

Review

Bench-to-bedside review: Potential strategies to protect or reverse mitochondrial dysfunction in sepsis-induced organ failureAlessandro Protti^{1,2} and Mervyn Singer¹¹Bloomsbury Institute of Intensive Care Medicine, Wolfson Institute for Biomedical Research and Department of Medicine, University College London, London, UK²Istituto di Anestesia e Rianimazione, Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena – IRCCS, Milan, ItalyCorresponding author: Mervyn Singer, m.singer@ucl.ac.uk

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Critical Care 2006, **10**:228 (doi:10.1186/cc5014)**Abstract**

The pathogenesis of sepsis-induced multiple organ failure may crucially depend on the development of mitochondrial dysfunction and consequent cellular energetic failure. According to this hypothesis, interventions aimed at preventing or reversing mitochondrial damage may have major clinical relevance, although the timing of such interventions will be critical to both ensuring benefit and avoiding harm. Early correction of tissue hypoxia, strict control of glycaemia, and modulation of oxidative and nitrosative stress may afford protection during the initial, acute systemic inflammatory response. The regulated induction of a hypometabolic state resembling hibernation may protect the cells from dying once energy failure has developed, allowing the possibility of functional recovery. Repair of damaged organelles through stimulation of mitochondrial biogenesis and reactivation of cellular metabolism may accelerate resolution of the multiple organ failure syndrome.

Introduction

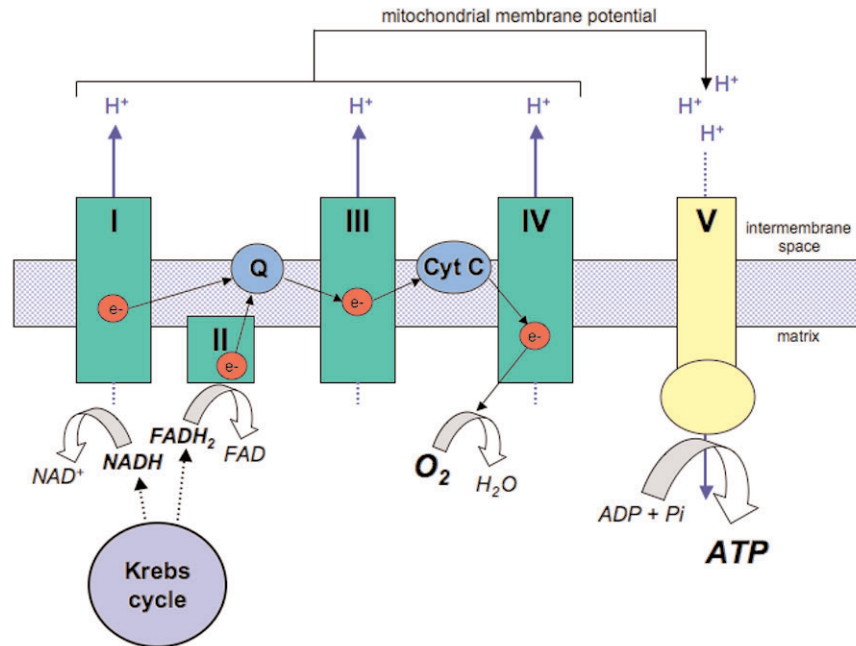
Sepsis is the systemic inflammatory response to infection and represents a major cause of morbidity and mortality in patients admitted to intensive care units (ICUs) [1]. However, despite decades of research, the pathophysiology of sepsis remains incompletely understood. A critical limitation of tissue oxygen delivery due to macrocirculatory or microcirculatory failure may play a role, especially in the early phase of the disease process before resuscitation has been initiated. Nonetheless, a growing body of evidence suggests that multiple organ failure (MOF) may develop during sepsis mainly as a consequence of impaired cellular oxygen utilization. Supportive data in patients include the following findings: total body oxygen consumption falls progressively with increasing severity of sepsis [2]; skeletal muscle tissue oxygen tension is abnormally high but normalizes during the recovery phase [3]; necrotic and apoptotic cell death is minimal, if it occurs at all, in most dysfunctioning organs [4]; and organs with limited regenerative capabilities, such as kidney, are usually able to

recover to such an extent that long-term support is usually not needed [5]. Sepsis-induced MOF may thus be related to a potentially reversible impairment in cellular function rather than any permanent structural damage.

The mitochondrion is the powerhouse of the cell [6]. Cellular energy production depends on three interconnected pathways: glycolysis within the cytoplasm, the Krebs cycle and the electron transport chain within the mitochondria (Figure 1). Glycolysis is a sequence of reactions that degrade glucose to pyruvate. In the presence of oxygen, pyruvate and other fuel molecules such as fatty acids and amino acids enter the mitochondria, where they are completely oxidized within the Krebs cycle. The reduced nicotinamide (NADH) and flavin (FADH₂) adenine dinucleotides transfer electrons to the respiratory enzyme complexes located in the inner mitochondrial membrane (electron transport chain) for the process of ATP generation by oxidative phosphorylation. NADH donates electrons specifically to complex I whereas FADH₂ reduces complex II. The electrons then flow via coenzyme Q (ubiquinone) to complex III, and are then transported via cytochrome C to reach complex IV (cytochrome oxidase). At this final stage, oxygen is reduced to water. Electron transfer through complexes I, III and IV generates a proton gradient across the inner mitochondrial membrane that is used by ATP synthase (complex V) to generate energy by phosphorylating ADP. The complete oxidation of one molecule of glucose yields 30-36 molecules of ATP, two of which come from glycolysis and two from the Krebs cycle. Glycolysis can also occur in the absence of oxygen. However, when oxygen is lacking, pyruvate can no longer be further oxidized within the mitochondria and is thus metabolized to lactate within the cytoplasm. Glycolysis represents a much less efficient metabolic pathway compared with the Krebs cycle and oxidative phosphorylation, because

FADH₂ = flavin adenine dinucleotide, reduced; ICU = intensive care unit; MOF = multiple organ failure; NADH = nicotinamide adenine dinucleotide, reduced; NO = nitric oxide; NOS = nitric oxide synthase; T₃ = tri-iodothyronine.

Figure 1



Schematic representation of oxidative phosphorylation within the mitochondria. Electrons donated from NADH and FADH₂ pass down the electron transport chain with oxygen being the terminal acceptor at complex IV. This movement of electrons results in a shift of protons across the inner mitochondrial membrane, generating the energy necessary for ATP synthase to produce ATP from ADP. FADH₂, flavin adenine dinucleotide, reduced; NADH, nicotinamide adenine dinucleotide, reduced.

there is net synthesis of only two molecules of ATP per molecule of glucose [7].

Because mitochondria utilize more than 90% of total body oxygen consumption to produce ATP, the abnormalities in oxygen consumption described during sepsis are likely to be associated with evidence of mitochondrial dysfunction. Studies conducted during the early phase of sepsis (within the first few hours) have produced conflicting results. Nonetheless, mitochondrial structure and function were consistently shown to be impaired in a severity-dependent manner in animal models lasting at least 12-16 hours [8]. Of note, ATP levels were variably affected, depending on the balance between energy production and consumption, the model and possibly the tissue under investigation. In septic shock patients studied within 24 hours of ICU admission, the degree of skeletal muscle mitochondrial dysfunction was associated with the severity of the disease [9]. In this work, tissue ATP levels were significantly lower in nonsurvivors than in an orthopaedic surgical control population, but they were maintained in those who survived sepsis.

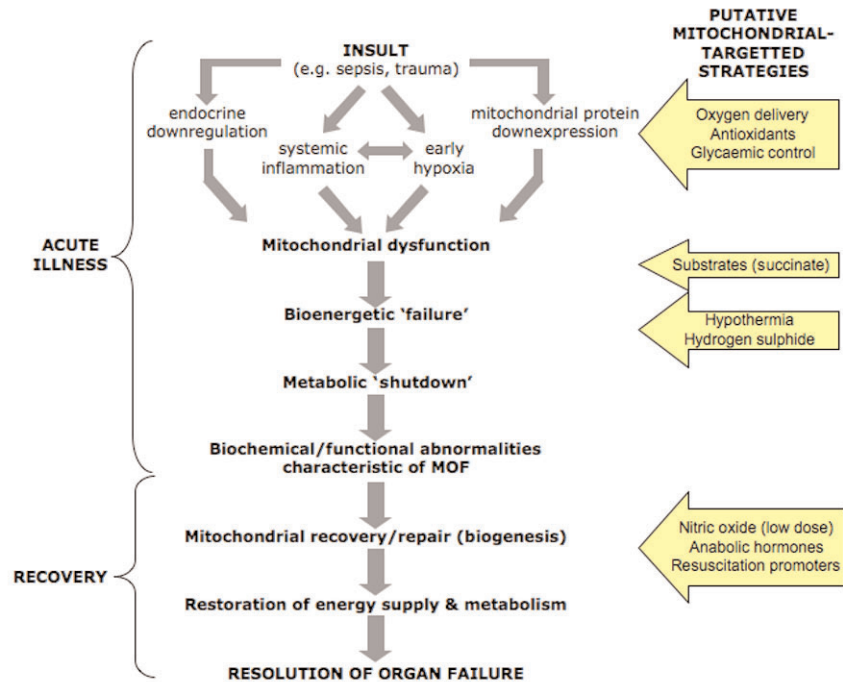
A reduction in energy consumption implies a reduction in cellular metabolism, which manifests clinically as organ dysfunction. Rather than being viewed negatively as 'failure', an alternative paradigm may be advanced whereby this metabolic shutdown represents an adaptive cellular strategy

[10]. In the face of persisting mitochondrial dysfunction and reduced ATP production, the cell may shift its focus to survival rather than aiming to continue normal functioning.

The pathogenesis of mitochondrial dysfunction during sepsis is likely to be highly complex. Nitric oxide (NO), reactive oxygen species and other inflammatory mediators are produced in excess and can directly inhibit mitochondrial respiration. NO competes with oxygen in binding to cytochrome oxidase (complex IV), thereby decreasing the activity of the enzyme. This will block the electron transport chain and lead to overproduction of superoxide. Superoxide will react with NO to generate peroxynitrite and other nitrogen species that are able to alter the structure and function of several other mitochondrial proteins, notably complex I [11]. Early cellular hypoxia may favour the competitive NO-mediated inhibition of cytochrome oxidase, contributing to the earlier, if not greater, development of mitochondrial dysfunction [12].

Endocrine changes that occur during sepsis are also likely to play a role. Among others, thyroid and sex hormones, insulin, glucocorticoids and leptin positively modulate mitochondrial energy production, protein synthesis and biogenesis [13-17]. Increased incidences of the low tri-iodothyronine (T₃) syndrome, hypogonadism, insulin resistance, adrenal insufficiency and decreased circulating leptin levels in nonsurvivors compared with survivors have been reported

Figure 2



Hypothesized role of mitochondria in the development of MOF and subsequent recovery. Potential therapeutic interventions are illustrated at the appropriate steps. MOF, multiple organ failure.

during prolonged sepsis and critical illness [18,19]. Accordingly, depletion of respiratory complex proteins has been described in the diaphragm in a rat model of sepsis [20].

A further mechanism could be represented by the down-regulated synthesis of new mitochondrial protein. In human volunteers, administration of bacterial endotoxin decreased blood leucocyte expression of mitochondrial respiratory chain complexes and ATP synthase genes [21].

Assuming that the pathogenesis of MOF during sepsis is contingent on development of mitochondrial dysfunction and cellular energetic failure, recovery is likely to occur when damaged organelles are repaired or replaced. Preliminary results have shown an association between progressive improvement in mitochondrial respiration and organ function in patients who survive their episode of septic shock [22].

Strategies aimed at preventing or reversing mitochondrial dysfunction and cellular energetic failure may thus represent a new therapeutic option in the treatment of sepsis (Figure 2).

Prevention and early reversal of mitochondrial dysfunction

Mitochondrial dysfunction in sepsis can occur even with aggressive fluid resuscitation [23] and adequate tissue oxygenation [24,25]. Derangement in liver metabolism possibly

due to mitochondrial damage was recently reported in a hyperdynamic, normotensive, mechanically ventilated, antibiotic-treated septic animal model, despite preserved microvascular perfusion [26]. Nonetheless, early cellular hypoxia can further limit aerobic production of ATP and contribute to the development of energy failure.

Optimization of oxygen delivery can ameliorate cellular energetic failure provided that mitochondria retain their ability to produce energy. Patients with severe sepsis or septic shock whose global oxygen delivery was optimized early after admission to an emergency room experienced better outcomes than did conventionally managed patients [27]. Conversely, no benefit [28] or even harm [29] was reported when a similar approach was adopted after admission to ICU, when organ failure had already become established. The same intervention, performed at different time points, had very different clinical impacts. In the early phase, when the cellular energetic machinery is still likely to be functional and oxygen supply may represent a limiting factor, reversal of tissue hypoxia may ameliorate the impending cellular energetic failure and reduce the incidence/severity of organ dysfunction. In a later phase, when mitochondrial damage has occurred and the cell has become intrinsically unable to utilize oxygen to produce ATP, a similar strategy may not provide any benefit. Lack of improvement in oxygen consumption despite a re-established oxygen supply has

been associated with unfavourable outcomes in patients with sepsis syndrome or septic shock [30].

Hyperglycaemia and insulin resistance are common among critically ill patients and represent an additional potential threat to mitochondrial integrity. Acute hyperglycaemia can dramatically increase the production of reactive oxygen species in normal bovine aortic endothelial cells [31]. Moreover, insulin stimulates mitochondrial protein synthesis and oxidative phosphorylation [15]. Maintenance of normoglycaemia with intensive insulin therapy during critical illness has been demonstrated to preserve hepatocyte mitochondrial ultrastructure and function [32] and improve outcome in both medical and surgical intensive care patients [33,34].

Reactive oxygen and nitrogen species are over-produced during sepsis, whereas mitochondrial antioxidants (reduced glutathione and manganese superoxide) are depleted. The membrane permeable glutathione ethyl ester can protect complex I from oxidative and nitrosative damage in an early phase [35]. Manganese-based superoxide dismutase mimetics may exert a similar protective effect, scavenging superoxide anions and preventing them from further reacting with NO to generate peroxynitrite within the mitochondria [36].

Prevention of cellular energetic failure in the presence of mitochondrial dysfunction

Once permanent mitochondrial dysfunction has developed, cellular optimization of any residual ability to produce energy and/or reduce metabolic requirements may prevent the ATP level from dropping below the threshold that stimulates initiation of cell death pathways.

Electron donors that are able to 'bypass' defective components of the respiratory chain may help in attaining the former objective. Within the inner mitochondrial membrane, complex II works in parallel with complex I, albeit to a lesser extent, transferring electrons from FADH₂ produced during the oxidation of succinate to coenzyme Q. Unlike complex I, the activity of complex II is relatively preserved during sepsis [9,23,37]. When complex I is inhibited, the administration of succinate may increase electron flow through the respiratory chain and thus increase generation of ATP, provided that any inhibition of the electron transport chain distal to complex II has not become rate-limiting. Preliminary data from our laboratory confirm this action. In two different animal models of sepsis, the infusion of succinate dimethyl ester prevented the fall in liver ATP content [38] and prolonged survival time [39].

Another possible strategy that could be pursued in the face of a severe and extended impairment in mitochondrial energy production is to reduce cellular energetic expenditure. Hibernating and aestivating animals reduce their metabolic rate in the face of climate change or drought. Similarly, oxygen-conforming organisms such as turtles and frogs can tolerate prolonged periods of hypoxia by suppressing ATP

turnover [40]. Humans do not hibernate or aestivate and have only a limited tolerance to inadequate oxygenation. Nonetheless, patients with chronic coronary artery disease frequently develop a myocardial contractile dysfunction – termed myocardial hibernation – that may represent an adaptive response to ischaemia, rather than depend on an ongoing energetic deficit, which will recover on restoration of adequate perfusion [41].

Mechanism(s) governing hibernation remain to be clarified. Carbon monoxide and NO may mediate the active decrease in energy demand that occurs in cells that lack oxygen [42,43]. The natural peptide 'hibernation induction trigger', its synthetic analogue [D-Ala², D-Leu⁶] enkephalin (DADLE) and other δ-opioids can also reduce cellular metabolism and protect organs against ischaemia [44]. Rapid induction of profound cerebral hypothermia in animals that do not normally hibernate may guarantee protection during prolonged cardio-circulatory arrest [45]. Mice exposed to hydrogen sulphide experience a dramatic decrease in their metabolic rate: within 6 hours, oxygen consumption and carbon dioxide production drop by around 90%, and body core temperature approaches that of the environment [46]. Such a suspended animation-like state fully reverses when the hydrogen sulphide is discontinued, without any permanent behavioural or functional damage. It is conceivable that, even during sepsis, induced hibernation may protect the organism from prolonged energetic failure and enable faster recovery on resolution of the inflammatory insult. Some caveats do need to be applied. For example, the hyperthermic response to infection activates the expression of cytoprotective heat shock proteins and may therefore be considered beneficial [47]. Therapeutic induction of hibernation may remove this intrinsic protective mechanism with potentially deleterious results.

The converse may also hold true. Premature stimulation of cellular metabolism before mitochondria have regained their ability to respond adequately in terms of energy production may lead to cellular compromise. Examples of harmful therapeutic approaches that may be invoked are the use of high-dose dobutamine [29], thyroxine [48] and growth hormone [49].

Resolution of mitochondrial dysfunction: arousal from 'hibernation'

Repair and replacement of damaged mitochondria are probably controlled at a transcriptional level, but proximal steps in the signalling pathway still need to be elucidated. NO was recently suggested to play a major role. Long-term exposure to a low concentration of the gas triggered expression of transcriptional factors that regulate mitochondrial proliferation and significantly increased mitochondrial mass in different cells in culture [50]. NO exerts different actions depending on the rate, amount and site of production. The large quantity synthesized by the inducible isoform of nitric oxide synthase (NOS) during the acute inflammatory response to sepsis blocks mitochondrial

respiration and can be cytotoxic. On the other hand, the smaller amounts of NO produced by the specific constitutive endothelial NOS may trigger mitochondrial biogenesis in a later phase. Nitration also dramatically accelerates mitochondrial protein turnover, from days to hours [51]. Taken together, these results suggest that recovery from mitochondrial dysfunction may depend on a NO-dependent signalling pathway. Specific inhibition of inducible NOS during sepsis may represent a potential therapeutic strategy [52-55], although dose selection will be critical. This is pertinent to the dose-related increase in mortality reported in a phase III trial of a nonspecific NOS inhibitor in septic shock patients [56]. Indeed, the overall negative outcome of this study camouflages the survival benefit seen with low doses.

Hormones may play an equally important role. Thyroid hormones stimulate mitochondrial activity. Injection of T₃ in hypothyroid rats upregulated mitochondrial biogenesis-related transcription factors [57]. In contrast to the acute response, persistently low circulating levels of T₃ during the prolonged phase of critical illness may be due to neuroendocrine dysfunction [18]. Replacement hormonal therapy given at the right time, when cells have regained the ability to both restore mitochondrial activity and increase metabolic rate, may beneficially arouse the cell and promote earlier organ recovery. However, as described above, thyroxine supplementation may prove dangerous [48], and so the right conditions must be in place.

Other hormones that could be considered in the treatment of sepsis are leptin and oestrogen. Leptin is a hormone secreted by adipose tissue. It regulates food intake and energy balance to maintain constancy of total body fat mass. In diabetic fatty rats, ectopic hyperleptinaemia triggered mitochondrial proliferation, transforming white adipocytes into mitochondria-rich, fat-oxidizing cells [17]. Administration of oestrogen or antiandrogen agents after trauma/haemorrhage also increased mitochondrial enzyme activities, protein synthesis and ATP levels relative to those in sham-operated controls [58].

A further biological equivalent to sepsis-induced hibernation is bacterial dormancy. This is a reversible, low-growth state well recognized in mycobacteria such as *Mycobacterium tuberculosis*. *Micrococcus luteus* can be aroused from its quiescent phase by an endogenous protein named 'resuscitation promoting factor' [59]. As mitochondria descend from a bacterial endosymbiont, the identification and application of a similar protein that can specifically stimulate mitochondrial activity may well yield beneficial results.

Conclusion

Mitochondrial dysfunction occurs during sepsis and may play a major role in the development of MOF.

Prevention and correction of mitochondrial dysfunction and cellular energetic failure represent novel strategies that may

improve clinical outcomes of septic patients. Timing of any intervention appears to be critical and the possibly adaptive role of some changes currently viewed as pathological must be considered. The regulated induction of a hypometabolic state resembling hibernation may help the cell in facing a reduced capacity to generate energy. The stimulation of mitochondrial activity and biogenesis during the late phase of sepsis may accelerate the recovery process. This increasing insight into underlying mechanisms promises to be an exciting era of novel therapeutic developments.

Competing interests

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