Alterations of mitochondrial function in sepsis and critical illness

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Purpose of review

Septic shock is the consequence of a conflict between a pathogenic agent and the immune system of the host. This conflict induces an immune-mediated cytokine storm, with a whole-body inflammatory response often leading to multiple organ failure. Although extensively studied, the pathophysiology of sepsis-associated multiorgan failure remains unknown. One postulated mechanism is changes in mitochondrial function with an inhibition of mitochondrial respiratory chain and a decrease of oxygen utilization.

Recent findings

Mitochondrion is a key organelle in supplying energy to the cell according to its metabolic need. Hypoxia and a number of the mediators implicated in sepsis and in the associated systemic inflammatory response have been demonstrated to directly impair mitochondrial function. A large body of evidence supports a key role of the peroxynitrite, which can react with most of the components of the electron transport chain, in the mitochondrial dysfunction.

Summary

A pivotal role is suggested for mitochondrial dysfunction during the occurrence of multiorgan failure. Understanding the precise effect of sepsis on the mitochondrial function and the involvement of mitochondria in the development of multiple organ failure is fundamental. More human studies are thus necessary to clarify the mitochondrial dysfunction in the various phases of sepsis (early and late phase) before testing therapeutic strategies targeting mitochondria.

Keywords

mitochondria, multiorgan failure, oxidative phosphorylation, oxidative stress, peroxynitrite, sepsis

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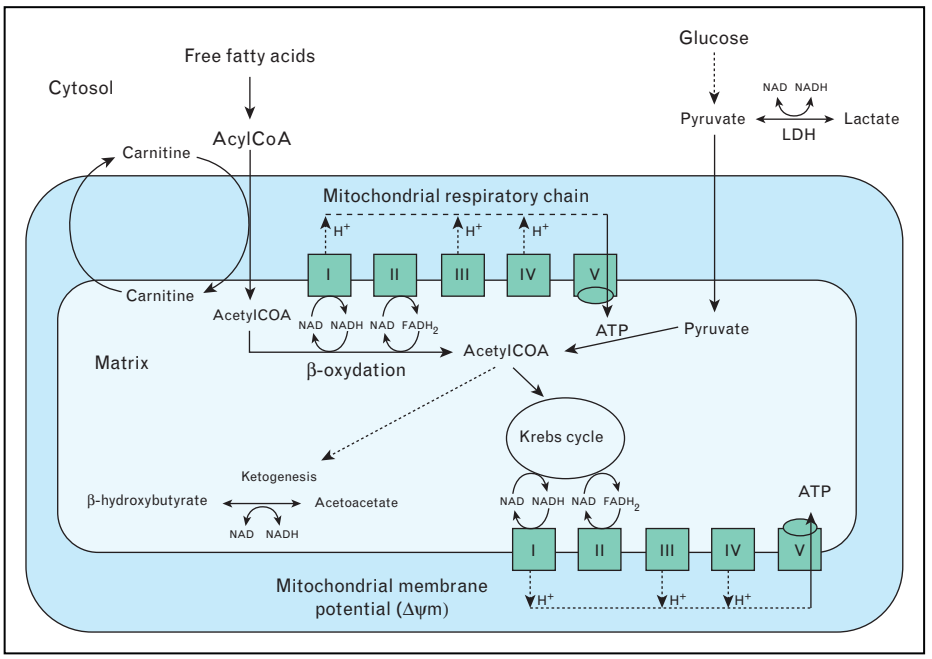
Introduction

In spite of the progress in the management of patients with septic shock, mortality remains very high. In the initial phase of septic shock, mortality is mostly due to the absence of haemodynamic control. In the late phase of septic shock, mortality is due to multiple organ failure (MOF). Septic shock is the consequence of a conflict between a pathogenic agent and the immune system of the host. This conflict induces an immune-mediated cytokine storm, with a whole-body inflammatory response often leading to MOF. Although extensively studied, the pathophysiology of sepsis-associated multiorgan failure remains unknown.

The respective roles of tissue hypoxia and cellular energetic metabolic dysfunction in the contribution to organ dysfunction in sepsis have been discussed continually for many years. One of the main characteristics of septic shock is an impairment of oxygen extraction, despite evidence of apparent tissue hypoperfusion. Two mechanisms have been postulated to explain this inability to extract oxygen. The first mechanism is a maldistribution of blood flow at either a macrovascular or a microvascular level with resulting tissue hypoperfusion. The second postulated mechanism is a change in mitochondrial function, leading to an inhibition of the mitochondrial respiratory chain and a decrease in oxygen utilization. Thus, it has been proposed that a key defect in sepsis is an interruption in oxidative phosphorylation within mitochondria. The result is an inability of the cell to use molecular oxygen for ATP production, despite adequate oxygen availability. This has been termed cytopathic hypoxia [1].

A number of the mediators implicated in septic shock have been demonstrated to directly impair mitochondrial function. For example, peroxynitrite ($ONOO^-$) can react with most of the components of the electron transport chain, including complexes I and III [2–4], and may mediate apoptosis by permeabilization of the outer mitochondrial membrane. Furthermore, $ONOO^-$ may induce DNA damage through activation of the DNA repair enzyme poly-ADP-ribosylpolymerase (PARP-1).

Figure 1 Schematic representation of the different energetic pathways described in mitochondria



I, II, III and IV are the complex I, II, III and IV of the mitochondrial respiratory chain. β oxydation and Krebs cycle produce NADH and FADH that donate electron to the complex I and II, respectively. Electron further transits to complex III and IV and generates a proton gradient across the inner membrane. Proton gradient is further transformed to ATP by complex V (ATP synthase). AcylCoA, acyl coenzyme A; FAD, flavine adenine dinucleotide (oxidized form); FADH, flavine adenine (reduced form); LDH, lactate dehydrogenase; NAD, nicotine adenine dinucleotide (oxidized form); NADH, nicotine adenine (reduced form).

Understanding the effect of sepsis on mitochondrial function and the role of mitochondria in the development of MOF is fundamental before testing therapeutic interventions stimulating or shutting down energetic metabolism. This article will address the changes in mitochondrial function occurring during sepsis and the key role of mitochondria in the pathogenesis of organ failure.

Mitochondrial function in physiological conditions

Mitochondria are essential for the conversion of latent energy, found in substrates provided by the oxidation of glucose, fats and amino acids to store energy in the form of ATP. In each eukaryotic cell, mitochondria are abundant, with the mitochondrial content being dependent on cell type and energy demand. Each mitochondrion has an outer membrane that is permeable to large molecules and an inner membrane that is impermeable to most solutes and contains the protein complexes involved in electron transport (complexes I, II, III and IV) and ATP synthesis [5]. The inner membrane also contains transport proteins involved in the movement of pyruvate, fatty acids, ATP, ADP and inorganic phosphate across the membrane. The Krebs cycle takes place within the interior of the mitochondrion (matrix), in which the mitochondrial DNA and ribosomes, that give the organelle the ability to make some of its own proteins, are located. The Krebs cycle produces nicotine adenine dinucleotide (NADH, three per molecule of acetyl-CoA) and flavine adenine dinucleotide ($FADH_2$, one per molecule of acetyl-CoA), which then donate electrons to the respiratory complexes I (NADH dehydrogenase) and II (succinate-coenzyme Q reductase), respectively. Complex I transfers electrons from NADH to coenzyme Q (CoQ). Complex II transfers electrons from $FADH_2$ to CoQ. Complex III (CoQ-cytochrome *c* reductase) accepts electrons from CoQ and passes them to cytochrome *c*. Complex IV (cytochrome oxidase) transfers electrons from cytochrome *c* to oxygen (Fig. 1). This flow of electrons between respiratory mitochondrial complexes I–IV provides energy to transfer protons (H^+) across the inner membrane from the matrix to the intermembrane space. The resulting electrochemical gradient, expressed as the mitochondrial membrane potential ($\Delta\Psi m$) (−150 to −180 mV) [6], is vital to both ATP production and Ca^{2+} accumulation and is, therefore, essential to the maintenance of mitochondrial homeostasis. This electrochemical gradient exerts a force called the proton motive force. This energy available in the proton motive force is used to drive the enzymatic synthesis of ATP. The protons are translocated back across the inner membrane by the F_0F_1-ATPase complex (complex V) anchored to the inner membrane. Thus, electron transport and ATP synthesis are tightly coupled [7]. As dissipation of the proton gradient occurs, ATP energy production (uncoupling) stops, whereas electron

and proton transport continue unabated (and may even increase due to the positive feedback from ADP). Dissipation of the proton gradient may be either due to uncoupling proteins (UCPs) or transition pore opening. A family of UCPs has been described in the inner mitochondrial membrane. By regulating mitochondrial biogenesis, calcium flux, free-radical production and local temperature, subsequent studies clearly showed that they can directly influence cell function. Electrons from NADH pass through all three ATP-generating complexes, generating three ATPs per molecule. Electrons from $FADH_2$ pass through only two ATP-generating complexes, generating two ATPs per molecule. Thus, the maximum ATP yield per molecule of glucose under aerobic conditions is 36 or 38 ATPs (10 molecules of NADH and two molecules of $FADH_2$ per molecule of glucose).

Mitochondria are not simply ATP-producing organelles, but they also play a key role in cell signalling. For example, reactive oxygen species (ROS) generated by mitochondria act as second messengers in the cellular response to hypoxia [8], and they also have several potentially important effects on vascular tone, angiogenesis, endothelial cell growth, migration, proliferation and survival [9].

Mitochondrial function in sepsis and critical illness

Most evidence suggesting changes in mitochondrial function during sepsis is coming from experimental studies. These studies describe sepsis-induced changes in mitochondrial respiratory chain and oxygen utilization and identify some of the mediators involved in the inhibition of mitochondrial function [for example tumour necrosis factor alpha (TNFα) and peroxynitrite].

Mediators implicated in sepsis and mitochondrial function

Some of the mediators implicated in sepsis and in the associated systemic inflammatory response have been demonstrated to directly impair mitochondrial function. Cellular hypoxia alone may modify mitochondrial respiratory chain function. During hypoxia, cells can downregulate energy requirements and ATP demand in response to the decrease in regional O_2 supply to maintain cell viability. This adaptive response is known as O_2 conformance or hibernation. Guzy and Schumacker [10] proposed that the mitochondrial electron transport chain acts as an O_2 sensor by releasing ROS in response to hypoxia.

The proinflammatory cytokine TNFα has been reported to increase ROS in the mitochondria [11], and there are suggestions that cytotoxic activity of TNFα is mediated by damage to mitochondria. It was indeed recently demonstrated that TNFα was able to inhibit oxidative phosphorylation at the level of cytochrome *c* oxidase [12••]. Using total hepatocyte homogenates, TNFα treatment led to a 60% reduction in cytochrome *c* oxidase activity and decreased the mitochondrial membrane potential by more than 60% of the cellular ATP content [12••]. In this study, TNFα decreased ATP concentration in a time-dependent fashion, and cells were almost energy depleted after 30 min.

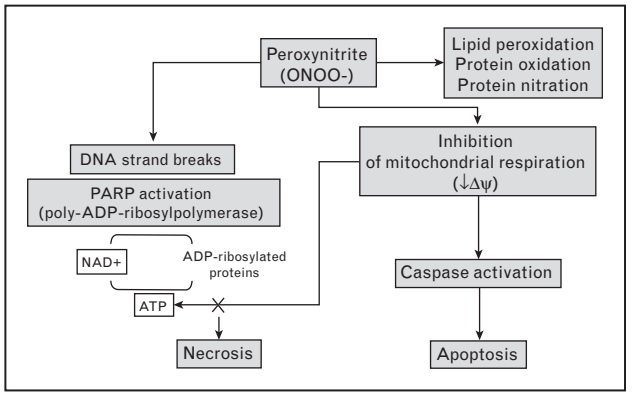
ROS and reactive nitrogen species (RNS) have several potentially important effects on mitochondrial function [9]. It is well established that nitric oxide is able to inhibit mitochondrial electron transport by decreasing the activity of cytochrome *c* oxidase (nitric oxide binds to ferrocytochrome a_3) [13]. The onset of this inhibition is very fast (inhibition of isolated cytochrome *c* oxidase ≈10 s), and nitric oxide competes with oxygen for the ferrocytochrome a_3 site. This suggests that under physiological conditions, when the oxygen concentration is low, nanomolar concentrations of nitric oxide can effectively act as a regulator of the mitochondrial respiratory chain to induce reversible inhibition of this chain. In this context, nitric oxide production during hypoxia may lower mitochondrial respiration as an adaptation to lower oxygen availability [8,14]. Decreased oxygen consumption was confirmed by inhibiting nitric oxide production in a model of rat peritonitis, suggesting an inhibition of respiratory chain activity in sepsis mediated by nitric oxide [15••]. However, higher nitric oxide concentrations result in irreversible cessation of the mitochondrial respiratory chain. When nitric oxide is in the high nanomolar range, it may outcompete superoxide dismutase (SOD) and react with the superoxide anion ($O_2^{\bullet -}$) to generate $ONOO^-$ ($O_2^{\bullet -}$ reacts with nitric oxide at a signicantly faster rate than with SOD, $k = 6.7 \times 10^9$ mol/l/s). Under proinflammatory conditions, simultaneous production of $O_2^{\bullet -}$ and nitric oxide can be strongly activated to increase production by 1000-fold, which will increase the formation of peroxynitrite by 1 000 000-fold [2]. Thus, even modest increases in the production of $O_2^{\bullet -}$ and nitric oxide will greatly stimulate the formation of peroxynitrite. This reaction is associated with a decrease in nitric oxide availability. A large body of evidence supports a key role of $ONOO^-$ in cell cytotoxicity [2,4,16]. The half-life of $ONOO^-$ is short (10–20 ms), but sufficient to cross biological membranes. Thus, $ONOO^-$ diffuses and reacts within one to two cell diameters. Peroxynitrite can react with most of the components of the electron transport chain, including complexes I and III [2,4].

Peroxynitrite may reach mitochondria from extramitochondrial compartments or may be directly produced within the mitochondria. Indeed, mitochondria can produce nitric oxide via the activity of a Ca^{2+}-sensitive

mitochondrial nitric oxide synthase (mtNOS) and superoxide due to the natural leak of electrons from the mitochondrial respiratory chain. Peroxynitrite may mediate apoptosis by permeabilization of the outer membrane. Mitochondrial outer membrane permeabilization allows the efflux of various proapoptotic signalling molecules, which promote cell death. Mitochondrial outer membrane permeabilization also facilitates the mitochondrial permeability transition (MPT). MPT describes the permeabilization of the inner mitochondrial membrane. The permeability transition pore results in the dissipation of mitochondrial membrane potential, yielding a cessation of electron transfer and ATP production [17,18]. In addition, $ONOO^-$ can directly oxidize low-molecular weight thiols, most notably reduced glutathione (GSH), which plays a major role in the cellular defence against oxidative stress. Moreover, $ONOO^-$ may induce DNA damage with activation of the DNA repair enzyme PARP-1. Upon severe DNA injury, overactivation of PARP-1 depletes the cellular stores of NAD^+, an essential cofactor of the glycolytic pathway, the TCA and the mitochondrial electron transport chain. As a result, the loss of NAD^+ leads to a marked decrease in the cellular pools of ATP, resulting in cellular dysfunction and cell death ('suicide hypothesis' after irreversible DNA injury) (Fig. 2). This point has been clearly demonstrated by Szabo *et al.* [19] in vascular smooth muscle cells exposed either to lipopolysaccharide (LPS) or interferon-gamma.

Catecholamines should be mentioned as they are the primary symptomatic treatment currently used for septic shock patients. Regueira *et al.* [20•] observed an amelioration of respiratory rate in liver at complexes I and II by using norepinephrine in a model of endotoxic shock in pigs. This amelioration was independent of hepatic blood flow modifications, and it suggests an action of catecholamine on mitochondrial respiratory chain complex activity. The mechanism remains to be elucidated.

Figure 2 Mechanisms of peroxynitrite-mediated cell death



NAD, nicotine adenine dinucleotide; PARP, poly-ADP-ribosylpolymerase.

Animal models of sepsis and mitochondrial function

Mitochondrial ultrastructural abnormalities have been described in numerous septic shock models [21–24]. For example, Welty-Wolf *et al.* [23] described ultrastructural mitochondrial injuries in skeletal muscle (distorted cristae, electron lucent areas within the matrix and fragmented inner membrane) in baboons 12 h after *Escherichia coli* injection (10^{10} CFU/kg). In a feline model of acute endotoxaemia, Crouser *et al.* [24] observed significant alterations of mitochondrial ultrastructure in liver samples (obtained 4 h after LPS injection), with mild to moderate mitochondrial swelling. Interestingly, despite the maintenance of tissue oxygen availability, these authors found a reduction of mitochondrial respiration, especially at complex IV (40% inhibition), and a partial uncoupling of mitochondrial oxidative phosphorylation. Finally, a significant correlation was demonstrated between the severity of ultrastructural injury and the magnitude of mitochondrial respiratory dysfunction. These mitochondrial injuries were prevented by pretreatment with cyclosporin A, a potent inhibitor of the MPT [18]. Other studies in septic animal models have reported significant decreases in mitochondrial oxidative phosphorylation, mostly at the level of complexes I, II and IV [3,25–28,29•,30•,31,32,33•]. However, other studies have found unaltered or increased mitochondrial function in septic models [34–38]. These variable findings may result from differences in species, the model, the severity of the induced sepsis, the degree of resuscitation or the timing of the analysis of mitochondrial function. Regarding this last point, more consistent findings of decreased function were reported in long-term models (≥24 h). In a long-term, fluid-resuscitated, faecal peritonitis rodent model, Brealey *et al.* [39] found that the severity of organ dysfunction and poor outcome were associated with nitric oxide overproduction and increasing mitochondrial dysfunction. The contribution of this study is important because this model can be considered as a representative model of human sepsis with 40% mortality and the development of organ failures. In this study, complex II, III and IV activities remained unchanged over time and with sepsis in both skeletal muscle and liver. Both skeletal muscle and liver complex I activity fell at 24 h (liver and muscle) and 48 h (muscle), with increasing disease severity in the septic rats and significant reduction of liver and muscle ATP concentrations. Remarkably, the authors found only mild or focal histological abnormalities. Neither apoptosis nor necrosis was a major feature, suggesting the possibility of an adaptive programmed shutdown of cellular function [39]. This result is in agreement with those reported by Hotchkiss *et al.* [40]. If cell death is not a major feature of sepsis, it can be postulated that the organ-system

dysfunction of sepsis and related inflammatory states represent a multiorgan hibernation-like state. However, as mentioned by Singer [41], although hibernation is adaptive and potentially protective during ischaemia and hypoxia, it may be pathologic during sepsis if persistent. Callahan and Supinski [42] reported a downregulation of genes encoding electron transport chain components and phosphofructokinase (PFK) (rate-limiting enzyme for glycolysis) that correlated with reductions in the expression levels of electron transport chain subunits, possibly explaining decreased respiratory chain activity [43].

Notably, mitochondrial regeneration, termed biogenesis, provides new functional mitochondria. This phenomenon could be implicated in the recovery from sepsis after the critical period. It seems to be triggered by nitric oxide production and mitochondrial DNA oxidative damage [44,45]. A recent study by Haden *et al.* [46••] demonstrated the tight association of mitochondrial biogenesis and the restoration of oxidative metabolism during the late phase of sepsis in a murine model of peritonitis.

Septic patients and mitochondrial function

Few human studies have examined mitochondrial function during sepsis. After in-vitro incubation of human umbilical endothelial cells with serum from septic shock patients, Boulos *et al.* [47] observed a significant depression of endothelial cell mitochondrial respiration (>60%). This decrease was prevented by pretreatment with 3-aminobenzamide, a poly(ADP-ribose) synthase inhibitor, or *NG*-methyl-L-arginine, a nonspecific NOS inhibitor. These data suggest that nitric oxide and poly(ADP-ribose) synthase activation may play an important role in the inhibition of mitochondrial respiration during septic shock. This result supports the hypothesized role of RNS. Nitric oxide interacts with superoxide to form peroxynitrite and impairs mitochondrial respiration in sepsis. Peroxynitrite may also cause DNA strand breakage, thereby activating PARP and depleting ATP.

Brealey *et al.* [48] obtained muscular biopsies from 28 patients in septic shock. During the first 24 h of patient management, the authors observed a decrease in complex I activity and decreased muscular ATP content in non-surviving patients as compared to a control group of patients undergoing elective hip surgery. Complex I activity had a significant inverse correlation with the severity of the septic shock (norepinephrine requirements) and nitrite/nitrate concentrations. There was a significant positive correlation of complex I activity with reduced glutathione concentrations (antioxidant depletion) and ATP.

Vanhorebeek *et al.* [49] described the ultrastructural morphology of mitochondria from liver and skeletal muscle biopsies obtained from 20 patients who died in ICUs, mainly from sepsis. Hypertrophic mitochondria, with increased numbers of abnormal and irregular cristae, were observed on liver biopsies, as well as a decrease in complex I and IV activity. No morphological or functional changes were confirmed in skeletal muscle. Liver alterations were prevented by intensive insulin therapy [49].

In 10 septic patients with at least two organ failures, Fredriksson *et al.* [50] observed a decrease in the activity of citrate synthase and complexes I and IV in intercostal and leg muscles. However, the activities of complexes I and IV did not seem modified when these activities were compared with the activity of citrate synthase. Thus, the authors suggested a decrease in the number of mitochondria (a two-fold decrease in mitochondrial content) in these patients with MOF. Thus, this study demonstrates a decrease in the number of mitochondria rather than a functional change in mitochondrial function. A decrease in ATP concentrations and a rise in lactate levels (increased anaerobic energy) were observed in leg muscles, but not in intercostal muscles. Moreover, these authors recently demonstrated that this reduced mitochondrial content is not associated with a global failure in mitochondrial biogenesis, as both in-vivo protein synthesis and mitochondrial-related mRNA abundance were sustained [51]. However, the loss of coordination among key elements of mitochondrial biogenesis was apparent, resulting in activation of some specific mitochondrial matrix proteases [51].

Conclusion

Changes in mitochondrial function during sepsis appear to play a key role in the pathogenesis of organ failure and in the recovery of septic patients. Hypoxia and a number of the mediators implicated in sepsis and in the associated systemic inflammatory response have been demonstrated to directly impair mitochondrial function. A large body of evidence supports a key role of $ONOO^-$ in mitochondrial dysfunction; more human studies are necessary to clarify mitochondrial dysfunction in the various phases of sepsis (early and late phase – MOF) before testing therapeutic strategies targeting mitochondria.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 314–315).

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