

Mitochondrial fatty acid metabolism in acute kidney injury

By Hee-Seong Jang¹, Babu J. Padanilam^{1,2,*}

¹Department of Cellular and Integrative Physiology, University of Nebraska Medical Center, Omaha, NE

²Department of Internal Medicine, Section of Nephrology, University of Nebraska Medical Center, Omaha, NE

Abstract Mitochondrial injury in renal tubule has been recognized as a major contributor in acute kidney injury (AKI) pathogenesis. Ischemic insult, nephrotoxin, endotoxin and contrast medium destroy mitochondrial structure and function as well as their biogenesis and dynamics, especially in renal proximal tubule, to elicit ATP depletion. Mitochondrial fatty acid β -oxidation (FAO) is the preferred source of ATP in the kidney, and its impairment is a critical factor in AKI pathogenesis. This review explores current knowledge of mitochondrial dysfunction and energy depletion in AKI and prospective views on developing therapeutic strategies targeting mitochondrial dysfunction in AKI.

Key words: Mitochondria, Energy metabolism, Fatty acid oxidation, ATP, Mitochondrial dysfunction, Ischemia/reperfusion injury, Nephrotoxin, Sepsis, Acute kidney injury

INTRODUCTION

Acute kidney injury (AKI) is characterized by rapid disruption of renal function that occurs in diverse insults, including sepsis, nephrotoxins and ischemia/reperfusion injury that may occur during organ transplantation and cardiothoracic, vascular and general surgery.¹⁾ AKI occurs in approximately 30% of all patients admitted to intensive care units and is associated with high mortality and morbidity.^{2,3)} Further, a number of recent experimental and clinical studies indicate that AKI is an independent risk factor for onset and deterioration of chronic kidney disease.⁴⁻⁶⁾ Currently, AKI remains a significant health burden with its undefined pathogenesis and is a major unmet medical need without any pharmacological treatments.^{7,8)}

Kidney is an organ with high metabolic demand due to active transport of glucose, ions and nutrients. Thus, renal tubular mitochondrial dysfunction and ATP depletion are critical factors inducing AKI.^{9,10)} Proximal tubules re-

quire more active transport mechanisms than other tubule types because they reabsorb 70% of the glomerular filtrate. Proximal tubule, particularly the S3 segment residing in the outer medullary region, is highly vulnerable to hypoxic condition like ischemia/reperfusion injury (IRI), since this region is exposed to the lowest oxygen pressure (tissue oxygen tension 10~20 mmHg) with only 5~10% of total renal blood flow.¹¹⁾ Therefore, sufficient energy supply from fatty acid β -oxidation (FAO) is critical to the normal function of proximal tubule.¹²⁾ Regardless of the etiology, AKI induces mitochondrial dysfunction in proximal tubule.^{13,14)} Pathological signals like elevated levels of intracellular and mitochondrial Ca^{2+} , increased production of reactive oxygen species (ROS), mitochondrial membrane permeabilization and depolarization, and loss of ATP are hallmarks of mitochondrial dysfunction.¹³⁾ Given that proximal tubule is highly sensitive to depletion of ATP, mitochondria is a potential therapeutic target for preventing renal tubular cell death.^{12,15)} Here, we review the current findings regarding molecular mechanism of mitochondrial dysfunction, primarily focused on impaired fatty acid metabolism and ATP depletion, and recent developments in mitochondria-targeted therapeutic strategies in AKI.

Received: September 1, 2018; Revised: October 5, 2018; Accepted: October 7, 2018

*Correspondence to : Babu J. Padanilam

Department of Cellular and Integrative Physiology, University of Nebraska Medical Center, Omaha, NE 68198-5850

Tel: 1-402-559-3575, FAX: 1-402-559-4438

E-mail: bpadanilam@unmc.edu

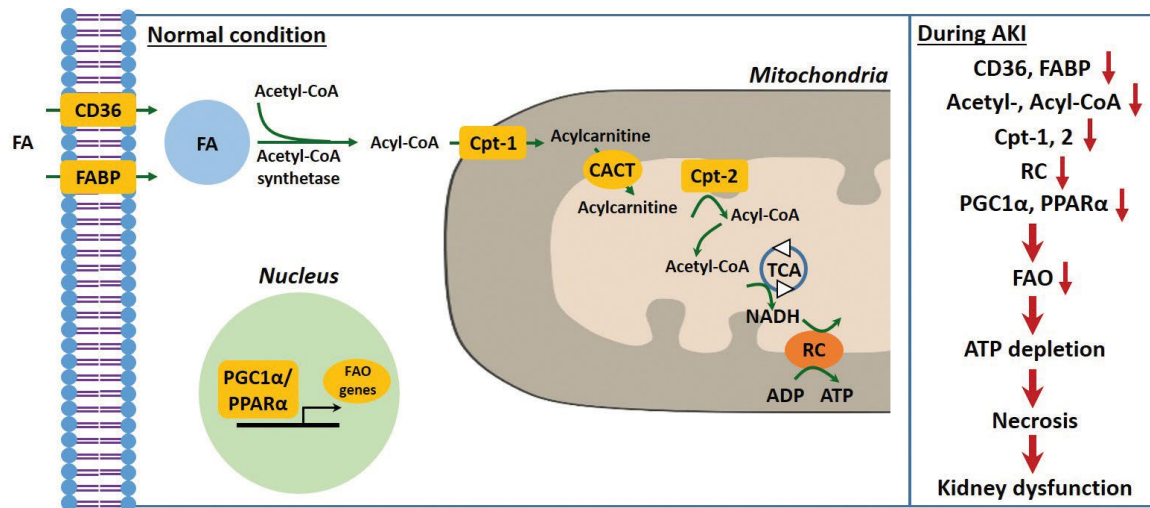


Figure 1. Mitochondrial fatty acid metabolism in AKI. FA enters into cytosol of renal proximal tubule cell (PTC) via FABP or CD36. In the cytosol, FA are transformed from acetyl-CoA to acyl-CoA by addition of acetyl-CoA through acetyl-CoA synthetase and then transferred to mitochondrial matrix by carnitine shuttle, Cpt-1, CACT and Cpt-2, step by step. Acyl-CoA undergo β -oxidation to produce acetyl-CoA for TCA. NADH generated by TCA is used as an electron donor for RC. During AKI, inhibition of genes related to fatty acid oxidation occurs to impair fatty acid oxidation and that in turn deplete ATP, resulting in PTC necrosis and kidney dysfunction. FA, fatty acid; FAO, fatty acid β -oxidation; Cpt, carnitine palmitoyltransferase; CACT, carnitine-acylcarnitine translocase; TCA, tricarboxylic acid cycle; RC, respiratory chain.^{12,13,18,20,21,25,26,29,31,32}

Mitochondrial energetics

Mitochondria are highly dynamic intracellular organelles and have a network with other organelles, including endoplasmic reticulum and nucleus. Mitochondria can undergo physical changes like fusion, fission, and movement, and serve as a power plant generating cellular energy.¹⁶⁾ The magnitude of generation of ATP is dependent on energy demand of the cell, based on their requirement for passive or active transport of ion, glucose, and nutrients. The principle role of mitochondria is to generate cellular energy via electron transport and oxidative phosphorylation, as well as calcium homeostasis and ROS generation. Kidney uses 95~99% of energy derived from mitochondrial oxidative metabolism.¹⁷⁾ Oxidative phosphorylation is more efficient by generation of 36 ATP per glucose, compared to 2 ATP by glycolysis. On the other hand, one fatty acid (FA) is more effective as it can generate 106 ATP via FAO. The proximal tubule has low glycolytic capacity, and prefers FAO for generation of its high ATP demand.^{18,19)} Although FAO is the most efficient mechanism for producing ATP in proximal tubules, it is important to note that due to the high consumption of oxygen by proximal tubules, they are more susceptible than other cell types to changes in oxygen levels.¹⁹⁾ The distal tubule, on the other hand, has higher glycolytic metabolism and could switch to anaerobic metabolism in AKI, and may explain why it is relatively resistant to AKI.¹⁹⁾

Mitochondrial fatty acid metabolism in AKI

FA uptake, oxidation and synthesis are tightly balanced to achieve lipid homeostasis and to prevent lipid accumulation in various disease conditions. In the ischemic kidney, defects in the transport and oxidation of fatty acids can result in accumulation of fatty acids in the cytoplasm which may contribute to the decrease in ATP production and mitochondrial energetics.¹²⁾ FA are taken up in proximal tubule cells via specialized transport proteins on the plasma membrane, such as CD36, and also by retrieval from the glomerular filtrate by receptor-mediated albumin endocytosis.^{18,20)} Since FA is impermeable to outer mitochondrial membrane (OMM), FAs are activated to acyl-CoA by acyl-CoA synthetases in the cytosol. Carnitine palmitoyltransferase-1 (CPT-1), the rate-limiting enzyme of carnitine shuttle on OMM, catalyzes the conversion of acyl-CoA to acylcarnitine, thus facilitating its passage through inner mitochondrial membrane (IMM) by carnitine-acylcarnitine translocase (CPT-2).^{18,20,21)} CPT-2 on the IMM reconverts the acylcarnitine into an acyl-CoA. The acyl-CoAs undergo FAO to generate acetyl-CoA, to fuel the tricarboxylic acid cycle and production of NADH and FADH₂, that serve as electron donors to the electron transport chain for ATP production.²⁰⁾ Deficiency of CPT-1 results in energy failure and diverse kidney diseases including diabetic nephropathy and chronic kidney disease.^{18,20)} In the ischemic kidney, the activity of CPT1 is

attenuated, resulting in reduced transport of FAs into the mitochondria, decreased β -oxidation and consequent lipid deposition.^{22,23)}

Although the causal relationship is unclear, a number of reports suggest that lipid accumulation in certain tissue and cell could be harmful, and is referred to as lipotoxicity.^{24,25)} Accumulation of triglyceride, which is produced by dysregulated glycerol and non-esterified fatty acid (NEFA) presumably derived from impaired FA transport and/or FAO in cytoplasm causes lipotoxicity, contributing to decreased production of ATP and mitochondrial energy metabolism.^{25,26)} NEFA triggers mitochondrial dysfunction as a cause of energetic failure of proximal tubules during hypoxia/reoxygenation, and intracellular accumulation of NEFA and triglycerides with downregulation of mitochondrial FAO.²⁵⁾ Accumulation of triglycerides is observed in tubule injured by ischemic, glycerol-induced and septic AKI, as well as in *in vitro* damaged proximal tubule cell.²⁶⁾ Lipid accumulation in ischemic proximal tubule may result in persistent energy depletion with NEFA-induced mitochondrial dysfunction.²⁵⁾ It is still under debate whether FA or triglyceride *per se* is toxic, but it is clear that intrarenal lipid accumulation, by as of yet undefined mechanisms, can represent characteristics of diseased status.^{24,25,27)} Some data show that, in two chronic kidney disease (CKD) mice models (diabetic nephropathy and folic acid nephropathy), cell-specific lipid accumulation by overexpression of CD36 in tubular epithelial cells did not generate renal fibrosis.²⁸⁾ It is proposed that mitochondrial defects in energy production are more detrimental than the lipid accumulation in the cytoplasm. Further studies to define the causal relationship between lipid accumulation and energy depletion and the effect of lipotoxicity during AKI are warranted.

PGC1-PPAR α axis is well described as a master regulator of FAO genes in diverse tissues, including kidney and its modulation affects the outcome of AKI.^{18,29,30)} Studies by Portilla et al.³¹⁻³⁴⁾ have demonstrated persisting disturbances of mitochondrial and peroxisomal FAO during both ischemic and cisplatin-induced AKI. These changes are proposed to be driven by downregulation of gene transcription and decreased DNA binding activity of PPAR α and decreased expression of its coactivator, peroxisome proliferator activated receptor-gamma coactivator-1 (PGC-1). Activation of PPAR α by fibrates and other PPAR α ligands led to improvement of renal function and prevention of necrotic and apoptotic tubule cell death in cisplatin

nephrotoxicity.³⁵⁾ Importantly, the PPAR α ligand effects were not seen in PPAR α null mice,³⁶⁾ supporting specific mediation by this pathway in cisplatin nephrotoxicity. However, the mechanisms that may lead to inhibition of PPAR α signaling pathways in AKI are not defined. Moreover, PPAR α activation through selective stimulation of FAO seems to eliminate potential toxic metabolites, such as ceramides, which are derived from impaired FAO, contributing to lipotoxicity.^{18,25,37)} In addition to its role in FAO, PGC-1 has been recognized as a master regulator of mitochondrial biogenesis and its metabolic switch to FAO to glycolysis.¹⁴⁾ Mitochondrial biogenesis and its regulatory mechanism were reviewed in detail elsewhere.^{12,30)}

Targeting mitochondrial dysfunction in AKI

Experimental and clinical studies were done or is ongoing for targeting mitochondrial dysfunction in both AKI and CKD.²⁰⁾ Strategies improving mitochondrial FAO is effective in suppression of albuminuria in type 2 diabetic patients, even though unwanted effects are seen in some renal diseases.^{38,39)} In addition, mitochondrial targeting for treatment of AKI is still attractive, since drugs, such as SS-31 (Szeto-Schiller 31) and MitoQ, in early clinical trials have shown beneficial effect in renal diseases.²⁰⁾ SS-31, which is a cell-permeable tetrapeptide targeting mitochondria, has a beneficial effect for preventing disruption of mitochondrial matrix and accelerating ATP recovery in experimental IRI model. MitoQ is a mitochondria-targeted lipophilic antioxidant and is shown to protect mitochondrial respiratory complex in septic AKI. However, it has potential side effects including, inhibition of oxidative phosphorylation and potential role as a pro-oxidant by its characteristics of a lipophilic cation. Indirect activation of CPT-1 by carnitine and 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR; activator of AMPK) protect ischemic AKI in mice.⁴⁰⁾ Similarly, propionyl L-carnitine also have a beneficial effect to prevent IRI through mitigation of oxidative stress and neutrophil infiltration.⁴¹⁾

Despite the fact that strategies are developed to preserve or restore mitochondrial function and FAO in AKI, major obstacles remain. Since mitochondria is a major organelle to generate ROS, maladaptive mitochondria might increase oxidative stress. Moreover, before attempting mitochondrial therapies, it is required to have a comprehensive understanding of mitochondrial energy metabolism, as well as redox status, in *in vivo* tubular cells and in diseased tissue.¹⁴⁾

CONCLUSION

Disruption of mitochondrial homeostasis, which is critical to ATP production, is associated with mitochondrial dysfunction and AKI pathogenesis. Persistent inhibition of FAO promotes ATP depletion and subsequent inflammation in AKI, resulting in lipotoxicity and CKD progression after AKI. A better understanding to mitochondrial energy metabolism in the setting of AKI may lead to development of effective therapeutics for prevention of ATP depletion and treatment of AKI.

ACKNOWLEDGEMENT

Preparation of this paper is supported by National Institutes of Health grant DK-083291 and DK-090332 and American Heart Association Grant in Aid 10GRNT4040022 and 15GRNT25080031 to BJP, and American Heart Association postdoctoral fellowship Grant 15POST25130003 to HSJ.

REFERENCES

- Eltzschig HK, Eckle T. Ischemia and reperfusion - from mechanism to translation. *Nat Med* 2011;17:1391-401.
- Schrier RW, Wang W, Poole B, Mitra A. Acute renal failure: definitions, diagnosis, pathogenesis, and therapy. *J Clin Invest* 2004;114:5-14.
- Case J, Khan S, Khalid R, Khan A. Epidemiology of acute kidney injury in the intensive care unit. *Crit Care Res Pract* 2013; 2013:479730.
- Coca SG, Singanamala S, Parikh CR. Chronic kidney disease after acute kidney injury: a systematic review and meta-analysis. *Kidney Int* 2012;81:442-8.
- He L, Wei Q, Liu J, Yi M, Liu Y, Liu H, et al. AKI on CKD: heightened injury, suppressed repair, and the underlying mechanisms. *Kidney Int* 2017;92:1071-83.
- Basile DP, Bonventre JV, Mehta R, Nangaku M, Unwin R, Rosner MH, et al. Progression after AKI: Understanding Maladaptive Repair Processes to Predict and Identify Therapeutic Treatments. *J Am Soc Nephrol* 2016;27:687-97.
- Molitoris BA. Therapeutic translation in acute kidney injury: the epithelial/endothelial axis. *J Clin Invest* 2014;124:2355-63.
- Agarwal A, Dong Z, Harris R, Murray P, Parikh SM, Rosner MH, et al. Cellular and Molecular Mechanisms of AKI. *J Am Soc Nephrol* 2016;27: 1288-99.
- Weinberg JM, Venkatachalam MA, Roeser NF, Saikumar P, Dong Z, Senter RA, et al. Anaerobic and aerobic pathways for salvage of proximal tubules from hypoxia-induced mitochondrial injury. *Am J Physiol Renal Physiol* 2000;279:F927-43.
- Basile DP, Anderson MD, Sutton TA. Pathophysiology of acute kidney injury. *Compr Physiol* 2012;2:1303-53.
- Nourbakhsh N, Singh P. Role of renal oxygenation and mitochondrial function in the pathophysiology of acute kidney injury. *Nephron Clin Pract* 2014;127:149-52.
- Bhargava P, Schnellmann RG. Mitochondrial energetics in the kidney. *Nat Rev Nephrol* 2017;13:629-46.
- Stallons LJ, Funk JA, Schnellmann RG. Mitochondrial Homeostasis in Acute Organ Failure. *Curr Pathobiol Rep* 2013;1:10.
- Hall AM, Schuh CD. Mitochondria as therapeutic targets in acute kidney injury. *Curr Opin Nephrol Hypertens* 2016;25:355-62.
- Forbes JM. Mitochondria-Power Players in Kidney Function? *Trends Endocrinol Metab* 2016;27:441-2.
- Chandel NS. Evolution of Mitochondria as Signaling Organelles. *Cell Metab* 2015;22:204-6.
- Mandel LJ. Primary active sodium transport, oxygen consumption, and ATP: coupling and regulation. *Kidney Int* 1986;29:3-9.
- Simon N, Hertig A. Alteration of Fatty Acid Oxidation in Tubular Epithelial Cells: From Acute Kidney Injury to Renal Fibrogenesis. *Front Med (Lausanne)* 2015;2:52.
- Bonventre JV, Yang L. Cellular pathophysiology of ischemic acute kidney injury. *J Clin Invest* 2011;121:4210-21.
- Szeto HH. Pharmacologic Approaches to Improve Mitochondrial Function in AKI and CKD. *J Am Soc Nephrol* 2017;28:2856-65.
- Aon MA, Bhatt N, Cortassa SC. Mitochondrial and cellular mechanisms for managing lipid excess. *Front Physiol* 2014;5:282.
- Idrovo JP, Yang WL, Nicastro J, Coppa GF, Wang P. Stimulation of carnitine palmitoyltransferase 1 improves renal function and attenuates tissue damage after ischemia/reperfusion. *J Surg Res* 2012;177:157-64.
- Portilla D. Role of fatty acid beta-oxidation and calcium-independent phospholipase A2 in ischemic acute renal failure. *Curr Opin Nephrol Hypertens* 1999;8:473-7.
- Bobulescu IA. Renal lipid metabolism and lipotoxicity. *Curr Opin Nephrol Hypertens* 2010;19:393-402.
- Weinberg JM. Lipotoxicity. *Kidney Int* 2006;70:1560-6.
- Zager RA, Johnson AC, Hanson SY. Renal tubular triglyceride accumulation following endotoxic, toxic, and ischemic injury. *Kidney Int* 2005;67:111-21.
- Listenberger LL, Han X, Lewis SE, Cases S, Farese RV Jr, Ory DS, et al. Triglyceride accumulation protects against fatty acid-induced lipotoxicity. *Proc Natl Acad Sci U S A* 2003;100:3077-82.
- Kang HM, Ahn SH, Choi P, Ko YA, Han SH, Chinga F, et al. Defective fatty acid oxidation in renal tubular epithelial cells has a key role in kidney fibrosis development. *Nat Med* 2015;21:37-46.
- Tran M, Parikh SM. Mitochondrial biogenesis in the acutely injured

- jured kidney. *Nephron Clin Pract* 2014;127:42-5.
30. Weinberg JM. Mitochondrial biogenesis in kidney disease. *J Am Soc Nephrol* 2011;22:431-6.
 31. Portilla D. Energy metabolism and cytotoxicity. *Semin Nephrol* 2003;23:432-8.
 32. Portilla D, Dai G, McClure T, Bates L, Kurten R, Megyesi J, et al. Alterations of PPARalpha and its coactivator PGC-1 in cisplatin-induced acute renal failure. *Kidney Int* 2002;62:1208-18.
 33. Portilla D, Dai G, Peters JM, Gonzalez FJ, Crew MD, Proia AD. Etomoxir-induced PPARalpha-modulated enzymes protect during acute renal failure. *Am J Physiol Renal Physiol* 2000;278:F667-75.
 34. Portilla D, Li S, Nagothu KK, Megyesi J, Kaissling B, Schnackenberg L, et al. Metabolomic study of cisplatin-induced nephrotoxicity. *Kidney Int* 2006;69:2194-204.
 35. Nagothu KK, Bhatt R, Kaushal GP, Portilla D. Fibrate prevents cisplatin-induced proximal tubule cell death. *Kidney Int* 2005;68:2680-93.
 36. Li S, Wu P, Yarlagadda P, Vadjunec NM, Proia AD, Harris RA, et al. PPAR alpha ligand protects during cisplatin-induced acute renal failure by preventing inhibition of renal FAO and PDC activity. *Am J Physiol Renal Physiol* 2004;286:F572-80.
 37. Kamijo Y, Hora K, Kono K, Takahashi K, Higuchi M, Ehara T, et al. PPARalpha protects proximal tubular cells from acute fatty acid toxicity. *J Am Soc Nephrol* 2007;18:3089-100.
 38. Keech A, Simes RJ, Barter P, Best J, Scott R, Taskinen MR, et al. Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. *Lancet* 2005;366:1849-61.
 39. Cases A, Coll E. Dyslipidemia and the progression of renal disease in chronic renal failure patients. *Kidney Int Suppl* 2005:S87-93.
 40. Idrovo JP, Yang WL, Matsuda A, Nicastro J, Coppa GF, Wang P. Post-treatment with the combination of 5-aminoimidazole-4-carboxamide ribonucleoside and carnitine improves renal function after ischemia/reperfusion injury. *Shock* 2012;37:39-46.
 41. Mister M, Noris M, Szymczuk J, Azzollini N, Aiello S, Abbate M, et al. Propionyl-L-carnitine prevents renal function deterioration due to ischemia/reperfusion. *Kidney Int* 2002;61:1064-78.