Mitochondria in Sepsis-Induced AKI

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ABSTRACT

AKI is a common clinical condition associated with the risk of developing CKD and ESKD. Sepsis is the leading cause of AKI in the intensive care unit (ICU) and accounts for nearly half of all AKI events. Patients with AKI who require dialysis have an unacceptably high mortality rate of 60%–80%. During sepsis, endothelial activation, increased microvascular permeability, changes in regional blood flow distribution with resulting areas of hypoperfusion, and hypoxemia can lead to AKI. No effective drugs to prevent or treat human sepsis-induced AKI are currently available. Recent research has identified dysfunction in energy metabolism as a critical contributor to the pathogenesis of AKI. Mitochondria, the center of energy metabolism, are increasingly recognized to be involved in the pathophysiology of sepsis-induced AKI and mitochondria could serve as a potential therapeutic target. In this review, we summarize the potential role of mitochondria in sepsis-induced AKI and identify future therapeutic approaches that target mitochondrial function in an effort to treat sepsis-induced AKI.

Sepsis is life-threatening organ dysfunction caused by dysregulated host response to infection.[1] As the leading cause of AKI in the intensive care unit, sepsis accounts for 45%–70% of all AKI cases.[2] A hemodynamic hallmark of sepsis is generalized vasodilation and decreased systemic vascular resistance.[3] Previous studies have suggested that hypoperfusion and intrarenal vasoconstriction, caused by activation of the sympathetic nervous system and the renin-angiotensin-aldosterone axis, and release of vasopressin may contribute to AKI in sepsis.[4–7] However, recent studies have questioned old paradigms and have demonstrated that sepsis-induced AKI develops with normal or even increased renal blood flow,[8–10] suggesting that blood flow redistribution, microvascular changes, and other causes may be critical in leading to injury.

During sepsis, upregulation of endothelial nitric oxide synthesis[11] can affect arterial vasodilatation and decrease systemic vascular resistance.[12] The vasodilatory effect of endothelial nitric oxide synthase within the kidney might be expected to lessen renal vasoconstriction induced by NE, angiotensin II, vasopressin, and endothelin during sepsis.[3,13] More than likely, these vasoactive substances lead to redistribution of flow away from the renal medulla to the renal cortex, leading to medullary ischemia in sepsis-induced AKI.[14–16] Furthermore, sepsis-related impairment of the endothelium may also attenuate or abolish the normal effect of endothelial nitric oxide synthase in the kidney to counteract vasoconstriction.[11] Another critical pathway that has been identified in sepsis-induced AKI is alterations in primary tubular metabolism which could secondarily affect the regional circulation through decreased levels of ATP and mitochondrial dysfunction.[17,18] Tran *et al.*[19] demonstrated that mitochondrial dysfunction, cellular swelling, and a pronounced accumulation of acylglycerols developed in tubules, which led to decreased PGE2 and promoted medullary vasoconstriction in ischemia AKI. Thus, studies on mitochondria might lead to greater insights into the mechanism of AKI. Furthermore, restoration of healthy mitochondrial function and mass is likely critical to the recovery of kidney function.[20]

The recent progress in understanding the role of mitochondria in sepsis-induced AKI has led to an array of potential applications for mitochondria as biomarkers of kidney injury as well as targets for novel therapeutic strategies. In this review, we describe mitochondrial metabolic

dysregulations in sepsis-induced AKI and discuss how this knowledge may guide the development of potential new therapies for sepsis-induced AKI.

METABOLIC REGULATIONS OF MITOCHONDRIA DURING SEPSIS-INDUCED AKI

A critical function of mitochondria is to provide energy (ATP) that is used by the kidney to remove waste products from the blood as well as to regulate fluid and electrolyte balance. Mitochondrial homeostasis is closely regulated by mitochondrial biogenesis. Mitochondrial biogenesis is the process by which cells increase mitochondrial mass and it is accompanied by increases in metabolic enzymes for glycolysis, oxidative phosphorylation (OXPHOS), and greater mitochondrial capacity for energy production. Under conditions such as oxidative stress and during sepsis, mitochondria can alter metabolic processes to adapt to these stressful conditions through a number of signaling pathways that maintain homeostasis.

Energy Metabolism of Mitochondria in Sepsis-Induced AKI

The provision of energy to the cell is through the electron transport chain in a process called OXPHOS. Glucose metabolism through glycolysis and β-oxidation of fatty acids (FAs) are the main energy substrates for the kidney. Energy production occurs through a series of electron transfers along the inner mitochondrial membrane leading to the eventual production of ATP. Under normal conditions, proximal tubular cells prefer FAs as the energy source and aerobic respiration is the primary mechanism of ATP production.[21] However, during sepsis and hypoxic conditions, instead of feeding pyruvate into the tricarboxylic acid cycle to generate ATP through OXPHOS, proximal tubule cells convert pyruvate to lactate, a less efficient mechanism to produce ATP.[22,23]

The metabolic shift during sepsis is driven by mammalian target of rapamycin complex 1 (mTORC1)–induced stabilization of hypoxia inducible factor–1α (HIF-1α) through the Akt/mTORC1/HIF-1α pathway (Figure 1).[22–24] Recent studies also suggest that, during LPS-induced AKI, hexokinase activation and increased glucose-6-phosphate dehydrogenase activity are linked to increased pentose phosphate pathway activity. Despite the fact that glycolysis provides less efficient energy generation, it can provide sufficient energy for cell survival as well as for maintenance of essential structural components[25] and it leads to a decrease in OXPHOS and the production of mitochondrial reactive oxygen species (ROS), at least during the early course of sepsis.
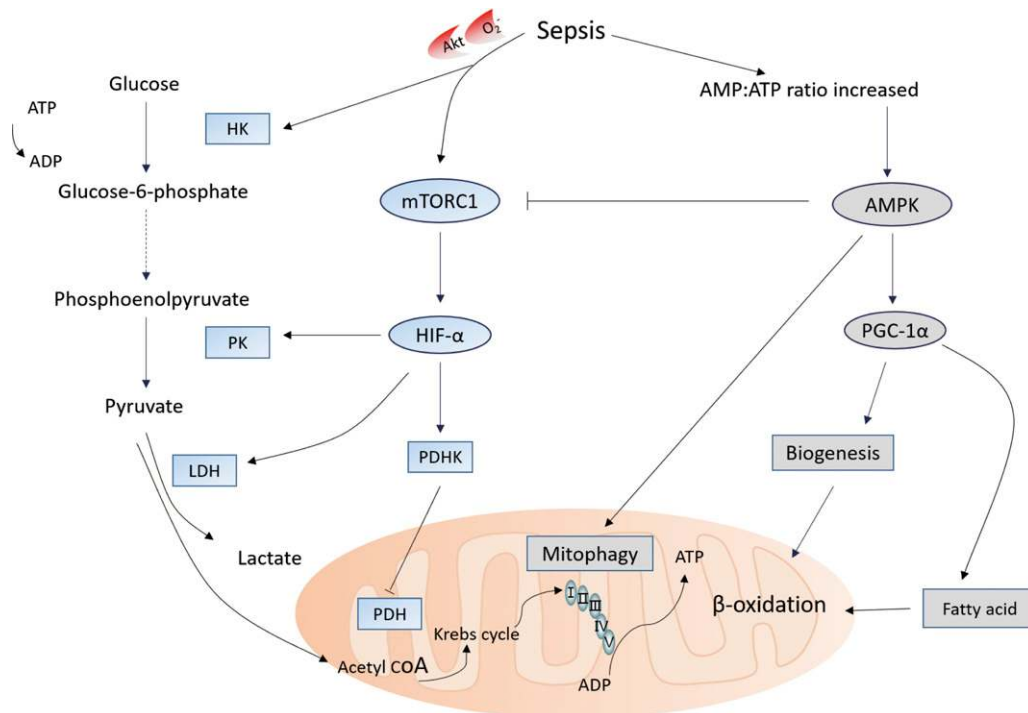
Figure 1. Energetic metabolic reprogramming in tubular epithelial cells during sepsis-induced AKI. Activated HIF-1α promotes the transformation of pyruvate into lactate, and inhibits the transformation of pyruvate into acetyl-CoA, thus blocking entry into the Krebs cycle. HIF-1α also induces the expression of PKM2, and slows down the conversion of phosphoenol pyruvate to pyruvate. HK is activated by Akt or O_2^- and increases glucose-6-phosphate dehydrogenase activity which is linked to increased PPP activity. As ATP levels decrease, AMPK is activated. AMPK leads to induction of mitochondrial biogenesis by PGC-1α, inhibits mTORC, and activates mitophagy by phosphorylating the serine/threonine protein kinase ULK1. Acetyl-CoA, acetyl-coenzyme A; PKM2, M2 isoform of pyruvate kinase; HK, hexokinase; PPP, pentose phosphate pathway; mTORC, mammalian target of rapamycin complex.

However, as ATP levels decrease, adenosine monophosphate–activated protein kinase (AMPK), a master sensor of energy catabolic status, is induced. Activation of AMPK can lead to the production of critical antioxidant enzymes and the induction of mitochondrial biogenesis by peroxisome proliferator–activated receptor (PPAR) γ coactivator–1α (PGC-1α). Moreover, activation of AMPK leads to an increase in glycolytic pathway flux, FA oxidation, and glucose transport. All of these events contribute to cell growth and an increase in cellular metabolism.[26,27] In addition, AMPK also inhibits mTORC and activates autophagy by phosphorylation of the serine/threonine protein kinase Unc-51–like kinase 1 (ULK1).[28] This AMPK-dependent response regulates metabolism, reprioritizes energy expenditure toward the functions necessary for survival, limits oxidative damage from dysfunctional mitochondria, and eventually stabilizes energy balance by mitochondrial biogenesis.

Thus, during sepsis-induced AKI, energy metabolism might first switch to glycolysis in order to decrease oxygen consumption and enhance the capacity of the cell to defend against oxidative damage through decreasing OXPHOS and mitochondrial ROS production. Later activation of AMPK may allow for cell survival and mitochondrial biogenesis.

Mitochondrial Biogenesis in Sepsis-Induced AKI

Mitochondrial biogenesis can increase ATP production in response to increasing energy demand by the generation of new and functional mitochondria. PGC-1α is a positive mitochondrial biogenesis regulator.[29] During sepsis, transient local ischemia and increased cytokine levels, especially TNF-α, can reduce PGC-1α expression in tubular cells and suppress kidney recovery.[30] Both mammalian target of mTORC and AMPK signaling pathways regulate mitochondrial biogenesis and help maintain healthy

mitochondria during AKI (Figure 2). Activated mTORC1 triggers pathways that lead to mitochondrial biogenesis by activating the transcriptional repressor yin and yang 1 (YY1).[31] AMPK can induce mitochondrial biogenesis by stimulating the transcription of the gene encoding PGC-1α (PPARGCIA) and by phosphorylating PGC-1α at Thr177 and Ser539 to increase its activity.[27]

Other pathways that stimulate mitochondrial biogenesis include sirtuins (SIRT), cAMP, and cyclic guanosine monophosphate (cGMP). The activity of SIRT1 is activated by NAD, leading to further activation of downstream targets such as PGC-1α.[32] PGC-1α is a pivotal determinant of renal recovery from injury by regulating NAD biosynthesis in ischemia-reperfusion and in AKI secondary to nephrotoxic drugs.[33] The NAD precursor niacinamide (NAM) can enhance NAD production and augment production of the fat breakdown product β-hydroxybutyrate (β-OHB), which
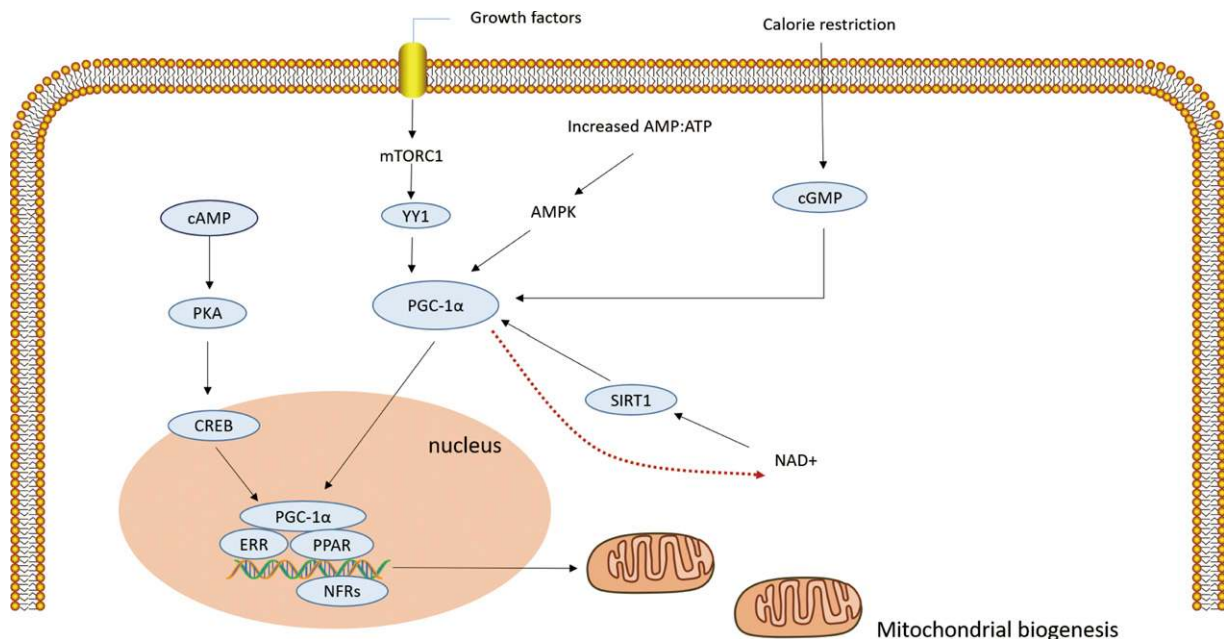
Figure 2. Mitochondrial biogenesis in sepsis-induced AKI. There are multiple pathways involved in mitochondrial biogenesis. Activated PGC-1α cooperates with NRFs and promotes the expression of multiple nuclear-encoded genes; PGC-1α activation promotes its translocation from the cytoplasm to the nucleus; activated mTOR1 triggers pathways by activating the transcriptional repressor YY1; AMPK can induce mitochondrial biogenesis; SIRT1 is activated by NAD$^+$, and then activates PGC-1α. Stimulation of adenylyl cyclase increases cAMP, which activates PKA that in turn phosphorylates CREB. CREB can stimulate mitochondrial biogenesis; caloric restriction increases levels of cGMP and inhibits phosphodiesterases which can stimulate PGC-1α activation and mitochondrial biogenesis *in vivo*. mTOR, mechanistic target of rapamycin; YY1, Yin and yang 1; SIRT, Sirtuins; NAD, nicotinamide adenine dinucleotide; PKA, protein kinase A; cGMP: cyclic guanosine monophosphate.

leads to increased vasodilatory prostanoids such as PGE2, a secreted autacoid that maintains renal perfusion.[34] After ischemia, mice deficient in PGC-1α (a downstream substrate of both SIRT and AMPK) develop local deficiency of the NAD$^+$ precursor niacinamide or NAM, accumulate fat, and fail to re-establish normal renal function.[19] Stimulation of adenylyl cyclase increases cAMP levels, which activates protein kinase A (PKA) that in turn phosphorylates cAMP-responsive element–binding protein (CREB).[35,36] CREB is also a transcriptional activator of PGC-1α and can thus also stimulate mitochondrial biogenesis through this pathway.[35] Finally, caloric restriction (food only provided on alternate days for 3 or 12 months) increases levels of cGMP and inhibits phosphodiesterases which can stimulate PGC-1α activation and mitochondrial biogenesis *in vivo* and folic acid–induced AKI.[37,38] In summary, these findings indicate that PGC-1α may be a critical factor in the recovery of sepsis-induced AKI through mitochondrial biogenesis, and mitochondrial regeneration may be a future therapeutic target for sepsis-induced AKI.

Superoxide Anion Metabolism in Sepsis-Induced AKI

Renal mitochondrial injury occurs early in the course of sepsis and is associated with cellular damage as a result of ROS generation. Much of the data in this area of study are derived from ischemia-reperfusion models of AKI and the applicability to sepsis models has not been as well studied; the causes of mitochondrial ROS production upon reperfusion after ischemia are still unclear. It has been assumed that ROS production during reperfusion is a nonspecific consequence of oxygen interacting with dysfunctional mitochondria upon reperfusion. Recently, this view has changed, suggesting that specific metabolic pathways may be operative in which superoxide is generated through reverse electron transport at complex I of the electron transport chain. Moreover, selective accumulation of the citric acid cycle intermediate succinate is a universal metabolic signature of ischemic tissues and is responsible for

mitochondrial ROS production during reperfusion.[39] Importantly, pharmacologically inhibiting succinate accumulation, or slowing succinate metabolism at reperfusion, has been shown to be protective in ischemia-reperfusion models.[40]

ROS are toxic to the endothelium and defense mechanisms are critical to maintain organ perfusion and function.[41] Mitochondria have intrinsic antioxidant mechanisms to protect against damage induced by ROS through a large array of mechanisms (*e.g.*, SOD, glutathione, thioredoxin).[42] In response to oxidative stress, NF erythroid 2–related factor 2 activates the transcription of genes encoding antioxidant enzymes such as glutathione peroxidase, SOD2, and catalase. In this manner, a mechanism to prevent ROS-induced injury is upregulated.[43] Glutathione is a tripeptide (γ-glutamyl-cysteinal-glycine) nucleophile capable of preventing damage to important cellular components caused by ROS. Mitochondria contain their own pool of glutathione (mGSH), which not only helps to decrease excessive ROS levels but also prevents the release of cytochrome c from the inner mitochondrial membrane.[44] mGSH directly interacts with superoxide anions and becomes oxidized to glutathione disulfide (GSSG).[44,45] GSSG cannot exit the mitochondria and is converted back to mGSH by glutathione reductase, for reuse or elimination from the mitochondria.[45] The conversion of GSSG to mGSH requires NADP (NADPH) that is produced by the pentose phosphate pathway. SOD2 is a mitochondrial enzyme that binds to the superoxide byproducts of oxidative phosphorylation and converts them to hydrogen peroxide and oxygen.[46]

Mitochondrial uncoupling protein 2 (UCP2) also plays a role in attenuating excessive ROS production. As levels of ROS increase, UCP2 is activated and acts to dissipate the proton motive force as heat and as a result reduces ROS production.[47,48] Together, these antioxidant systems can maintain optimal ATP production, and sustain mitochondrial function.

Fusion and Fission

Fusion is the combining of two mitochondria, and fission is the splitting of

mitochondria into two. Mitochondria are highly dynamic organelles that exchange genetic and other information by coordinated fusion and fission. Under physiologic conditions, the processes of fusion and fission are necessary to maintain mitochondrial homeostasis and play an important role in the quality control of mitochondria.[49]

Mitochondria fuse together *via* mitofusin 1 and 2 (Mfn1 and 2) on the outer membrane and require activation of dynamin-like 120 kD (OPA1) on the inner membrane. Fusion can maintain ATP production and redistribute mitochondrial proteins. Mitochondria divide *via* dynamin-related protein 1 (Drp1) on the outer membrane. Fission can isolate damaged mitochondria from the mitochondrial network. Dysfunctional mitochondria, which are toxic for tubular cells, are removed by mitophagy. If the daughter mitochondria produced by fission are unbalanced and depolarized, they are targeted for mitophagy.[50]

In response to insults such as ischemia-reperfusion injury or sepsis, the balance between mitochondrial fusion and fission shifts to mitochondrial fission. The mitochondria become fragmented in sepsis.[51,52] Changes in mitochondrial morphology, characterized by initial fragmentation of the organelles followed by ultrastructural alterations resulting in mitochondrial swelling and cristae deformation, are observed in renal tubular cells during septic AKI.[51] The disruption of mitochondrial dynamics at both outer and inner membranes plays a crucial role in mitochondrial dysfunction and tubular cell injury and death in ischemia-reperfusion and cisplatin-induced AKI.[53] It is likely that the same is true in sepsis.[52]

ROS Scavenging by Mitochondria in Sepsis-Induced AKI

Ultrastructural changes are observed in mitochondria during sepsis-induced AKI. Mitophagy (autophagy of mitochondria) protects stressed cells from death due to mitochondrial fragmentation and can ameliorate AKI.[54] Importantly, despite ATP depletion and ROS generation, the necrotic and apoptotic responses of mitochondria are limited

in sepsis-induced AKI. Supporting this is that mitophagy is rapidly induced during various models of AKI and plays important roles in renal protection.[54] Parikh et al.[20] demonstrated that mitophagy removed damaged mitochondria in tubular epithelial cells during sepsis-induced AKI. Two main mechanisms of mitophagy have been described. In the first mechanism, mitophagy is mediated by the PTEN-induced putative kinase protein 1 (Pink1)–PARKIN mechanism. The second mechanism of mitophagy involves two Bcl-2 family proteins: BCL2/adenovirus E1B 19-kD protein–interacting protein 3 (BNIP3) and Nip3-like protein X (NIX, also known as BNIP3L).

Energy-sensitive kinases mTORC1 and AMPK are known to regulate mitophagy.[55–59] mTORC1 phosphorylates ULK1/2 and autophagy-related gene (Atg) 13 which inhibit ULK1/2 kinase activity,[57,60] which negatively regulates autophagy. AMPK can inhibit mTORC1 and activate mitophagy by phosphorylating the serine/threonine protein kinase ULK1.[56] Some studies have reported

that inhibition of the mTORC pathway can impair tubular proliferation and delay recovery of kidney function during ischemia-reperfusion injury models.[61–64] Additional studies are required to investigate the role of mTORC1-mediated induction of autophagy in sepsis-induced AKI. Other molecules such as antiapoptotic members of the Bcl-2 family, BNIP3, HIF, and p53 also induce autophagy in AKI (Figure 3).[54]

The increase in permeability of the outer mitochondrial membrane can release these proapoptotic factors into the cytoplasm. Jiang et al.[65] demonstrated that mitophagy occurred before tissue damage or tubular apoptosis. In these cases, excessive mitophagy can "spill over" and digest normal components in the cell causing lethal injury, whereas, on the other hand, insufficient mitophagy can also release proapoptotic substances. The signaling pathways between autophagy and apoptosis in AKI need to be further studied.

Mitochondrial homeostasis is critical because rapid recovery of ATP levels is essential for cell survival. In this regard,

the various processes of mitochondrial scavenging (mitophagy and apoptosis) and biogenesis are key steps to ensure cellular function and survival.

THERAPEUTIC TARGETING OF MITOCHONDRIA IN SEPSIS-INDUCED AKI

Understanding mitochondrial metabolic regulation during sepsis-induced AKI may lead to new therapeutic targets. This section summarizes recent findings regarding the manipulation of mitochondrial metabolism, homeostasis, and recovery (Table 1).

Targeting Mitochondrial Energy Pathways
Akt/mTORC/HIF-1α Pathway
The Akt/mTORC/HIF-1α pathway can regulate glucose metabolism and mitochondrial function. Activation of Akt/ mTORC/HIF-1α can switch tubular epithelial cell metabolism to aerobic glycolysis in response to hypoxia and sepsis. Activation of this pathway decreases
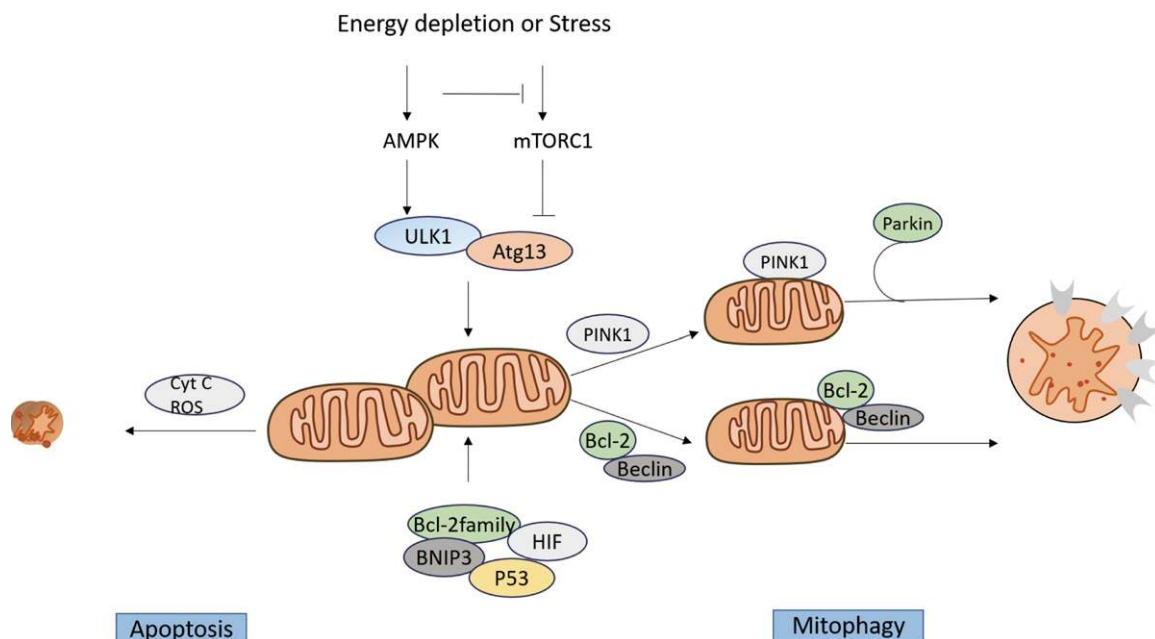
Figure 3. ROS scavenging by mitochondria in sepsis-induced AKI. AMPK can inhibit mTORC1 and activate mitophagy by phosphorylating the serine/threonine protein kinase ULK. mTORC1 phosphorylates ULK1/2 and Atg13 which negatively regulates autophagy. Other molecules such as antiapoptotic members of the Bcl-2 family, BNIP3, HIF, and p53 also induce autophagy in AKI. The increase in the permeability of the outer mitochondrial membrane can release proapoptotic factors into the cytoplasm. These factors activate the caspase-dependent or -nondependent cascade reaction mechanism. mTORC, mechanistic target of rapamycin complex; Atg, Autophagy-related gene.

Table 1. Pharmacologic approaches targeting mitochondria for kidney injury

Drugs	Pathway/Target	Process	Effect	Species	Reference
Antracyclines	Akt/mTORC/HIF-1α pathway	Energy pathway	Ameliorate injury	Animals	[67]
β-glucan	Akt/mTORC/HIF-1α pathway	Energy pathway	Ameliorate injury	Animals	[23]
AICAR	AMPK/Sirt1–6 pathway	Energy pathway	Decrease tubular damage	Animals	[68,69]
Formoterol	PGC1α	Biogenesis	Restore renal function	Animals	[78]
LY344864	PGC1α	Biogenesis	Improve renal function	Animals	[79]
MA-5	Mitofilin/Mic60	Biogenesis	Decrease plasma BUN	Humans	[97,98]
Levosimendan	Mitochondrial ATP-sensitive K$^+$ channels	Biogenesis	Restore renal function	Humans	[100]
Bendavia (SS-31)		Antioxidation	Reduced serum creatinine and BUN	Humans	[80–82]
Mito Q	Chain-breaking antioxidant ubiquinol	Antioxidation	Improve renal function	Humans	[83]
Mdivi-1	Drp1	Fusion and fission	Ameliorate injury	Animals	[53,87]
Antymycin A	p53	Mitophagy	Protect renal tubular cells	Animals	[90]
Ambra1	Parkin	Mitophagy	Ameliorate injury	Animals	[92]
Cyclophilin-D		MPT	Protect against renal injury	Animals	[99]
CsA	Drp1	MPT	Ameliorate podocyte damage	Humans	[88,89]

CsA, cyclosporine A; Drp1, dynamin-related protein 1; Mdivi-1, mitochondrial division inhibitor–1; MA-5, mitochonic acid 5; Mito Q, mitoquinone; MPT, mitochondrial permeability.

oxygen consumption and enhances the capacity of the cell to cope with oxidative damage. In addition, aerobic glycolysis supports the ability of the innate immune system to develop memory and modify the response to future insults.[23,66]

Treatment of rodents with antracyclines inhibits mTOR and thus activates autophagy, which limits AKI and improves survival in a model of cecal ligation and puncture (CLP)–induced sepsis.[67] In monocytes, prestimulation of the Akt/mTORC1/HIF-1α pathway with β-glucan (by exposure to sublethal concentrations of *Candida albicans*) results in increased secretion of TNF and IL-6, and such prestimulation results in improved survival.[23] The protection achieved by pretreatment with β-glucan was lost when Akt, mTOR, or HIF-1α was inhibited. This suggests that the Akt/mTORC1/HIF-1α pathway, which is involved in metabolic reprogramming and regulation during infection, is a promising target to ameliorate sepsis-induced AKI.

AMPK/Sirt1–6 Pathway
AMPK targets a number of proteins, the phosphorylation of which leads to the production of antioxidant enzymes; the induction of mitochondrial biogenesis; and an increase in glycolytic flux, FA oxidation, and glucose transport; all of these events contribute to cell growth and an increase in cellular metabolism. AMPK/SIRT1–6 pathway signaling has

been implicated as a target for correcting metabolic and mitochondrial function in AKI. AMPK is a master sensor of energy status that stimulates catabolic processes. A high AMP/ATP ratio activates AMPK to stimulate cell growth and metabolism.

5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) acts as an activator of AMPK and can increase the levels of PGC-1α and other mitochondrial proteins while reducing ROS production in a diabetic mouse model.[68] Stimulation of AMPK with AICAR before CLP-induced sepsis was associated with significant protection from AKI and with decreased inflammatory molecules such as IL-6, IL-10, and TNF-α,[69] suggesting that AMPK may play a protective role in the early response to sepsis. There is a crosstalk between AMPK and SIRT3 signaling.[60,70] SIRT1 and SIRT3 are protein deacetylases that have roles in many mitochondrial processes, such as the electron transport chain, the tricarboxylic acid cycle, FA oxidation, and mitochondrial biogenesis.[71] SIRT1 and SIRT3 are activated by NAD$^+$.[72]

Administration of AICAR to cisplatin-treated mice attenuated the decreases in SIRT3 expression, phosphorylated AMPK level, and tubular damage.[73] Compared with saline-treated control mice, cisplatin-treated mice have decreased expression and lower protein levels of SIRT3, increased tubular damage, and decreased levels of phosphorylated AMPK.[73] These studies

provide a therapeutic rationale for targeting AMPK to improve outcomes in AKI. The relationship between AMPK activity and SIRT in mitochondrial protection needs to be further studied.

Targeting Mitochondrial Biogenesis
Carbon monoxide upregulates mitochondrial biogenesis *via* activation of redox-regulated NF-E2–related factor transcription factor, nuclear respiratory factor (NRF1, NRF2), and PGC-1α. Carbon monoxide is released endogenously by activation of hemeoxygenase-1. Induction of hemeoxygenase-1 in sepsis models has shown protective effects through NRF-2 and mitochondrial biogenesis.[74] PGC-1α, which is expressed highly in the kidney, is capable of driving all steps of mitochondrial biogenesis. It is an attractive pharmacologic target for kidney protection.[33]

After renal ischemia, PGC-1$\alpha^{-/-}$ mice developed local deficiency of NAM, marked fat accumulation, and failure to re-establish normal function.[19] Kidney-specific PGC-1α gene–knockout mice exhibited persistent AKI after LPS-induced sepsis.[75] Remarkably, exogenous NAM improved local NAD levels, fat accumulation, and kidney function in postischemic PGC-1$\alpha^{-/-}$ mice. Recent studies have demonstrated that supplementation with a NAD precursor such as nicotinamide mononucleotide or NAM can contribute to kidney protection in

cisplatin- or ischemia-induced AKI.[76,77] These findings imply that PGC-1α is necessary for recovery of kidney function in AKI.

In addition, two different G protein–coupled receptors, the β2 adrenergic receptor (β2AR) and the 5-hydroxytryptamine receptor 1F (5-HT$_{1F}$), can restore mitochondrial function and stimulate kidney recovery by inducing mitochondrial biogenesis after IR-induced injury. Formoterol, a β2AR agonist, stimulates mitochondrial biogenesis by upregulating PGC-1α through the cAMP/PKA/CREB axis in kidney proximal tubular cells and can accelerate the recovery of mitochondrial and kidney function in mouse models of ischemia-reperfusion AKI.[78] LY344864, a potent 5-HT$_{1F}$ agonist, induces mitochondrial biogenesis in naive mice and accelerates the recovery of mitochondrial and kidney function in the same AKI murine model.[79]

Antioxidant Therapy

Antioxidants that specifically target mitochondria have shown promising effects in ameliorating AKI. The Szeto-Schiller (SS) peptide selectively interacts with cardiolipin to stabilize the mitochondrial inner membrane and directly deliver antioxidants to the mitochondria.[80] The peptide SS-31 (Bendavia) ameliorates mitochondrial dysfunction by reducing mitochondrial cristae disorganization in proximal tubules during ischemia and has been shown to protect mitochondrial structure and function in an *in vivo* study of an ischemia-reperfusion–induced AKI model in rats. Furthermore, SS-31 also ameliorated reperfusion-induced oxidative stress and accelerated the recovery of mitochondrial structure and ATP levels, which in turn preserved proximal tubular cell structure, decreased tubular cell apoptosis, and partially preserved kidney function.[80] When administered immediately after CLP in mice, SS-31 normalized kidney ATP content, decreased apoptosis, reduced histologic injury score, and reduced serum creatinine and BUN levels.[81] A human clinical trial is investigating the efficacy of SS-31 in ameliorating ischemia-reperfusion (NCT02436447).[82] In addition, a phase 1

study is investigating the safety and pharmacokinetics of SS-31 (MTP-131) in human subjects with impaired kidney function.

It has been demonstrated that inducible nitric oxide synthase inhibitors or antioxidants (L-glutathione, ebselen, or MitoQ) can specifically target mitochondria and reduce oxidative damage, preserve cytochrome c oxidase activity, prevent mitochondrial membrane potential dissipation, and improve kidney function *in vitro* and in LPS-[83,84] and CLP[85]-induced sepsis models. Other compounds such as acetyl- L-carnitine, a mitochondrial antioxidant activity modulator, can ameliorate tubular injury and improve kidney function in cisplatin-induced AKI in mice.[73,86]

Targeting Mitochondrial Fusion and Fission

Mitochondrial division inhibitor–1 (Mdivi-1), a pharmacologic inhibitor of Drp1, blocks mitochondrial fragmentation and subsequent AKI progression in cisplatin-induced AKI[53] as well as rhabdomyolysis-induced AKI[87] in mice. Cho et al.[88] demonstrated that cyclosporine A suppresses Drp1 dephosphorylation and prevents mitochondrial fragmentation, Bax accumulation, cytochrome c release, and apoptosis after hypoxia-induced ATP depletion in kidney proximal tubular cells *in vitro*. The *in vivo* pretreatment with Drp1 inhibitor significantly attenuated mitochondrial dysfunction and abnormal fusion-to-fission balance in a CLP-induced sepsis model.[52] A phase 2 clinical trial to assess the reno-protective effect of cyclosporine A in cardiac surgery is now ongoing (NCT02397213).[89]

Targeting Mitophagy Activator Therapy

As described above, mitophagy can remove damaged mitochondria. Mitophagy is induced in AKI and pharmacologic enhancement of this process could minimize cell injury and accelerate recovery. In this regard, antymycin A or myxothiazol, inhibitors of the mitochondrial respiration complex, can ameliorate cisplatin-induced p53 activation and

exert cytoprotective effects in cultured kidney tubular cells.[90] Additionally, rapamycin protected against gentamicin-induced AKI in pigs by inducing mitophagy.[91] Another compound of interest, Ambra 1, induces mitophagy by interacting with Parkin. Ambra 1 may be a therapeutic agent to induce mitophagy.[92]

Although the induction of mitophagy seems to ameliorate AKI, some studies have reported conflicting results, and the role of mitophagy in AKI remains a subject of debate.[93] Drugs that induce mitophagy also induce mitochondrial depolarization. Mitochondrial membrane depolarization suppresses fusion, leading to fragmentation of the mitochondrial network. Fusion suppression is mostly led by the Overlapping with the m-AAA protease homolog (OMA1). After mitochondrial depolarization, PINK1 is exposed on the outer mitochondrial membrane of depolarized mitochondria in its full-length form[94,95] and triggers mitophagy.[95,96]

Resolving these issues and conducting clinical trials on these compounds will be important to determine the ultimate role of mitophagy-targeted therapy in AKI.

Other Therapeutic Approaches Targeting Mitochondria in AKI

As presented above, there are other ways that mitochondria may be targeted for therapeutic benefit.

Mitochonic acid 5 (MA-5), a derivative of the plant hormone indole-3-acetic acid, is one of the newest class of mitochondria-targeted agents. It can reduce tubular necrosis and decrease plasma BUN after cisplatin administration.[97] MA-5 is proposed to target the mitochondrial protein mitofilin/Mic60 at the cristae junction of the inner mitochondrial membrane and facilitates oligomerization of ATP synthase and super complex formation.[97,98]

Cyclophilin-D, a key regulator of mitochondrial permeability, protects against kidney injury in multiple experimental models of AKI.[99] Levosimendan is a Ca^{2+} sensitizer–positive inotropic and vasodilator drug used to treat heart failure. It has renoprotective effect after cardiopulmonary bypass surgery.[100] The molecular

mechanism of levosimendan-evoked protection might include an interaction with mitochondrial energy conservation through mitochondrial ATP-sensitive K^+ channels. A clinical study of levosimendan, investigating the safety and efficacy in intensive care patients with AKI, is now ongoing (clinicaltrials.gov identifier NCT01720030).

MITOCHONDRIAL BIOMARKERS FOR SEPSIS-INDUCED AKI

As mitochondrial dysfunction initiates and accelerates kidney injury in sepsis, disruption of mitochondrial integrity in kidney tubular cells seems to be a hallmark in diverse forms of AKI.[20] Mitochondrial DNA (mtDNA) is a circular double-stranded DNA inherited maternally which is housed in the mitochondrial matrix, encased within a double-membrane system composed of the outer and inner mitochondrial membranes.[101] Increased mitochondrial ROS generation can decrease mitochondrial membrane potential, leading to impairment of membrane integrity.[102,103] These changes could subsequently permit leakage of mtDNA into the cytosol. Furthermore, one of the proposed mechanisms by which mtDNA is translocated to the extracellular space is vianecroptosis.[104,105] Therefore, in the kidney, disruption of mitochondrial integrity may result in release of mtDNA fragments, known as mitochondrial damage-associated molecular patterns, from the matrix into the urine, where they serve as surrogate markers of kidney mitochondrial injury.[106]

Therefore, recent studies evaluated the efficiency of urinary mtDNA (UmtDNA) as a marker of kidney injury in patients with AKI with various causes.[107–110] Currently, because of diverse patient cohorts, and small sample sizes with type 2 statistical error, the utility of UmtDNA is unclear. Hu et al.[110] reported that UmtDNA identified renal dysfunction and mitochondrial damage in sepsis-induced AKI. However, UmtDNA may serve as a valuable biomarker for the development and testing of mitochondria-targeted therapies in AKI. UmtDNA is a surrogate marker of mitochondrial integrity. Because

of the role of mitochondrial function and tissue bioenergetics in tissue repair processes, UmtDNA may aid the development of new mitochondrial-targeted therapies for sepsis-induced AKI.

CONCLUSIONS

Mitochondria, given their central role in energy metabolism, play a key role in the pathophysiology of sepsis-induced AKI. Multiple targets for therapy have been identified in preclinical work but further research is needed to identify the most promising targets with the aim of improving AKI outcomes.

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DISCLOSURES

None.

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