The potential mechanism of mitochondrial dysfunction in septic cardiomyopathy

Pan Pan, Xiaoting Wang and Dawei Liu

Abstract
Septic cardiomyopathy is one of the most serious complications of sepsis or septic shock. Basic and clinical research has studied the mechanism of cardiac dysfunction for more than five decades. It has become clear that myocardial depression is not related to hypoperfusion. As the heart is highly dependent on abundant adenosine triphosphate (ATP) levels to maintain its contraction and diastolic function, impaired mitochondrial function is lethally detrimental to the heart. Research has shown that mitochondria play an important role in organ damage during sepsis. The mitochondria-related mechanisms in septic cardiomyopathy have been discussed in terms of restoring mitochondrial function. Mitochondrial uncoupling proteins located in the mitochondrial inner membrane can promote proton leakage across the mitochondrial inner membrane. Recent studies have demonstrated that proton leakage is the essential regulator of mitochondrial membrane potential and the generation of reactive oxygen species (ROS) and ATP. Other mechanisms involved in septic cardiomyopathy include mitochondrial ROS production and oxidative stress, mitochondria Ca\(^{2+}\) handling, mitochondrial DNA in sepsis, mitochondrial fission and fusion, mitochondrial biogenesis, mitochondrial gene regulation and mitochondria autophagy. This review will provide an overview of recent insights into the factors contributing to septic cardiomyopathy.

Keywords
Sepsis, cardiomyopathy, mitochondrial, uncoupling proteins

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Introduction

Sepsis is defined as an immune and inflammatory response that is capable of inducing multi-organ dysfunction. Recent data indicate that mortality rate from sepsis or septic shock is approximately 40% in intensive care units (ICU). It is the main cause of death among patients in hospital and the mortality is as high as 28.7% despite progress having been made in its treatment. In the US, the mortality rate due to sepsis is higher than that from prostate cancer, breast cancer and AIDS combined, and this figure is growing annually. The high morbidity and mortality associated with sepsis and septic shock make them the 10th most common cause of death in the US. An important organ system frequently involved in sepsis is the cardiovascular system, with septic cardiomyopathy being one of the most serious complications associated with sepsis or septic shock, which may progress to left and right heart systolic and diastolic failure. Basic and clinical research has studied its mechanism of dysfunction for more than five decades.

In 1951, a hyperdynamic state was identified in patients with sepsis or septic shock, which was the first cardiovascular event shown to be caused by sepsis. In subsequent famous studies, fluid therapy was attempted in these patients and appropriate and sufficient volume resuscitation was demonstrated to be one of the most effective therapeutic treatments for sepsis. However, in the mid-1980s, many clinicians found that some septic patients had normal or even a slightly higher cardiac output with descended ejection fraction and stroke volume. It was also shown that septic patients with cardiovascular dysfunction had a higher mortality rate than those without cardiovascular dysfunction. From then on, researchers paid more and more attention to septic cardiomyopathy and attempted to clarify the mechanism of this critical manifestation.

A number of studies identified that myocardial depression was not related to hypoperfusion as an adequate oxygen supply had already been proved in experiments on human and animals, while a circulating depressant factor in septic shock, which was first proposed fifty years ago, must play an important role in heart dysfunction. Other mechanisms like mitochondrial dysfunction/apoptosis, cellular damage, cell signalling, autonomic dysfunction, decreased coronary blood flow, increased heat shock protein or adhesion molecules and myocardial hibernation phenomenon were proposed with the development of scientific theory and technology. Nevertheless, an increasing number of studies had focused on myocardial energy metabolism as cells, which seemed unable to maintain proper metabolism in septic patients. Consequently, this imbalance led to energy failure and even death. It was demonstrated that cardiomyocyte injury occurred in sepsis-induced cardiac dysfunction, but there was almost no cell death. It is particularly important to find the correct treatment to repair cell function. More specifically, many researchers identified mitochondrial dysfunction as the key pathological change in septic cardiomyopathy. Since the heart is one of the organs that is highly dependent on abundant adenosine triphosphate (ATP) levels to maintain its contraction and diastolic function, impaired mitochondrial function is lethally detrimental to the heart. According to cardiac pathophysiology, energy depletion resulting from mitochondrial dysfunction would contribute to significant myocardial damage, such as diabetic cardiomyopathy, ischaemic reperfusion injury and heart failure. A series of mitochondria-related mechanisms in septic cardiomyopathy have been explored in order to find a way to restore mitochondrial function. This
review will provide an overview of recent insights into the factors contributing to septic cardiomyopathy.

**Mechanism of mitochondrial dysfunction in septic cardiomyopathy**

Mitochondrial dysfunction in sepsis can cause continuous damage to cells and organs. Reactive oxygen species (ROS) like superoxide are increased during sepsis and evoke oxidative and nitrosative injury. Increased superoxide can result in the inhibition of oxidative phosphorylation complexes, decreased O$_2$ consumption and mitochondrial membrane potential ($\Delta\Psi$). As a consequence, the release of ROS increases. In addition, the increased levels of uncoupling proteins increase the extent of proton leakage. Mitochondrial permeability transition pores (mPTP) open due to increased Ca$^{2+}$. Hence, oxidative damage happens to the inner mitochondrial membrane. In addition, inappropriate mitochondrial autophagy (mitophagy) leads to the reduction of mitochondrial mass and dysfunctional mitochondria. In summary, ATP regeneration is a complex process, and once the heart lacks energy, damage to cardiac function may follow.

**Mitochondrial ROS production and oxidative stress**

Complexes I and III of the respiratory chain in mitochondria produce small amounts of ROS physiologically. However, sepsis is a disease accompanied by increased oxidative stress, and large amounts of ROS come from activated neutrophils. In addition, azotized stress leads to the oxidation of xanthine and its reactive nitrite increased in plasma. While in serum, the antioxidant capacity is diluted because of decreased levels of antioxidants like vitamin C, vitamin E, unconjugated bilirubin, uric acid, and other unknown factors. Intracellurally, the concentration of oxidized glutathione dimmers increase while amounts of glutathione drop. A large body of evidence strongly suggests that ROS and reactive nitrogen species led to specific impairments of oxidative phosphorylation in the septic myocardium (e.g. complex I, complex IV, F0F1 dysfunctions), especially myocardial cell mitochondria. Research has demonstrated that the activity of inducible mitochondrial nitric oxide synthase was obviously increased in a septic mouse model, which led to the growth of peroxynitrite ONOO$^-$. It is clear that nitric oxide (NO) and its derivatives play an indisputable role in the regulation of cardiovascular function and vascular tone. Research has shown that ONOO$^-$ has a negative influence on myocardial mitochondrial dysfunction in sepsis. Nevertheless, we should realize that there is a casual relationship between NO and heart function, since NO is not only produced by cardiac mitochondria but it is also found in other intracellular locations and it is produced in different cell types. To prevent and alleviate oxidative damage, researchers have been searching for suitable antioxidants, such as mitochondria-targeted vitamin E, mitochondria-targeted antioxidant MitoQ, and vitamin C. These antioxidants represent an attractive treatment for mitochondrial injury. However, in order to gain wider acceptance, more clinical experiments are needed to confirm the practical use of antioxidants.

**Mitochondrial Ca$^{2+}$ handling**

One of the most important steps in regenerating ATP is building up a proton gradient that is dependent on the impermeability of the inner mitochondrial membrane (IMM). Evidence shows that the electron transport chain that pumps protons to maintain the chemiosmotic energy gradient
is based on the impermeability of the IMM. Although mPTP is formed due to a sudden change in the IMM and the influence of other substances, as it is located in the inner mitochondrial membrane it is mainly mediated by Ca$^{2+}$. During sepsis, mitochondrial Ca$^{2+}$ content is raised by increased Ca$^{2+}$ leaking from the sarcoplasmic reticulum and decreased Ca$^{2+}$ uptake into the same organelle. Researchers found that inhibition of nicotinamide adenine dinucleotide phosphate oxidase 2 preserved intracellular calcium handling, mitochondrial function and played a protective role in sepsis-induced cardiomyopathy. Ca$^{2+}$ overload can result in the mPTPs opening and becomes the trigger of sequential pathological changes; and for myocardial cells, mPTP opening can lead to activation of caspase proteins, and ultimately to cardiomyocyte contractile dysfunction. For the heart in sepsis, the abnormal transport of calcium is an important factor affecting heart function. An animal study showed that cytokines like tumour necrosis factor-$\alpha$ and interleukin-1$\beta$ were released and affected calcium leakage. This disorder proved that sepsis can weaken cardiomyocyte contractility in isolated rat heart model, and there was no difference in the damage between right and left ventricles. Prevention of mPTP opening through decreased calcium leakage can reduce the activation of cytochrome c release.

**Mitochondrial DNA in sepsis**

With the development of molecular biology, researchers now recognize the role of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) in the occurrence and development of disease. Among the known DAMPs, mitochondrial DNA (mtDNA) has become the focus of considerable research. MtDNA is a circular molecule that encodes the key proteins involved in the oxidative phosphorylation system. In addition to its coding function, mtDNA is also involved in cellular immune functions. Like bacteria, mtDNA is a component that can be recognized as a DAMP by the immune system and triggers or promotes a series of defence reactions. Multiple *in vivo* and *in vitro* studies have demonstrated that mtDNA can be transferred from mitochondria to the cytosol via mPTPs, and thus any pathological changes leading mPTP opening will increase the leakage of mtDNA. In 2013, the first study of mtDNA in ICU patients found that the levels of circulating mtDNA were significantly higher in non-survivors than survivors. Subsequently, another study found that plasma mtDNA levels in patients with sepsis was greater than in healthy controls. Consequently, the authors demonstrated via an *in vivo* experiment that the high concentration of mtDNA was able to increase neutrophil viability. However, delayed neutrophils apoptosis and local accumulation were associated the poor outcome in patients with sepsis.

**Mitochondrial fission and fusion**

It is widely known that mitochondria are hyperdynamic organelles and that their morphology is inextricably linked to their function. Fission and fusion are the determinative factors in mitochondrial morphology. Balanced and proper mitochondrial membrane fission and fusion support the reliable production of mitochondria, while abnormal morphology cannot meet the metabolic demands. Usually, the changes of structures caused by the fusion/fission processes are observed within 24 h. Very recent research has demonstrated that proper mitochondrial fusion and fission can regulate mitochondrial function and maintain heart development.
Different inner or outer membrane fusion and fission depends on proteins encoded by different genes (outer membrane fusion: mitofusin-1 and mitofusin-2 \([MFN1\) and \(MFN2\) genes], phospholipase D family member 6 \([PLD6\) gene]; inner membrane fusion: mitochondrial dynamin like GTPase \([L-OPA1\) gene]; outer membrane fission: death associated protein kinase 2 \([DAPK2\) gene, also known as \(DRP1\)); inner membrane fission: mitochondrial dynamin like GTPase \([S-OPA1\) gene], mitochondrial fission process 1 \([MTFP1\) gene, also known as \(MTP18\)). In a septic mouse model, scientists found that thioredoxin 1 overexpression can alter the ultrastructure in the mitochondrial cristae accompanied by increased expression of mitochondrial dynamin like GTPase \((OPA1\) gene) and activation of the dynamin 1-like \((DNM1L\) gene, also known as \(DRP1\)). In addition, it has been proved that the inhibition of unbalanced mitochondrial fission through the inhibitor Mdivi-1 can protect organs function in endotoxaemia.65

**Mitochondrial biogenesis**

In addition to mitochondrial fission and fusion, mitochondrial biogenesis is the other main component of the mitochondrial mass control system. Physiologically, creation of new and healthy mitochondria in terms of biogenesis is important to meet cellular metabolic energy demands.67 It has been reported that mitochondrial biogenesis may partially counteract mitochondrial protein depletion, helping to maintain functionality and energetic status in the critically ill patients.68 During sepsis, excess ROS and free radical generation damage mitochondria and result in impaired mitochondrial synthesis, while biogenesis becomes decreased in early sepsis and increased in later sepsis.32 Endotoxin causes the activation of oestrogen-related receptor alpha, peroxisome proliferator-activated receptor gamma coactivator 1-alpha \((PGC-1\alpha)\).69 Mitochondrial synthesis can be affected by regulating the above substances. A study suggested that acetylcholine promoted mitochondria biogenesis via the PGC-1\(\alpha\) pathway and improved mitochondrial function.70 Some other studies also have demonstrated that increased biogenesis can improve the prognosis in sepsis, and the inhibition of biogenesis can increase mortality.71,72 However, it appears that redundant biogenesis can aggravate mitochondrial function. For example, a study demonstrated that the overexpression of PGC1-\(\alpha\) resulted in an over-dose of biogenesis and led to heart failure.73 Thus, we need to do further research on biogenesis in order to more clearly understand its effect on mitochondrial and organ function.

**Mitochondrial genes**

One of the molecular mechanisms that occurs in mitochondria during trauma or sepsis is mitochondrial gene modification, though there are limited publications in this field. In a mouse model of haemorrhage trauma, the transcriptional profile of mitochondria genes was changed by the trauma and led to worsened heart function.74 To date, no specific gene changes have been identified in cardiac muscle cells during sepsis. However, in a hepatic model, a mutation in the ATPase subunit-8 partially protected mice against endotoxaemic stress, most probably leading to better hepatic energy status despite elevated oxidative stress.75 It has become popular to research the circadian rhythms and to some extent clock genes appear to control mitochondrial function. For example, disruption of the clock genes affects the immune response, which in turn induces proinflammatory mediators, leading to bioenergetic decay and formation of ROS.76 More basic and clinical research is required to determine
the role of mitochondrial and clock genes in the prediction of disease development and prognosis. More importantly, evidence if required to determine whether these two types of genes can work as effective treatments for diseases.

**Mitochondrial autophagy**

As discussed above, mitochondrial impairment can be lethal to cells, initiating the necrotic or apoptotic cell death pathways. The body has a series of mechanisms that it can use to correct sepsis-related organ dysfunction caused by abnormal mitochondria. Apart from generation of new and functionally normal mitochondria (biogenesis), removal of dysfunctional mitochondria is another key mechanism of organ recovery. This removal of mitochondria via autophagy is known as mitophagy. Damaged mitochondria are isolated by autophagosomes and ultimately degraded by fusion with lysosomes. Morphological and biochemical evidence indicates that mitophagy is associated with recovery, suggesting that this process has something to do with cardiac recovery from sepsis. Mitochondrial autophagy was activated during sepsis in \textit{PARK2}-deficient mice and \textit{PARK2} exerted additional protective roles in sepsis-induced mitochondrial and cardiac contractile dysfunction. Many studies put mitophagy as a therapeutic target to improve heart function. Current data demonstrate that the hypophosphorylated form of IkappaB\(\beta\) (an inhibitor of nuclear factor kappa B) at Ser313 is beneficial to the heart in sepsis through enhancement of autophagy and inhibition of apoptosis. Other research indicates that fasudil prevented lipopolysaccharide-induced heart oxidative stress by inhibiting RhoA/ROCK from activating the autophagic processes. In addition, lysosome reformation mediated by cobalt protoporphyrin IX or transcription factor EB may be involved in cardioprotection against lipopolysaccharide-induced septic insults, and may be a novel mechanism for protecting the heart against oxidative stress.

**Uncoupling proteins in mitochondria**

Mitochondrial uncoupling proteins (UCPs) located in the mitochondrial inner membrane can promote the leakage of protons across the mitochondrial inner membrane. It is an essential regulator of mitochondrial membrane potential, which can disperse the mitochondrial proton gradient by translocating H\(^+\) across the inner membrane, and finally influencing ATP generation. Physiologically, uncoupling can decrease mitochondrial ROS production and increase heat generation. UCPs are part of a protein family consisting of five subtypes. The UCP molecule is composed of six hydrophobic membrane-spanning \(\alpha\)-helices, which are responsible for creating the channel within the inner membrane. Furthermore, the \(\alpha\)-helices are arranged into three cassettes; the latter ones being connected by amino, carboxyl termini and two loops. The loops are implicated in the control of access to the channel. UCPs possess a binding site for purine nucleotides in order to inhibit the uncoupling activity physically. The essential function of UCP1 is to produce the heat from brown adipose tissue (BAT) to maintain body temperature. UCP2 through to UCP5 have been found in fungi, plants and animals. All five subtypes of UCPs can be expressed in mammalian cells and have different tissue distributions. UCP1 is mainly distributed in BAT, but is also found in other places such as white adipose tissue, pancreatic \(\beta\) cells, retinal cells and skeletal muscle. It plays an important role in glucose metabolism. UCP2 is the most common protein in this family, as it is
found in various tissues, such as the central nerve system, kidney, heart, liver, pancreas, spleen, thymus and macrophages. UCP3 is mostly found in BAT and skeletal muscle, and despite of high sequence similarity with UCP1, it has no thermoregulation properties and its physiological functions remain unknown. The UCP4 and UCP5 genes have less sequence similarity with UCP1, and they are mostly expressed in the brain. Recently, it was hypothesized that the UCP4 and UCP5 genes originate from a common ancestral gene and are probably responsible for ATP transportation.

Uncoupling protein 2 can be regulated at various levels; at the molecular level (gene, mRNA and protein transcriptional, translational, turn-over), proton conductance and by pharmacological regulation. Recent research identified two of the most common gene polymorphisms: the promoter variant –866G>A and the codon 55 missense polymorphism. The former polymorphism can promote higher UCP2 mRNA expression, while the latter polymorphism can reduce the degree of uncoupling in pathological process. To date, the four most common transcription regulatory proteins and their relative transcription factor binding sites that are involved in the regulation of human UCP2 transcription are the peroxisomal proliferator-activated receptors, PGC-1α, forkhead box protein A1, and the SMAD family. MicroRNAs and heterogeneous nuclear ribonucleoprotein K induce a totally new layer of protein regulation after transcription. It has been found that UCP2 expression can be modulated by different drugs. For example, adenosine monophosphate-activated protein kinase activator can up-regulate UCP2 activation. However, some chemotherapeutic drugs like doxorubicin and taxol can down-regulate UCP2 expression and influence the cardiac function.

In contrast to the single function of UCP1, UCP2 is more related to organ function as it can be found in several tissue and organs. The wide distribution of UCP2 means that it is involved in regulating metabolism, including ROS production, food intake, glucose control, and immunity; and some pathologies, such as heart failure, diabetes, and cancer. A number of studies highlight the importance of UCP2 in cardiovascular diseases. ROS are elevated in some pathological processes, including sepsis-induced cardiomyopathy, cardiac reperfusion injury, and diabetic cardiomyopathy. Redundant ROS can stimulate proton leakage, thus leading to decreased UCP2 activity and reduced generation of ROS. UCP2 plays a protective role in the heart via this negative feedback loop. For the human heart, modulation of UCP2 level appears increased in oxidative stress status. Uncoupling of oxidative phosphorylation diminishes superoxide formation by complex I. Evidence suggests that UCP2 has a protective effect on myocardial damage and down-regulated UCP2 is associated with a failing heart. UCP2 up-regulation attenuated ROS generation and prevented mitochondrial Ca\(^{2+}\) overload, significantly suppressing markers of cell death. In a UCP2 gene silencing animal model, UCP2–/– mice were more likely to have damaged mitochondrial morphology and function, suggesting UCP2 may play a protective role in cardiomyocytes under septic conditions. Decreased membrane potential and ATP content, depletion of mtDNA and increased ROS were aggravated by silencing of UCP2. In addition, researchers found that UCP2 had a regulatory role in the activation of p38 mitogen-activated protein kinase, nuclear factor kappa B and
the expression of downstream inflammatory mediators in H9C2 cells stimulated with septic serum.109

Conclusions
Mitochondria account for one third of the volume of cardiomyocytes and have an important role to play in the regulation of ROS generation and ATP production, which is important for maintaining heart function and cardiomyocyte survival. Therefore, any modification of mitochondria may contribute to cardiovascular diseases. There is considerable research evidence to show that UCP2 acts as an essential protein in mitochondrial function, by decreasing ROS generation and increasing ATP production. This protective feedback loop helps organs to recover their functions after sustaining damage. More research is needed to develop new drugs or promising therapeutic approaches that could potentially be used to reverse the mitochondrial damage associated with several diseases. Further research about UCP activity and regulation could advance our understanding of myocardial depression. The increasing interest in sepsis will allow novel research tools to be used to develop effective treatments in the future.

Declaration of conflicting interests
The authors declare that there are no conflicts of interest.

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