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Key Points:

Abstract:

Disclosures: Both Christof Westenfelder (CW) and Anna Gooch are employees and officers at SymbioCellTech. They have ownership interests in SymbioCellTech and hold patents. CW is Professor emeritus at the University of Utah Health Sciences Center, Department of Medicine, Salt Lake City, UT.

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Heme Protein-Induced Acute Kidney Injury is caused by Disruption of Mitochondrial Homeostasis in Proximal Tubular Cells

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It is now well established that many forms of Acute Kidney Injury (AKI) such as those induced by ischemia/reperfusion, cis-Pt, sepsis, cardiopulmonary bypass surgery, and others are characterized by critical disturbances in primarily proximal tubular mitochondrial dynamics, i.e., increased mitochondrial fission and decreased fusion, boosted mitophagy and reduced mitochondrial biogenesis, together resulting in inadequate energy metabolism [1,2]. The cellular stresses of these insults lead to reduced oxygen consumption and ATP and NAD⁺ generation, boosted ROS production, free ferro, ferric and ferryl iron-induced lipid peroxidation and ferroptosis [3], mitochondrial fragmentation and increased mito- and autophagy. This loss in proximal tubular ATP generation disables tubular transport and various other ATP-dependent cellular functions that result in Fanconi-like transport defects that reduce the reabsorption of electrolytes, glucose, bicarbonate, phosphate, amino acids, peptides and other ultrafiltrate constituents. These re-absorptive disturbances are combined with defective secretion of uric acid and other compounds, significantly mediated by a substantial loss of specific Na-K-ATPase activity in depolarized tubular cells [4]. In addition, decreased renal blood flow, tubular leaks, interstitial accumulation of proinflammatory cells, complement activation and obstruction are important contributors to the loss of renal function in these forms of AKI.

Importantly, impaired function of mitochondria-associated Endoplasmatic Reticulum (ER) Membranes (MAMs) impairs mitochondrial quality control, further induces oxidative stress and apoptosis, disturbs ER-mitochondrial Ca²⁺ crosstalk, induces ER stress (UPR, unfolded/misfolded protein response) and inflammasome (NLRP3) activation, mediated by release of PAMPs, DAMPs and HAMPS, which will result in the secretion of potent pro-inflammatory IL-1β and IL-18 by macrophages and dendritic cells [5–7]. The cell intrinsic cytoprotective mechanisms provided by Heme-Oxygenase I and ferritin will be overwhelmed when the tubular cell injury is too severe [8]. Cellular necroptosis, necrosis, apoptosis and ferroptosis result, and with incomplete recovery the associated interstitial fibrosis mediates the transition to CKD [7]. See Summary Figure 1.

Of note is the fact that ischemic and toxic forms of AKI are also shown to exhibit increases in intracellular levels of heme proteins, which includes cytochrome p450-derived heme.

Mitochondrial dynamics in cells with healthy mitochondria consist of balanced mitochondrial fission and fusion activities and physiological mitochondrial biogenesis and mitophagy [1,2]. Definition of these highly complex mitochondria-targeted pathogenic mechanisms of acute proximal tubular injury includes the full characterization of intrinsic cytoprotective and cellular repair capabilities. Rebalancing of mitochondrial fission and fusion, mitophagy and mitogenesis are critical steps. Execution of required autophagy and cellular apoptosis and inactivation of the inflammasome and complement system will reduce ROS production and reestablish physiological ATP and NAD⁺ production, i.e., normalization of the needed energy metabolism. This is followed by re-differentiation of tubular cells and their proliferation at sites of tubular cell loss, improved renal blood flow and GFR (Fig. 1). Preclinical studies have shown that small molecular interventions that effectively target IL-1β and IL-18 or their receptors have promise, while no clinical data for the treatment of HP-AKI and other forms of AKI are currently available [7]. As elegantly demonstrated by Parikh et al., administration of nicotinamide boosts the synthesis of NAD⁺ and promises to become a simple but effective intervention in the management/prevention of AKI by enhancing the
cellular metabolic activity [8].

In the current *Kidney360* article, Nath and colleagues, experts in the field of clinically important Pigment Nephropathies, conducted detailed mitochondrial studies in the glycerol model of rhabdomyolysis/myoglobin-induced AKI in mice [9]. The authors pointed out that such mitochondrially focused studies had not been conducted in Heme Protein-induced AKI (HP-AKI), while a large body of respective data exists in ischemic and toxic forms of AKI (see above). In order to arrive at the development of effective interventions for this form of AKI, the authors posited that the underlying mitochondrial manifestations in proximal tubular cells must be fully defined.

When Hemoglobin or Myoglobin-derived Heme Protein levels are increased in the circulation, due to hemolysis or muscle injury, respectively, these freely filtered lipophilic Heme Proteins are taken up by the megalin/cubilin-transporter on proximal tubular cells and interfere with mitochondrial function/respiration by their intercalation in lipid rich mitochondrial membranes [10]. In addition, heme stimulates lipid peroxidation by hydrogen peroxide generation. This deleterious interaction results in the massive disruption of mitochondrial dynamics that is characterized by decreased ATP generation, decreased NAD+ and NAD+/NADH levels, and the generation of ROS.

Nath and colleagues [8] used the glycerol model of rhabdomyolysis/myoglobin-induced AKI in mice and exposure of murine and human proximal tubular cells in vitro to heme. They demonstrated that ATP and NAD+ content and the NAD+/NADH ratio were significantly reduced within 8 hrs. of injury/exposure, and pro-inflammatory cytokines were highly upregulated. The expression of proteins that regulate mitochondrial biogenesis (PGC-1alpha, NRF1 and TFAM) and fusion (MFN2), critical for positive mitochondrial quality control, was impaired, paralleled by the downregulation of key proteins responsible for the maintenance of outer and inner mitochondrial membrane integrity and polarization (VDAC, Tom20, Tim23). There was concurrent upregulation of DRP1, the critical protein for the execution of mitochondrial fission that critically reduces the functional mitochondrial mass. Elegant studies of murine renal cortical tissue, employing TME and novel 3D imaging, demonstrated major changes in mitochondrial structure, mitochondrial fragmentation, distortion of mitochondrial cristae, swelling and mitophagy, changes closely correlated with the molecular observations. When severe, such damage will lead to tubular cell apoptosis, ferroptosis [3] and necroptosis, together resulting in cell death and permanent loss of kidney function (Fig. 1). Exposure of murine and human proximal tubular cells in vitro to relevant concentrations of heme demonstrated parallel changes to those seen in vivo: suppression of PGC-1alpha (mitochondrial biogenesis) and upregulation of p-DRP1 (mitochondrial fission).

These detailed studies by Nath and colleagues [9] significantly expand our knowledge of the central pathogenic role that mitochondrial damage plays in HP-AKI. Nath and colleagues’ extensive previous work in the field provides a solid basis for the current investigations. These observations will also be highly pertinent to the development of novel therapeutic interventions in AKI, i.e., interventions that will have significant clinical relevance.

Accordingly, knowledge of intrinsic defense mechanisms in proximal tubules combined with novel therapeutic interventions to prevent AP-AKI and stimulate repair and functional recovery are of significant translational importance (Fig. 1). The cytoprotective anti-oxidant and other effects of upregulated heme oxygenase-1 (HO-1) in AP-AKI and other forms of AKI are well established [11]. Another recently discovered endogenous defense
mechanism and various novel therapies also work via upregulation of HO-1, which includes the actions of both the intrinsic and administered NGAL:siderophore:Fe complex [12] and the administration of Angiotensin 1-7 that activates the Mas Receptor[13]. In HP-AKI renal Angiotensin-II levels are increased which causes renal vasoconstriction, while Angiotensin 1-7 levels that oppose Angiotensin-II’s effects are decreased. The Angiotensin 1-7 therapy reduces vasoconstriction through eNOS, downregulates proinflammatory TLR-4, NF-κB, iNOS, and upregulates the cytoprotective Nrf-2/HO-1 pathway. Previously, largely identical therapeutic benefits of Angiotensin 1-7 were shown in ischemia-reperfusion AKI [14]. Of particular promise may be the therapeutic use of Cilastatin, an FDA approved compound, that blocks the megalin-mediated endocytosis of myoglobin and hemoglobin by proximal tubular cells and thereby provides significant protection against both HP-AKI [15] and other forms of toxic AKI induced by cis-Pt, vancomycin, colistin and aminoglycosides, and this without affecting the specific pharmacologic actions of these drugs [16].

In conclusion, these detailed studies by Nath and colleagues [9] significantly expand our knowledge of the central pathogenic role that mitochondrial damage plays in HP AKI. Specifically, the current studies demonstrate that the concepts of mitochondrial pathology that are well defined in ischemic and toxic forms of AKI do also apply to HP-AKI. A full understanding of these mechanistic details will be needed for the eventual design of effective therapies to prevent HP-AKI, hasten the recovery process and also block the development of CKD. It is finally expected that future work by these investigators will include a focus on the contributions that defective MAMs and ER stress (UPR, unfolded/misfolded Protein response) make to the mitochondrial pathology in HP-AKI.

Disclosures
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Author Contributions
CW and AG conceptualized the editorial, wrote the original draft, and reviewed and edited the manuscript.
References


Figure 1

Legend to Fig. 1. Heme Protein-Induced Acute Kidney Injury causes the catastrophic Disruption of Mitochondrial Homeostasis in Proximal Tubular Cells.

(A) In normal proximal tubular cells, Mitochondrial Dynamics (Fusion and Fission) and Quality Control (Mitogenesis and Mitophagy) are balanced and maintain normal ATP synthesis and cellular functions. The activity of Autophagy is physiological. (B) Heme Protein induces proximal tubular cell injury and AKI by collapsing Mitochondrial Dynamics and Quality Control, resulting in decreased O$_2$ consumption and boosted ROS production, leading to decreased ATP and NAD$^+$ levels, lipoxygenation, ferroptosis and other forms of cell death. The inflammasome (NLRP3) is activated, i.e. Ca$^{++}$ and free iron are increased. Autophagy is boosted. The potential role of ER stress and dysfunctional MAMs has not been investigated. (Right) OUTCOMES: (C) Irreversible Cell Death and loss of Kidney Function. Incomplete repair, interstitial fibrosis microvascular rarefaction, transition to CKD. (D) Recovery and Repair mechanisms, Return of Kidney Function. (E) Endogenous Defenses and novel Therapies.
Endogenous Defenses:
- **HO-1**: Anti-oxidant, anti-inflammatory, anti-apoptotic (11)
- **NGAL:Siderophore:Fe**: upregulates HO-1, inhibits Cell Death (12)

**THERAPY**: Prevention & Support for Recovery/Repair
- **Angiotensin 1-7 Administration - Mas Receptor Activation**: reduces vasoconstriction through eNOS, downregulates TLR-4, NF-κB, iNOS, upregulates Nrf-2/HO-1 pathway (13, 14)
- **Megalin Blockade with Cilastin**: reduces prox. tubular Myoglobin Uptake (15, 16)

**Normal Proximal Tubular Cell**
- Physiological Mitochondrial Dynamics and Quality Control:
  - Fusion ↔ Fission
  - Mitogenesis ↔ Mitophagy
  - Normal ATP synthesis
  - No ER stress or Apoptosis
  - Physiological Autophagy

**Heme Protein-induced Proximal Tubular Cell Injury and AKI**
- Decreased O2 consumption, ATP & NAD+ synthesis, increased ROS and lipoxygenation, Ferroptosis, other

**Collapse of Mitochondrial Dynamics and Quality Control**
- Fission >> Fusion
- Mitophagy >> Mitogenesis
- Dysfunctional MAMs?
- ER stress?
- Apoptosis
- Mitochondrial Fragmentation
- Increased Autophagy
- Increased i.c. Ca++ and free iron
- Activated Inflammasome

**Irreversible Cell Death and loss of Kidney Function**
- Necrosis
- Apoptosis
- Ferroptosis
- Necroptosis

**Incomplete Repair**
- Transition to CKD

**Recovery & Repair**
- Normalization of Mitochondrial Dynamics and Quality Control: Increased Mitogenesis
- Return of ATP synthesis
- Replenishment of lost tubular cells

**Return of Kidney Function**