Human AKI and Heme Oxygenase-1

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Findings derived from animal models of AKI gain clinical credence and appeal when translational studies corroborate their occurrence in human AKI. In the current issue of JASN, Zager et al.1 take important steps in addressing the clinical applicability of the conclusion drawn from animal models that induction of heme oxygenase-1 (HO-1) is a protective response in AKI.

The major heme-degrading mechanism in tissues, heme oxygenase has its origin in studies probing the metabolic fate of heme, particularly the metabolic sources of carbon monoxide. 11–12 First, HO activity generates three oxygenase has its origin in studies probing the metabolic fate of heme, particularly the metabolic sources of carbon monoxide. 11–12 First, HO activity generates three products: biliverdin, ferrous iron, and carbon monoxide. 11–13 The products of HO-1 induction have distinct cellular and physiological actions. Biliverdin, the initial product of heme degradation, is converted to bilirubin by biliverdin reductase. 11–14 Biliverdin and bilirubin are major pigmentary forms of heme in the reticuloendothelial system. 11 It was then realized that there were two isoforms with heme oxygenase activity, the constitutive isoform, HO-2, and the inducible isoform, HO-1; the latter was identified as a cytoprotective gene in a model of AKI induced by heme proteins. 15 Subsequently, induction of HO-1 was recognized as a protective response that can occur in all organs and tissues and against virtually any insult. 11,12,14–16

At least four features of HO-1 underlie its salutary effects in stressed tissues. 11,12 First, HO activity generates three chemically distinct products—carbon monoxide, bile pigments, and iron—each of which can participate in specific cellular processes; induced HO-1 thus transmits broad-based and far-ranging signals through its tripartite products and their respective downstream effects. Second, all products of HO activity are involved in a number of homeostatic mechanisms; induced HO-1 thus readily communicates with networks that influence cell survival. Third, ischemic and toxic insults can destabilize intracellular heme proteins (for example, cytochrome p450), and heme that is released can itself perpetrate cell injury; induced HO-1 thus vitiates secondary toxicants.

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pathways of cellular injury mediated by free heme. Fourth, HO-1 is a finely regulated gene readily responsive to diverse stimuli. It rapidly appears in injured cells and recedes as repair and renewal of tissues occur; expression of HO-1 thus occurs when and where needed.

Induction of HO-1 in the kidney consistently occurs in models of AKI and confers a beneficial response.1-3,11 Whether such induction occurs in human AKI is thus of interest. However, addressing this issue in human AKI is challenging, not only because of the general unavailability of human kidney tissue in human AKI, but also because measurements of HO-1 levels in extracellular fluid venture into largely uncharted areas in the heme oxygenase field. The heme oxygenase literature regards the expression of HO-1 as a cellular phenomenon involving mainly the endoplasmic reticulum; it provides little information on the presence of HO-1 in extracellular compartments and none on the route HO-1 may take to get there.

In addressing this question, Zager et al. first determined whether the appearance of HO-1 in plasma and urine would provide a faithful readout of intracellular HO-1 in the injured kidney.1 Their approach quite cleverly exploited the specific features of different models of AKI. In two models characterized by early-onset, sublytic cell injury and necrosis (ischemia and glycerol-induced AKI), immunoreactive HO-1 (by ELISA) was detected in plasma and urine within 4 hours after the insult. In cisplatin-induced AKI, a model characterized by relatively delayed-onset sublytic cell injury and necrosis, plasma and urinary levels of HO-1 were elevated at 24 hours and antedated the increase in BUN. The importance of sublytic cell injury and necrosis, as occurs in these AKI models, in effecting such elevation in plasma and urinary HO-1 was underscored by studies in AKI induced by urinary tract obstruction. Urinary tract obstruction leads to flattening and atrophy of the tubular epithelium, and if epithelial cell death occurs, it primarily involves apoptosis and its containing effect and not necrosis with its spillage of cellular debris; urinary tract obstruction was attended by a lesser rise in plasma and urine HO-1 levels. Studies undertaken in vitro in proximal tubular epithelial cells injured by iron reveal the presence of immunoreactive HO-1 in the extracellular supernatant concomitant with intracellular induction of HO-1. Finally, to address whether the increased plasma levels of HO-1 reflect sources other than the kidney, Zager et al. evaluated HO-1 gene expression in extrarenal organs in glycerol-induced and cisplatin-induced AKI at the 24-hour time point; in these studies, induction of HO-1 in extrarenal organs was not observed. These findings led to the conclusion that HO-1 protein, induced in injured tubular epithelial cells, may either exit across a leaky apical plasma membrane to appear in urine or across a porous basolateral membrane to eventually appear in plasma.

These studies were then followed by measurements undertaken in critically ill patients with and without AKI.1 The findings in patients with AKI recapitulate experimental observations; namely, the presence of AKI leads to markedly higher levels of plasma and urinary HO-1 compared with patients without AKI. Moreover, this elevation in plasma and urinary HO-1 in patients with AKI is not observed in patients with CKD or ESRD, thus demonstrating that uremia, when chronically imposed, is not attended by increased levels of plasma HO-1. Thus, the markedly increased concentrations of HO-1 in urine and plasma in human AKI, in conjunction with accompanying analyses in disease models, support the view that HO-1 is induced in the kidney in human AKI.

Zager et al. countenance the possibility that extrarenal production of HO-1 may contribute to HO-1 appearing in plasma. In this regard, induction of HO-1 has been described in the liver within 6 hours in the glycerol model13 and by 24 hours in the cisplatin model.14 Presumably, such hepatic HO-1 induction reflects, at least in part, heme proteins delivered to the liver as occurs in the glycerol model or the direct pro-oxidant effects of cisplatin incorporated in the liver. Extrarenal HO-1 production is of particular interest in ischemia-induced AKI because any such induction would reflect long-range effects of localized ischemia and not a direct effect of the imposed insult: induction of HO-1 has been detected in the heart within 4 hours and in the aorta within 24 hours after renal ischemia.15 Thus, the contribution of the injured kidney to plasma levels of HO-1 may be supplemented by extrarenal sources.

The increased appearance of HO-1 in plasma in AKI seems especially relevant to the concept that AKI instigates renal and systemic inflammation and adverse distant effects.16 These regional and long-range effects contribute to AKI-associated mortality and involve the elaboration of cytokines, including IL-6. Plasma levels of IL-6 are increased in human AKI and are a predictor of mortality,17 whereas in murine ischemic AKI, IL-6 is substantially induced and underlies such injury.18 HO-17-9 mice, subjected to renal ischemia, exhibit increased plasma IL-6 levels, heightened IL-6 mRNA expression in the kidney and other organs, worse renal function, and increased mortality; administering an IL-6 antibody reduces mortality and improves renal function.19,20 It is thus conceivable that increased plasma levels of HO-1 in human AKI, as observed by Zager et al., represent a countervailing, anti-inflammatory response to inimical events enveloping the kidney and extending outward through the systemic circulation to distant organs. This possibility is supported by the fact that substantially higher plasma levels of HO-1 were observed in AKI models characterized by sublytic cell injury and necrosis, processes that are decidedly proinflammatory. A final consideration is that a similar, counter-regulatory role in AKI, as envisioned for HO-1, has been accorded to IL-10, an anti-inflammatory cytokine that is protective in experimental AKI and elevated in plasma in human AKI.17,21 Interestingly, IL-10 induces HO-1, and products of heme oxygenase, such as carbon monoxide, can, in turn, upregulate IL-10.11

The present findings raise additional lines of inquiry regarding the nature of the immunoreactive HO-1 protein present in plasma and urine—specifically, whether this protein possesses HO activity and whether it exhibits sequence homology with native HO-1 protein. It is notable that the HO-1 protein...
detected in urine exists as a 16-kD moiety, whereas intact HO-1 is a 32-kD protein, consisting of two helical loops. It is possible that this cleavage of HO-1 is incurred by proteolytic enzymes released from injured cells or by hydrogen peroxide, which may attain micromolar levels in urine.22

In conclusion, Zager et al provide the first concerted analysis of HO-1 in plasma and urine in human AKI and elucidate the significance of these findings by their discerning application of relevant in vivo and in vitro models. Such translational analyses, recently used by their laboratory with regard to MCP-1 and AKI, serve to validate or repudiate the clinical applicability of findings derived from animal models of AKI. The demonstration that increased amounts of HO-1 appear in the regional and systemic milieu of human AKI raises the possibility that this inducible protein may subserve a protective role in human AKI; additionally, these findings introduce a new candidate for consideration in the biomarker field. Finally, these findings are of therapeutic significance. Novel inducers of HO-1, such as bardoxolone methyl, are not only protective in experimental AKI24 but also show early promise in human diabetic nephropathy.25 Based on these and the current findings, such compounds offer the exciting prospect for a new preventive or therapeutic strategy in human AKI.

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DISCLOSURES

None.

REFERENCES


**On-Line Hemodiafiltration: Not a Self-Fulfilling Prophecy**

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As the quest for better dialysis outcomes continues, the focus is shifting to a better removal of uremic toxins in the middle molecular weight range. Years ago, both the American Hemodialysis¹ and the European Membrane Permeability Outcome² studies failed to demonstrate a survival advantage for high-flux over low-flux hemodialysis (HD), although in both studies removal of the classic middle molecule β2-microglobulin (β2M) was significantly better with high-flux membranes. However, in both trials, specific subgroups were identified, for which high-flux treatment was associated with improved outcome, including patients with dialysis vintage >3.7 years, patients with diabetes, and patients with serum albumin levels <40 g/dl. It was argued that removal of middle molecular uremic toxins by high-flux HD may not have been effective enough to show a general survival difference and that more intense removal of those putatively atherogenic uremic toxins would certainly improve outcomes—just a question of time until that would be demonstrated.

The basic understanding that better removal of uremic middle molecular weight substances may only be achieved by enhanced convective transport led to the development of new technologies for the on-line production of sterile, nonpyrogenic dialysate that can also be safely used as infusate. Among modern blood purification therapies, on-line hemodiafiltration (HDF) equipment allows much higher convection and substitution volumes than the earlier, fluid bag based HDF technology. On-line HDF therefore has the potential to provide the largest removal of the widest range of solutes. Documented advantages of on-line HDF include a higher removal rate for creatinine, phosphate, β2M, and some protein-bound uremic compounds.³ Several nonrandomized studies showed this may translate into potential clinical benefit, as demonstrated by sustained lower β2M and phosphate levels.³ On-line HDF is further associated with a lower incidence of intradialytic hypotension, improvements in erythropoietin responsiveness and nutritional status, prevention of inflammation, and better preservation of residual renal function.⁴,⁵ However, because of the lack of larger randomized studies, it remains a matter of debate whether these latter effects may have been related primarily to the treatment mode itself or secondary to improved dialysate purity.

Despite these encouraging data, there was still a lack of convincing evidence of a survival benefit of HDF compared with standard conventional HD. This has now changed with the publication of the Convective Transport Study (CONTRAST) by Grooteman et al. in this issue of the *JASN*.⁷ The results of the CONTRAST trial are the first to be published out of a series of four randomized controlled studies comparing on-line HDF versus low- or high-flux HD, which were initiated some years ago in several European countries (The Netherlands, Turkey, France, and Spain).

In CONTRAST, a total of 714 chronic HD patients were randomly assigned to either thrice-weekly 4-hour on-line HDF in postdilution mode or thrice-weekly 4-hour low-flux HD using on-line produced sterile dialysate. The results of the study, which was event driven with all-cause mortality as primary and the composite of fatal and nonfatal major adverse cardiac events as secondary end points, are somewhat puzzling: After a mean follow-up of almost 3 years, there was no difference in any of the predefined end points between the two treatment groups. Not even the predefined subgroup analysis according to age, sex, serum albumin, diabetes, residual renal function, type of vascular access, and dialysis vintage showed any differences in outcome. However, in secondary on-treatment analysis, a significantly better outcome was found for the subgroup with a mean delivered convective volume exceeding 22 L/treatment, and this difference remained unaltered even after intense adjustment for potential confounders. Very similar findings have been reported from the randomized controlled Turkish Hemodiafiltration Study at the 2011 EDTA meeting in Prague, where no overall advantage for on-line HDF over high-flux HD was found in the total study population, whereas after on-treatment analysis, a significantly improved outcome was observed in the subgroup of patients treated with the highest convection volumes.

The findings of the on-treatment analysis may be the clue for interpretation of the CONTRAST data. According to the study protocol, on-line HDF was strictly performed in the postdilution mode with a target convective volume of 24 L/treatment delivered within an average of 4 hours of treatment time. Because there was no evidence regarding the relation between convection volume and outcome, these treatment targets were chosen arbitrarily based on maximal achievable convection volumes derived by modeling calculations. As treatment time was fixed, the major modifiable variables were dialyzer surface area, filtration fraction, and extracorporeal...