Heme Proteins and Kidney Injury: Beyond Rhabdomyolysis

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Abstract
Heme proteins, the stuff of life, represent an ingenious biologic strategy that capitalizes on the biochemical versatility of heme, and yet is one that avoids the inherent risks to cellular vitality posed by unfettered and promiscuously reactive heme. Heme proteins, however, may be a double-edged sword because they can damage the kidney in certain settings. Although such injury is often viewed mainly within the context of rhabdomyolysis and the nephrotoxicity of myoglobin, an increasing literature now attests to the fact that involvement of heme proteins in renal injury ranges well beyond the confines of this single disease (and its analog, hemolysis); indeed, through the release of the defining heme motif, destabilization of intracellular heme proteins may be a common pathway for acute kidney injury, in general, and irrespective of the underlying insult. This brief review outlines current understanding regarding processes underlying such heme protein-induced acute kidney injury (AKI) and chronic kidney disease (CKD). Topics covered include, among others, the basis for renal injury after the exposure of the kidney to and its incorporation of myoglobin and hemoglobin; auto-oxidation of myoglobin and hemoglobin; destabilization of heme proteins and the release of heme; heme/iron/oxidant pathways of renal injury; generation of reactive oxygen species and reactive nitrogen species by NOX, iNOS, and myeloperoxidase; and the role of circulating cell-free hemoglobin in AKI and CKD. Also covered are the characteristics of the kidney that render this organ uniquely vulnerable to injury after myolysis and hemolysis, and pathobiologic effects emanating from free, labile heme. Mechanisms that defend against the toxicity of heme proteins are discussed, and the review concludes by outlining the therapeutic strategies that have arisen from current understanding of mechanisms of renal injury caused by heme proteins and how such mechanisms may be interrupted.

Introduction
Heme proteins (HPs) are defined by a tetrapyrrole (heme) prosthetic group liganded to a specific protein moiety. Numbering in the hundreds, HPs are characterized by ubiquity, heterogeneity, versatility, and biochemical ingenuity. HPs are present in virtually all cellular and extracellular compartments and are involved in essential cellular processes that maintain homeostasis and health (Table 1). HPs represent an ingenious chemical strategy because they capitalize on the biochemical versatility of heme but circumvent the intrinsic toxicity and insolubility of free heme (1): the heme prosthetic group with its central iron atom can shuttle electrons and engage in assorted oxidation-reduction and catalytic processes; heme possesses the ability to bind and transport various gases (oxygen, nitric oxide, carbon monoxide); and heme may influence gene expression. However, when unliganded, free, and “labile,” heme is toxic (Table 2), and because of its lipophilicity, heme can intercalate and thereby access plasma and cell membranes and cellular compartments (1). This article provides an overview of kidney injury caused by HPs.

Overview of HP-Based Processes that Injure the Kidney
Table 3 broadly summarizes how HPs are involved in kidney injury, and this is detailed below.

Destabilization of HPs
HPs can be destabilized when cells are stressed, leading to the weakening of the union between heme and protein moieties, with the resulting release of heme (1–3). Indeed, renal free heme content is increased not only in AKI induced by myoglobin (Mb) and hemoglobin (Hb) (4,5), but also in AKI caused by “non-HP-related” insults such as ischemia-reperfusion injury (IRI) (6) and cisplatin (7). Such scission of HPs may especially apply to the relatively unstable cytochrome p450 enzymes (8), proteins abundant in the kidney and needed for diverse metabolic processes. Studies almost 30 years ago called attention to the involvement of catalytic iron, released from destabilized cytochrome p450 enzymes, in the pathogenesis of AKI (8). Destabilized intracellular HPs and attendant increased levels of free heme/Fe may thus provide one of the final common pathways for AKI, irrespective of the original insult. The following support this concept (1): (1) intracellular...
heme is generally elevated in AKI, and worsening of AKI occurs when heme degradation is compromised by impairment in or deficiency of heme oxygenase-1 or heme oxygenase-2 (4,9,10); (2) free heme is damaging to the kidney and other organs and tissues (Table 2) (1,3,11–14); and (3) heme, when used in large amounts to abort human porphyria, causes fulminant AKI (15).

**Cellular Egress and Renal Delivery**

The nephrotoxicity of Mb and Hb has been superbly discussed by a prior comprehensive review of the pathogenesis and salient clinical features of HP-AKI (16). The molecular weight (MW) of Mb, released during myolysis, enables rapid filtration into the urinary space. Hb, released during hemolysis, is bound initially to haptoglobin (Hpt) after which the free tetrameric Hb (MW 64 kD) dissociates into dimers (MW 32 kD), the latter also filtered into the urinary space, albeit less easily compared with Mb (1,17). Megalin and cubilin, receptors on the apical surface of the renal proximal tubule, reclaim Mb and Hb present in urine (18,19).

**Aberrant/Inordinate Activation of Oxidant-Generating HPs**

Inducible nitric oxide synthase (iNOS) and NADPH oxidase (NOX) are inducible HPs that kill microbes, the former by generating peroxynitrite, the latter by generating reactive oxygen species (ROS). However, in disease states, these HPs may be aberrantly or inordinately activated, in the absence of an infectious process, for example, iNOS by numerous cytokines (20), and NOX by angiotensin II (21).

**HP Transfer from Mitochondria to the Cytosol**

Cytochrome c is an essential member of the mitochondrial electron transport chain. When mitochondria are injured, as invariably occurs in AKI, cytochrome c is released into the cytosol. When present in the cytosol, cytochrome c activates Apaf-1 and caspases 9 and 3, which may culminate in apoptosis (22).

**Degranulation of Neutrophils and Monocytes**

Activation of these cells releases myeloperoxidase in the extracellular space (23,24). Myeloperoxidase generates abundant ROS and reactive nitrogen species and, in particular, powerful oxidants such as hypohalous acid (from hydrogen peroxide and chloride). Hypohalous acid generates damaging products such as chloramines and glycated proteins (23,24). Myeloperoxidase is required in the formation of neutrophil extracellular traps (25). Myeloperoxidase contributes to renal injury in renal vasculitides and glomerulonephritides, and is implicated in IRI and the pathogenesis of diabetic nephropathy (24).

**Rhabdomyolysis/Hemolysis as a Paradigm for HP-Induced AKI**

An intriguing aspect of rhabdomyolysis is why the kidney is singled out such that this organ bears the brunt of this disease, a systemic one caused by a toxin released into the circulation (extracellular Mb). Table 4 summarizes the basis for the vulnerability of the kidney to acute injury caused by Mb or Hb. Figure 1 outlines the intrarenal pathways involved in AKI caused by Mb (and likely applies to Hb-AKI) and is detailed as follows.

**Renal Vasodistion**

Vasodilation of the intact kidney is largely dependent on production of nitric oxide (and, to a much less extent, carbon monoxide). Mb and Hb induce renal vasodistion through three main processes. First, the heme prosthetic group of these HPs avidly binds nitric oxide and carbon monoxide, thereby siphoning off these vasodilators

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### Table 1. Functions of heme proteins

| Function                                      | Relevant Heme Protein                                      |
|----------------------------------------------|-----------------------------------------------------------|
| Oxygen transport and storage                 | Hemoglobin, myoglobin, neuroglobin                        |
| Mitochondrial respiration                    | Mitochondrial cytochromes                                 |
| Cellular metabolism and detoxification       | Cytochrome P450 enzymes                                    |
| Vasodilation                                 | eNOS, cyclooxygenase                                       |
| Endothelial and vascular integrity          | eNOS                                                       |
| Antioxidant function                         | Glutathione peroxide, catalase                            |
| Cellular signaling                           | cGMP                                                       |
| Immune regulation                            | Indoleamine 2,3-dioxygenase                                |
| Antimicrobial defense                        | iNOS, NADPH oxidase, myeloperoxidase                      |

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### Table 2. Toxic effects of free heme

- Acute renal vasoconstriction
- Stimulates lipid peroxidation and generation of hydrogen peroxide
- Functions as a Damage-Associated Molecular Pattern (DAMP)
- Impairs glucose-6-phosphate dehydrogenase
- Perturbs certain cytoskeletal proteins
- Inhibits cell proliferation
- Damages mitochondria
- Induces cell death
- Activates NF-κB and induces proinflammatory cytokines
- Signals through the TLR4 receptor
- Activates the inflammasome
- Chemotaxtrakcent for leukocytes
- Promotes leukocyte adhesion to the endothelium
- Promotes formation of NETs
- Induces platelets to discharge their Weibel–Palade bodies
- Induces platelets to activate macrophages
- Induces tissue factor expression
- Thrombogenic

NETs, neutrophil extracellular traps.
from the renal vasculature with resulting renal vasoconstriction (17, 26). These HPs, when in plasma, also undergo auto-oxidation with the generation of superoxide anion (see below), which further depletes vascular nitric oxide because superoxide anion avidly interacts with nitric oxide to generate peroxynitrite. Second, Mb and Hb also stimulate the production of potent vasoconstrictors in the kidney, including isoprostanes (27, 28), endothelin-1 (29), and thromboxanes (30), in large part via the oxidative stress imposed by these HPs; notably, the kidney may be particularly sensitive to vasoconstrictors such as endothelin-1 and isoprostanes (28, 31). Glyc erol-induced AKI (G-AKI), a commonly used model of HP-AKI, reflects the pathogenetic involvement of isoprostanes (27, 28) and endothelin-1 (32, 33). Third, Mb potentiates the vasoconstrictive effects of angiotensin II produced by the kidney (34). Renal vasoconstriction not only impairs renal delivery of oxygen and nutrients but also imposes renal ischemia. Ischemia exerts adverse metabolic effects, including the reduction in kidney content of ATP, NAD^+, and glutathione (GSH, a major component of the antioxidant GSH/glutathione peroxidase system) (35), all of which would heighten the sensitivity of the renal proximal tubule to injury when subsequently exposed to Mb or Hb.

**Auto-Oxidation of Mb/Hb**

Figure 2 broadly summarizes these biochemical processes, as previously discussed (2, 36, 37). When Mb and Hb are released into the circulation, they undergo auto-oxidation because they are no longer surrounded by the reducing and antioxidant intracellular milieu of myocytes and red blood cells (RBCs); such an intracellular milieu maintains the heme iron of these HPs in the ferrous (Fe^{2+}) state needed for oxygen transport and storage. Extracellularly, however, the HP-Fe^{2+} species is oxidized (by oxygen and/or hydrogen peroxide) first to HP-Fe^{3+}, accompanied by the generation of superoxide anion (and subsequently hydrogen peroxide). HP-Fe^{3+} is further oxidized by hydrogen peroxide to HP-Fe^{4+} and other radical species, which induce lipid peroxidation and the formation of isoprostanes; redox cycling occurs between HP-Fe^{3+} and HP-Fe^{3+}.

Two unique attributes of the kidney promote Mb/Hb auto-oxidization and its harmful effects. First, even in the presence of normal kidney function, urine contains hydrogen...
Table 4. Vulnerability of the kidney to AKI after myolysis or hemolysis

- Renal O2 consumption, second highest among tissues, relies on high renal blood flow rates. The kidney is particularly sensitive to the vasoconstricting actions of HPs.
- The proximal tubule expresses megalin/cubilin, which reclaim HPs filtered into urine.
- The proximal tubule has the second highest mitochondrial volume density of all organs/tissues; mitochondria are vulnerable to the damaging effects of heme.
- The kidney is enriched in cytochrome p450 enzymes, which are prone to destabilization under stress; heme and iron may be thereby released.
- The kidney uniquely produces uromodulin, a glycoprotein with a proclivity for forming casts with Mb and Hb.
- H2O2, present in urine (in μM amounts), promotes auto-oxidation of Mb and Hb.
- Urinary acidification promotes uromodulin-HP cast formation.
- Urinary acidification promotes lipid peroxidation by HP-Fe4+

HPs, heme proteins; Mb, myoglobin; Hb, hemoglobin.

peroxide in micromolar amounts (38). As shown in Figure 2, hydrogen peroxide promotes the auto-oxidation of HPs; redox cycling between their ferric and ferryl species; and the degradation, release, or transfer of heme in HP-Fe4+. Second, urinary acidification heightens lipid peroxidation caused by HP-Fe4+ and the accompanying generation of the potent renal vasoconstrictor, isoprostanes, an effect that underlies, in part, the protective effects of alkali in HP-AKI (27). Through such Mb/Hb auto-oxidation, occurring in plasma and urine, ROS/heme/Fe-mediated damage may occur in the kidney.

**Impaired Glomerular Filtration Barrier and Proteinuria**

Approximately 50% or more of patients with rhabdomyolysis may be proteinuric, with substantial subsets being frankly nephrotic (39). In traversing the glomerular filtration barrier, Mb and Hb may be incorporated by the podocyte, which, like the proximal tubule, has an active endocytic process (40). Such Mb/Hb incorporation may damage the podocyte by processes akin to those in the proximal tubule, thereby impairing glomerular permselectivity with attendant proteinuria (40). For example, Hb is endocytosed via megalin/cubilin receptors by podocytes in vitro and in vivo, and in the course of the metabolism of Hb, cellular oxidative stress occurs with attendant podocyte injury and apoptosis of podocytes (40). Glomerular leakage of protein may contribute to tubular injury in HP-AKI because proteinuria is a risk factor for tubulointerstitial injury for virtually all kidney diseases (41).

**Proximal Tubule Epithelial Cell Injury**

The proximal tubule bears the brunt of functional impairment and structural damage in G-AKI (42). Present on the apical surface of the proximal tubule, megalin/cubilin receptors are a Trojan horse that incorporate Mb and Hb intracellularly. Studies based on an innovative, inducible, proximal tubule-specific genetic deficiency of megalin in mice demonstrate that such mice are markedly protected against G-AKI, and that the administration of cilastin, an inhibitor of megalin, similarly confers protection in G-AKI (18). These HPs are then split intracellularly into their heme and protein moieties. The rise in renal intracellular heme levels reflects not only heme originating from Mb and Hb, but also heme derived from destabilized cytochrome p450 HPs (1). Heme also causes tubular injury through the release of iron when the heme ring is degraded (43); increased intracellular levels of free iron is reactive, catalyzing the Fenton reaction and the generation of ROS (44,45). Abundant evidence indicates the involvement of iron and ROS in HP-AKI. In G-AKI, there is increased generation of hydrogen peroxide (46), increased lipid peroxidation (47), depletion of GSH (48), and increased amounts of catalytic iron (49), whereas scavengers of hydrogen peroxide (50), inhibitors of lipid peroxidation (28,51), GSH repletion (48), scavengers of the hydroxyl radical (49), iron chelation (49), the potent antioxidant thioredoxin (52), and antioxidant anti-inflammatory nutrients (53) are all protective in G-AKI.

**Heme-Dependent Mitochondrial Injury**

The fundamental role of acute mitochondrial injury in driving IRI is now supported by abundant evidence (54–56). Some 25 years ago, we posited that intracellular free heme targets mitochondria because heme would permeate their lipid-rich outer membrane, whereas heme’s toxicity would be amplified by the low-level mitochondrial generation of hydrogen peroxide (5). In G-AKI, studied at

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Figure 2. | Auto-oxidation of HPs (myoglobin/hemoglobin). Please see relevant text for details.
NF-kB and clear evidence supports the role of specific proinflammatory cytokines such as TNF-α in G-AKI (60). Proinflammatory cytokines are commonly induced by the transcription factor NF-κB, and studies in vitro demonstrate that NF-κB is activated by heme, reactive iron, and ROS (61). However, although there is evidence that protective strategies in G-AKI are attended by decreased activation of NF-κB (62,63), there do not appear to be studies examining the effect of directly suppressing activation of NF-κB per se on G-AKI. NF-κB activation occurs, in part, after signaling through the TLR4 receptor (3), and studies in vitro demonstrate that the proinflammatory effects of heme occur through the TLR4 receptor (3,64). However, studies in vivo using TLR4−/− mice or an inhibitor of the TLR4 receptor have failed to demonstrate a protective effect of these strategies in G-AKI (64). Two investigative lines regarding inflammation and AKI induced by Mb or Hb merit emphasis. First, infiltrating macrophages contribute to the severity of G-AKI (65) because these macrophages are polarized to a proinflammatory (and injurious) M1 phenotype. Additionally, platelets, activated by heme, engage macrophage receptors to generate ROS and form macrophage extracellular traps, which, in turn, lead to TLR-dependent pathways of AKI (66). The other investigative line explored in AKI induced by Mb or Hb attests to activation of the NLRP3 inflammasome and the attendant production of proinflammatory cytokines and the instigation of pathways of cell death (67–71). Finally, it should be noted that not all macrophages or cytokines are injurious in G-AKI (65). For example, erythropoietin is protective in G-AKI by inducing polarization of macrophages to the potentially protective M2 phenotype (72), whereas the cytokine G-CSF protects against G-AKI (73).

Complement-Mediated Renal Injury

Heme activates the alternative complement pathway (26). Recent studies demonstrate that complement activation occurs in both humans with rhabdomyolysis and rodents with G-AKI (74,75). Complement depletion by cobra venom factor protects against G-AKI, as do genetic deficiency of C3 and strategies that bind heme (74,75). Activation of complement occurs via two pathways: the lectin pathway and the alternative complement pathway (74).

Tubular Cast Formation

The thick ascending limb uniquely generates abundant amounts of uromodulin (Tamm–Horsfall protein), a glycoprotein with a strong proclivity for forming casts with Mb or Hb, especially under acidic conditions and when urinary flow rate is low (76). Cast formation promotes renal injury for three reasons (Figure 1). First, this impedes the urinary clearance of Mb and Hb. Second, this promotes auto-oxidation of HPs by urinary hydrogen peroxide. Third, nephron obstruction prolongs the exposure of proximal tubules to Mb and Hb, thereby promoting cellular uptake of Mb and Hb and tubular damage. Proximal tubule cell injury and the sloughing of cells and their fragments into the urinary space foster cast formation; proteins leaked into the urinary space because of impaired glomerular permselectivity also promote cast formation. However,
such involvement of uromodulin in AKI induced by Mb and Hb must be viewed within the context of recent seminal insights regarding uromodulin (77,78). Uromodulin serves assorted homeostatic functions in the healthy kidney and protects against IRI by suppressing the IL-23/IL-17 axis and neutrophil recruitment, among other actions (77,78). Uromodulin’s interaction with Mb or Hb and its resulting adverse effects thus mask and overwhelm the homeostatic and otherwise nephroprotectant effects of this unique protein.

Table 5. Therapeutic strategies in preclinical studies that reduce AKI induced by HPs

| Therapeutic策略                  | Ref. |
|----------------------------------|------|
| Vasodilators (27,28,32,33,51)    |      |
| Bicarbonate (27,76)              |      |
| Antioxidants (28,48,49,50,52)    |      |
| Curcumin (53,114)               |      |
| Haptoglobin (94,95)             |      |
| Hemopexin (74)                  |      |
| α1-microglobulin (83,97)        |      |
| Hepcidin (99)                   |      |
| Ferritin (106)                  |      |
| Iron-binding agents (49)        |      |
| Inhibitors of megalin (18)      |      |
| Inhibitors of complement (74,75)|      |
| Anti-inflammatory compounds (60,62,63,70,71,73) | |
| Acetaminophen (51)             |      |
| Erythropoietin (72)             |      |
| Inducers of HO-1/HO products (9,106–108) | |
| Activators of Nrf2 (111–115)   |      |
| Preconditioning (115,116)       |      |
| Mesenchymal stromal cells and extracellular vesicles (118) | |

HPs, heme proteins.

Circulating Cell-Free Hb and AKI

In hemolytic states, circulating cell-free Hb (CFH) is detected and is implicated in the pathogenesis of AKI. Salient examples include cardiopulmonary bypass surgery (79), sickle cell anemia (80), paroxysmal nocturnal hemoglobinuria (81), transfusion reactions (82), infectious processes (82), preeclampsia and the HELLP syndrome (83), thrombotic microangiopathy (82), and sepsis (82). Sepsis is a major cause of AKI (84), and the appearance of CFH in sepsis may reflect either the underlying cause for sepsis or adverse effects of sepsis on RBCs (82). Sepsis leads to impaired deformability of RBCs and RBC damage in the turbulence-prone areas of the microcirculation and in the constraining luminal space of the microcirculation (82,85). Additionally, the inflammatory and prothrombotic milieu of sepsis can provoke eryptosis (RBC cell death) and/or disseminated intravascular coagulation, either of which may lead to CFH (82). CFH, in turn, exacerbates the severity of AKI caused by sepsis (86). A proximate step in sepsis-associated damage to the kidney and other organs is endothelial injury. Such injury is characterized by degradation of the glycocalyx lining the endothelium, increased endothelial leakiness with transendothelial egress of fluids and proteins from the circulation, and an endothelium that promotes inflammation, leukocyte adhesion, and thrombosis (87). Such endothelial changes may all result from the damaging effects of auto-oxidation of CFH and the release of heme (88). Heme is not only proinflammatory but also thrombogenic (89).

HPs/Heme and CKD

Recurrent Exposure to Rhabdomyolysis/Hemolysis

We previously described a triphasic response when the kidney is exposed intermittently to HPs: first, AKI; second, acquired resistance to AKI; and third, CKD (90). These studies articulated the concept that AKI can eventuate in CKD (90). Such occurrence of CKD represents the culmination of recurrent episodes of ischemia and oxidative stress imposed by HPs and is likely mediated through upregulation of oxidant-inducible cytokines such as MCP-1 and TGF-β1 (81,90).

Hematuric Glomerular Disease

Thirty years ago, we postulated that hematuria (a source of Hb) can contribute to CKD (41), a thesis based on studies demonstrating that hematuric glomerulonephritis leads to endocytosis by and degradation of RBCs within the proximal tubule (91), and that microinjection of RBCs into the tubular lumen of proximal tubules leads, progressively, to endocytosis of RBCs and their intracellular destruction, proximal tubule injury, peritubular interstitial inflammation and fibrosis, and CKD (92). In support of this thesis of hematuria-instigated, Hb-driven CKD is the demonstration in large national databases that persistent asymptomatic isolated microscopic hematuria is a risk factor for progressive CKD and ESKD (93).

Tubulointerstitial Disease

Activated leukocytes infiltrating the tubulointerstitium can upregulate iNOS and NOX, and/or release myeloperoxidase that injures the kidney as previously discussed. Renal tubules, when stimulated by cytokines or angiotensin II, may upregulate iNOS and NOX, and thereby serve as additional sources for ROS and reactive nitrogen species.

Defense against HPs

Such defenses include superoxide/hydrogen peroxide-scavenging systems and those that are specifically geared to Hb, heme, and iron (Figure 3). Superoxide dismutase converts superoxide anion to hydrogen peroxide, whereas the latter is degraded by the glutathione/glutathione peroxidase system and by catalase. Nonenzymatic degradation of hydrogen peroxide also occurs by α-keto acids, and, pyruvate, an α-keto acid, protects against G-AKI (50). Ascorbate and uric acid provide antioxidant defense in plasma, whereas α-tocopherol and ubiquinone reduce membrane lipid peroxidation.

The toxicity of CFH is interrupted by the binding of Hb to Hpt, the complex then incorporated by phagocytes via the CD163 receptor (2,17). Such binding negates Hb auto-oxidation and Hb’s permeation into the urinary space. The significance of Hb-Hpt binding is reflected by the increased sensitivity of Hpt−/− mice to AKI after hemolysis (94) and that the administration of Hpt to individuals undergoing cardiac surgery (a CFH-associated condition) reduces the
risk for AKI (95). Free heme is bound to proteins and/or degraded. Hemopexin (HPX) is, overwhelmingly, the dominant heme-binding protein; albumin binds heme but only quite weakly (2,17). Genetic deficiency of HPX promotes AKI in sickle cell disease, the latter attended by CFH; administration of HPX reduces such injury in sickle cell disease (96). Alpha1-microglobulin also binds heme and scavenges ROS and protects against heme-mediated injury in vitro and in a model of preeclampsia (83,97). Hepcidin, a protein involved in iron homeostasis and one that may suppress inflammation, is protective in IRI (98), and, interestingly, hepcidin protects cells of the distal nephron against cell death caused by Hb/heme (99).

Studies in G-AKI were the first to demonstrate the cytoprotective actions of HO-1 in any tissue (9) and were predicated on the long and singular history in nephrology of seeking to understand the basis of tissue resistance to injury (100). HO catalyzes heme to biliverdin during which iron is released from the heme ring and carbon monoxide evolves; biliverdin is then converted to bilirubin via biliverdin reductase. HO-1 and HO-2 are the inducible and constitutive isoforms, respectively. HO-2 provides an already existing mechanism for catabolizing heme, whereas HO-1 induction, commonly facile and fulminating, markedly increases the cellular capacity for degrading heme. Induction of HO-1 was subsequently shown to protect against other forms of renal injury (101–103) and contribute to acquired resistance to renal injury (104). Induction of HO-1 is coupled to increased synthesis of ferritin, the dominant iron-binding protein (9,105). The virtue of this system is that it ensures not only the removal of heme but also the safe sequestration by ferritin of iron released from the heme ring. Ferritin not only binds iron with very high capacity (one molecule of ferritin binds 4500 atoms of iron), but the ferritin h-chain prevents the redox cycling of iron and thus the propagation of oxidative stress (105,106). If, however, the increase in free heme overwhelms the existing or induced heme oxygenase-ferritin system, heme- and iron-dependent pathways of injury will ensue. Heme can be degraded nonenzymatically (e.g., by ROS) that circumvents the seamlessly protective coupling of induction of ferritin to that of HO-1, and iron-mediated oxidant damage may occur. The protective effects of the HO system arise not only from degrading heme but also from the intrinsic protective effects of ferritin, bile pigments, and carbon monoxide. Biliverdin and bilirubin are anti-inflammatory and antioxidant, and, in relatively low amounts, are cytoprotective. Carbon monoxide, in low concentrations, is vasorelaxant, antioxidant, anti-inflammatory, and anti-apoptotic. In G-AKI, persuasive evidence attests to the protective effects of ferritin (106) and carbon monoxide (107). In an in vitro model of HP-induced renal injury, bilirubin, in micromolar amounts, exerts dose-dependent cytoprotection (108). The cytoprotective effects of the HO-1/ferritin system ramify widely; for example, they enable the kidney to degrade safely free labile heme appearing during hemolytic episodes caused by malaria (109). Upstream of HO-1 is the transcription factor Nrf2, which is readily activated by heme (110). Activation of Nrf2 is protective in HP-AKI (111–115) via the induction of HO-1 and other antioxidant enzymes. An innovative approach to activating Nrf2 in G-AKI involves the inhibition of HO activity and the administration of iron, in essence exerting renal stress, which then elicits robust Nrf2 activation and induction of assorted cytoprotective genes, including HO-1 gene/protein (115,116).

A repressor of HO-1 expression is the protein bach1 (117). Increased amounts of heme release HO-1 expression from repression by bach1, with the attendant induction of HO-1 mRNA. The applicability of this construct for HO-1 induction has been shown in G-AKI.

G-AKI is attenuated not only by constitutive and inducible defense mechanisms but also by regenerative and reparative responses; such responses and attendant AKI recovery are fostered by mesenchymal stromal cells and derived extracellular vesicles (118).

Conclusions
This brief review outlines current understanding of the pathogenetic processes underlying such HP-induced kidney damage and from which diverse therapeutic strategies have been uncovered (Table 5). Translating these preclinical strategies into therapeutic realities is, indubitably, the challenge that lies ahead, and will likely involve a multi-pronged approach.

Disclosures
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Author Contributions
A.J. Croatt, K.A. Nath, and R.D. Singh wrote the original draft of the manuscript; K.A. Nath was responsible for the formal analysis, funding acquisition, and supervision; K.A. Nath and R.D. Singh were responsible for the conceptualization; R.D. Singh and K.A. Nath were responsible for the methodology and visualization; and all authors reviewed and edited the manuscript.

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