

Mitochondria — key roles in sepsis*

Mitochondrie — rôles clés dans le sepsis

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Abstract The pathophysiological mechanisms underpinning the development of, and recovery from, sepsis-induced organ failure require further delineation. Mitochondrial dysfunction may well play a key role. This review will therefore consider mitochondria's function in normal physiology, evidence linking bioenergetic alterations to organ dysfunction after severe and prolonged inflammation, and potential therapeutic strategies that may be applied.

Keywords Mitochondria · Sepsis · Multi-organ failure · Pathophysiology · Therapeutics

Résumé Au cours du sepsis, les mécanismes physiopathologiques sous-tendant le développement et la récupération des dysfonctions d'organe sont encore mal compris. La dysfonction mitochondriale pourrait jouer un rôle majeur. Cette mise au point décrit la physiologie normale des mitochondries, les arguments reliant l'altération bioénergétique mitochondriale aux dysfonctions d'organes après des périodes d'inflammation sévère et prolongée. Enfin, les perspectives thérapeutiques envisageables seront abordées.

Mots clés Mitochondrie · Sepsis · Défaillance multiviscérale · Physiopathologie · Traitement

Introduction

The systemic inflammatory response to infection and severe sepsis may progress to multi-organ failure and carries with it

a high morbidity and mortality [1,2]. Precise pathophysiological mechanisms remain elusive. The contribution of an impaired circulation leading to tissue hypoperfusion is well established, but an important role of bioenergetic dysfunction is also emerging. An association is found between the degree of mitochondrial dysfunction and outcomes in patients with sepsis-induced multi-organ failure [3]. While this does not confirm cause-and-effect, it does nevertheless suggest a new route for therapeutic intervention focused on either protection or acceleration of the recovery process. This is particularly pertinent in light of the multiple trial failures related to immunomodulatory therapies. This overview will provide an insight into mitochondrial biology, its relevance to sepsis, and possible therapeutic opportunities that emerge.

Mitochondria in health

The physiological roles of mitochondria

Virtually all cell types possess mitochondria, the notable exception being erythrocytes. Most cell types rely upon mitochondria to provide the bulk of the energy requirement [in the currency of adenosine triphosphate (ATP)] needed to enable normal cellular functioning, and to be able to respond to any intrinsic or extrinsic physiological or pathophysiological stress. Mitochondria utilize approximately 98% of total body oxygen consumption and generate 90% of human power by proton transfer through ATP [4,5]. Proton transfer occurs through a series of enzymatic steps that occur within the electron transfer chain located in the inner mitochondrial membrane, leading to oxidative phosphorylation of adenosine diphosphate (ADP).

Mitochondria also have roles in cell signaling and triggering of cell death pathways. The co-ordinated release of cytochrome c from mitochondria activates intrinsic pathways of programmed cell death, apoptosis, whereas necrosis can be triggered when the ATP level falls below a certain threshold. Reactive oxygen species (ROS), produced as a 'by-product'

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of oxidative phosphorylation, plays an important role in maintaining vascular tone, oxygen sensing and, possibly, glucose regulation during skeletal muscle contraction [6]. Indeed, mitochondria are the predominant source of ROS production within the body. The three endogenous gases—nitric oxide (NO), carbon monoxide, and hydrogen sulphide—are also important regulators of mitochondrial signaling in health. Their higher concentrations in disease states such as sepsis have progressively greater inhibitory effects on mitochondrial respiration and ROS generation.

Other functions of the mitochondrion include the site of production (e.g. cortisol) or action (e.g. triiodothyronine) of many hormones, the biosynthesis of heme and iron-sulphur clusters, and heat generation.

Energy generation by mitochondria

In the cytosol, glucose is metabolized to pyruvate by glycolysis. Pyruvate is transported into the mitochondria, through an antiporter with hydroxide ions, for conversion by pyruvate dehydrogenase to acetyl coenzyme A (acetyl CoA). Fatty acids are esterified to fatty acyl coA in the cytosol. Medium chain fatty acids (C8 to 10) can diffuse through the mitochondrial membrane, whereas long chain fatty acids rely on the carnitine pathway. Carnitine palmitoyltransferase (CPT)-1 in the mitochondrial membrane exchanges carnitine for CoA attached to the fatty acid and a conjugate form. This conjugate is transported into the matrix where CPT-2 breaks the conjugate, allowing the fatty acid CoA to reform and undergo beta-oxidation within the mitochondrial matrix.

Acetyl-CoA feeds into the tricarboxylic acid (TCA) or Krebs' cycle, generating nicotinamide and flavin adenine dinucleotide (NADH and FADH₂). As summarized in the Figure 1, NADH passes electrons to complex I (NADH dehydrogenase) of the electron transport chain, becoming oxidized to NAD⁺, while FADH₂ donates electrons to complex II (succinate dehydrogenase). Electrons are then passed onto ubiquinone (coenzyme Q), before moving on to complex III (cytochrome bc1 complex), cytochrome c, and then complex IV (cytochrome a, a₃, cytochrome c oxidase). Oxygen is the terminal electron acceptor of the chain at this enzyme complex, being reduced to water. If oxygen is prematurely or incompletely reduced, an increase in superoxide radical (ROS) production occurs, particularly at complexes III and I. The mitochondrion deals with ROS production through its large array of antioxidants, such as superoxide dismutase, catalase, glutathione peroxidase, and peroxiredoxins. This can, however, be overwhelmed in pathological processes generating large amounts of ROS.

As the electrons transfer down the chain, protons move across the inner mitochondrial membrane generating an electrochemical gradient. This 'chemiosmotic gradient' provides the energy to drive ATP synthase (complex V) to produce ATP from ADP. ATP is transported out of the mitochondria and ADP moves back in via the adenine nucleotide translocase (ANT).

The process is not 100% 'efficient' in terms of ATP production. Some of the proton gradient is dissipated before oxidative phosphorylation is complete. This 'uncoupling' is due to a variety of mechanisms, including specialized uncoupling proteins within the inner mitochondrial membrane.

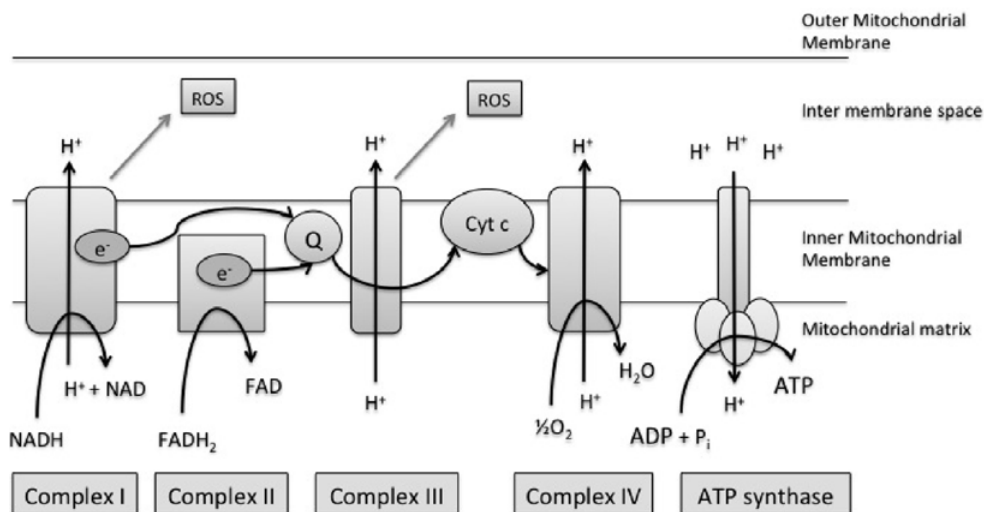


Fig. 1 The mitochondrial electron transport chain. A number of redox reactions enable the generation of a proton (H⁺) gradient to generate ATP. Electron (e⁻) transfer is shown along with main sites of reactive oxygen species (ROS) production. NADH: nicotinamide adenine dinucleotide; FADH₁: flavin adenine dinucleotide; Q: ubiquinone, or coenzyme Q; Cyt c: cytochrome c; ADP: adenosine diphosphate; P_i: Phosphate; ATP: adenosine triphosphate

Uncoupling has particular importance in heat generation and hibernation.

The mitochondrial life cycle

Mitochondrial biogenesis is the production of new mitochondria/mitochondrial protein occurring with, and independent of, cell mitosis. In the non-dividing cell, biogenesis improves the capacity for energy production if energy demands increase. The process involves production of mitochondrial proteins encoded either by the cell nucleus with subsequent import and integration into the mitochondria, or via mitochondrial deoxyribonucleic acid (DNA) which encodes 13 proteins that are mainly situated within the oxidative phosphorylation pathway.

A key player that orchestrates mitochondrial biogenesis is the peroxisome proliferator-activated receptor gamma coactivator (PGC)-1alpha, a co-activator of nuclear transcription factors such as nuclear respiratory factors 1 and 2 (NRF-1 and -2) that upregulate nuclear production of mitochondrial proteins [7–9]. NRF-1 also increases expression of Tfam (transcription factor A for the mitochondrion), which, once transported into the mitochondrion, stimulates transcription of mitochondrial DNA [10].

Numerous influences on PGC-1alpha occur in response to physiological (e.g. exercise) and pathophysiological (e.g. hypoxia) stimuli. AMP (adenosine monophosphate)/ATP ratios are known inducers in brown adipose tissue and liver via beta-adrenergic/cyclic AMP pathways [8]. In skeletal muscle, the calcineurin A, CaMK (Ca²⁺/calmodulin-dependent kinase), p38 MAPK (mitogen-activated protein kinase), and AMPK (AMP-activated protein kinase) pathways have been implicated [11–14], as well as sirtuins (enzyme deacylators) [15]. Negative regulators influence energy balance via endogenous RIP140 (nuclear receptor-interacting protein 1), the p160 myb-binding protein, and the GCN5 acetyltransferase complex [16–18]. Interaction also occurs with thyroid, glucocorticoid, estrogen, and estrogen-related receptors [19].

An association is also emerging between NO and mitochondrial biogenesis. Endogenous NO upregulates PGC-1alpha mRNA expression [20]. NO donors can increase mitochondrial DNA in cell cultures, while mice depleted of endothelial nitric oxide synthase show reduced mitochondrial biogenesis, mitochondrial mass, basal oxygen consumption, and ATP levels [21].

During their lifetime, mitochondria undergo numerous morphological changes during fusion and fission events. These mitochondrial dynamics are primarily affected by GTPases; this links with roles in cell division and proliferation as well as self-directed removal of damaged or surplus mitochondria, a process known as mitophagy. Mitofusin-2 and OPA-1 (optic atrophy-1), proteins driving fusion

events, and DRP-1 (dynamin-related protein-1), a protein that influences fission, have been associated with altered mitochondrial membrane potential and reduced oxygen consumption [22].

Mitochondrial variation in tissue types

Mitochondria have an intricate, sophisticated, and complex purpose within the cell and in overall tissue physiology. In skeletal muscle, mitochondria exist as reticular networks located near the sarcolemma and also within muscle fibres [23]. Heart and skeletal muscles have more mitochondrial content, including respiratory chain subunits, than in liver, kidney, and brain tissue. Heart muscle mitochondria also have more cristae (folds) per surface area [24].

Morphological variation is seen in human hepatocytes, neuronal cells, and umbilical vein endothelial cells in terms of shape, number of cristae, and distribution within the cell [25]. Even within the same cell type, variable membrane potentials are seen, being higher in peripherally located mitochondria and possibly related to calcium sequestration. It is likely that mitochondrial antioxidant capacity also varies between cell types, making some more vulnerable to oxidative stress.

Mitochondrial dysfunction in sepsis and multi-organ failure

The systemic inflammatory response syndrome is triggered by microbial antigens (sepsis) or other factors (e.g. trauma, hemorrhage, burn injury). Micro-organisms or their constituents are recognized by specialized pattern recognition receptors (PRRs) situated either on or inside immune, endothelial, and epithelial cells. The best characterized set of PRRs is the toll-like receptors (TLRs). PRRs recognize both pathogen-associated molecular patterns (PAMPs) on invading organisms, as well as host-derived danger-associated molecular patterns (DAMPs) released in response to stress, tissue injury, or cell death. Mitochondria released into the circulation due to tissue damage act as a DAMP.

The subsequent release of pro-inflammatory cytokines and other mediators leads to activation or suppression of multiple pathways involving cardiovascular, immunological, hormonal, coagulation, metabolic, and bioenergetic systems. This leads to organ dysfunction that is manifest clinically as impaired physiological or biochemical activity of that organ. Severe dysfunction leads to a state of failure that may require significant levels of pharmacological or mechanical organ support to maintain an acceptable level of homeostasis compatible with continued survival.

Impaired perfusion early in the septic process (due to intrinsic and extrinsic fluid losses and decreased intake)

can lead to tissue hypoxia and amplification of the systemic inflammatory response. While early and aggressive correction of the tissue oxygen debt may prove clinically beneficial [26], attempts to correct cellular hypoxia when organ dysfunction was established proved fruitless, or even harmful [27,28]. This suggests an important temporal component to the pathophysiology of sepsis. Indeed, it appears that the condition shifts from hypoxia to dysoxia. In other words, there is availability yet an inability of cells and tissues to use oxygen. This was termed cytopathic hypoxia but, more accurately, should be labeled cytopathic dysoxia [29]. The data in support of this are the findings of (i) progressive reductions in oxygen consumption with increasing sepsis severity [30]; yet (ii) maintained, or even elevated levels of oxygen at the tissue level found in both humans and animal models [31,32]; and (iii) a remarkable lack (or minimal presence) of cell death, apoptotic or necrotic, in the failed organs that supports the notion that oxygen lack and tissue hypoperfusion is a continuing and pathophysiologically significant problem. The parallel findings of oxygen availability yet decreased utilization infer a new metabolic steady state has been achieved. As mitochondria are the predominant utilizers of oxygen, their likely importance in septic multi-organ failure should be considered. Although there may be an as yet unrecognized mechanism invoked by prolonged and severe inflammation that directly inhibits metabolism, there are well-defined mechanisms by which ATP production is impaired, leading to a secondary shutdown of metabolism, and thus cell functioning, akin to hibernation or estivation. Inflammatory processes that directly target mitochondria work via inhibition of their activity, through direct damage from reactive species, and by a decrease in biogenesis through decreased transcripts of genes encoding for respiratory complex proteins.

Respiratory chain activity

Animal models of sepsis have been predominantly used to study mitochondrial function. Short-term models lasting several hours demonstrate highly variable results of sepsis on oxidative phosphorylation. As previously reviewed, increased, unchanged, or reduced respiratory activity have all been reported [33]. This may reflect differences in species, in tissues studied, in the forms and severity of the septic insult administered, and in the *ex vivo* techniques used to prepare samples and measure activity. The majority of long-term sepsis models (lasting >16 hours) do however demonstrate changes in respiratory chain activity, morphology, or mitochondrial mass. This has been found in various tissues taken from different animal species, e.g. heart, muscle and liver, and brain [34–37]. Patient samples, including muscle, diaphragm, liver, and monocytes, have all shown decreased mitochondrial enzyme activity, membrane poten-

tial, and histological changes [3,38–42]. Mitochondrial respiratory complex I is most often found to be inhibited. We performed vastus lateralis skeletal muscle biopsies in patients soon after admission to intensive care with septic shock [3]. Associations were found between complex I dysfunction, ATP depletion, glutathione depletion, excess NO production, and mortality. A Swedish study reported reduced mitochondrial enzyme activity in biopsies taken from leg and intercostal muscles of critically ill patients, whereas mitochondrial activity was increased in leg skeletal muscle of biopsy taken two hours after an injection of endotoxin [43]. This emphasizes the varying pathophysiological changes that occur over the course of sepsis.

Impact of altered redox state

Sepsis produces a large amount of ROS and reactive nitrogen species (RNS). This is partly due to activation of immune and endothelial cells, but also due to increased production of mitochondrial superoxide. NO, itself a reversible inhibitor of mitochondrial respiratory complex IV, can react with superoxide to form peroxynitrite which has a more prolonged and potentially irreversible inhibitory effect on respiratory complexes via nitration, particularly affecting complex I [44,45]. Direct damage to mitochondrial DNA may also produce ROS [46].

Altered substrate provision

Early work did not demonstrate any alterations in Krebs' cycle intermediaries in cardiac tissue of an animal model [47]. However, Vary reported inhibition of pyruvate dehydrogenase, preventing pyruvate entry into the mitochondrion and thereby leading to an increase in lactate levels [48]. More recently, Mason and Stofan showed reduced activity of the Krebs' cycle enzyme, aconitase, in association with reduced mitochondrial respiration [49].

Altered mitochondrial biogenesis

Calvano et al administered endotoxin to healthy volunteers and performed transcriptomics on white cells sampled thereafter [50]. Notably, they described decreased expression of genes encoding for mitochondrial respiratory proteins. We reported a similar finding in the transcriptome of muscle biopsies taken from critically ill patients, with a significantly lower gene transcript in those patients who subsequently died [40]. This also correlated with a decrease in complex I activity. Furthermore, PGC-1 α transcript levels were raised above normal in those patients who survived, whereas levels in non-survivors were similar to those in healthy control samples. Another study in patients with established septic multi-organ failure in the ICU showed upregulation

of NRF2alpha (nuclear factor (erythroid-derived 2)-like 2alpha)/GABP (GA-binding protein) genes, suggestive of biogenesis. Here, protein synthesis was preserved within the mitochondria, as were genes related to energy metabolism. Interestingly, when considering genes associated with muscle turnover, presence of those linked with catabolism, atrogens, was noted [51]. In a long-term mouse model of *S. aureus* peritonitis, organ dysfunction and clinical illness was accompanied by a fall in metabolic rate and a decrease in mitochondrial mass. Recovery of metabolic activity and organ function, and clinical improvement were preceded by an upregulation of markers of mitochondrial biogenesis such as PGC-1alpha, Tfam (transcription factor A, mitochondrial) and NRF-1, and suppression of RIP140, an endogenous co-repressor [52]. In a recent study of endotoxic mice, locomotor muscles were found to be more susceptible to mitochondrial injury compared to ventilatory muscles, with decreased biogenesis and an increase in autophagy [53].

Putative mitochondrially-targeted therapies in sepsis-induced multi-organ failure

A variety of strategies are available, for example, to protect mitochondria from injury, or to increase biogenesis with the aim of accelerating recovery.

Antioxidants

Preclinical trials using antioxidants show promise. In a murine model using N-acetylcysteine and deferoxamine after cecal ligation and puncture (CLP) induction, a significant improvement was seen in oxidant profile and mortality [54]. Antioxidants targeted specifically to mitochondria such as MitoQ and MitoVitE show improved mitochondrial activity and a reduction in organ failure severity [55–57]. Melatonin has antioxidant effects and has also improved redox outcomes and mortality in animal models [58]. Notably, its circadian variation in plasma is altered in critical illness [59]. Its use in neonates with sepsis has also shown benefit with reduced oxidative stress; however, larger trials in adults are lacking [60]. Exploitation of antioxidant therapies has been considered in detail elsewhere [61].

Inducing ‘suspended animation’

Decreasing metabolic rate is an established therapeutic practice achieved by inducing therapeutic hypothermia in cardiac arrest survivors and in infants with anoxic encephalopathy. Carbon monoxide and hydrogen sulphide are potential gasotransmitters that may have similar effects to induce the hibernation state alluded to earlier. While high levels of carbon monoxide can dangerously inhibit complex IV, at lower

concentrations it enabled tissue protection in animal models of sepsis [62]. A water-soluble carbon monoxide releasing agent given after induction of sepsis in a mouse model improved survival rates; this was accompanied by an increase in mitochondrial respiration, in PGC-1alpha expression, and in mitochondrial DNA copy number [63]. Carbon monoxide is released endogenously after activation on heme oxygenase (HO)-1. Induction of HO-1 in sepsis models has shown an action through NRF-2, linking it to mitochondrial biogenesis [64,65].

Hydrogen sulphide, also an inhibitor of complex IV, reduced oxygen consumption in mice and induced a reversible state of ‘suspended animation’ [66]. Pre-treatment has shown a survival benefit in lethal hypoxemia and hemorrhage [67]. There is potential utility in sepsis with improved neutrophil migration and decreased mortality reported through its use in septic mice [67,68].

Stimulating mitochondrial biogenesis

The role of biogenesis and its association with survival benefits in critically ill patients opens new avenues for research into using it as a treatment modality. Its association with the HO-1/carbon monoxide pathway has been mentioned earlier. Recently, Thomas et al used a recombinant human Tfam and found improved redox and mitochondrial activity profiles in both cultured mouse fibroblasts and a murine model of sepsis, in which survival was also enhanced [69]. Their murine model of Parkinson’s disease also showed recombinant human mitochondrial transcription factor A protein (rhTFAM) improved motor function. This may have implications in severe sepsis as muscle wasting occurs early and with significant subsequent impact on return to normal function [70–72].

Conclusion

In summary, there is significant evidence that implicates mitochondrial dysfunction in septic organ dysfunction. Whether this is causative or epiphenomenal is less clear. However, survivors have better preservation of ATP and biogenesis markers. Multi-organ failure may represent a mechanism through which the likelihood of eventual survival is enhanced in those hardy enough to survive. Cells may enter a ‘hibernating’ state in the face of overwhelming inflammation. This adaptive strategy may have evolved in response to effects on cellular bioenergetics.

Conflict of interest: None.

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