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REVIEW ARTICLE

Metabolic Resuscitation Strategies to Prevent Organ Dysfunction in Sepsis

Vera A. Reitsema,^{1,*} Bastiaan S. Star,^{1,*} Vincent D. de Jager,¹ Matijs van Meurs,²
Robert H. Henning,¹ and Hjalmar R. Bouma^{1,3}

Abstract

Significance: Sepsis is the main cause of death among patients admitted to the intensive care unit. As current treatment is limited to antimicrobial therapy and supportive care, mortality remains high, which warrants efforts to find novel therapies.

Recent Advances: Mitochondrial dysfunction is emerging as a key process in the induction of organ dysfunction during sepsis, and metabolic resuscitation might reveal to be a novel cornerstone in the treatment of sepsis.

Critical Issues: Here, we review novel strategies to maintain organ function in sepsis by precluding mitochondrial dysfunction by lowering energetic demand to allow preservation of adenosine triphosphate-levels, while reducing free radical generation. As the most common strategy to suppress metabolism, that is, cooling, does not reveal unequivocal beneficial effects and may even increase mortality, caloric restriction or modulation of energy-sensing pathways (*i.e.*, sirtuins and AMP-activated protein kinase) may offer safe alternatives. Similar effects may be offered when mimicking hibernation by hydrogen sulfide (H₂S). In addition H₂S may also confer beneficial effects through upregulation of antioxidant mechanisms, similar to the other gaso-transmitters nitric oxide and carbon monoxide, which display antioxidant and anti-inflammatory effects in sepsis. In addition, oxidative stress may be averted by systemic or mitochondria-targeted antioxidants, of which a wide range are able to lower inflammation, as well as reduce organ dysfunction and mortality from sepsis.

Future Directions: Mitochondrial dysfunction plays a key role in the pathophysiology of sepsis. As a consequence, metabolic resuscitation might reveal to be a novel cornerstone in the treatment of sepsis. *Antioxid. Redox Signal.* 31, 134–152.

Keywords: sepsis, oxidative phosphorylation, metabolism, hypothermia, caloric restriction, hibernation, hydrogen sulfide

Mitochondrial Dysfunction Plays a Key Role in the Pathophysiology of Sepsis-Associated Organ Injury

SEPSIS IS A POTENTIALLY life-threatening syndrome that is characterized by multiorgan dysfunction caused by a dysregulated host response to infection (147). Clinical manifestations are highly variable, depending on the health status of the patient, underlying cause of sepsis, and organs involved, but they may comprise signs of infection (*i.e.*, fever or hypothermia, productive cough, and dysuria), hyperdy-

namic circulation with signs of poor tissue perfusion (*i.e.*, tachycardia, hypotension, cold, and mottled skin), tachypnea, and altered mental status. Sepsis severity can be estimated by using the sequential (sepsis-related) organ failure assessment (SOFA) score, comprising cut-off values for dysfunction of coagulation, cardiovascular, neuronal, mental, and renal systems (147). Current treatment principles of sepsis consist of source control by early broad-spectrum antibiotic treatment and/or source drainage, combined with hemodynamic resuscitation by fluid supplementation and vasopressors. Despite efforts to improve treatment, sepsis remains the main

Departments of ¹Clinical Pharmacy and Pharmacology, ²Critical Care, and ³Internal Medicine, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands.

*These authors contributed equally to this article.

cause of death among patients admitted to the intensive care unit (ICU) with an in-hospital mortality rate of 18%–30% (98).

The pathophysiology of sepsis is complex and incompletely understood. Traditionally, organ failure was viewed to result from inflammation leading to tissue hypoxia, thus precipitating apoptosis and necrosis. However, this theory has been largely abandoned, as histological appearances of failing organs from patients who died of sepsis show only minimal signs of cell death, clearly insufficient to explain the extensive organ failure (80, 157). More recently, mitochondrial dysfunction, defined as failure to produce adenosine triphosphate (ATP) by mitochondria, while generating increased amounts of free radicals such as reactive oxygen species, is emerging as a key process in the induction of organ dysfunction during sepsis.

The mitochondrial membrane potential ($\Delta\Psi_m$) is the driving force to produce ATP during oxidative phosphorylation by F_1F_0 -ATP synthase. However, severe sepsis is associated with a reduced $\Delta\Psi_m$ in platelets (68) (Fig. 1) and increased oxidative stress due to higher levels of free radical production, whereas systemic antioxidant capacity is reduced (35, 37). Failure to recover mitochondrial function, as measured by a persisting reduction in $\Delta\Psi_m$, antioxidant capacity, and ATP-levels, while levels of oxidative stress are increased, is associated with mortality from sepsis (23, 35, 36, 68). More evidence about the role of mitochondria in the pathophysiology of sepsis stems from animal experiments, as treatment of septic mice with a mitochondria-targeted antioxidant (*i.e.*, MitoTEMPO) not only restores mitochondrial function but also precludes acute kidney injury (AKI) and prolongs survival (127). Together, although the pathophysiology of sepsis is not yet fully unraveled, clinical data reveal derangement of mitochondrial function that is associated with poor outcome—in line with these observations, preclinical experiments reveal a key role for mitochondrial dysfunction in the pathophysiology of sepsis.

The initial trigger that causes mitochondrial dysfunction in sepsis is not yet clear. However, activation of neutrophils by the infection could represent an early event that leads to the initiation of damage, due to release of proinflammatory mediators, proteases, and free radicals (a.o. O_2^- , H_2O_2 , and $OH^\cdot$) that not only aid in bacterial clearance but also induce oxidative damage and increase vascular permeability (78). In addition, activated neutrophils, but also endothelial cells and macrophages, produce nitric oxide (NO) that induces vasodilation, reacts with superoxide (O_2^-) to form highly reactive free radicals such as peroxynitrite ($ONOO^-$) (17), inhibits cytochrome C oxidase (complex IV) (132), and triggers opening of mitochondrial permeability transition pores (MPTPs), which leads to a loss of $\Delta\Psi_m$ (171) (Fig. 2).

The reduced $\Delta\Psi_m$ hampers ATP production, while augmenting the generation of free radicals by mitochondria. Subsequently, failure to produce ATP *via* oxidative phosphorylation diverts metabolism to aerobic glycolysis to convert pyruvate into lactate as an alternative route to generate ATP, which is termed the “Warburg” effect (63). In turn, lactate can serve as fuel for oxidative metabolism and a substrate for gluconeogenesis in a process termed “the lactate shuttle” (63). Despite its properties that are potentially protective in nature, lactate is classically seen as a stress biomarker and increased levels are associated with organ dysfunction and mortality in patients with sepsis (63). Since oxidative phosphorylation is responsible for >90% of the total oxygen (O_2) consumption, the induction of mitochondrial dysfunction reduces cellular O_2 consumption in sepsis, which is known as “cytopathic hypoxia”: a reduced cellular O_2 extraction from the blood, thereby effectively leading to cellular hypoxia, in spite of normal blood O_2 levels and hemoglobin saturation (55). Thus, activation of the innate immune system seems to spark the generation of free radicals, which, together with NO, inflicts damage to

FIG. 1. Severe sepsis is associated with a reduced $\Delta\Psi_m$ on admission, whereas failure to recover the $\Delta\Psi_m$ is associated with mortality from sepsis. The platelet $\Delta\Psi_m$ -index (Mmp-index) is reduced in patients with severe sepsis ($n=17$), compared with patients with sepsis ($n=9$) and controls without infection ($n=17$) (A). Although the platelet Mmp-index is not different between survivors ($n=10$) and nonsurvivors ($n=7$) of severe sepsis on admission (<48 hours), only survivors are able to recover the platelet Mmp-index to values that are comparable to controls (B). Box margins identify the upper and lower quartile, the horizontal line marks the median, and *whiskers* indicate minimal and maximal values. $*p < 0.05$ [Kruskal–Wallis, *post hoc* Mann–Whitney *U* (A) and Wilcoxon signed-rank (B)]. (A, B) is reprinted with permission from Gründler *et al.*, *Critical Care*, 2014, originally published by BioMed Central (68). $\Delta\Psi_m$, mitochondrial membrane potential.

FIG. 2. Mitochondria play a key role in the pathophysiology of multiple organ dysfunction syndrome in sepsis. Under healthy circumstances, the generation of small amounts of free radicals by the electron transport chain is counterbalanced by antioxidant enzymes and molecules. However, sepsis is associated with a decrease in total antioxidant capacity and an increase in overall free radical production (*i.e.*, $O_2^{\cdot-}$, NO, and $ONOO^-$) generated as by-products of the increased mitochondrial respiration during the hypermetabolic state triggered by sepsis and dysregulation of mitochondrial function due to opening of MPTPs. In turn, mitochondrial dysfunction leads to a reduced antioxidant defense, increased free radical formation, and a reduced ATP production, which progresses into loss of cellular homeostasis. Finally, damaged mitochondria can induce not only apoptosis through release of cytochrome *c* but also remote organ injury by releasing proinflammatory damage-associated molecular patterns. ATP, adenosine triphosphate; MPTPs, mitochondrial permeability transition pores; NO, nitric oxide; $O_2^{\cdot-}$, superoxide; $ONOO^-$, peroxynitrite. Color images are available online.

mitochondria. In turn, damaged mitochondria produce lower levels of ATP secondary to the loss of $\Delta\Psi_m$, whereas generation of free radicals increases (Fig. 2) (23, 126). Taken together, as mitochondrial dysfunction is emerging as a key process in the pathophysiology of sepsis, strategies that protect mitochondria, which may be termed “metabolic resuscitation” as coined by Leite and de Lima (98), represent a promising treatment strategy for sepsis (Fig. 3; Supplementary Tables S1 and S2).

Preventing an Energetic Burn-Out: Strategies to Reduce Metabolism in Sepsis

Loss of cellular homeostasis in sepsis is likely be mediated by cellular ATP depletion and oxidative damage due to mitochondrial dysfunction. Consequently, preservation of cellular ATP levels, by either increasing ATP generation or lowering its demand, might thus preclude organ injury. Metabolic suppression can lower ATP demand by slowing down cellular energy-consuming processes. Further, metabolic suppression may indirectly lower free radical generation that occurs as a by-product of mitochondrial ATP generation. The metabolic rate can be reduced by cooling (*i.e.*, physically or pharmacologically), which is generally termed “targeted temperature management.” Alternatively, molecular switches sensing low cellular energy status can be activated by caloric restriction (CR) or pharmacological mimetics, thereby resulting in a decreased ATP demand.

Targeted temperature management in sepsis

Lowering the body temperature may be key to preclude organ dysfunction by reducing energy demand, thereby preserving cellular energy levels in sepsis. Indeed, cooling rats

to 32°C–34°C reduces levels of lactate, interleukin (IL)-6, capillary leakage and tissue edema (45), as well as liver injury (96) during sepsis induced by cecal ligation and puncture (CLP). Physical cooling (target 36.5°C–37°C) in febrile patients with septic shock lowers the requirements for vasopressors and reduces early 14-day mortality [randomized controlled trial (RCT); $n = 197$] (144). A *post hoc* analysis of this study revealed that the time spent at a body temperature below 38.4°C is associated with better survival (143). However, these protective effects of hypothermia have not been reproduced by other studies yet.

Lowering the body temperature from 39.1°C before cooling to 37.1°C after cooling is associated with a reduced energy expenditure, but it does not affect hemodynamics or gas exchange, in a small unblinded crossover study ($n = 20$) in critically ill febrile patients (131). The authors further conclude that the reduction in energy expenditure seems to be mainly attributable to sedation, rather than cooling itself (131). Recently, the multicenter Cooling and Surviving Septic Shock Study trial was not able to demonstrate beneficial effects of hypothermia (target 32°C–34°C, for 24 hours) followed by normothermia (target 36°C–38°C, for 48 hours) on 30-day all-cause mortality among 436 patients with severe sepsis or septic shock (85). Further, it should be noted that physical cooling may even increase metabolic rate in endothermic organisms when trying to maintain euthermy in response to forced cooling. Together, current evidence does not support the use of physical cooling to improve sepsis outcome.

An alternative approach to lower temperature in sepsis consists of pharmacological antipyretic treatment. A small randomized trial with 30 febrile patients (body temperature >38.5°C) revealed that the antipyretic drugs metamizole and

FIG. 3. Strategies to prevent mitochondrial meltdown in sepsis. Sepsis is associated with a hypermetabolic state and increased ATP consumption, which might be dampened by therapeutic hypothermia. In turn, consumption of ATP leads to an increased ratio of [AMP]:[ATP], which activates AMPK that signals toward reduced energy consumption, while inducing ATP generation through glycolysis and oxidative phosphorylation. Activation of AMPK can be induced by AICAR and metformin, as well as through Sirt-1 by resveratrol, caloric restriction, and SRT1720. PDC is the final step of the glycolytic pathway, converting pyruvate into Acetyl-CoA to fuel the Krebs cycle. PDK is an inhibitor of PDC that can be inhibited by dichloroacetate, whereas thiamine is an essential cofactor for the function of PDC. Oxidative phosphorylation (*i.e.*, ETC complex IV) is inhibited by NO, whose generation can be precluded by NOS inhibitors. NAC, 7-NI, and aminoguanide augment generation of GSH, a potent antioxidant that scavenges free radicals, such as alpha-lipoic acid, Tempol, and H₂S/NaHS. MitoTEMPO, MitoQ, SS-31, and XJB-5-131 are examples of antioxidant drugs that accumulate within mitochondria. Mitochondrial damage in sepsis induces fission, which leads to a fragmented mitochondrial network and may be precluded by Mdivi-1. 7-NI, 7-nitroindazole; AICAR, 5-aminoimidazole-4-carboxamide ribonucleotide; AMPK, AMP-activated protein kinase; ETC, electron transport chain; GSH, glutathione; H₂S, hydrogen sulfide; Mdivi, mitochondrial division inhibitor; NAC, N-acetylcysteine; NaHS, sodium hydrosulfide; NOS, nitric oxide synthase; PDC, pyruvate dehydrogenase complex; PDK, pyruvate dehydrokinase; Sirt-1, Sirtuin-1; Tempol, 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl; XJB-5-131, mitochondrial antioxidant coupled to gramicidin S. Color images are available online.

propacetamol (a prodrug of paracetamol) reduce energy expenditure and O₂ consumption by about 7% with every 1°C decrease in temperature (66). However, inflammatory parameters and cytokine levels are not affected by administration of these antipyretic drugs (66). Moreover, a large database study comprising 15,268 adult patients with sepsis revealed an increased mortality by antipyretic medication among patients with a body temperature >39°C (182). A retrospective study in 606 ICU patients with sepsis demonstrated nonsteroidal anti-inflammatory drug (NSAID) and paracetamol use to be associated with increased 28-day mortality, after adjusting for age, disease severity, mechanical ventilation, comorbidity, and maximum body temperature during ICU stay (95). A later prospective RCT in 700 febrile ICU patients who received paracetamol or placebo demonstrated no effects on mortality, despite a minor decrease in peak (−0.25°C) and average (−0.28°C) body temperature induced by paracetamol as compared with placebo (174). In conclusion, although targeted temperature man-

agement reduces the metabolic demands, studies on its effects on sepsis are inconclusive and mortality may even increase when body temperature drops below euthermia.

CR may restore energetic balance in sepsis

CR can reduce O₂ consumption and free radical production, while stimulating mitochondrial biogenesis, maintaining ATP production, and increasing antioxidant capacity (103, 114). Thereby, CR may preclude mitochondrial dysfunction and allow preservation of cellular ATP levels, while averting oxidative stress in sepsis. Indeed, short-term CR (40% for 3 weeks) (151) and alternate day fasting (72) before the induction of sepsis by an injection of cecal slurry or lipopolysaccharide (LPS) intraperitoneally lowers biomarkers for inflammation and reduces mortality (151). Unfortunately, no preclinical study analyzed the effects of CR after induction of sepsis. An observational study involving 1004 ICU patients (*i.e.*, sepsis and nonsepsis) revealed a J-shaped

relation between caloric intake *versus* length-of-stay in ICU/hospital and in-hospital mortality, even after adjustment for age, type of admission, and disease severity (38). These findings are in line with an earlier prospective multicenter study that comprises 187 ICU patients and revealed that an intake of 33%–65% of estimated caloric needs to be associated with better outcome, whereas an intake of >66% of estimated caloric needs was associated with increased in-hospital mortality (92).

In contrast, another observational study among 2772 mechanically ventilated ICU patients revealed a reduction in mortality with increased caloric intake (5). To determine whether or not CR affects mortality, a prospective multicenter RCT comprising 894 ICU patients randomized patients to permissive underfeeding (40%–60% of estimated caloric requirements) or standard enteral feeding (70%–100% of estimated caloric requirements) for up to 14 days and demonstrated no effect on length-of-stay in ICU/hospital or in-hospital mortality (13). In conclusion, current evidence does not support CR in patients with sepsis.

Pharmacological modulating of cellular energy sensing

Targeted temperature management and CR may be pharmacologically exploited by activating molecular energy sensors, such as AMP-activated protein kinase (AMPK), a key molecular energy sensor whose activation leads to a reduction in anabolic energy-consuming processes, while stimulating catabolic processes, thus refueling the energy storage of the cell (52). Activation of AMPK by the specific agonist 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) in sepsis induced by CLP or LPS in mice lowers endothelial activation and neutrophil adhesion, reduces levels of circulating cytokines, and precludes AKI (52, 101).

AMPK can also be activated by metformin, which lowers levels of circulating cytokines during endotoxemia in mice (164). Preadmission metformin use is associated with lower mortality rates among ICU patients with type 2 diabetes ($n=7404$), though not significant for the subgroup with sepsis ($n=255$) (34). Patients with septic shock and a lactate >10 mmol/L have a better survival when they use metformin preadmission ($n=44$) as compared with nonusers ($n=118$) (46). In contrast to these retrospective studies, van Vught *et al.* (165) were not able to reveal an association between diabetes mellitus, preadmission use of insulin or metformin and disease presentation, and mortality or host response in a prospective observational study comprising 1104 ICU patients with sepsis. Hence, the protective effects of AMPK activation in preclinical models using specific AMPK agonists cannot be mimicked by metformin in patients with sepsis.

Indirect activation of AMPK by modulating upstream targets

Similar to AMPK, sirtuins are energy-sensing molecules (136). Sirtuin-1 (Sirt-1) is an nicotinamide adenine dinucleotide (NAD)⁺-dependent histone/protein deacetylase of the sirtuins family that is activated in response to lowered energy availability during CR or starvation, but it can also be activated by polyphenols. Sirt-1 stimulates the activation of AMPK, which, in turn, enhances the activity of Sirt-1, thereby constituting a positive feedback loop (136). Activation of Sirt-1 by CR or specific agonists during sepsis might

allow preservation of cellular homeostasis by reducing metabolic demand, through activation of AMPK. Pretreatment with Sirt-1 activating polyphenols (*i.e.*, resveratrol and polydatin) or using specific Sirt-1 activators (*i.e.*, SRT1720) before CLP in rodents reduces oxidative stress, improves mitochondrial function, lowers proinflammatory chemokines, protects against liver, heart, and brain injury, and increases survival (62, 90, 172). Surprisingly, however, despite these protective effects of sirtuin activation, administration of the selective Sirt-1 inhibitor EX-527 in severe murine CLP-induced sepsis also decreases the level of circulating cytokines and increases survival (184). Taken together, preclinical data suggest protective, although incompletely understood, effects of activating the molecular energy sensors AMPK and Sirt-1 in rodent models of sepsis.

Hibernators are able to safely modulate metabolism

A superior method to safely preserve energy during sepsis might be adapted from hibernating animals: masters of metabolic reduction, while maintaining ATP levels and averting oxidative stress. Hibernation consists of a phase called “torpor” that is characterized by a profound reduction in metabolic rate and body temperature, lasting 6–30 days (depending on the species), which are interspersed by euthermic “arousal” periods (21). The suppressed mitochondrial respiration during torpor leads to an O₂ consumption of <10% of euthermic values (25, 117). Remarkably, despite these profound physiological changes, no signs of organ injury can be found (21).

The molecular mechanisms that induce torpor and preclude organ injury are not fully understood. However, several mechanisms have been coined, including thyroid hormones, 5'-adenosine monophosphate, and hydrogen sulfide (H₂S) (22). Of note, H₂S can mimic a torpor-like state in non-hibernators and the increased resistance to oxidative stress during hibernation is, at least in part, due to the cytoprotective effects of H₂S (Fig. 4) (22, 158). Whether hibernators are also relatively resistant to mitochondrial dysfunction induced by sepsis remains to be studied. Unraveling the molecular mechanisms that induce torpor and preclude organ injury during torpor arousal cycles might lead to the discovery of novel pharmacological targets to protect mitochondria in sepsis.

Balance Energy Levels by Supporting Mitochondrial Function in Sepsis

Reducing energy expenditure in sepsis may not only allow preservation of cellular ATP levels but also reduce the generation of free radicals as by-products of mitochondrial ATP production. However, current attempts to improve sepsis outcome by reducing energy expenditure (*i.e.*, physical or pharmacological temperature management, CR, and targeting molecular energy sensors) have not been uniformly successful thus far. These strategies have in common that they indirectly affect mitochondrial function, whereas strategies that directly affect mitochondrial function might be more effective in precluding mitochondrial dysfunction in sepsis. Such strategies comprise diverting fuel and O₂ supply to mitochondria, modulating mitochondrial dynamics, and averting oxidative damage to mitochondria, and they will be discussed in the upcoming sections.

FIG. 4. Endogenous H₂S protects against mitochondrial dysfunction. Endogenous H₂S is mainly produced by the transsulfuration pathway consisting of cystathionine- β -synthase, as first and rate-limiting step, and cystathionine- γ -lyase (6, 84). Endogenous H₂S precludes the formation of MPTPs, upregulates antioxidant mechanisms, scavenges free radicals, and stimulates ATP production by donating electrons in the respiratory chain. Color images are available online.

Fueling mitochondria throughout sepsis

Mitochondria depend on the availability of Acetyl-CoA to fuel oxidative phosphorylation, which is generated from pyruvate in the glycolytic pathway by pyruvate dehydrogenase complex (PDC) that depends on thiamine pyrophosphate (ThPP; derived from thiamine) for its function (98). Severe sepsis in patients is associated with thiamine deficiency in 10% of the cases on presentation and 30% after 72 hours (48). Potentially, lack of thiamine contributes to the reduced PDC activity, as revealed in peripheral mononuclear cells in sepsis, which is associated with increased mortality (123).

Thiamine deficiency in mice undergoing CLP increases levels of 4-hydroxy-2-nonenal (a marker of oxidative stress), tumor necrosis factor (TNF)- α , and monocyte chemoattractant protein-1 (42). Consequently, direct activation of PDC or supplementation of thiamine may preclude mitochondrial function in sepsis by generating Acetyl-CoA to fuel mitochondrial metabolism. Activity of PDC can be enhanced by dichloroacetate, which lowers concentrations of lactate without affecting hemodynamics or survival as compared with placebo in an RCT ($n=252$) in patients with lactate acidosis due to hemorrhage, myocardial failure, or sepsis (150).

The effects of thiamine supplementation in patients with sepsis are contradicting. Thiamine levels in patients with septic shock without thiamine deficiency do not correlate with lactic acid acidosis (48), and administration of thiamine (200 mg twice daily for 7 days) does not improve lactate levels or outcome in patients as demonstrated in an RCT ($n=88$). However, supplementation of thiamine in patients with thiamine deficiency does lower lactate levels (47). Thiamine supplementation (200 mg twice daily for 7 days) lowers the incidence of AKI and reduces the need for renal replacement therapy in patients with septic shock, as demonstrated in a placebo-controlled RCT ($n=70$) (116).

Marik *et al.* (110) revealed a drastic reduction in organ dysfunction and mortality after administration of thiamine (200 mg every 12 hours for 4 days), ascorbic acid (1500 mg every 6 hours for 4 days), and hydrocortisone (50 mg every 6 hours for 7 days) to patients with sepsis ($n=47$) in a retrospective before-after study. However, due to the cocktail

that was administered, it remains to be unraveled whether the individual components govern protective effects and/or whether they may have acted synergistically. Thus, although thiamine deficiency may negatively affect outcome in sepsis, it remains to be unraveled whether administration of thiamine may govern protective effects in all patients with sepsis.

O₂: a little less saturation?

Failure to increase O₂ consumption and improve O₂ extraction, despite extensive resuscitation with fluids and vasopressors such as dobutamine, is associated with mortality from sepsis (73). Clinically, the phenomenon of cytopathic hypoxia (*i.e.*, impaired O₂ extraction) can be observed as a relatively high (central) venous O₂ saturation (ScvO₂) as compared with the arterial O₂ saturation (54). Interestingly, the ScvO₂ is associated with impaired survival, as demonstrated in 619 patients suspected of having sepsis in the emergency department (ED) (133). However, if mitochondrial dysfunction leads to impaired O₂ extraction and does not result from reduced arterial oxygenation, then administration of higher levels of O₂ would not be expected to have beneficial effects on sepsis outcome.

In contrast with this hypothesis, O₂ supplementation (100% O₂ for 2–3 hours) in septic mice induced by zymosan lowers levels of proinflammatory cytokines, while augmenting levels of anti-inflammatory cytokines and antioxidants and reducing heart, lung, liver, and kidney injury (81). In line with these findings, O₂ supplementation (titrated at partial pressure of oxygen [PaO₂] 90–120 mmHg) to sheep with fecal peritonitis increased stroke volume, mean arterial pressure (MAP) and improved renal and cerebral perfusion, leading to a reduced [lactate]:[pyruvate] ratio (74). These findings might be explained by reduced NO production that may contribute to (micro)vascular dysfunction, as suggested by the reduced nitrite (NO₂⁻) levels after the O₂ treatment in sheep (74).

In contrast to these preclinical data, administration of high levels of O₂ to patients does not affect clinically relevant outcomes: Liberal O₂ therapy (10 L 40% O₂/minute) in patients suspected of sepsis in the ED does result in hyperoxemia

in 64% of patients, but it does not affect mortality (152). Moreover, a brief period of hyperoxemia, by administration of 100% O₂ for 20 minutes in ventilated patients with severe sepsis may even further impair O₂ delivery due to vasoactive effects of O₂, leading to vasoconstriction (135). A *post hoc* analysis of two ICU trials comprising 3933 patients with severe sepsis revealed high levels of O₂ supplementation (fraction of inspired oxygen [FiO₂] ≥60%), hypoxemia (PaO₂ <8 kPa), and hyperoxemia (PaO₂ ≥16 kPa) to be associated with increased mortality (41). However, these studies do not directly imply toxic effects of O₂ therapy, since a higher level of cytopathic hypoxia will also result in a higher ScvO₂. To our knowledge, two randomized studies are currently ongoing that will study the effects of O₂ supplementation: one comparing O₂ supplementation titrated with a PaO₂ of 105–135 mmHg versus 60–90 mmHg (ClinicalTrials.gov: NCT02321072) and one comparing O₂ therapy at 15 L/minute with no supplemental O₂ (ClinicalTrials.gov: NCT02378545). Potentially, these studies may elucidate whether or not patients with sepsis may benefit from (liberal) O₂ supplementation.

Keeping it together by mitochondrial fission and fusion

The efficiency of oxidative phosphorylation is dependent on a patent mitochondrial network (65). Mitochondria are dynamic organelles that constantly undergo fission and fusion in response to stress, damage, and changes in energetic demand: a critical feature to maintain mitochondrial network patency (18). The fission and fusion part of the mitochondrial quality control program exchanges damaged and healthy components between mitochondria, whereas mitochondria that have accumulated too much damaged components will be subjected to mitophagy (173). Consequently, effective mitochondrial quality control in sepsis might augment oxidative phosphorylation efficiency, while reducing free radical formation by damaged mitochondria.

Murine sepsis induced by CLP leads to a reduced amount of optic atrophy 1 (Opa1; required for fusion) and activation of GTPase dynamin related protein (Drp1; required for fusion), which suggests a shift toward mitochondrial fission (139). Similar effects are observed after CLP in the rat, which leads to a reduced mitochondrial mass with fragmentation of the mitochondrial network with a decreased expression of Mitofusin 2 (required for fusion) and increased expression of the fusion-protein Drp1 (65). Pretreatment of rats with Mdivi (mitochondrial division inhibitor)-1, an inhibitor of the fission-protein Drp1, before CLP, improved mitochondrial function and reduced apoptosis (65). Further, Mdivi-1 attenuates LPS-induced sepsis-associated encephalopathy, potentially *via* protection against apoptosis by preventing fission, while inducing fusion by activating Opa1 (44).

Protein and transcriptome-based analysis of skeletal muscle biopsies from 17 patients with sepsis showed that mitochondrial protein synthesis and mitochondrial genes are not comprised as compared with age-matched healthy controls (58). Despite activation of mitochondrial biogenesis (*i.e.*, upregulation of NRF2 α /GABP, TFAM, TFB1M, and TFB2M) in sepsis, this response fails to maintain mitochondrial function and mitochondrial mass (58). Hence, a reduction of mitochondrial mass is not due to catabolic processes *per se*, but it may imply an active remodeling process of the tissue (58). Modulation of the signaling routes involved may

allow reshaping of the mitochondrial network in sepsis. In conclusion, sepsis leads to a shift toward mitochondrial fission, which leads to mitochondrial dysfunction and cellular apoptosis, as demonstrated in preclinical studies.

Mitochondrial Effects of Gasotransmitters

The gasotransmitters H₂S, carbon monoxide (CO), and NO are produced endogenously, have important signaling functions, and are important modulators of mitochondrial function. Consequently, studying the effects of sepsis on the levels of these gasotransmitters and its association with mitochondrial function may allow the discovery of novel pharmacological targets, which might comprise modulation of endogenous production of these gasotransmitters or administration of exogenous H₂S, CO, or NO.

Antioxidant and immunomodulatory effects of H₂S

H₂S has a broad range of effects, varying from immunomodulatory effects to modulation of hemodynamics and preservation of mitochondrial homeostasis. H₂S can maintain mitochondrial homeostasis by targeting free radicals, MPTP formation, and upregulating antioxidant pathways, which may boost ATP production and preserve cellular homeostasis (29, 75, 153). Sepsis is associated with increased levels of H₂S in plasma, as demonstrated in LPS-challenged mice (3), as well as in septic shock in humans (100).

Endogenous H₂S is mainly produced by cystathionine- β -synthase (CBS), cystathionine- γ -lyase, and 3-mercaptopyruvate sulfurtransferase (3-MST) (84). Knocking out major H₂S-producing enzymes (*a.o.* CSE^{-/-}, Δ 3MST, and CBS^{+/-} mice) does not seem to have a large effect on sepsis in mice, as malondialdehyde (MDA; marker for oxidative stress) in the lung and overall survival are unaffected. However, levels of circulating TNF- α , IL-10, and IL-12 are lower in all transgenic mice studied and the renal function (*i.e.*, blood urea nitrogen) was slightly better in CBS^{+/-} mice (3). Unfortunately, levels of H₂S were not measured and it remains speculative whether or not these (congenital) knockouts of a single H₂S-producing enzyme had led to a compensatory increase of H₂S production by other H₂S-producing enzymes. Thus, although sepsis augments endogenous levels of H₂S in plasma, its role in the pathophysiology remains to be unraveled.

Inhalation of exogenous H₂S after induction of sepsis by CLP reduces levels of circulating TNF- α and IL-6, precludes the upregulation of heme oxygenase-1 (HO-1), and induces activation of nuclear factor- κ B (NF- κ B) in the lung, of which the latter may aid in faster resolution of inflammation and, as a consequence, lower cytokine levels (166). In line with these findings, administration of exogenous H₂S by sodium hydrosulfide (NaHS; 100 μ mol/kg $\equiv$ 5.6 mg/kg, *s.c.*) to mice with sepsis induced by CLP lowers levels of circulating TNF- α and IL-10, decreases the activity of myeloperoxidase in the lung, lowers caspase activity in the spleen, accelerates bacterial clearance, and increases survival from 20% to 80% at day 3 (53). The protective effects of H₂S are governed by precluding the upregulation of C/EBP-homologous protein (CHOP) induced by sepsis, as demonstrated in CHOP^{-/-} mice (53).

In contrast to these protective effects, administration of a higher dosage of NaHS (10 mg/kg, *i.p.*) augments activation of stimulated NF- κ B activity through activation of extracellular-signal regulated kinase 1/2 by substance P,

leading to downstream activation of cyclooxygenase-2 and production of prostaglandin E2 in the lung and liver that was associated with increased expression of different proinflammatory cytokines, chemokines (10, 179), and adhesion molecules (10) after CLP-induced sepsis in mice. Together, H₂S has pleiotropic effects and although the role of endogenous H₂S in sepsis remain to be unraveled, administration of exogenous H₂S shows both protective antioxidative and anti-inflammatory effects as well as proinflammatory effects. Potentially, differences in the route of administration, dosage, and the narrow therapeutic window of H₂S might explain the contradictory effects of H₂S in sepsis.

Despite the potential protective effects of H₂S in sepsis, administration of exogenous H₂S by inhalation or using soluble H₂S donors (*e.g.*, NaHS) may, however, induce major side effects, comprising hypotension and immune suppression that occurs at higher concentrations (>500 nm) (155) that might impair the protective effects of H₂S in sepsis. A safer strategy might be to increase endogenous production of H₂S, as can be induced by CR (77), or pharmacologically by serotonin or dopamine (158). Administration of dopamine reduces free radical formation and cell death during hypothermia/rewarming *in vitro* (158) and *in vivo* (51) by activating CBS. However, administration of dopamine, which is commonly used as a vasoactive catecholamine to avert symptoms of shock, is associated with a higher mortality risk in sepsis, independent of shock severity (138). Taken together, although inducing endogenous H₂S production seems a promising strategy to improve sepsis outcome, it remains to be studied whether boosting of endogenous H₂S production has any potential for clinical application, due to its pleiotropic effects and potentially toxic side effects.

CO boosts mitochondrial respiration within a small therapeutic range

High levels of CO lead to impairment of mitochondrial respiration, whereas low concentrations of CO boost mitochondrial respiration (93, 162). Further, CO stimulates mitochondrial biogenesis by activating nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathways (167) and governs antiapoptotic effects by inhibiting Fas/FasL ligands, caspases, cytochrome *c* release from mitochondria, poly (ADP-ribose) polymerase cleavage, and Bcl-2 (180, 181). The catalytic breakdown of heme by HO-1 is the major intracellular pathway that releases CO (160). Hence, HO-1 might represent a druggable target to stimulate mitochondrial function and preclude organ dysfunction by releasing CO, in addition to administration of CO.

Stimulation of cultured macrophages with LPS increases the expression of HO-1, free radical production and induces excessive mitochondrial fission (175). Administration of exogenous CO by CO-releasing molecule (CORM), however, increases mitochondrial superoxide dismutase (SOD) activity and $\Delta\Psi_m$ (146), lowers free radical production, and precludes excessive mitochondrial fission (146, 175) in macrophages and alveolar cells on LPS challenge *in vitro*. Administration of CORM-2 can rescue mice from lethal endotoxemia and CLP-induced sepsis (161, 162, 168). Inhalation of CO activates anti-inflammatory pathways, while reducing levels of different proinflammatory cytokines and increasing levels of the anti-inflammatory cytokine IL-10

after an injection of LPS in mice through activation of MAP-kinases (125). Similar anti-inflammatory effects are observed in pigs (113) and monkeys (115).

Despite these promising effects in preclinical models of sepsis, the effect of CO in human sepsis remains less clear. Sepsis induces endogenous CO production in patients (142), which is associated with survival from severe sepsis (178). In contrast, however, inhalation of CO (500 ppm, 1 hour) does not affect levels of circulating TNF- α , IL-6, and IL-8 in LPS-challenged healthy human volunteers (112). Thus, although the therapeutic range of gaseous CO itself is small, increasing levels of CO by administration of CORMs or enhancing endogenous production of CO by stimulating HO-1 shows promising effects in preclinical models of sepsis.

Lowering NO levels minimizes free radical release

NO is an important signaling molecule that increases blood flow by inducing smooth muscle cell relaxation and counteracts the endothelial inflammatory response (43). NO is synthesized from L-arginine by nitric oxide synthase (NOS), which is distinguished in three isoforms: neuronal NOS, endothelial NOS, and the inflammatory activated inducible NOS (iNOS) (9). Sepsis is associated with an increase in iNOS and release of large amounts of NO (128). As described earlier, NO can interact with superoxide (O₂⁻) to form the highly reactive free radical ONOO⁻, inhibit cytochrome C oxidase (complex IV) (132), and trigger opening of MPTP, leading to loss of $\Delta\Psi_m$ (171) (Fig. 3). Precluding NO formation might well limit mitochondrial dysfunction in sepsis. Inhibitors of iNOS [*i.e.*, aminoguanidine, N-(3-(aminomethyl)benzyl)acetamide (1400 W)] prevent lipid oxidation and tyrosine nitration, improve mitochondrial function, thereby reducing tissue injury (39, 91, 121). In contrast to the effects of selective iNOS inhibitors, nonselective inhibition of NOS using N(G)-nitro-L-arginine methyl ester (L-NAME) before CLP in rats reduces oxidative stress (122) and inflammation (83), while increasing organ injury and mortality on LPS challenge in rats (145). Thus, iNOS/NO-derived peroxynitrite formation seems to play an important role in the initiation of organ injury in sepsis and inhibiting iNOS in rodent models of sepsis reveals protective effects.

Infusion of the nonspecific NOS-inhibitor L-NAME in patients with septic shock improves arterial pressure and oxygenation, allowing lower catecholamine dosages, without affecting mortality in a nonrandomized uncontrolled trial in 11 patients with septic shock (15). Treatment with the non-selective iNOS inhibitor L-NG-methylarginine hydrochloride (546C88) in an RCT that included 312 patients with septic shock promoted the resolution of shock, but it did not affect 28-day survival (16). A subsequent multicenter RCT that studied the effects of 546C88 in septic shock (*n*=797) demonstrated an increased mortality in sepsis, due to a higher proportion of cardiovascular deaths, although it did decrease the incidence of deaths caused by multiple organ failure resulting from sepsis (104). However, to our knowledge, the effect of selective iNOS blockade in patients with sepsis has not been studied thus far. However, selective inhibition of iNOS by aminoguanidine in LPS-challenged healthy volunteers lowers TNF- α levels, whereas levels of IL-6 and IL-10 are not affected (50). In conclusion, although overproduction of NO appears to be an important mechanism in septic shock,

it remains to be studied whether (selective) modulation of NOS governs protective effects in sepsis. Although iNOS inhibition showed promising preclinical effects, the effects in clinical trials seem limited.

Once the Mitochondria Overheat: Boosting Antioxidants to Avert Oxidative Damage to Mitochondria

Antioxidants might be critical in precluding mitochondrial dysfunction in sepsis, as oxidative stress seems to play a key role in initiating mitochondrial damage. Further, strategies that aim at increasing levels of cellular ATP by augmenting mitochondrial respiration do not only stimulate ATP production but also induce oxidative stress due to the formation of free radicals as by-products of mitochondrial respiration. Consequently, the optimal therapy to preclude mitochondrial meltdown in sepsis might comprise stimulation of mitochondrial function to facilitate ATP production, combined with therapies that avert oxidative damage. Importantly, sepsis is associated with increased free radical production, as evidenced by higher levels of lipid peroxidation and protein oxidation (137) and reduced antioxidant defense mechanisms [*i.e.*, glutathione (GSH) and SOD] in patients with sepsis (30). This disbalance between free radical formation and antioxidant mechanisms results in more free radicals that can directly cause oxidative damage to lipids, proteins, and DNA, as well as the mitochondrial electron transport chain (ETC)—thereby impairing the ability of mitochondria to produce ATP (59). As a consequence, boosting the antioxidant capacity or scavenging free radicals may allow preservation of mitochondrial function.

Alpha-lipoic acid

Alpha-lipoic acid is a naturally occurring dithiol compound, soluble in water and fat, and an essential cofactor for mitochondrial bio-energetic enzymes. Alpha-lipoic acid and its reduced form, dihydrolipoic acid, are scavengers of free radicals and can increase endogenous antioxidant capacity by reduction of GSH (87) and increasing the activity of antioxidant enzymes SOD and catalase in septic rats (129). Thereby, alpha-lipoic acid inhibits and lowers oxidative stress, inflammation, and AKI, while improving survival from sepsis in rats and mice (8, 26, 88, 99). In a trial of patients with atrial fibrillation undergoing catheter ablation, alpha-lipoic acid lowered the inflammatory markers C-reactive protein (CRP), TNF- α as well as the oxidative stress marker nitrotyrosine at 12 months after ablation, indicating that alpha-lipoic acid may be a safe and effective treatment for patients with sepsis as well (140). To our knowledge, currently, no studies on alpha-lipoic acid are conducted in patients with sepsis.

Ascorbic acid

Ascorbic acid, or vitamin C, is a water-soluble vitamin and an essential cofactor for iron- and copper-containing enzymes with immune-modulating effects (159). Although ascorbic acid is a free radical scavenger, its antioxidant effects are mainly attributed to changes in the redox signaling of nicotinamide adenine dinucleotide phosphate oxidase, which reduces free radical production (170). Among patients at the ICU, levels of ascorbic acid are significantly lower in patients with organ failure as compared with patients without

organ failure (20). Ascorbic acid treatment in severe sepsis demonstrated to be safe and lowered proinflammatory biomarkers (*i.e.*, CRP and procalcitonin), leading to preservation of organ function, as demonstrated by reduced SOFA scores (12.5 or 50 mg/kg every 6 hours for 4 days) (57) and a reduction in vasopressor need and 28-day mortality in patients with septic shock, without affecting length of ICU stay (25 mg/kg every 6 hours for 72 hours) (176).

The combination of high-dose ascorbic acid (1500 mg every 6 hours for 4 days or until ICU discharge) with hydrocortisone (50 mg every 6 hours for 7 days or until ICU discharge, followed by a taper over 3 days) and thiamine (200 mg every 12 hours for 4 days or until ICU discharge) reduces SOFA scores and the need for vasopressors, and it drastically lowers hospital mortality from 40% to 9% in patients with severe sepsis (110). In conclusion, ascorbic acid effectively counteracts oxidative stress in patients with sepsis, presumably by scavenging free radicals and upregulating antioxidant mechanisms. Given the safety and relatively low price of ascorbic acid, it seems reasonable to administer ascorbic acid to patients with (severe) sepsis in an attempt to limit organ dysfunction. However, further trials are warranted to confirm these results and to define the optimal treatment regimen before implementing this treatment into clinical treatment guidelines.

Glutathione

GSH is synthesized from the amino acids cysteine, glutamate, and glycine, and it contains a thiol group ($-SH$) that can scavenge free radicals (86). GSH is the most abundant mitochondrial antioxidant and plays an important role in maintaining mitochondrial function and protecting mitochondrial DNA against oxidative damage (79). GSH levels can be increased by administration of N-acetylcysteine (NAC), a synthetic precursor of GSH. Administration of NAC [150 mg/kg loading dose, followed by 15 mg/(kg·h)] to volunteers challenged with LPS prevents fever and the increase in TNF- α , while increasing renal blood flow and stabilizing blood pressure (141). Both GSH [70 mg/(kg·d), $n=10$] and NAC [75 mg/(kg·d), $n=10$] increase plasma levels of GSH, reduce oxidative damage, and improve organ function as compared with the placebo group ($n=10$) in patients with septic shock (124).

However, a meta-analysis of 41 studies ($n=2,768$) was not able to demonstrate beneficial effects of NAC during sepsis on duration of mechanical ventilation, incidence of organ failure, length of ICU stay, or mortality (156). Moreover, administration of NAC 24 hours after onset of sepsis was associated with cardiovascular instability, potentially by formation of toxic metabolites (*i.e.*, thiol and glutathionyl free radicals, glutathione disulfide, cysteine) (70), or the eradication, rather than normalization, of free radicals that may also serve important signaling roles in sepsis. Thus, although the mechanisms of NAC- and GSH-induced protection are well understood, the side effects limit its use for treatment of sepsis.

Melatonin

Melatonin is primarily secreted in the pineal gland and has anti-inflammatory effects, while simultaneously acting as a free radical scavenger (149). An *ex vivo* study with blood from healthy volunteers showed that melatonin prevents

LPS-induced mitochondrial dysfunction, reduces oxidative stress, and inhibits cytokine release (60). Similar effects of melatonin were seen in healthy volunteers on LPS challenge (4). Administration of 10 mg melatonin twice daily to human newborns ($n = 10$) with sepsis lowered biomarkers of oxidative stress [*i.e.*, MDA and 4-hydroxylalkenals], numbers of circulating neutrophils and CRP level, and reduced mortality as compared with untreated septic newborns (0/10 *vs.* 3/10) (64). Hence, melatonin scavenges free radicals, lowers inflammation, preserves mitochondrial function, and can reduce mortality from sepsis in newborns; however, whether these findings can be reproduced in other populations with sepsis remains to be determined.

Selenium

Selenium is an essential nutrient that is incorporated in selenoproteins, which play an essential role in antioxidant defense. Human plasma contains around 25 different selenoproteins that have selenocysteine at their active side and are able to catalyze redox reactions (134). Most selenium in plasma, however, is present in three forms: selenoprotein-P (50%), glutathione peroxidase (GSH-Px) (20%–40%), and albumin (9%) (71). Selenium is necessary not only for the formation of selenoproteins but also for GSH-Px that depends on selenium for its generation (177). Sepsis is associated with reduced plasma concentrations of selenium and selenoproteins (56). Low selenium and GSH-Px levels are associated with an increased frequency of organ failure and mortality in critically ill patients (109).

However, the effects of selenium administration on patients with sepsis are unequivocal: One study (open-label RCT, $n = 21$) demonstrates a better clinical outcome and a lower incidence of AKI (12), whereas another study (blinded RCT, $n = 29$) shows no effects on inflammation or mortality, but it did show a reduced incidence of ventilator-associated pneumonia (32). A meta-analysis of RCTs ($n = 792$ patients) demonstrates that administration of selenium reduced mortality in patients with sepsis, although the length of stay at the ICU or incidence of pneumonia was not affected (7). Together, selenium is an important precursor for different antioxidant enzymes; limited clinical evidence demonstrates promising protective effects of normalizing the reduced plasma levels of selenium in sepsis.

Tempol

Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl) contains a redox cycling nitroxide and is a membrane-permeable SOD mimetic (120). Tempol is able to prevent radical production by mitochondrial complex I in cardiac cells on incubation with TNF- α (69). Tempol prevents organ dysfunction in rodent and pig sepsis models (40,102) and improves survival after CLP in rats (75). However, tempol does not affect mortality in zymosan-induced peritonitis (40). In conclusion, tempol reveals protective effects in preclinical models of sepsis, warranting further clinical studies.

Improving Antioxidant Efficacy by Targeting Mitochondria

The effects of antioxidant treatments on clinical outcomes are unequivocal and sometimes very limited, which is po-

tentially due to the broad tissue distribution of these drugs with very low concentrations in mitochondria. Specifically targeting antioxidants toward mitochondria may well increase the efficacy of antioxidants by precluding mitochondrial dysfunction in sepsis (119) (Fig. 5).

Coenzyme Q

Coenzyme Q (ubiquinone) is an electron carrier in the mitochondrial inner membrane, accepting electrons from complex I and II and transporting them to complex III, subsequently translocating protons across the mitochondrial inner membrane (19). Further, coenzyme Q exhibits strong antioxidant effects by regenerating the potent antioxidant α -tocopherol (vitamin E) from tocopheroxyl radicals (94). Consequently, boosting levels of coenzyme Q may boost mitochondrial function and reduce oxidative stress. Septic shock is associated with a lowered plasma level of coenzyme Q (29). Administration of coenzyme Q before administration of LPS in rat reduces free radical release from neutrophils, lowers oxidative stress and histological signs of organ damage, but does not influence mortality (1). Importantly, the use of coenzyme Q in clinical practice is limited by its long supplementation period needed to increase tissue levels (*i.e.*, 13 weeks) and biased distribution to plasma, liver, and spleen (94), thereby limiting its bioavailability in other organs.

MitoQ

MitoQ (Mitoquinone) is a ubiquinone derivative, consisting of a lipophilic cation group (*i.e.*, triphenyl phosphonium, TPP) coupled to coenzyme Q, which is readily taken up (driven by $\Delta\Psi_m$) and inserted into the mitochondrial membrane (89). Preclinical data from rodent endotoxemia models reveal that MitoQ reduces the formation of free radicals, maintains $\Delta\Psi_m$ and function, reduces levels of proinflammatory cytokines in plasma, and limits cardiac dysfunction, as well as AKI and liver injury (106, 107). Although MitoQ has not yet been tested in clinical trials of sepsis, clinical trials in healthy volunteers and patients with hepatitis C revealed no clinically relevant adverse events (61). However, a potential limitation of MitoQ is its potential to increase production of free radicals by ETC complex I as demonstrated in isolated mitochondria and induce apoptosis in endothelial cells *in vitro*, which might implicate that MitoQ may have a narrow therapeutic range (49). In addition, the reduced $\Delta\Psi_m$ in sepsis (2) might limit the uptake of MitoQ, as the uptake of MitoQ depends on this potential (89).

MitoTEMPO

MitoTEMPO is an SOD mimetic that possesses superoxide and alkyl radical scavenging properties, that is, similar to MitoQ, conjugated to TPP (118, 119). Thereby, concentrations of MitoTEMPO within mitochondria are 100–1000 times higher than outside mitochondria (118). Administration of MitoTEMPO to mice restores mitochondrial function, precludes AKI, and prolongs survival after CLP (127). Although MitoTEMPO might be a promising treatment of sepsis, only limited data are available. Further, similar to MitoQ, uptake into mitochondria might be limited due to the reduced $\Delta\Psi_m$ in sepsis.

FIG. 5. Targeting mitochondria. Coenzyme Q is an electron carrier in the mitochondrial inner membrane and can also be administered as a strong antioxidant that is readily taken up by mitochondria. Uptake of drugs can be facilitated by various routes. Antioxidants can be encapsulated in vesicles that fuse with the outer mitochondrial membrane, delivering its content. Coupling to MTS directs the transport of antioxidants toward the mitochondrial matrix. Driven by their high affinity for the inner mitochondrial membrane phospholipids, GS-based antioxidants can deliver antioxidants into the mitochondrial matrix (e.g., XJB-5-131). Alternatively, drugs (e.g., antioxidants) can be coupled cationic agents, for example, TPP or choline esthers (e.g., NAC and GSH). TPP is a lipophilic cation that is rapidly taken up by mitochondria driven by the membrane potential ($\Delta\Psi_m$). Examples of TPP-conjugated drugs are MitoQ and MitoTempo. Finally, drugs can be conjugated to small, positively charged peptides with an amino sequence directly targeting the mitochondrial matrix, also called SS-peptides (S-20 or S-31). GS, gramicin S; MTS, mitochondrial targeting sequences; SS, Szeto-Schiller; TPP, triphenyl phosphonium. Color images are available online.

Mitochondria-targeted peptides that incorporate antioxidants

An alternative method to target mitochondria is through Szeto-Schiller (SS)-peptides: small, positively charged peptides with an amino sequence directly targeting the mitochondrial matrix. In contrast to TPP, uptake of SS-peptides does not depend on membrane potential (118, 183). SS-peptides protect against oxidative stress in isolated mitochondria (183), although their underlying molecular mechanism remains unclear (24). SS-31 is a stable, water-soluble compound designed to resist peptidase degradation (183) that accumulates 1000-fold within mitochondria, where it accumulates in the inner mitochondrial membrane (183). Administration of SS-31 in a mouse model of ischemia/reperfusion diminishes oxidative stress, while augmenting levels of ATP (27).

Another peptide that targets mitochondria is gramicidin S: a peptide that is commonly used to target antibiotics to bacteria (108). Mitochondrial antioxidant coupled to gramicidin S. (XJB-5-131) consists of the SOD-mimetic and electron scavenger 4-NH₂-TEMPO that is conjugated to a modified peptide of gramicidin S. Administration of XJB-5-131 to rats with hemorrhagic shock lowers oxidative stress, reduces caspase activity, and improves survival (108). To our knowledge, despite its promising effects, neither SS-31 nor XJB-5-131 has been tested in models of sepsis yet.

Conclusion

In conclusion, mitochondrial dysfunction plays a key role in the pathophysiology of sepsis and is characterized by a reduced production of ATP, whereas oxidative stress is

augmented. Strategies to preclude mitochondrial dysfunction comprise lowering the energetic demand, as may be achieved by targeted temperature management (*i.e.*, physical or pharmacological cooling) or CR. However, although preclinical data reveal some protective, but unequivocal results, clinical studies do not confirm these protective effects. Although precluding high fever is beneficial in patients with sepsis, forced hypothermia may even increase mortality. Modulation of molecular energy sensors might represent a successful strategy to pharmacologically exploit the benefits of CR, but this remains to be tested in clinical studies. In contrast to these strategies that indirectly affect mitochondrial function, strategies that directly affect mitochondrial function comprise modulation of mitochondrial dynamics to ensure a patent mitochondrial network, preserving mitochondrial fuel supply and precluding oxidative damage. The gasotransmitters H₂S and CO deserve special attention, as these may not only scavenge free radicals but can also augment mitochondrial respiration (and thereby, ATP production), while lowering inflammation in preclinical models of sepsis.

Although stimulating mitochondrial function may boost ATP production, it simultaneously augments the release of free radicals. Consequently, for optimal efficacy, such a therapy should be combined with antioxidants to successfully reduce mitochondrial meltdown in sepsis. In preclinical models of sepsis, antioxidants lower inflammation, preclude organ dysfunction, and reduce mortality; scarce data reveal protective effects in humans with sepsis as well. The efficacy of antioxidants can be increased by delivering the compounds into mitochondria, which lowers oxidative damage, inflammation, organ injury, and mortality in preclinical models of sepsis. Modulating mitochondrial function to augment the generation of ATP, while averting oxidative stress, might be a promising metabolic resuscitation strategy to preclude organ injury and lower mortality in sepsis.

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Address correspondence to:

Hjalmar R. Bouma, MD, PhD

Department of Clinical Pharmacy and Pharmacology

University Medical Center Groningen

University of Groningen

P.O. Box 30.001, EB70

9700 RB Groningen

The Netherlands

E-mail: h.r.bouma@umcg.nl

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Abbreviations Used

$\Delta\psi_m$ = mitochondrial membrane potential
 3-MST = 3-mercaptopyruvate sulfurtransferase
 546C88 = L-NG-methylarginine hydrochloride
 AICAR = 5-aminoimidazole-4-carboxamide ribonucleotide
 AKI = acute kidney injury
 AMPK = AMP-activated protein kinase
 ATP = adenosine triphosphate
 CBS = cystathionine- β -synthase
 CHOP = C/EBP-homologous protein
 CLP = cecal ligation and puncture
 CO = carbon monoxide
 CORM = carbon monoxide-releasing molecule
 CR = caloric restriction
 CRP = C-reactive protein
 Drp1 = dynamin-related protein 1
 ED = emergency department
 ETC = electron transport chain
 GSH = glutathione
 GSH-Px = glutathione peroxidase
 H₂S = hydrogen sulfide
 HO-1 = heme oxygenase-1
 ICU = intensive care unit
 IL = interleukin
 iNOS = inducible NOS
 L-NAME = N(G)-nitro-L-arginine methyl ester
 LPS = lipopolysaccharide
 MAP = mean arterial pressure
 MDA = malondialdehyde
 Mdivi = mitochondrial division inhibitor
 MPTPs = mitochondrial permeability transition pores
 NAC = N-acetylcysteine
 NaHS = sodium hydrosulfide
 NF- κ B = nuclear factor- κ B
 NO = nitric oxide
 NOS = nitric oxide synthase

Abbreviations Used (Cont.)

O₂ = oxygen
O₂⁻ = superoxide
ONOO⁻ = peroxynitrite
Opa1 = optic atrophy 1
PaO₂ = partial pressure of oxygen
PDC = pyruvate dehydrogenase complex
RCT = randomized controlled trial
ScvO₂ = high (central) venous O₂ saturation
Sirt-1 = sirtuin-1

SOD = superoxide dismutase
SOFA = sequential (sepsis-related) organ
failure assessment
SS = Szeto-Schiller
Tempol = 4-hydroxy-2,2,6,6-tetramethylpiperidine-
1-oxyl
TNF = tumor necrosis factor
TPP = triphenyl phosphonium
XJB-5-131 = mitochondrial antioxidant coupled
to gramicidin S.

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