



Role of Mitochondrial Oxidative Stress in Sepsis

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Mitochondria are considered the power house of the cell and are an essential part of the cellular infrastructure, serving as the primary site for adenosine triphosphate production via oxidative phosphorylation. These organelles also release reactive oxygen species (ROS), which are normal byproducts of metabolism at physiological levels; however, overproduction of ROS under pathophysiological conditions is considered part of a disease process, as in sepsis. The inflammatory response inherent in sepsis initiates changes in normal mitochondrial functions that may result in organ damage. There is a complex system of interacting antioxidant defenses that normally function to combat oxidative stress and prevent damage to the mitochondria. It is widely accepted that oxidative stress-mediated injury plays an important role in the development of organ failure; however, conclusive evidence of any beneficial effect of systemic antioxidant supplementation in patients with sepsis and organ dysfunction is lacking. Nevertheless, it has been suggested that antioxidant therapy delivered specifically to the mitochondria may be useful.

Key Words: mitochondria; oxidative stress; sepsis

INTRODUCTION

Despite the availability of advanced treatments, sepsis is one of the leading causes of death in intensive care units [1,2]. Multiple factors contribute to the development of this condition; both extrinsic and intrinsic mechanisms are involved in hemodynamic collapse [3]. Extrinsic mechanisms involve endotoxins, viruses, fungi, or other toxins, while intrinsic mechanisms include proinflammatory mediators released by host immune cells. Both of these mechanisms induce a complex cascade of cellular events, resulting in the release of proinflammatory and anti-inflammatory mediators.

Sepsis develops after an initial host response to an infection becomes amplified and dysregulated, which leads to circulatory changes and septic shock. The most common consequences are impaired vascular permeability, cardiac malfunction, and mitochondrial dysfunction leading to impaired respiration [4]. The pathogenesis of sepsis-induced myocardial injury remains unclear, but the mitochondrial dysfunction of myocardial cells plays a very important role in the pathophysiological mechanism [5]; oxidants and antioxidants also play a key role. Normally, there is a balance between the oxidant and antioxidant systems in the body; oxidative stress occurs when oxidant levels exceed those of antioxidants, which contributes to the septic process and may lead to organ damage. The inflammatory response and persistent oxidative

Received on April 25, 2018 Accepted on May 8, 2018

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*No potential conflict of interest relevant to this article was reported.

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stress seen during sepsis-induced organ failure induce changes in the mitochondria that ultimately lead to mitochondrial dysfunction and cell death. The generally accepted theory is that sepsis represents an uncontrolled inflammatory response to a pathogen [6]. While numerous studies have been performed using anti-inflammatory agents, most have failed to identify any beneficial effect and thus have called into question the aforementioned hypothesis of hyper-inflammation [7-10].

The protocol followed in the treatment of sepsis or hemodynamic collapse is not consistent among studies. However, general guidelines recommend early goal-directed therapy and supportive measures for hemodynamic collapse. Until recently, therapies focused on addressing macrocirculatory failure, which often exhibits decreased cardiac output or decreased mean arterial pressure. However, immunohistochemical analyses have revealed that apoptosis is not widespread in sepsis, suggesting that mechanisms other than cell death are responsible for the condition's associated mortality [11]. A growing body of evidence indicates that the pathogenesis of sepsis involves an inability of the cell to consume oxygen. Since mitochondrial molecular oxygen (O_2) consumption accounts for 90% of the body's O_2 usage, impaired O_2 utilization and mitochondrial dysfunction may play a key role in the pathogenesis of sepsis. Additionally, excessive oxidative stress is a feature of sepsis, and redox homeostasis may therefore be involved; consequently, therapies targeting redox abnormalities could be useful for improving the management of septic patients.

INVOLVEMENT OF MITOCHONDRIAL DYSFUNCTION IN SEPSIS PATHOGENESIS

The mitochondrion serves as the central source of reactive oxygen species (ROS) in normal physiological conditions. Oxidative phosphorylation (OXPHOS) takes place in the inner mitochondrial membrane. The mitochondrial electron transport chain present in the inner mitochondrial

membrane consists of five OXPHOS complexes (I–V). Electrons are transferred from complex I to II–IV, leading to adenosine triphosphate (ATP) generation in complex V (ATP synthase). When there is a defective reduction of O_2 to H_2O_2 , it results in an excess generation of superoxide usually at the complex IV of the electron transport chain [12]. Superoxide is usually converted to hydrogen peroxide (H_2O_2) by manganese-containing superoxide dismutase (Mn-SOD) and thereafter to H_2O by catalase (CAT). The various markers of oxidative stress involved in the process of sepsis are an increased SOD/CAT ratio, which results in the accumulation of H_2O_2 in cells [13-17], decreased level of glutathione, increased level of malondialdehyde, and an increase in protein carbonyl groups. Thus, any changes in the SOD:CAT ratio, like an inhibition of CAT or an overexpression of SOD, will lead to an increase in the oxidative stress levels and morbidity in sepsis [18]. Selenium-containing glutathione peroxidase also plays a role in the catalytic conversion of H_2O_2 to water (H_2O) [19,20].

As O_2 is the final receptor of electrons in the electron transport chain, measurement of oxygen consumption is a good option to assess mitochondrial function. The peripheral blood of septic patients show normal PO_2 levels even though the oxygen consumption by cells might be reduced [21,22]. This phenomenon, known as “cytopathic hypoxia,” is a condition when the septic cells are unable to utilize oxygen [23,24]. It was previously demonstrated that, during sepsis, cellular energetic failure due to mitochondrial dysfunction is the main reason for poorer outcomes of critically ill patients [25]; improving mitochondrion biogenesis may lead to increased patient survival [26]. The pathological effects of mitochondrial dysfunction result from the excessive production of ROS, ATP depletion, the release of proapoptotic proteins, and a disturbance in Ca^{2+} homeostasis [27]. Cells also show inflammatory responses that are triggered by oxidative stress via the activation of redox pathways. A rise in the levels of intracellular adhesion molecules I and IV, along with elevation of monocyte chemoattractant protein 1 are considered as important inflammatory markers of sepsis.

Phosphorylated and transcriptionally active nuclear factor-kappa B (NF- κ B) levels also increase, along with those of circulating inflammatory mediators, such as cytokines, in patients with sepsis.

TARGETING ANTIOXIDANTS TO MITOCHONDRIA

Although moderate levels of ROS are required for proper cell function, particularly in immune cells, excessive mitochondrial oxidative stress is the main cause of a number of cell pathologies, including sepsis. Therefore, targeting antioxidants to mitochondria can be a useful therapeutic method for treating sepsis. Strategies to deliver antioxidants to mitochondria or accelerate ROS scavenging by antioxidants augment the capacity of the antioxidant defenses of mitochondria by increasing the expression of endogenous antioxidant enzymes (Figure 1).

Elevating Endogenous Antioxidant Protein Levels

The main antioxidant proteins, including SOD, glutathione, CAT, thioredoxin, and peroxiredoxin, regulate the redox balance in mitochondria. Therefore, increasing these endogenous mitochondrial antioxidant proteins can elim-

inate the overproduction of ROS and prevent damage to the mitochondria. SOD converts the superoxide radical and the singlet oxygen radical to H_2O_2 and O_2 , respectively, and serves as the first line of antioxidant defense in biological systems. The level of SOD in plasma is significantly decreased in patients with severe sepsis [28]. Macarthur et al. [29] reported that intravenous infusion of the SOD mimetic M40401 protected against hypotension, decreased inflammatory cytokines, and improved mortality in a rat model of septic shock. The upregulation of SOD2 induced by insulin also protected against mitochondrial oxidative stress in a septic acute kidney injury rat model [30]. These studies suggest that SOD regulation could have therapeutic value in sepsis. Apart from SOD, glutathione is the most abundant water-soluble antioxidant; it plays an important role in maintaining redox homeostasis and is synthesized in the cytoplasm and then transported into the mitochondria. N-acetylcysteine is a hydrophilic antioxidant that elevates glutathione level. Glutathione peroxidase catalyzes the conversion of H_2O_2 into water. CAT also catalyzes the breakdown of H_2O_2 . There are several other enzymes and small molecules that act as scavengers and comprise the antioxidant defense system, such as ascorbic acid (Table 1).

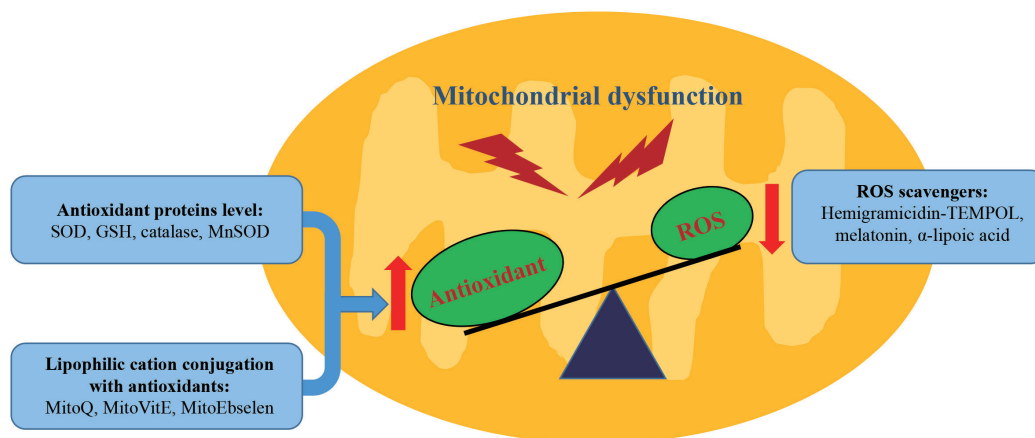


Figure 1. Overview of the process of drawing antioxidants and reactive oxygen species (ROS) to mitochondria in sepsis. ROS production within the mitochondria destroys the redox system so that the existing antioxidants are insufficient to eliminate any overproduced ROS. Delivering antioxidants to the mitochondria and scavenging ROS are beneficial aspects of sepsis treatment. SOD: superoxide dismutase; GSH: glutathione; MnSOD: manganese-containing superoxide dismutase; TEMPOL: 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl; MitoQ: 10-(6'-ubiquinonyl)decyltriphenylphosphonium bromide; MitoVitE: mitochondria-targeted antioxidant.

Table 1. Summary of antioxidants and their effects

Antioxidant (endogenous component)	Diet-derived component	Mechanism of action
Glutathione peroxidase	Tocopherols and tocotrienols (vitamin E)	H ₂ O ₂ to H ₂ O
Superoxide dismutase (Mn-, Zn-, or Cu-dependent)	Vitamin A and carotenoids (β-carotene, lycopene, lutein, etc.)	O ²⁻ to O ₂
Catalase	Ascorbate (vitamin C, those species lacking synthetic capacity, e.g., human)	H ₂ O ₂ to H ₂ O and O ₂
Glutathione	Tocopherols and tocotrienols (vitamin E)	Antioxidant scavenger, DNA repair, cofactor for enzymes
Ascorbic acid	Selenium, zinc, manganese, copper, phytochemicals with antioxidant activity	Acts against oxidation of lipids, proteins, and DNA

Lipophilic Cation Conjugation with Small Molecule: MitoQ

The most widely used strategy to transport small molecules to the mitochondria *in vivo* is by conjugation of an antioxidant to a lipophilic cation [31,32]. The construction of a lipophilic cation allows it to pass through membranes easily and gather in the mitochondrial matrix without a carrier. 10-(6'-ubiquinonyl)decyltriphenylphosphonium bromide (MitoQ; ubiquinone attached to the lipophilic triphenylphosphonium cation) has been used to protect cells from oxidative damage and apoptosis by activating ubiquinol and therefore restoring its antioxidant efficacy in the respiratory chain [33,34]. Lowes et al. [35] first reported that MitoQ suppressed proinflammatory cytokine levels and increased anti-inflammatory cytokine levels in sepsis models, both *in vitro* and *in vivo*. Studies in humans have shown that MitoQ exerts antioxidant and anti-inflammatory effects on the leukocytes of type 2 diabetes patients by decreasing ROS production and tumor necrosis factor alpha via suppression of NF-κB activation [36]. Another study revealed that administration of MitoQ impeded endotoxin-induced cardiac dysfunction in a rat model of sepsis [37]. Assessment of MitoQ in a human phase II Parkinson disease trial confirmed that it is safe to administer to patients [38]. Nevertheless, MitoQ has not been utilized in clinical studies on sepsis. Other compounds conjugated to the triphenylphosphonium cation, such as vitamin E, peroxidase, or ebselen, also have an effect on the elimination of mitochondrial oxidative stress [39]. Further human studies will be required to demonstrate a beneficial effect of lipo-

philic cation conjugates.

Mitochondria-Targeted ROS Scavengers: Hemigramicidin-TEMPOL

The mechanism of 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPOL) in the elimination of oxidative stress is as follows: TEMPOL limits formation of the highly toxic hydroxyl radical from H₂O₂ and removes the superoxide anion. There are two portions in TEMPOL (the targeting portion and the ROS-scavenging portion), which have been extensively demonstrated to be effective cytoprotectors in different oxidative stress models [40,41]. XJB-5-131, one of the hemigramicidin-TEMPOL conjugates, ameliorated intestinal mucosal injury and prolonged survival in a rat model of hemorrhagic shock and had a greater effect than non-targeted TEMPOL [42,43]. In cecal ligation and puncture murine models of sepsis, Mito-TEMPOL attenuated sepsis-induced acute kidney injury, decreased mitochondrial superoxide levels, improved renal microcirculation and the glomerular filtration rate, and significantly increased the rate of survival [44].

Other Methods: Melatonin and Lipoic Acid

Melatonin, a potent antioxidant, has a higher concentration in the mitochondria than in other cellular organelles. Melatonin reduces oxidative stress by scavenging endogenous free radicals, such as the hydroxyl radical, H₂O₂, peroxyxynitrite anion, and nitric oxide [45]. Several animal studies have demonstrated that melatonin attenuates mitochondrial dysfunction in septic mice [46] and

peritonitis-induced septic shock in rats [47]. Clinical studies have indicated that melatonin exerts beneficial effects in humans. Gitto et al. [48] showed that melatonin administration reduced the septic newborn death rate by decreasing the concentrations of lipid peroxidation products. In another study, melatonin had beneficial effects on sepsis-induced mitochondrial dysfunction, oxidative stress, and cytokine responses [49]. Alpha-lipoic acid is another metabolic antioxidant; its reduced form, dihydrolipoic acid, plays an important role in mitochondrial dehydrogenase reactions; directly scavenges ROS, such as superoxide radicals, hydroxyl radicals, and peroxy radicals; and exerts an anti-inflammatory effect by inhibiting the transcriptional activator NF- κ B [50]. A recent study suggested that α -lipoic acid decreases neutrophil infiltration, lipid peroxidation, protein carbonylation and increases SOD and CAT activity, which contributed to reduction in inflammation and oxidative stress in a rat model of sepsis [51].

CONCLUSION

It is widely accepted that oxidative stress is involved in the pathogenesis of sepsis. The exact mechanism underlying oxidative stress in sepsis evolution is quite complicated, but it is well-known that mitochondrial dysfunction, an energetic deficit of cells, and reduced vascular tone are the major factors involved in multi-organ failure and poorer outcomes or mortality in septic patients. Numerous therapies for sepsis have been clinically tested over the years. Several lines of evidence suggest that antioxidants are beneficial for sepsis treatment. In particular, mitochondrion-targeted antioxidants seem promising, as they accumulate inside the inner membrane of the mitochondria. However, the results of clinical trials are not sufficient for implementation of these therapies in a clinical setting because animal models do not correspond completely to human sepsis. Further research is needed to completely understand the mechanism underlying sepsis.

Acknowledgments

This work was supported by the research fund of Chungnam National University, Daejeon, Korea.

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