Oxidative Stress in Sepsis

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Sepsis is the leading cause of acute kidney injury (AKI) and leads to increased morbidity and mortality in intensive care units. Current treatments for septic AKI are largely supportive and are not targeted towards its pathophysiology. Sepsis is commonly characterized by systemic inflammation and increased production of reactive oxygen species (ROS), particularly superoxide. Concomitantly released nitric oxide (NO) then reacts with superoxide, leading to the formation of reactive nitrogen species (RNS), predominantly peroxynitrite. Sepsis-induced ROS and RNS can reduce the bioavailability of NO, mediating renal microcirculatory abnormalities, localized tissue hypoxia and mitochondrial dysfunction, thereby initiating a propagating cycle of cellular injury culminating in AKI.

septic acute kidney injury | oxidative stress | sepsis

1. Introduction

Sepsis is the leading cause of acute kidney injury (AKI), accounting for approximately 50% of cases of renal dysfunction in intensive care units [1]. Development of AKI during sepsis is both a significant and an independent prognostic factor for prolonged hospitalization and in-hospital death [2]. There is also mounting epidemiological evidence that survivors of either mild or short episodes of AKI are predisposed to greater risk of developing chronic kidney disease (CKD) and end-stage renal disease in later life [3]. Antibiotics and resuscitation with fluids and vasopressors are currently the mandated treatments for human sepsis. Renal replacement therapy (RRT) is recommended for patients who develop severe septic AKI [4]. However, these are mainly palliative interventions aimed at keeping the patients alive in the hope that the kidneys recover. Accordingly, a better understanding of the pathophysiology of septic AKI is required to formulate effective mechanism-guided interventional strategies.

Although global renal ischemia has been proposed as a cause of septic AKI [5], there is both experimental and clinical evidence challenging this dogma. Histopathological investigations performed on post-mortem kidney tissue from patients that succumbed to septic AKI demonstrate heterogenous focal, patchy tubular injury, with minimal tubule-epithelial death (<5%), apical vacuolization and minor focal mesangial expansion, which are not characteristic of severe renal ischemic injury [6][7]. In addition, there is compelling evidence from clinically relevant ovine and porcine models of sepsis that AKI develops even in the absence of global renal ischemia [8][9][10]. Similarly to the histopathological findings in human sepsis, acute tubular necrosis and tubular cell apoptosis were not characteristic of AKI in such large mammalian models of hyperdynamic sepsis [9][11]. During inflammatory conditions such as sepsis, there appears to be an uncoupling of the renal microcirculation from the macrocirculation [12]. In ovine sepsis, selective renal medullary tissue ischemia and hypoxia precede the

development of AKI by 12–24 h, despite increases in renal blood flow and renal cortical perfusion and oxygenation [13][14][15]. Oxidative stress plays a critical role in promoting adaptive responses to localized tissue hypoxia by stabilization of hypoxia inducible factors (HIF), which promotes the transcription of multiple genes [16]. However, in sepsis there is an imbalance between reactive oxygen species (ROS) and reactive nitrogen species (RNS) and the host's antioxidant defense mechanisms.

2. Interactions between the Septic Inflammatory Cascade and Oxidative Stress

The inflammatory response is the body's first line of defense against invading pathogens, but this can also be a critical initiating factor for renal injury. In sepsis, inflammatory mediators, including pathogen- and damage-associated molecular patterns, are released into the intravascular area and are detected by Toll-like receptors on tubular and endothelial cells [17]. Activation of these receptors subsequently propagates a myriad of downstream processes contributing to tubular reparation, vascular rarefaction and amplification of pro-inflammatory immune modulators at sites of injury, leading to vascular congestion and endothelial dysfunction [18]. These processes appear to converge to stimulate superoxide-induced amplification of tissue hypoxia and cellular injury (**Figure 1**).

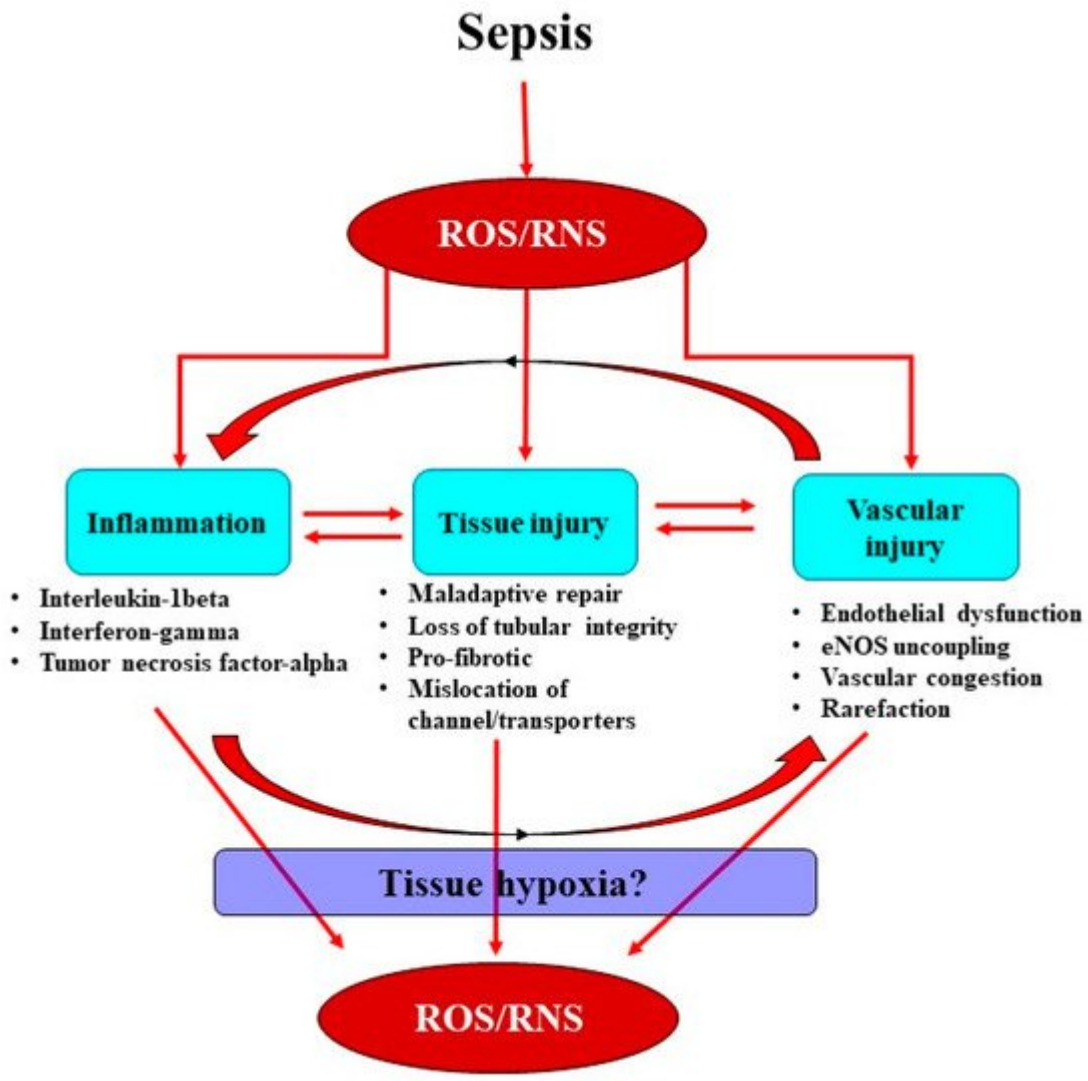

Figure 1. Schematic of the proposed relationships among sepsis-induced production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and inflammation, tissue, and vascular injury. ROS and RNS result in enhanced production of immune-modulatory cells at sites of injury in vessels and tubules, thereby initiating a complex cascade of inflammation and injury. Importantly, tissue and vascular injuries in severe sepsis can have deleterious consequences, as they can contribute to an uncoupling of endothelial nitric oxide synthase (eNOS), mediating endothelial dysfunction and tissue hypoxia, thereby enhancing the accumulation of ROS and RNS.

Sepsis-induced tubular and vascular injuries, in concert with oxidative stress, trigger the recruitment of polymorphonuclear neutrophils, setting in motion a cascade of immunomodulatory events which leads to downstream production of ROS and RNS, further propagating injury [19]. Neutrophils have the ability to generate superoxide through a complex process known as the “oxidative burst” [20][21]. Neutrophils express multiple receptors, including the β1, β2 and β3 integrins, which when activated bind to fibronectin, fibrinogen and collagen, mediating their translocation to the extracellular matrix by P-selectins and E-selectins [22]. Here, a series of signaling cascades ultimately leads to the downstream release of intracellular calcium and the formation of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex, resulting in the production of ROS [20][21]. Furthermore, inducible nitric oxide synthase (iNOS) activity is upregulated within immune cells (**Figure 2**). This increases the production of nitric oxide (NO), which can react with the ROS produced by NADPH oxidase to form the RNS peroxynitrite (**Figure 2**). Peroxynitrite contributes to nitrosative damage, such as S-nitrosylation of proteins, thereby affecting normal functioning of proteins. The notion of ROS-induced vascular injury is supported by the observation in patients with sepsis of extensive production of superoxide from leucocyte microparticles, which in turn enhances adhesion molecules’ activity and endothelial activation [23]. Moreover, high plasma levels of lipid peroxidation markers (F2-isoprostanes and isofurans) have been strongly associated with AKI in patients with sepsis [24].

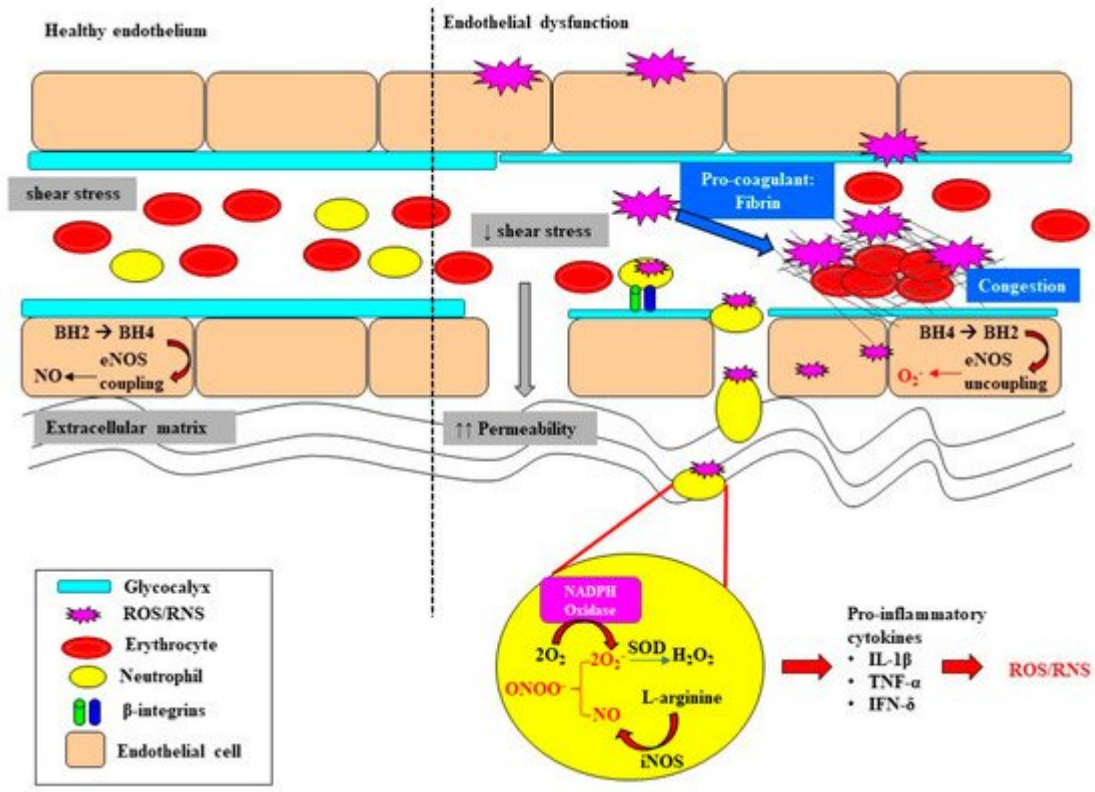

Figure 2. A schematic outlining the initiation of oxidative damage to the endothelium, the downstream consequences for endothelial dysfunction and the propagation of oxidative stress. The layer of glycocalyx lining the apical surface of endothelial cells is important for the maintenance of shear-stress and flow-mediated nitric oxide (NO) release and vasodilation of the endothelium. Sepsis-induced oxidative stress leads to glycocalyx thinning and shedding, resulting in the loss of gap junctions, and subsequently increased permeability and vascular leakage. Loss of gap junctions also greatly facilitates extravasation of neutrophils in the circulation, mediated by β-integrins, through to the extracellular matrix. The resultant superoxide (O_2^-) that is formed can interact with nitrosative species generated by inducible nitric oxide synthase (iNOS), forming the highly reactive peroxynitrite ($ONOO^-$) and then hydrogen peroxide (H_2O_2). Oxidative bursts from neutrophils contribute to enhanced oxidative damage and the downstream production of pro-inflammatory cytokines which have the intrinsic capability of producing reactive oxygen species (ROS) and reactive nitrogen species (RNS). Oxidative stress can also induce the recruitment of pro-coagulants to the site of injury, resulting in vascular congestion, ultimately impeding blood flow. Increased oxidative stress at the endothelium depletes and oxidizes the pool of tetrahydrobiopterin (BH4), resulting in the uncoupling of endothelial nitric oxide synthase (eNOS) in endothelial cells, thereby enhancing the production of ROS. Interleukin-1beta (IL-β); tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-δ).

The generation of oxidative stimuli by neutrophils further attracts the pro-inflammatory chemokine ligand-5 and intracellular adhesion molecule-1, which are important factors that facilitate the recruitment of leukocytes to sites of tissue injury [25][26]. Mobilized and activated leukocytes initiate a cytokine storm involving the recruitment of pro-inflammatory cytokines, including interleukin-1-beta (IL-1β), interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) [27][28], which can lead to the further production of ROS (**Figure 1**). Indeed, inflammatory and oxidative stress biomarkers such as TNF-α, IL-1β, myeloperoxidase activity, malondialdehyde and hydrogen

peroxide (H_2O_2) are all reported to be significantly elevated in patients with septic AKI [29]. Collectively, the initial inflammatory cascade in sepsis appears to be a critical initiating factor in the propagation of oxidative stress, which can have deleterious effects on renal microcirculation.

3. Oxidative Stress Exacerbates Microcirculatory Abnormalities and Vascular Rarefaction

Endothelial dysfunction and microvascular rarefaction have been described as common pathophysiological features of AKI and are postulated to be critical factors mediating progression to CKD following recovery from AKI [30][31][32]. The NO system is an important regulator of vascular tone within the renal microcirculation, but it can be deleteriously affected in sepsis (**Figure 2**). In the healthy state, the biosynthesis of NO by vascular endothelial cells is dependent on the coupling state of endothelial nitric oxide synthase (eNOS) and the bioavailability of the co-factor tetrahydrobiopterin (BH4) [33]. When endogenous levels of the co-factor BH4 are sufficient, L-arginine is coupled with the reduction of oxygen, leading to the production of the potent vasodilator NO [33][34]. However, when BH4 levels are low, eNOS is uncoupled and superoxide is produced instead. Furthermore, BH4 is highly susceptible to oxidization to BH2 when levels of superoxide are high, further depleting the pool of the rate limiting co-factor BH4 [35][36]. Uncoupling of eNOS is reported to contribute to the pathophysiology of a myriad of kidney diseases arising from diabetes, hypertension and ischemia-reperfusion injury [37][38]. Intravenous supplementation with BH4 in an ovine model of sepsis improved microvascular dysfunction via increasing the number of perfused vessels, the proportion of perfused small vessels and the microvascular index within the sublingual circulation [39]. In an ovine model of severe septic AKI induced by intravenous infusion of live *Escherichia Coli* for 48 h, eNOS gene expression was selectively down regulated in the renal medulla, but not the renal cortex [11]. However, whether an uncoupling of eNOS contributes to the early onset of microcirculatory abnormalities reported within the renal medulla in ovine septic AKI [13] warrants further investigation.

In sepsis, excessive superoxide generation and accumulation, in tandem with inflammation, also results in direct structural damage to the vasculature, resulting in vascular leakage and tissue edema (**Figure 2**) [40].

The damaged endothelium also attracts leukocytes to the site of injury, as part of the innate immune response facilitated by the exposed intercellular and vascular cell adhesion molecules. This homing of pro-inflammatory cells, in conjunction with compromised gap junctions, leads to extravasation of the pro-inflammatory cells from the endothelium into the surrounding tissue, contributing to persistent inflammation [41]. Notably, inflammatory cells can generate ROS themselves and so reduce NO bioavailability [41], thereby contributing to the extensive pool of superoxide, essentially setting up a vicious cycle of oxidative stress, inflammation and vascular injury [19] (**Figure 1**). Sepsis-induced microvascular injury can also release microparticles into the systemic circulation.

4. Renal Medullary Tissue Hypoxia: A Critical Event in Acute Kidney Injury?

Renal medullary hypoxia is emerging as a common pathophysiological feature of AKI arising from sepsis [13][42], cardiopulmonary bypass [43][44] and radiocontrast-induced nephropathy [45]. Furthermore, renal medullary hypoxia has been implicated as an important driver in the transition and/or propensity for progression from AKI to CKD [46][47]. The relatively high metabolic requirements of the tubular elements in the renal medulla, coupled with the topography of vascular and tubular architecture within the medulla, result in a steep oxygen gradient between the capillaries (vasa recta) and both the thick and thin ascending limbs of the loop of Henle and the collecting ducts [48]. There is also the potential for diffusive oxygen shunting in the renal medullary microcirculation (from descending to ascending vasa recta), which could further compromise renal medullary oxygen delivery [49]. In healthy sheep, graded occlusion of the renal artery and thus progressive reductions in renal blood flow resulted in proportionally greater degrees of renal medullary ischemia and hypoxia compared with a renal cortex indicative of an intrinsic deficit in the autoregulatory capacity of the renal medullary microcirculation [50]. Accordingly, under pathophysiological settings such as sepsis, renal medullary microcirculatory perturbations leading to even modest reductions in medullary oxygen delivery or increases in oxygen consumption can have adverse consequences for medullary tissue oxygenation.

Renal medullary hypoxia can be a major driver of a cascade of events leading to cellular injury, vascular injury and tubular dysfunction [51]. Acute renal insults, including endotoxemia, can both increase renal tissue oxygen consumption and reduce tissue oxygen delivery. For example, these changes can result in tubular injury and obstruction, and mislocalization of Na/K-ATPase and transport proteins within renal tubular epithelial cells, thereby reducing the efficiency of oxygen utilization for sodium reabsorption [52].

5. Sepsis-Induced Mitochondrial Dysfunction Activates Production of ROS

Mitochondrial dysfunction is proposed to be both a cause and consequence of renal hypoxia in the pathogenesis of septic AKI [53][54]. Mitochondria are the main consumers of oxygen within the kidneys. Thus, the production of physiological levels of mitochondrial ROS in the mitochondrial matrix is important because ROS serve as signals and regulators for a myriad of biological processes.

However, as cells experience prolonged periods of hypoxia, there is a change in metabolism and poor utilization of the available oxygen for ATP production in the mitochondrial electron transport chain, resulting in increased leakage of electrons and elevated production of free radicals/ROS [55][56]. Mitochondria use oxygen as the final acceptor of the respiratory chain, but its incomplete reduction can also produce ROS, especially superoxide [57]. Complex III of the electron transport chain is the inherent oxygen sensor during acute hypoxia, and it regulates the production of superoxide inversely with oxygen availability [58]. The transition of complex I from the active to “de-active” form was also reported to have the capacity to produce ROS outbursts during acute hypoxia [59]. Patients with septic AKI have elevated levels of receptor-interacting protein kinase-3 (RIPK3) in urine and plasma [60]. RIPK3 promotes oxidative stress and mitochondrial dysfunction in kidney tubular epithelial cells by increasing the expression and mitochondrial translocation of NADPH oxidase 4 and inhibition of mitochondrial complexes I and III

[60][61][62]. It is therefore not surprising that mitochondrial injury has been commonly related to multi-organ dysfunction in patients with sepsis [6][63].

There are pre-clinical and clinical studies demonstrating that the adaptive processes of mitochondrial fission are downregulated in sepsis, which likely contributes to the loss of mitochondrial mass, thereby propagating ROS-induced damage during septic AKI. Sepsis is associated with considerable morphological changes in mitochondria. These changes include reduced numbers of cristae due to swelling of the inter-cristae space and the mitochondrial matrix, and vacuolation within the mitochondria space [60][64]. Depending on the severity of mitochondrial damage, the removal of mitochondria can be carried out by two pathways: mitophagy and apoptosis (**Figure 3**).

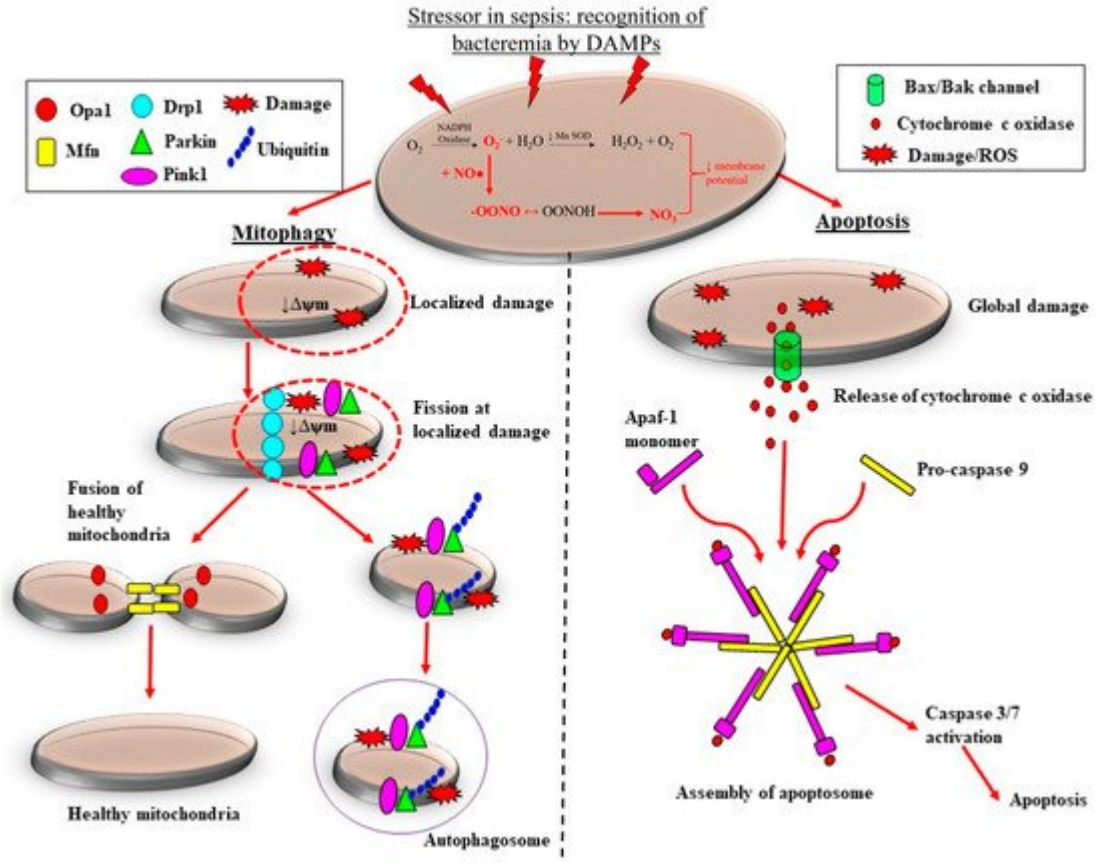

Figure 3. Adaptive and maladaptive responses of the mitochondria to sepsis-induced oxidative stress. Increased production of superoxide (O_2^-) by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase coupled with the reduced activity of manganese superoxide dismutase (MnSOD) results in the accumulation of O_2^-. Cytosolic nitric oxide (NO) produced by inducible nitric oxide synthase (iNOS) reacts with O_2^-, forming the highly reactive peroxynitrite ($ONOO^-$). The accumulation of O_2^- and $ONOO^-$ results in persistent oxidative stress and a reduction in mitochondrial membrane potential (ψm) and so mitochondrial dysfunction. In the case of localized mitochondrial damage, mitochondrial quality control mechanisms are activated which arrest mitochondrial dysfunction and limit excessive mitochondrial loss. Recruitment of mitochondrial fission proteins to sites of injury targets the damaged portions of the mitochondrion for fission. Subsequently, ubiquitin, PTEN-induced kinase (PINK1) and E3 ubiquitin-protein ligase (Parkin) proteins are recruited to the damaged mitochondrion, removed by mitochondrial fission and interact with phagophore, consequently forming an autophagosome. The healthy portion of the mitochondrion

undergoes mitochondrial fusion, adding to the existing mitochondrial pool and limiting excessive mitochondrial loss. On the other hand, extensive damage to mitochondria in severe sepsis results in the release of cytochrome c oxidase, and in the formation of an apoptosome when it interacts with apoptotic protease activating factor-1 (Apaf-1) monomers and pro-caspase 9. This leads to downstream activation of caspase 3/7, ultimately resulting in the containment of sepsis-induced damage via apoptosis. Mitochondrial dynamin-like GTPase (OPA-1); dynamin related protein-1 (DRP-1); damage-associated molecular patterns (DAMPs).

Extensive damage to the mitochondria results in global reduction of mitochondrial permeability, which can lead to accumulation of superoxide eventually, causing the opening of mitochondrial membrane channels. These injuries trigger a series of downstream events, starting with the release of the pro-apoptotic factors Bcl-2-associated X protein BAX and B-cell lymphoma-2 protein. This mediates the release of cytochrome c, which in turn interacts with apoptotic protease activating factor-1 proteins, ultimately forming an apoptosome that triggers a procaspase-9-mediated downstream intrinsic apoptosis cascade [65][66] (**Figure 3**). This process facilitates the removal of whole mitochondria in severe sepsis and results in a reduction of mitochondrial mass [67], which can further compromise the production of host antioxidants and enhance the production of superoxide (**Figure 3**). Therefore, ROS-induced damage to the mitochondria in sepsis can result in enhanced ROS production and accumulation, which can contribute to the vicious propagating cycle of oxidative stress, microvascular injury and cellular injury, culminating in AKI.

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