Published in final edited form as:

Nephron. 2018 ; 140(2): 156–159. doi:10.1159/000490808.

Iron Homeostasis Pathways as Therapeutic Targets in Acute Kidney Injury

Sundararaman Swaminathan¹

¹Division of Nephrology and Center for Immunity, Inflammation and Regenerative Medicine, University of Virginia, P.O. Box 800133, Charlottesville, VA 22908, USA

Abstract

Background—Iron is critical for fundamental biologic functions such as cell division and mitochondrial electron transport. However, by the virtue of its ability to donate electrons, iron is probably the most effective oxidant in biologic systems.

Summary—To avoid damage from iron-mediated oxidative injury or ferroptosis, multiple defense mechanisms exist including iron binding proteins and robust glutathione-dependent intracellular pathways. Hepcidin, through its ability to sequester iron within macrophages and induce H-ferritin, serves as an endogenous protective molecule against ferroptosis.

Key messages—Recent studies have demonstrated protective role of Hepcidin in both ischemia-reperfusion injury and heme-mediated models of acute kidney injury. Ferroptosis-inhibiting drugs and Hepcidin offer exciting novel prospects to treat acute kidney injury.

Keywords

Iron; oxidative stress; acute kidney injury; Hepcidin; ferroptosis

Introduction

Iron serves many fundamental biologic roles such as cell cycle progression, DNA synthesis and repair, mitochondrial function, and regulation of inflammation. However, iron has been implicated in oxidative stress by the virtue of its ability to donate free electrons to oxygen molecule and thus in generation reactive oxygen species (ROS). Both iron and ROS have been implicated in acute kidney injury (AKI). In this review, we will present emerging knowledge in this area with a special emphasis on the role of Hepcidin and ferroptosis in AKI.

Iron homeostasis and its regulation

It is important in hemoglobin synthesis and hence in tissue oxygen delivery. Most of iron in the body is derived from recycling of senescent red blood cells through erythrophagocytosis. In macrophages, heme undergoes degradation by heme oxygenase-1 to release ferrous iron,

carbon monoxide and bilirubin as its byproducts. Iron is either stored in intracellular ferritin or is exported out of the cell through the only known iron exporter, Ferroportin. ¹ Exported ferrous iron is oxidized by Hephaestin or Ceruloplasmin to ferric iron which can then be bound to transferrin for consumption by cells through transferrin receptor-mediated uptake.

Hepcidin, the master regulator of iron homeostasis, is primarily produced by hepatocytes and to small extent by other cells such as macrophages, bile duct epithelium and distal nephron of the kidney. Hepcidin binds to ferroportin through a disulfide bond to result in internalization of Hepcidin-ferroportin complex and to undergo lysosomal degradation. ¹ The end result of Hepcidin effect is reduced ferroportin expression and intracellular iron retention. Positive regulators of Hepcidin include iron and inflammatory cytokines such as IL-6. ² Thus, under condition of chronic inflammation, persistent induction of Hepcidin results in reticuloendothelial iron overload and reduced iron delivery for erythropoiesis to result in anemia of chronic inflammation.

Labile iron and acute kidney injury

Normally iron exists bound to proteins such as transferrin, ferritin, and NGAL and in the Fe^{3+} (ferric) state. However, under pathological conditions it can easily undergo a single electron transfer reaction and convert to Fe^{2+} (ferrous), which can be highly reactive and toxic. Catalytic or labile iron (which is primarily in ferrous form) is a small transitional pool of extracellular and intracellular iron. Unlike transferrin bound iron (TBI), this species of iron is loosely bound to serum albumin or endogenous chelators, such as citrate, acetate, malate, phosphate, and adenine nucleotides. Labile iron is capable of reacting with oxygen and generates reactive oxygen species such as hydroxyl radical and hydrogen peroxide that are implicated in lipid peroxidation and tissue injury through Fenton chemistry. ³ The partial reduction of molecular oxygen into water leads to formation of superoxide radical that is subsequently converted to hydrogen peroxide by superoxide dismutase. The Haber–Weiss reaction that can occur in cells generates hydroxyl radicals ($\bullet\text{OH}$) from H_2O_2 and superoxide ($\bullet\text{O}_2^-$) and is a potential source of oxidative stress. However, this is a slow reaction and is catalyzed by iron (Figure 1). Because of the potential reactivity of labile iron, its availability in the body is tightly regulated by multiple iron-handling proteins. Being one of the major metals in mammals, a small percentage increase in labile iron could induce cascading ROS-mediated injuries. ^{4,5}

Animal studies have shown the importance of non-heme iron in AKI more than two decades ago. ⁶ The ability of iron chelators to protect in animal models of AKI provided direct evidence on the importance of iron in mediating AKI. ⁷ The role of catalytic iron has since been demonstrated in multiples models of AKI such as hemoglobinuria, aminoglycoside exposure, ischemia-reperfusion injury, rhabdomyolysis, and cisplatin toxicity.

Human studies have shown a strong association between increased catalytic iron and progression to AKI. Leaf et. al. observed a significant increase in plasma catalytic iron following cardiopulmonary bypass (CPB). ⁸ Plasma catalytic iron levels were significantly higher at the end of CPB compared with preoperative levels. Furthermore, catalytic iron levels at the end of CPB and on postoperative day 1 were higher in patients who develop in-

hospital death or need for renal replacement therapy. The same authors demonstrated that higher plasma catalytic iron levels were associated with a greater risk of death/renal replacement therapy, AKI, and other adverse events, in critically ill patients admitted to medical or surgical intensive care units.⁹ Lele et al. showed a strong association between serum catalytic iron levels and progression to AKI in patients with acute coronary syndrome who underwent coronary angiography using iodinated radio-contrast.¹⁰ Patients with contrast-induced AKI had higher catalytic iron levels at 24 and 48 h. Thus, progression to AKI increases linearly with the presence of increased catalytic iron both in patients undergoing elective procedures and in critically ill patients.

Proximal tubule H-ferritin

In kidney, proximal tubules express H-ferritin (FtH) to effectively sequester iron and limits free iron-mediated toxicity. Ferritin-H (FtH) subunit exhibit ferroxidase activity, converting Fe^{+2} to Fe^{+3} so that iron may be stored in the ferritin mineral core. Each molecule of H-ferritin can bind to 4500 Fe^{2+} ions, making it an important endogenous iron chelator.¹¹ FtH is upregulated with HO-1 to store the ferrous iron derived from the reaction.¹² Further, HO-1 induced protection is dependent on its ability to upregulate FtH. Zarjou et al used proximal tubule-specific FtH knockout mice to study its role in rhabdomyolysis and cisplatin-induced AKI.¹³ Loss of FtH from proximal renal tubules worsened renal injury and increased mortality despite significantly higher expression of HO-1. When compared to littermate controls, proximal tubule specific H-ferritin knockout mice demonstrated increased urinary levels of iron acceptor proteins such as NGAL, hemopexin, and transferrin, after AKI. Thus, FtH in proximal tubule plays an important protective role in AKI.

Ferroptosis in Kidney Injury

As we discussed above, labile iron is implicated in diverse forms of AKI and iron chelators have been shown to protect against kidney injury. Intracellular glutathione serves as an important defense mechanism against iron-mediated oxidative injury and lipid peroxidation. Recently, pathways involved in iron-mediated tissue cell death or ferroptosis have been more carefully elucidated.¹⁴ Linkermann and colleagues demonstrated a key role for ferroptosis in renal tubular cells undergoes sequential death following ischemic kidney injury.¹⁵ In their study, inhibition of ferroptosis using a selective chemical inhibitor-ferrostatin 1 blocked ferroptosis *in vitro* and prevented murine renal ischemic reperfusion injury. Glutathione peroxidase 4 (GPX4) is an antioxidant enzyme that catalyzes reduction of lipid peroxides at the expense of reduced glutathione to protect against oxidative damage and ferroptosis. Oxidized glutathione generated by this reaction is recycled by glutathione reductase and NADPH. GPX4 is vital for survival. In an inducible GPX4 knockout mice, lack of GPX4 resulted in spontaneous acute renal failure and early mortality.¹⁶ Further studies in GPX4 knockout cells showed the importance of lipid mediators such as 5-, 12- and 15-hydroperoxyeicosotetraenoic acid (HpETE) and oxidized phospholipids. Ultrastructurally, ferroptosis involves rupture of outer mitochondrial membrane. By testing 40,000 drug-like small molecules, authors were able to identify a spiroquinoxalinamine derivative, Liproxstatin-1, as a novel inhibitor of ferroptosis to prevent AKI. More recently, oxidized

phosphatidyl ethanolamine -(C18:0/C20:4) have been shown to accumulate in the kidneys of GPX4 knockout mice. Suppression of esterification of acyl arachidonoyl (AA) and adrenoyl (AdA) by inhibition of acyl-CoA synthase 4 (ACSL4) led to prevention of ferroptosis *in vitro*.¹⁷ Thiazolidinediones inhibit ACSL4 and partly reduces mortality in inducible GPX4 knockout mice suggesting their potential benefit in AKI.¹⁸ These findings demonstrate the emerging importance of ferroptosis in AKI.

Hepcidin as a protective molecule in Acute Kidney Injury

Hepcidin induces iron sequestration primarily in macrophages by downregulating ferroportin expression and cellular iron export. In response to iron sequestration, cells induce the protective molecule, H-ferritin.

Cardiopulmonary bypass (CPB) is the second most common cause of AKI in intensive care units. Heme iron and reperfusion injury are implicated in the pathogenesis of AKI in this setting.¹⁹ In a nested case-control study of CPB patients and using urine mass-spectrometric assays, Ho et al. identified that urinary Hepcidin-25 levels significantly increases in patients who do not develop AKI as compared to those who develop AKI.²⁰ Prowle et al., showed that urinary Hepcidin normalized to creatinine in first 24 hours after CPB surgery inversely correlated with the risk of AKI in the first five post-operative days.²¹ Serum Hepcidin was higher in AKI but did not correlate with clinical outcomes.

As discussed earlier, ROS and ferroptosis are implicated in ischemia-reperfusion injury. In our original studies, we utilized Hepcidin-dependent endogenous iron homeostatic pathways to therapeutically target iron-mediated injury in AKI. We have observed that Hepcidin given 24–48 hours before IRI ameliorated kidney injury and this protection was associated with ferroportin downregulation, splenic iron retention, increased H-ferritin in the kidney and spleen, and reduced systemic and renal inflammatory response.²² In an independent recent study, protective effect of Hepcidin has been demonstrated in a murine model of hemoglobin-induced AKI.²³

Conclusion

Iron is important for critical cellular functions such as in erythropoiesis, hypoxia signaling, mitochondrial function, and DNA synthesis and repair. As free iron is toxic, iron metabolism is highly regulated. In AKI, ferroptosis is a significant contributor to cell death. Ferroptosis inhibition and Hepcidin administration are promising novel strategies to prevent and treat AKI (Figure 2), particularly in conditions such as AKI associated with cardiac surgery where ischemia-reperfusion and heme- and non-heme iron mediated toxicity play prominent pathogenic roles.

References

1. Nemeth E, Tuttle MS, Powelson J, et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science*. 2004; 306(5704):2090–2093. [PubMed: 15514116]
2. Wang CY, Babitt JL. Hepcidin regulation in the anemia of inflammation. *Curr Opin Hematol*. 2016; 23(3):189–197. [PubMed: 26886082]

3. Halliwell B. Reactive oxygen species in living systems: source, biochemistry, and role in human disease. *Am J Med.* 1991; 91(3C):14S–22S.
4. Braughler JM, Duncan LA, Chase RL. The involvement of iron in lipid peroxidation. Importance of ferric to ferrous ratios in initiation. *J Biol Chem.* 1986; 261(22):10282–10289. [PubMed: 3015924]
5. Walter PB, Knutson MD, Paler-Martinez A, et al. Iron deficiency and iron excess damage mitochondria and mitochondrial DNA in rats. *Proc Natl Acad Sci U S A.* 2002; 99(4):2264–2269. [PubMed: 11854522]
6. Baliga R, Ueda N, Shah SV. Increase in bleomycin-detectable iron in ischaemia/reperfusion injury to rat kidneys. *Biochem J.* 1993; 291(Pt 3):901–905. [PubMed: 7683877]
7. Shah SV, Rajapurkar MM, Baliga R. The role of catalytic iron in acute kidney injury. *Clin J Am Soc Nephrol.* 2011; 6(10):2329–2331. [PubMed: 21979910]
8. Leaf DE, Rajapurkar M, Lele SS, et al. Increased plasma catalytic iron in patients may mediate acute kidney injury and death following cardiac surgery. *Kidney Int.* 2015; 87(5):1046–1054. [PubMed: 25565307]
9. Leaf DE, Rajapurkar M, Lele SS, Mukhopadhyay B, Waikar SS. Plasma catalytic iron, AKI, and death among critically ill patients. *Clin J Am Soc Nephrol.* 2014; 9(11):1849–1856. [PubMed: 25189925]
10. Lele SS, Mukhopadhyay BN, Mardikar MM, et al. Impact of catalytic iron on mortality in patients with acute coronary syndrome exposed to iodinated radiocontrast-The Iscom Study. *Am Heart J.* 2013; 165(5):744–751. [PubMed: 23622911]
11. Harrison PM, Arosio P. The ferritins: molecular properties, iron storage function and cellular regulation. *Biochim Biophys Acta.* 1996; 1275(3):161–203. [PubMed: 8695634]
12. Nath KA, Balla G, Vercellotti GM, et al. Induction of heme oxygenase is a rapid, protective response in rhabdomyolysis in the rat. *J Clin Invest.* 1992; 90(1):267–270. [PubMed: 1634613]
13. Zarjou A, Bolisetty S, Joseph R, et al. Proximal tubule H-ferritin mediates iron trafficking in acute kidney injury. *J Clin Invest.* 2013; 123(10):4423–4434. [PubMed: 24018561]
14. Dixon SJ, Lemberg KM, Lamprecht MR, et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell.* 2012; 149(5):1060–1072. [PubMed: 22632970]
15. Linkermann A, Skouta R, Himmerkus N, et al. Synchronized renal tubular cell death involves ferroptosis. *Proc Natl Acad Sci U S A.* 2014; 111(47):16836–16841. [PubMed: 25385600]
16. Friedmann Angeli JP, Schneider M, Proneth B, et al. Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice. *Nat Cell Biol.* 2014; 16(12):1180–1191. [PubMed: 25402683]
17. Kagan VE, Mao G, Qu F, et al. Oxidized arachidonic and adrenic PEs navigate cells to ferroptosis. *Nat Chem Biol.* 2017; 13(1):81–90. [PubMed: 27842066]
18. Doll S, Proneth B, Tyurina YY, et al. ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. *Nat Chem Biol.* 2017; 13(1):91–98. [PubMed: 27842070]
19. Haase M, Haase-Fielitz A, Bellomo R. Cardiopulmonary bypass, hemolysis, free iron, acute kidney injury and the impact of bicarbonate. *Contrib Nephrol.* 2010; 165:28–32. [PubMed: 20427952]
20. Ho J, Lucy M, Krokhin O, et al. Mass spectrometry-based proteomic analysis of urine in acute kidney injury following cardiopulmonary bypass: a nested case-control study. *Am J Kidney Dis.* 2009; 53(4):584–595. [PubMed: 19070948]
21. Prowle JR, Ostland V, Calzavacca P, et al. Greater increase in urinary Heparin predicts protection from acute kidney injury after cardiopulmonary bypass. *Nephrol Dial Transplant.* 2012; 27(2): 595–602. [PubMed: 21804084]
22. Scindia Y, Dey P, Thirunagari A, et al. Heparin Mitigates Renal Ischemia-Reperfusion Injury by Modulating Systemic Iron Homeostasis. *J Am Soc Nephrol.* 2015; 26(11):2800–2814. [PubMed: 25788528]
23. van Swelm RP, Wetzels JF, Verweij VG, et al. Renal Handling of Circulating and Renal-Synthesized Heparin and Its Protective Effects against Hemoglobin-Mediated Kidney Injury. *J Am Soc Nephrol.* 2016; 27(9):2720–2732. [PubMed: 26825531]

In the first step, ferric ion is reduced to ferrous:

The second step involves generation of the highly reactive hydroxyl radical

Net reaction:

Figure 1.

Iron-catalyzed generation of reactive oxygen species

Figure 2.

Iron plays a key role in inducing cell death in acute kidney injury. Drugs targeting ferroptosis and Hepcidin are attractive targets to prevent and treat AKI.