as determined by morphological examination and by measurements of serum creatinine concentrations (16, 19). We have in fact confirmed a transient but strong induction of HO-1 mRNA in the kidney of rats following ischemia/reperfusion (16). SnCl₂ treatment prior to ischemia/reperfusion, which is known to induce HO-1 specifically in the kidney, improved renal dysfunction, as judged by a decreased serum creatinine concentration (Fig. 1). In animals pretreated with SnCl₂, the proximal tubular epithelial cells in the cortex were much less affected than SnCl₂-untreated controls, and they were nearly normal (Fig. 1) (19). In

Fig. 1. Effect of SnCl₂ or Sn-MP administration on serum creatinine and kidney tissue changes in rats with IARF. Rats were uninephrectomized and subjected to unilateral ischemia for 40 min to produce IARF. SnCl₂ (10 mg/100 g body weight) was administered subcutaneously, and Sn-MP (1 μ mol/kg body weight) was administered intravenously 24 h prior to the uninephrectomy. After the initiation of reperfusion, whole blood was collected for the determination of serum creatinine concentrations at 0, 6, 12, 18 and 24 h. They are shown as the mean $\pm$ S.E.M. (n = 6). Shown in the inset are histochemical changes in the renal cortex of rats following various treatments. Left: Kidney sections stained with hematoxylin and eosin. Extensive necrosis with cast formation in the proximal tubular cells was observed in IARF with vehicle treatment. It was aggravated in IARF with Sn-MP, in contrast to the relief from the injury in IARF with SnCl₂ pretreatment. Right: Kidney sections stained immunohistochemically using anti-rat HO-1 as a primary antibody. After IARF, HO-1 protein was induced in tubular epithelial cells, while the induction became obvious in IARF with SnCl₂ pretreatment. The bar represents 100 μ m.

contrast, inhibition of HO activity by the administration of Sn-MP to SnCl₂-pretreated animals abolished the beneficial effect of the SnCl₂ pretreatment on ischemic renal dysfunction, indicating the fundamental protective role of HO-1 in the renal epithelial cells against oxidative stresses (19).

Although HO-1 mRNA in the kidney of untreated rats was hardly detectable, it was markedly increased in the kidney following SnCl₂ treatment, while it was barely detectable in the liver, lung, heart and small intestine. This result indicates that HO-1 mRNA is induced by SnCl₂ treatment in a highly kidney-specific manner (19). Following SnCl₂ treatment, a marked elevation of renal HO-1 mRNA was observed, followed by increases in HO-1 protein expression and HO activity (19). HO-1 protein accumulated also specifically in the renal tubular epithelial cells, following SnCl₂ treatment (Fig. 1). Since the renal tubular epithelial cells are the target cells in the renal

ischemia/reperfusion injury, induction of HO-1 specifically in these cells indicates its critical association to the oxidative stress in these cells.

Ischemia/reperfusion was found to rapidly increase microsomal free heme concentrations in saline-treated rats, whereas SnCl₂-pretreatment prevented it from occurring. Tin pretreatment also significantly attenuated the severity of ischemic renal injury. In contrast, when SnCl₂-treated animals were treated with Sn-MP, microsomal heme concentrations were elevated and resulted in an aggravation of renal function (19). Thus, HO-1 induced by SnCl₂ pretreatment effectively decreased the excess amount of toxic "free heme", resulting in the amelioration of the ischemic renal injury.

4. Mechanisms of *ho-1* gene induction in IARF

Although the precise mechanism(s) of renal injury caused

by ischemia/reperfusion remains elusive, ROS produced by reperfusion following ischemia appears to be one of the important mediators of tissue injury in IARF (6). It was found that there is a rapid increase in microsomal heme concentration, which is thought to be liberated from cytochrome P450 (12), and occurs in the kidney immediately after reperfusion (16). This form of heme is apparently not associated with apoproteins, since it acts as a potent pro-oxidant, leading to the generation of ROS and aggravates the ischemic renal injury. ROS also induces the breakdown of hemoproteins which further aggravates oxidative tissue damages by liberating more heme. Under these circumstances, *ho-1* gene expression is transcriptionally increased in various cell types (20). In contrast, HO-1 deficient embryonic fibroblasts are known to be hypersensitive to the cytotoxicity of both hemin and hydrogen peroxide (21).

Recent evidence points to the fact that a group of oxidative stress inducible genes is under the immediate transcrip-

tional control of Nrf2-small Maf heterodimer-regulatory protein (Fig. 2). Nrf2 forms a heterodimer with a small Maf protein, interacts with an anti-oxidant responsive element (ARE), and induces transcription of a set of genes that encode anti-oxidant functions. Nrf2 thus regulates stress inducible protein genes via ARE (Fig. 2). For example, Nrf2-deficient cells have been shown to be hypersensitive to oxidative stresses (22). Various ROS-inducing agents increase the DNA binding activity of Nrf2 in the nucleus without influencing its mRNA level (23). In this manner, Nrf2 regulates a wide-range of metabolic responses to oxidative stress, which includes, among others, *ho-1* (23). The ARE cognate sequence shares a high degree of homology to the consensus Maf recognition element (MARE) sequence, permitting AREs to be competitively bound by a number of bZip transcription factors in the Maf, Jun, Fos and Cap'n'Collar families (23) (Fig. 2). Because of their responsiveness to a wide variety of stress agents including oxidative stresses, the MAREs were also named

Fig. 2. Hypothetical mechanism of HO-1 induction in IARF. Oxidative stresses liberate Nrf2 from the cytoskeleton into the nucleus (32). Nrf2 then forms a heterodimer with a small Maf protein, interacts with ARE, and induces transcription of a set of genes that encode antioxidant activities (23), one of which is *ho-1*. Cytosolic free heme contributes to the generation of ROS, which in turn facilitates the formation of a Nrf2/small Maf heterodimer. Induction of HO-1 protein results in the removal of the pro-oxidant free heme, in the production of the anti-oxidant bile pigments, and in the formation of the anti-apoptotic CO. When the synthesis of ROS exceeds the level of detoxifying capacity of the renal proximal tubular cell, IARF ensues as the consequence of the reperfusion injury. CYP: cytochrome P450.

stress-responsive elements (StREs) (24). Multiple StREs exist in *ho-1* enhancers, and they have been shown to play important roles in the regulation of *ho-1* gene expression by various oxidative stresses, ROS, heavy metals and LPS (23). For example, the DNA binding activity of transcription factor AP-1 was inhibited when AP-1 was exposed to oxidative stress in vitro (25), which in turn may allow the competitive binding of other activating transcription factors, such as an Nrf2-small Maf heterodimer, to ARE and hence induce genes with protective properties against oxidative stress; e.g., *ho-1* (23). While exposure of human MCF-7 cells to CdCl₂, a potent inducer of HO-1, stimulates phosphorylation of ERK, JNK and p38 mitogen-activated kinases, an inhibitor of p38, or co-expression of a dominant-negative mutant of p38 α , but not of ERK1, ERK2, JNK1 or JNK2, decreased HO-1 mRNA levels significantly (26). A dominant negative mutant of Nrf2 (a CNC-bZip member), but not of c-Jun or C/EBP β , inhibited Cd-mediated transactivation (26). These findings, also together with others, suggest that an oxidative stress induces *ho-1* gene expression via sequential activation of the p38 kinase pathway and Nrf2 (26).

5. Metabolic consequences of HO-1 induction

The immediate and specific adaptive response of HO-1 expression on the wide variety of oxidative stimuli suggests an important role of HO-1 in the protection against oxidative stress conditions, in addition to its key role in oxidative heme catabolism. The importance of HO-1 in the protection from inflammation is now amply documented in mice and humans deficient in HO-1 (21, 27). The absence of HO-1 results in an abnormally elevated serum heme concentrations (approx. 0.5 mM) and various oxidative and inflammatory complications (21, 27).

Free heme, largely liberated from hemeproteins under oxidative conditions, is highly lipophilic and will likely intercalate into the lipid bilayers in adjacent cells. Exposure of cells to heme is known to stimulate the expression of adhesion molecules ICAM-1, VCAM-1 and E-selectin on endothelial cells in vitro, probably through heme-mediated generation of ROS, which underscores reactive inflammatory changes (4). HO breaks down the pro-oxidant heme into three elements; i.e., iron, biliverdin IX α and carbon monoxide (CO). Iron, which is an oxidant, is directly sequestered and inactivated by co-induced ferritin (28). Biliverdin IX α is rapidly converted by biliverdin reductase to bilirubin IX α , which is an anti-oxidant (29). CO produced from heme by HO can suppress apoptosis of endothelial cells via the activation of p38 MAPK (30). Thus, all these metabolites of the HO reaction act as a member of the protective response against oxidative stimuli and contribute to suppress a series of oxidative tissue damages that are associated with IARF.

6. Conclusion

In this review, recent evidence suggesting the fundamental role of HO-1 in anti-oxidative responses is summarized. Both inhibition of HO activity and suppression of *ho-1* gene expression lead to aggravation of oxidative tissue injuries. In contrast, exogenous administration of HO-1 by gene transfer confers significant protection in a rat model of inflammation or hemoglobin-heme toxicity (31). These findings suggest that tissue-specific *ho-1* expression, either by tissue-specific induction of HO-1 by chemicals or tissue-specific *ho-1* gene overexpression, may hold therapeutic usefulness. Thus, tin salts, which have been simply thought to be toxic, may offer a new mode of treatment of IARF, because of their highly kidney-specific HO-1 inducing property.

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