

REVIEW

Targeting mitochondrial function in sepsis

H Aslami¹, NP Juffermans^{1,2}

¹ Laboratory of Experimental Intensive Care and Anesthesiology (L.E.I.C.A.), Academic Medical Center, Amsterdam, The Netherlands

² Department of Intensive Care Medicine, Academic Medical Center, Amsterdam, The Netherlands

Abstract - Sepsis is characterized by an exaggerated inflammatory response, with mobilization of bodily energy reserves to combat the invading microorganism. Despite increasing knowledge on pathophysiology and improved treatment, mortality of sepsis is increasing and calling for new therapeutic strategies. Mitochondria may be a target in reducing organ failure in sepsis, as the severity of mitochondrial dysfunction has been linked to the severity of sepsis. Pre-clinical and clinical data suggest that mitochondrial 'resuscitation' is beneficial in sepsis, by improving substrate utilization resulting in an improvement in local energy expenditures. In this review, we will discuss metabolic changes occurring during the course of sepsis and discuss possible strategies that target mitochondria which may be a novel way of reducing organ failure and thereby mortality in sepsis.

Keywords - sepsis, multiple organ failure, mitochondria and oxidative phosphorylation.

Introduction

Sepsis is the systemic inflammatory response in the presence of infection [1]. Current treatment for sepsis is supportive and includes antibiotics, fluid resuscitation and vasopressor agents to maintain adequate organ perfusion, as well as the use of organ replacement therapy. Despite extensive research and the exploration of various therapeutic possibilities, sepsis is still the leading cause of death in the critically ill patient and the incidence of sepsis-related death is rising [2] which calls for new therapeutic targets.

The inflammatory response to infection is initiated by the binding of bacterial microbial products to recognition receptors on immune cells, with activation of nuclear transcription factors leading to the production of tumour necrosis factor and various other acute phase cytokines. Together with the activation of the complement system, a pro-coagulant state is present [3]. In response to invading microorganisms, circulating immune cells, epithelial and endothelial cells are stimulated to produce reactive oxygen species (ROS) [4]. ROS not only damages the invading micro-organism, but also healthy tissue. With the pro-inflammatory response, a compensatory anti-inflammatory response is simultaneously initiated [3]. A dysregulated immune response, in which the pro-inflammatory response overwhelms the anti-inflammatory response, can lead to multiple organ failure [3]. At the later phase of sepsis, when anti-inflammatory responses ensue, a state of "immune paralysis" develops, in which patients are more vulnerable to secondary infections [5]. Furthermore, metabolic [6] and endocrine [7] disturbances are frequently found in sepsis and are thought to contribute to organ failure.

The mechanisms of sepsis-induced organ failure remain controversial. The correlation between high plasma lactate levels

and mortality in septic shock patients has led to the conclusion that oxygen debt was likely to contribute to organ failure. Indeed, adequate oxygenation as part of early goal directed therapy was found to improve outcome of sepsis [8]. However, increasing oxygen delivery failed to improve outcome in sepsis. On the contrary, boosting oxygen delivery may even be detrimental in sepsis patients [9], suggesting a failure in oxygen utilization rather than impairment in delivery. Mitochondria utilize 90% of total bodily oxygen need and have been shown to play a crucial role in the so-called "cytopathic hypoxia".

Indeed, recent research indicates that mitochondrial dysfunction is a feature of organ injury in sepsis [10,11], even in the presence of adequate tissue oxygenation [12]. It has been hypothesized that a decrease in mitochondrial oxygen consumption is a functional response to the overwhelming inflammatory response in sepsis [13,14]. In this view, mitochondrial 'shut-down' with concomitant hypo-metabolism may increase the chance of survival of cells or recovery of mitochondria after sustaining an intense inflammatory insult, thereby contributing to recovery of organ failure in sepsis. This hypothesis is strengthened by the finding that cellular hypoxia during sepsis is absent, oxygen consumption is reduced [15] and cell death in failing organs is practically not observed [16,17]. In particular, histological examination of failing organs is often remarkably normal, with minimal or no apoptosis or necrosis, even in those who die. Alternatively, reduced mitochondrial activity may be due to mitochondrial damage. In line with this, increased levels of cytokines and the generation of ROS have been found to induce damage of mitochondrial respiratory complexes as well as other vital mitochondrial proteins. In addition, increased lactate levels are found in sepsis, associated with adverse outcome [18]. This may suggest an ATP demand at the cellular level. Of note, survival of critically ill patients improved by increasing intracellular glucose levels by insulin, which resulted in increased activity of mitochondrial respiratory complexes [19], the product

Correspondence

H Aslami

E-mail: h.aslami@amc.uva.nl

of which is ATP. Also, mitochondria in muscle of sepsis patients who progress to death appear much more swollen compared to mitochondria in those who survive [20]. Thereby, mitochondrial dysfunction may not be an adaptive process but rather a result of injury inflicted by excessive inflammation. Regardless the hypothesis, it is clear that mitochondria are important players in the progression of sepsis. Several clinical studies have suggested that mitochondrial structure [19,21,22] as well as mitochondrial function can be improved by modulating mitochondrial substrate [23]. Thereby, mitochondria may be a promising target in combating sepsis. In the following section, we will discuss mitochondrial abnormalities in sepsis and possible strategies to improve mitochondrial function.

Methods

The following keywords or MeSH terms were used to obtain papers published in the Medline database: Mitochondria OR mitochondrial dysfunction OR mitochondrial therapy OR metabolism OR oxidative phosphorylation were combined with sepsis OR severe sepsis OR multiple organ failure OR shock OR critical illness OR antioxidants OR hypothermia OR suspended animation like state OR hydrogen sulfide. The relevance of each paper was assessed using the online abstracts. In addition, the reference list of the retrieved papers was screened for potentially important papers.

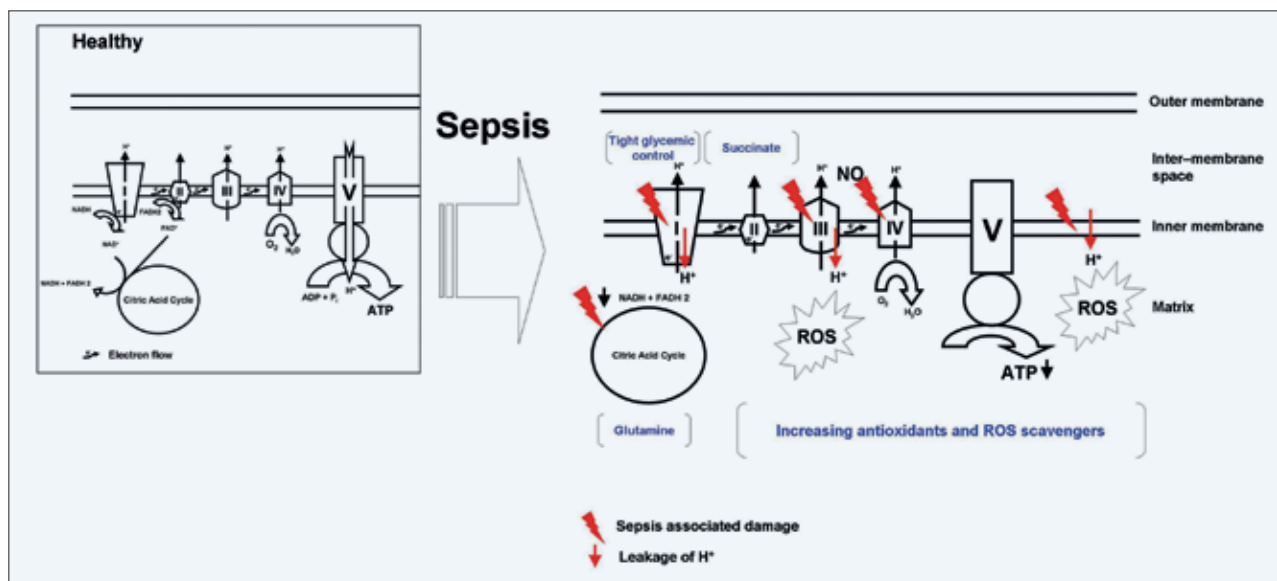
Mitochondrial energy production in health and disease

Mitochondria are membrane-bound organelles found in most cells. They consist of an inner membrane, an outer membrane, an inter-membrane space, cristae and the matrix. The inner membrane has

a large surface area, which contains the 5 respiratory complexes involved in the generation of energy in the form of ATP through oxidative phosphorylation (Figure 1). Nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH₂), two highly energetic molecules produced in the citric acid cycle, deliver electrons to respiratory complexes I and II respectively. The electrons pass through from one respiratory complex to the other, thereby generating a force which is used by the complexes to pump H⁺ from the matrix into the inter-membrane space. The generated mitochondrial current is used by complex V to generate ATP by coupling ADP with phosphate (Figure 1). The generated ATP exit mitochondria through ATP-transporters and is available for cellular metabolism. Daily mitochondrial ATP production and consumption is incredibly high and has been estimated to equal the body weight of a grown man [24]. Mitochondrial function can be estimated *in vitro* by stimulating mitochondrial enzymes involved in the citric acid cycle. Also, mitochondrial respiration can be measured in isolated mitochondria, as well as ATP, ADP, phosphate, creatinine phosphate and lactate levels as markers of mitochondrial functionality *in vivo*. The number and structure of mitochondria, as well as their function, are regulated by mitochondrial biogenesis [25]. This is a complex cellular program between mitochondrial DNA and the nucleus of the cell, which regulates cellular energy production. Synthesis of mitochondrial proteins and components is enhanced when energy demand is high. Synthesis is counterbalanced by mitophagy, a process which involves selective removal of mitochondria when energy demand is low or when mitochondria are damaged [26].

In animal models of sepsis caused by endotoxin [27] or live bacteria [28], functional and structural mitochondrial

Figure 1. Schematic representations of mitochondrial ATP production through oxidative phosphorylation starting with production of highly energetic molecules in the Krebs cycles in healthy mitochondria. Sepsis associated damage and potential strategies to resuscitate mitochondria are shown in red and blue color respectively in the right section of the figure. See text for a more detailed description.



abnormalities have been described. Also in patients with sepsis, electron microscopy images of mitochondria in muscle tissue appear to be swollen with destructed cristae, associated with organ failure [10]. It is not entirely clear how mitochondrial abnormalities are initiated, but nitric oxide (NO) may be a main pathway (Figure 1) [29]. In sepsis, NO is produced in excess, due to pro-inflammatory cytokines which promote NO formation via inducible nitric oxide synthase (iNOS) [30]. NO has been shown to competitively block mitochondrial complex I [31] and IV [32], resulting in diminished oxygen consumption. In septic patients, elevated muscle oxygen levels can be observed [9]. When intracellular oxygen levels increase, non-enzymatic production of ROS at complex I and III can occur (Figure 1). The increased ROS can react and thereby directly damage mitochondrial proteins, lipids and DNA [11,27,28]. Mitochondrial abnormalities, reflected by reduced tissue ATP/ADP ratios, may inhibit ATP-dependent reactions, in particular an adequate host response to invading organisms. Decreased ATP content has been shown to be negatively associated with resolution of organ dysfunction in a long term rat model of sepsis [11] as well as with increased mortality in muscle biopsies taken from septic patients [10]. Besides the inflammatory response, a number of medications that are frequently administered during sepsis may decrease mitochondrial respiration, including antibiotics [33].

Data regarding mitochondrial abnormalities in sepsis are, however, not all consistent. In some models of sepsis, damage to muscle mitochondria or alteration in ATP or creatinine phosphate concentrations were not observed [34,35]. Contrasting results may be explained by methodological differences with regard to sepsis severity, sepsis duration and species used in the models. The metabolic responses in sepsis which aim to mobilize bodily energy reserves suggest a compensatory response to local low ATP levels. The response may, however, be exaggerated, as suggested by both clinical and preclinical studies, with concomitant organ failure and increased mortality when left untreated. Whether this response is effective, contributing to improved host response, or exaggerated, contributing to adverse outcome, is discussed in the following section.

Changes in energy and substrate metabolism in sepsis

In sepsis, energy expenditure is usually increased by mobilizing bodily energy reserves, in order to maintain an adequate host response [36]. Plasma glucose levels increase due to insulin resistance and increased gluconeogenesis, initiated by pro-inflammatory cytokines [37]. The release of glucagon, cortisol, epinephrine and growth hormone further contribute to an increase in plasma glucose levels. Amino acids and fatty acid levels are increased by the breakdown of muscle and fat respectively. The amino acids are essentially used in the liver to fuel the synthesis of acute phase proteins and for gluconeogenesis, while fatty acids are important substrates for formation of prostaglandins and leukotrienes at the site of infection.

The mobilization of body reserves and the breakdown of proteins can be seen as a general response to stress, which, in a broad way, releases substrates to organs to support vital

functions. From an evolutionary standpoint, re-allocating energy reserves to combat the infection has more priority than storage and growth. In line with this view, insulin resistance is also seen during starvation and growth (e.g. pregnancy and puberty) [38]. Thereby, it can be hypothesized that insulin resistance may be a beneficial adaptation that secures survival or growth of the organism. In sepsis however, this response may be exaggerated, leading to enhanced wasting. Insulin resistance in sepsis is supposedly an unwanted change in glucose metabolism and attempts are then made to correct hyperglycaemia [39], albeit with conflicting results on outcome [40]. It seems clear, however, that gross hyperglycaemia is associated with adverse outcome in sepsis [39,41]. Similarly to the detrimental effects of hyperglycaemia, enhanced lipid breakdown could also be damaging. The enhanced lipid breakdown observed in sepsis results in an increase in triglyceride levels, with high levels of very-low-density lipoprotein (VLDL) and reduced levels of high density lipoprotein (HDL), thereby not only promoting atherogenesis, but also aggravating the course of sepsis [42], as HDL is considered to contribute to scavenging of bacterial toxins. Furthermore, low cholesterol levels have also been associated with increased risk of infection, leading to prolonged hospital stay [43]. Enhanced protein breakdown results in muscle wasting and fatigue. Muscle weakness acquired in the ICU not only increases length of stay, but also time on the mechanical ventilator, and is associated with increased mortality [44,45]. Thereby, mobilization of body reserves may be detrimental in sepsis.

Contrary to the belief that organ dysfunction is a result of a failure to increase energy expenditure, is the hypothesis that a decrease in mitochondrial activity and subsequent cellular processes is a functional response to overwhelming infection. An observation that supports this notion is that organ damage is mostly reversible, including organs with poor regenerative capacity, such as the liver. Both decreased mitochondrial functionality as well as the regenerative capacity of mitochondria have been found to be associated with improved survival in patients with sepsis [10,20]. However, whereas these observations point out the important role of mitochondria in the pathogenesis of organ failure, they do not determine whether recovery of mitochondrial function is a result of a functional mitochondrial 'shut-down' or of resolving mitochondrial damage. Direct measurement of ATP turnover and thus cellular metabolism in patients which may provide more insight into this issue, remains a challenge.

As the body copes with altered energy levels, influencing mitochondrial substrate utilization, or more general, improving mitochondrial function, may represent a candidate mechanism of improvement or resolution of sepsis-induced organ failure.

Influencing mitochondrial substrate utilization in sepsis

Carbohydrates

The most efficient pathway to produce ATP is through oxidative phosphorylation in the mitochondria. An alternative pathway is anaerobic glycolysis, thereby forming lactate. As discussed, plasma levels of lactate are increased in sepsis even in the absence of hypoxia. Mitochondrial complex I deficiency is

common, while complex II is well preserved in sepsis [10, 23]. Thus, when electrons flow through complex I is compromised, the addition of complex II substrate might be a strategy to improve electron flow and thus enhance ATP production in sepsis. In line with this thought, adding succinate as substrate reversed the decline in mitochondrial oxygen consumption in a rat model of abdominal sepsis [23] by approximately 40% (Figure 1). In this model, mitochondrial oxygen consumption stimulated with glutamate and malate was reduced, suggesting a complex I deficiency, which correlated with sepsis severity. Similarly, infusion of succinate prevented a decrease in liver ATP content and prolonged survival in murine endotoxemia as well as in a model of abdominal sepsis [46,47]. Also, normoglycemia achieved by infusion of insulin was found to improve mitochondrial integrity in the liver of patients with sepsis [19]. On electron microscopy imaging, liver mitochondria from patients who were assigned intensive insulin therapy showed less morphological abnormalities when compared to patient's assigned to conventional therapy [19]. Also, mitochondrial complexes I and IV showed significantly higher activity in the intensive insulin therapy group compared to conventional therapy (Figure 1) [19]. The effects on outcome are however conflicting [40,49].

Amino acids

Experimental data suggest impairment of enzymes involved in the citric acid cycle in sepsis, which catalyze degradation of carbohydrates to form NADH and FADH₂ [50] (Figure 1). A drop in NADH and FADH₂ can lead to reduction in electron flow through the respiratory complexes, thereby diminishing ATP production. Glutamine, the most abundant circulatory amino acid, is converted to glutamate and α -ketoglutarate, thereby entering the citric acid cycle at a different location than carbohydrates and boosting up the formation of electron donating molecules resulting in enhanced ATP production through oxidative phosphorylation. This was proven in a rat model of sepsis-induced by cecal ligation and puncture. Administration of glutamine in this model resulted in increased oxygen extraction and mitochondrial function, associated with improvement in cytochrome c oxidase activity [51]. Beside glutamine, arginine has also been studied extensively in the septic patient. Arginine is a non-essential amino acid. In sepsis, levels of arginine are decreased, presumably due to enhanced demand caused by increased formation of NO from arginine [52] and by starvation due to limited nutritional supply. Supplementing arginine was shown to improve immunologic response by enhancing macrophage phagocytic activity and ROS production in animal studies [53], but also to contribute to mortality in a canine model of peritonitis [54]. In clinical trials, the aggregated outcome data of septic patients who received supplemental arginine were unfavourably affected. Thereby, the use of arginine during sepsis was discouraged. Recently, however, it was found that low arginine levels in relation to its inhibitor are associated with disease severity and higher mortality [55]. To date, it is undecided whether improving arginine bioavailability is beneficial in sepsis. Also, it is not known whether the effects of glutamine are manifested by altering mitochondrial function.

Targeting oxidative stress in sepsis

Sepsis is associated with massive oxidative stress, defined as an excessive production of ROS (Figure 1). ROS is the most important antimicrobial defence mechanism [56]. However, ROS can also exacerbate organ injury [57]. Nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase and proton leak across the inner mitochondrial membrane are the main pathways for ROS formation. ROS which is formed by a leakage of protons across the inner mitochondrial membrane is presumably a result of damage to the respiratory complexes [56]. Tissue damage due to ROS is counter balanced by antioxidants and by ROS scavenging enzymes [57]. In sepsis, however, this balance is disturbed, due to increased ROS production and exhausted antioxidant pools. Alterations in these systems are associated with disease severity [4,58]. In septic patients, low plasma levels of antioxidants are found, especially selenium and ascorbic acid. Also, lipid peroxidation levels, which are a marker for oxidative damage, are high in sepsis, correlating with organ failure [58]. As the degree of oxidative stress correlates with disease severity [4], a supplement of antioxidants or ROS scavengers as a therapeutic strategy to reduce ROS damage in sepsis is a logical approach.

Vitamins

Uncontrolled ROS formation in the mitochondria can be scavenged by increasing the levels of antioxidants in sepsis. Vitamin A has been known for its antioxidant and immunomodulatory properties. When given to septic animals, a reduction in inflammation was observed [59]. Vitamin E and C are other antioxidants, which are depleted in sepsis [60]. Vitamin C acts on vascular endothelial cells through an inhibitory effect on iNOS, thereby reducing levels of NO in sepsis [61]. Additionally, supplementation with vitamin C was shown to induce apoptosis in circulating neutrophils in patients with abdominal sepsis [62]. The clinical benefit of vitamin C and E supplementation was shown in double-blind, placebo-controlled trials, showing a reduction in mortality, organ failure, duration of mechanical ventilation and length of ICU stay [21,63]. Similarly, early enteral supplementation with key pharmac-nutrients, including vitamins and glutamine, was associated with faster recovery of organ function compared to control [22].

Selenium

Selenium is an important component of enzymes involved in ROS degradation [64]. Selenium plasma levels tend to be low in sepsis and to correlate with disease severity [65]. In a double blind, randomized placebo-controlled multicenter trial in septic patients, continuous infusion of selenium reduced the rate of new infections, as well as mortality [65,66]. However, these beneficial effects were not confirmed in another randomized trial [67]. Probably, dose and timing of selenium supplementation played an important role. In the positive trial, lower doses were infused for a longer period, while in the negative trial, higher doses were administered during a shorter period. Importantly, selenium in combination with glutamine possibly increased the number of mitochondria, measured as an increase in mitochondrial DNA copy number [68].

Delivery of antioxidants in the mitochondria

Direct delivery of antioxidants at the site of ROS generation in the mitochondria can be achieved with MitoQ [69]. In an animal model of sepsis, administration of MitoQ reduced organ dysfunction [70]. Thus far, phase II clinical trials have been performed in chronic hepatitis and in Parkinson's disease [71], but not yet in sepsis patients.

Taken together, antioxidant therapy in the early phase of sepsis seems promising to prevent or attenuate the progression of organ failure in sepsis. However, studies of antioxidant administration in patients with sepsis have not been convincing to date and more research on this issue is warranted [72].

Inducing a hypo-metabolic state in sepsis

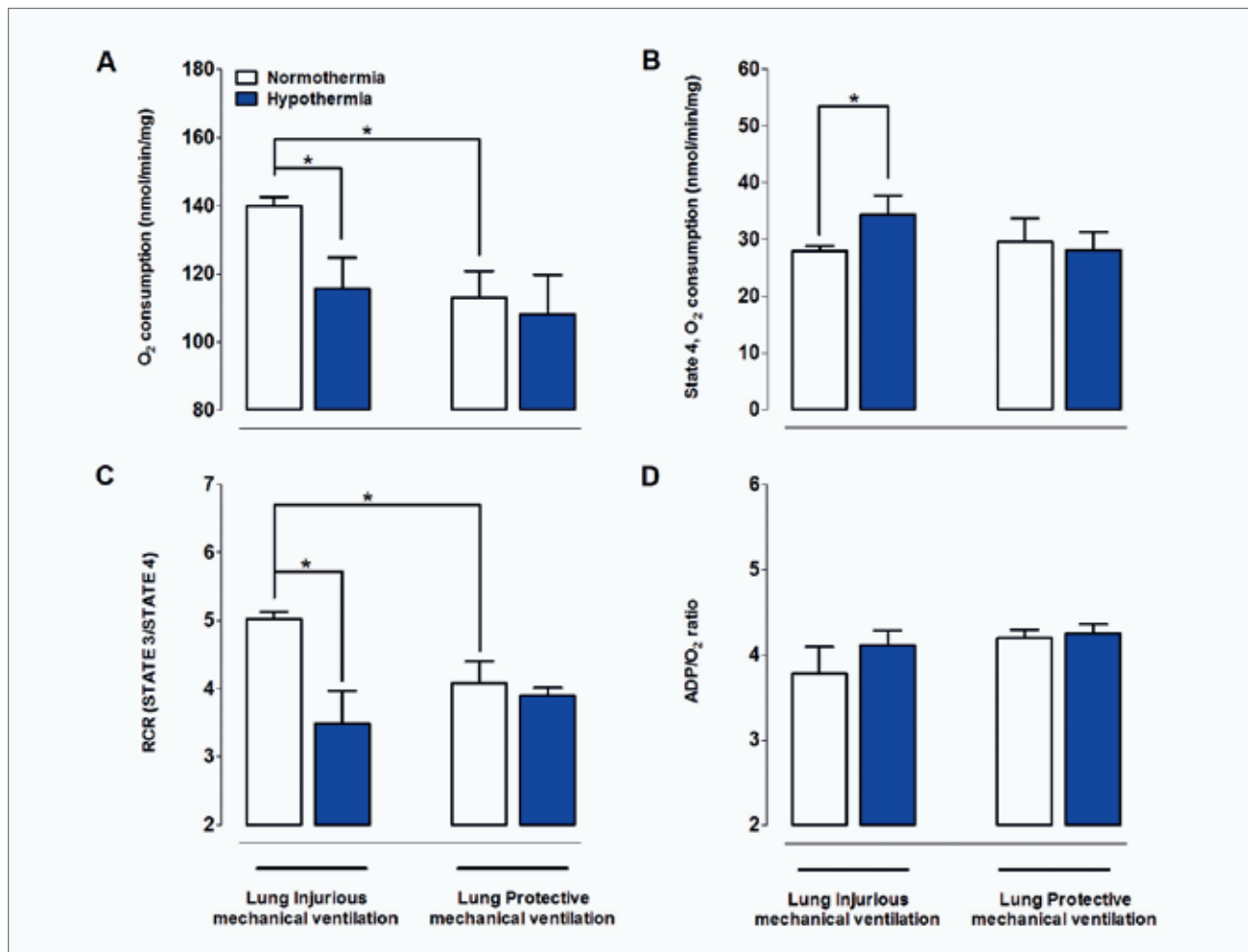
Sepsis is characterized by hypermetabolism, in which bodily fuel reserves are mobilized in order to cope with the overwhelming

insult, as described in the previous sections. Thus far, therapies are supportive, aimed at supplementing substrates or at increasing oxygen delivery to meet high demands, [3]. An alternative way of reducing organ failure and thereby mortality rate would be to reduce metabolic demand at the cellular level, a state as observed in naturally hibernating mammals. Humans do not hibernate and have only a limited tolerance to hypoxia. Nevertheless, myocardial hibernation, which can occur in patients with myocardial contractile dysfunction due to ischemic heart disease, is thought to provide an adaptive response to hypoxia. Down regulation of myocardial contraction may result in reduced energy expenditure, thereby preserving cellular integrity and viability [73].

Hypothermia

Hypothermia is a well-known therapeutic strategy to reduce organ injury. This approach is used during cardiothoracic surgery

Figure 2. The effect of hypothermia on mitochondrial oxygen consumption (A), state 4 respiration (B), respiratory control ratio (RCR) (C) and ADP/O₂ ratio (D) during oxidative phosphorylation of mitochondrial complex II substrate succinate and complex I blocker rotenone in animals after lung protective or injurious mechanical ventilation. State 4 respiration, RCR (mitochondrial oxygen consumption / state 4) and ADP/O₂ ratios are functional parameters for mitochondrial uncoupling, coupling between respiration and oxidative phosphorylation and mitochondrial efficiency respectively. Data are means \pm SEM.



and organ transplantation [74]. Also, hypothermia reduces brain injury in patients after a cardiac arrest [75]. Hypothermia reduces metabolism by 7% per grade reduction, thereby reducing oxygen requirement and carbon dioxide production, leading to decreasing ATP requirements. In patients with severe ARDS associated with sepsis, induced hypothermia applied as a last resort reduced mortality [76]. Also, hypothermia is able to reduce inflammation and prevent the production of superoxide and subsequent formation of reactive oxygen and nitrogen species during ischemia [74]. In a model of ventilator-induced lung injury [77], we found increased mitochondrial oxygen consumption compared to animals subjected to lung protective mechanical ventilation (Figure 2). As injurious mechanical ventilation is characterized by increased production of inflammatory cytokines [78], increase in oxygen consumption suggests increased ATP demand to maintain the pro-inflammatory state. When the body temperature was reduced to 32 °C, oxidative phosphorylation decreased (Figure 2). This may reflect less oxygen consumption *in vivo*, in line with a previous report [79]. Also, respiratory control ratio, a parameter of mitochondrial coupling between respiration and oxidative phosphorylation, was decreased, while state 4 respiration increased, which suggests mitochondrial uncoupling during hypothermia, as seen before [79]. Uncoupling induced by hypothermia prevents the production of ROS that mediates organ damage [80]. Of note, detrimental effects of hypothermia are described, in particular increased risk for infection, coagulation disorders and arrhythmia [74]. Therefore, besides hypothermia, other novel strategies to reduce metabolism are needed.

Suspended animation like state

Hydrogen sulphide (H₂S), commonly considered to be an environmental hazard, has been shown to induce a hibernation-like state in naturally non-hibernating animals [81], characterized

by reduction in body temperature, carbon dioxide production and oxygen consumption. Similar physiological changes were observed, when NaHS, a H₂S donor, was infused in rats [82]. Physiological changes were associated with reduced lung injury inflicted by mechanical ventilation. Furthermore, H₂S protected against ischemia reperfusion injury in an animal model of myocardial ischemia by preservation of mitochondrial structure and function [83]. Other mechanisms of protection are attributed via a vaso-relaxant effect, attenuation of inflammation and reduction of apoptosis [84]. In addition, an antioxidant effect of H₂S was observed [85]. However, pro-inflammatory and pro-apoptotic effects have also been described [84,86,87]. These contrasting results may depend on differences in dose, route of application and on tissue. Despite the disadvantages related to H₂S toxicity, its use as a potential therapy in battle wounds has raised the attention of the US military forces. Battle wounds are associated with extreme haemorrhagic shock, multiple organ failure and high mortality [88]. Survival from battle wounds depends on rapid damage control surgery. The hypothesis that treatment with H₂S could buy time to reach a hospital by lowering metabolism and tissue oxygen demand in the wounded soldier is currently under investigation [89, 90].

Conclusion

Despite advances in understanding the pathophysiology of sepsis-induced organ failure, mortality and morbidity remain high. Impaired mitochondrial function leading to a fall in organ ATP levels is probably a main pathway. Thus far, strategies targeting mitochondrial function seem promising, as well as reducing collateral damage caused by excessive ROS formation by antioxidants in sepsis. Combining antioxidant therapy with mitochondrial substrate is worth investigating in future trials. Timing and dosing of these interventions need further investigation.

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