Mitochondrial Dysfunction during Sepsis

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Abstract: Sepsis and multiple organ failure remain leading causes of death in intensive care patients. Recent advances in our understanding of the pathophysiology of these syndromes include a likely prominent role for mitochondria. Patient studies have shown that the degree of mitochondrial dysfunction is related to the eventual outcome. Associated mechanisms include damage to mitochondria or inhibition of the electron transport chain enzymes by nitric oxide and other reactive oxygen species (the effects of which are amplified by co-existing tissue hypoxia), hormonal influences that decrease mitochondrial activity, and downregulation of mitochondrial protein expression. Notably, despite these findings, there is minimal cell death seen in most affected organs, and these organs generally regain reasonably normal function should the patient survive. It is thus plausible that multiple organ failure following sepsis may actually represent an adaptive state whereby the organs temporarily 'shut down' their normal metabolic functions in order to protect themselves from an overwhelming and prolonged insult. A decrease in energy supply due to mitochondrial inhibition or injury may trigger this hibernation/estivation-like state.

Likewise, organ recovery may depend on restoration of normal mitochondrial respiration. Data from animal studies show histological recovery of mitochondria after a septic insult that precedes clinical improvement. Stimulation of mitochondrial biogenesis could offer a new therapeutic approach for patients in multi-organ failure.

This review will cover basic aspects of mitochondrial function, mechanisms of mitochondrial dysfunction in sepsis, and approaches to prevent, mitigate or speed recovery from mitochondrial injury.

Keywords: Mitochondria, nitric oxide, reactive oxygen species, sepsis, septic shock.

PHYSIOLOGICAL MITOCHONDRIAL FUNCTION

Mitochondria, as the powerhouse of the cells, convert over 90% of the available oxygen into water through a series of reactions with an intrinsic ATP production in the process. Along with energy production, mitochondria regulate several physiologic and pathologic reactions, including apoptosis, reactive oxygen species (ROS) generation and detoxication [1], dynamics of intracellular calcium and may also act as oxygen concentration sensor in vascular cells [2]. These organelles are composed of two specialized (inner and outer) membranes. The mitochondrial matrix is the space within the inner mitochondrial membrane and in these two locations the reactions associated with electron transport chain usually occur. The electron transport chain is composed of four individual complexes (complexes I to IV) that transfer electrons from a redox gradient to NAD+/NADH or fumarate/succinate couple to the O_2/H_2O couple.

This chain operates as a series of electron transfer reactions with the subsequent oxidation and reduction of the donor and the acceptor. Besides the four enzymatic complexes, respiratory chain is also composed of two mobile electron carriers (coenzyme Q – ubiquinol and cytochrome c) and the enzyme responsible for the conversion of ADP and inorganic phosphate to ATP (F1F0 –ATPase). During the electron transport chain, the electrons from NADH or $FADH_2$ are donated to complex I and complex II (*via* succinate) and them transferred to ubiquinol *via* coenzyme Q and them ubisemiquinone [3]. Ubiquinol donates electrons to complex III, which, in turn, transfers electrons to cytochrome c. The next step is the transfer of electrons from cytochrome c to complex IV where they are used for the conversion of molecular oxygen to H_2O. The motion of electrons through the series of redox reactions allows for the extrusion of protons from the matrix to the intermembrane space. This transfer occurs at complexes I, III, IV and creates a transmembrane electrochemical gradient. The proton motive force (composed by the electrochemical gradient and the pH gradient due to extrusion of H^+) is used for the formation of ATP at complex V as well as for ATP/ADP exchange by adenine nucleotide translocase (ANT) in the inner membrane.

LABORATORY AND CLINICAL EVIDENCE OF MITOCHONDRIAL DYSFUNCTION IN SEPSIS

Mitochondrial abnormalities in sepsis – both ultrastructural and biochemical– have been reported as long as 30 years ago [4]. However, there is considerable variability among the results of the studies in the literature, with reports of increased, decreased or unaltered mitochondrial function in sepsis. These discrepancies may be attributed to species diversity, different tissues investigated, differences in the degree of the injury and presence (or not) of resuscitation. Other possible explanation for these conflicting results lies in the technique used to measure mitochondrial function in tis-

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sues slices, tissue homogenates or isolated mitochondria. Techniques used to isolate mitochondria can select specific mitochondrial subpopulations (such as the less damaged ones) removing more deranged organelles which could interfere in the functional results [5]. Overall, the studies demonstrating variability in mitochondrial function were performed in short-term models, with data from long-term studies depicting more consistent and less variable results and mainly evidencing a decrease in mitochondrial function [4].

The electronic microscopy studies of mitochondria in sepsis have demonstrated structural abnormalities in these organelles after endotoxemia or septic insult in several different tissues. Liver isolated from septic animals showed a large degree of heterogeneity, with evidence of mitochondrial swelling, loss of crystal structure and disruption of the matrix. Importantly, abnormal mitochondria were present in cells that did not show evidence of necrosis or apoptosis [5]. In skeletal muscle of baboons submitted to *E.coli* challenge, mitochondria became enlarged and with distorted cristae and, as injury progresses, fragmentation of inner membrane was a common finding [6]. In a feline resuscitated model of endotoxemia, Crouser *et al.* demonstrated early as 4 hours alterations in mitochondrial structure, with mitochondrial swelling characterized by an increase in area and rounding of the mitochondria with a substantial decrease in intramitochondrial density. These effects were abolished by cyclosporine A, thus demonstrating a role for mitochondrial permeability transition pore in this process [7].

As already stated, the studies evaluating mitochondrial function exhibited disparate results. For instance, in a rat cecal ligation and puncture model, Kantrow *et al.* demonstrated reduced hepatocyte oxygen consumption after 16 hours of sepsis, but the further investigation of respiration rate of isolated hepatocytes from the septic animals evidenced an increase in both complex I and II mediated respiration in states 3 and 4 [5]. In a more recent study in a long-term pig model of endotoxemia, Porta *et al.* demonstrated impairment in respiratory control ratio in the liver, but not at skeletal muscle or kidney [8]. The mixed findings of these studies may be related to the technique of measuring mitochondrial respiration in isolated mitochondria, as described previously. Studies demonstrating an increase in mitochondrial activity after sepsis have also been reported. Perfused livers isolated from a rat model of fecal peritonitis demonstrated enhanced oxygen consumption at rest [9] and, in a rat model of massive endotoxemia, skeletal muscle and cardiac mitochondria showed an early rise in respiratory control ratio compared to controls [10].

Despite the contradictory results, most of the studies on mitochondrial function in sepsis, notably the ones with long-term models, showed a decrease in respiratory control ratio, mitochondrial enzymatic activities or ATP generation. One early study with endotoxemic and hemorrhagic animals demonstrated impaired mitochondrial respiration, as well as ultrastructural damage to mitochondria [11]. In a subsequent study in a chronic model of sepsis, Tavakoli and Mela depicted that, after 6 days of low grade sepsis, hepatic and skeletal muscle mitochondrial functions were disturbed as characterized by significant decreases in ATP synthesis and respiratory control ratios [12]. In an endotoxic rat model, state 3 respiration of mitochondria isolated from the diaphragm was reported to be diminished after 48 hours of challenge [13]. Likewise, the decrease in oxygen consumption at state 3 was also reported in myocardial mitochondria from rabbits after 24 hours of endotoxemia [14]. In a series of studies using near-infrared spectrophotometry, Schaefer *et al.* documented impairments in oxidative phosphorylation in endotoxemic rats. Moreover, the decrease in cytochrome aa3 redox state in one study was correlated with decreases in blood pressure and flow [15-17]. A recent study also demonstrated a decreased cerebral mitochondrial function in mice made septic by cecal ligation and perforation [18]. Likewise, in a fluid-resuscitated, long-term rat model of peritoneal sepsis with organ dysfunction, a decreased complex I activity and ATP production in liver and skeletal muscle was found [19]. Another interesting finding of this model was the depletion of glutathione content in muscle and liver, thus demonstrating a reduction in antioxidant defenses, which correlates with increased cellular oxidative stress during sepsis [19].

The data regarding mitochondrial dysfunction in human sepsis are still scarce. Despite some variability in the results, most of the human studies demonstrated the same derangement in mitochondrial function as laboratory studies. In an early study, Poderoso *et al.* demonstrated in skeletal muscle from septic shock patients a lower respiratory control ratio in the presence of malate-glutamate when compared to controls and no difference in succinate-derived respiration, thus suggesting a role for impairment of complex I activity in the genesis of sepsis [20]. Boulos *et al.* incubated cultured endothelial cells with plasma from septic shock patients and demonstrated that sepsis causes a decrease in mitochondrial function reported as a lower rate of mitochondrial respiration and reduced cellular ATP levels. Interestingly, the authors described a correlation between mitochondrial function in the exposed cells and hemodynamic variables of the patients such as cardiac output and mixed venous oxygen saturation [21].

Nucleotide measurements in human septic shock, although evaluated in few studies, have consistently showed a decrease in ATP levels. Severe trauma patients who subsequently developed sepsis were characterized by an important fall in ATP, phosphocreatine and ADP [22]. In a more recent study, patients with septic shock and diverse degrees of critical illness were submitted to biopsies from intercostal and leg muscles and a decrease in energy rich-phosphates was described in addition to an increased anaerobic energy production in leg muscle, although similar findings were not demonstrated in the intercostals muscles [23].

Finally, in a larger series, Brealey *et al.* demonstrated that patients not surviving to a septic shock episode submitted to leg muscle biopsy within 24 hours of intensive care presented mitochondrial dysfunction evidenced by inhibition in complex I and reduced ATP levels. This mitochondrial dysfunction was clearly correlated to severity of shock and to plasma levels of NO metabolites. Differently from the non-survivors, septic survivors had ATP levels preserved when compared with orthopedic controls [24]. Taken together, these results may associate the mitochondria to the pathogenesis of multiple organ failure (MOF) in septic shock, al-

though a definitive causal relationship remains to be proven, since all these alterations can simply be epiphenomena.

MITOCHONDRIAL DYSFUNCTION IN SEPSIS AS A SUSPENDED ANIMATION LIKE-STATE

The previously described pathogenesis of sepsis always contemplated an excessive inflammatory response, associated with activation and recruitment of leukocytes to the inflammatory tissues. In these tissues, leukocytes (mainly neutrophils and monocytes) would release inflammatory mediators and reactive oxygen and nitrogen species (ROS/RNS) that would be responsible for fighting the microorganisms although the inflammation generated by this process would damage the tissues, induce necrosis/apoptosis and subsequently MOF.

However, several puzzling concerns with this hypothesis have been raised. First, some studies in animals with resuscitated models of sepsis demonstrated a lack of important necrosis or apoptosis in organs with laboratorial evidence of organ failure [19]. In addition, an elegant study carried out by performing autopsy in septic patients demonstrated apoptosis in lymphoid organs but, remarkably, absence of apoptosis in solid organs like kidney or liver, despite clinical evidence of organ dysfunction [25]. Another matter of concern with this pathogenetic theory of sepsis is related to the functional recovery of the organs after the septic episode (for instance, recovery from a septic acute renal failure) even in tissues with poor regenerative capacity. Indeed, it is uncommon that a patient without previous organ failure needs long-term support of failed organs after a septic episode. It sounds unlikely that if massive necrosis or apoptosis were present in these organs, they would be able to recover functional capacity during the convalescence period.

All these data lead to the discussion that the pathogenetic defect in sepsis is probably functional rather than structural. Initially, altered tissue perfusion caused by microvascular derangements may take place. As sepsis evolves, this early hypoxia and the release of inflammatory mediators may induce mitochondrial inhibition with subsequent bioenergetic failure (Fig. (**1**)). This theory may explain the progressive reduction in tissue oxygen consumption associated with the rise in tissue oxygen tension as sepsis progresses [26] and may lead to the suggestion that, if early sepsis is associated with microcirculatory disturbances, in late course of the disease the problem seems to be related more to oxygen utilization than to oxygen availability. These data lead to the hypothesis that sepsis-induced MOF could represent an adaptive state, into which affected organs 'shutdown' as a defense mechanism to survive during hostile environment (Fig. (**1**)). The metabolic shutdown mechanism could be related to the alterations in cellular bioenergetics described in the last section inasmuch as mitochondrial dysfunction, maintenance in ATP levels and reduction in oxygen consumption suggest a decrease in ATP turnover during sepsis. This mechanism may resemble the hibernation and aestivation characteristics of some animals and of human myocardium after ischemia and this metabolic downregulation may enhance the chance of recovery of organ function if the patient survives. Thus, the recovery of sepsis could represent the arousal of cells from this state of dormancy with a compatible recuperation of cellular bioenergetics. This may be achieved by recovery of dormant mitochondria or by generation of new organelles. The process of generating new mitochondria is termed mitochondrial biogenesis and will be discussed in details later in this review.

The down-regulation of the cellular metabolism after hypoxia is a known response. Hepatocytes, for instance,

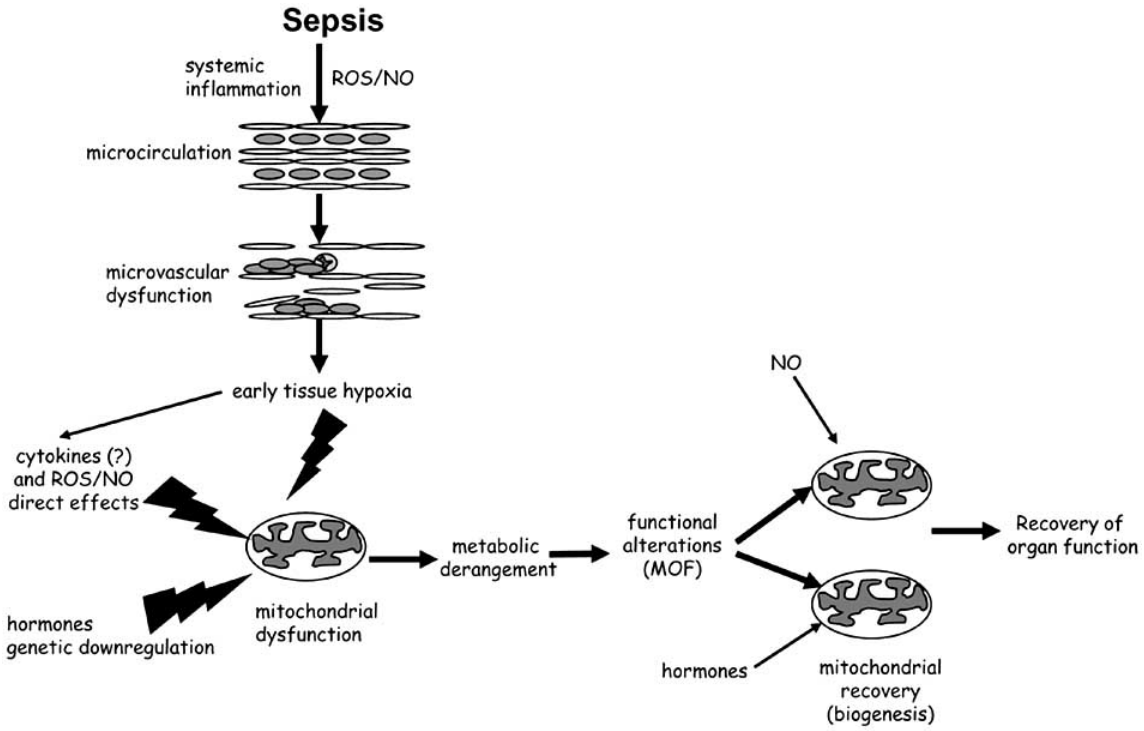

Fig. (1). Pathophysiology of mitochondrial and microcirculatory dysfunction in sepsis.

when submitted to prolonged (3 hours) of moderate hypoxia (PO_2=20-50 mmHg) respond with reduction in oxygen consumption and decrease in adenine nucleotides. Remarkably, cell viability evaluated by membrane Na^+/K^+ ATPase activity was maintained and non-essential processes (evaluated in this study by acetaminophen metabolism reactions) were reduced [27]. These data suggest that, under "hostile" milieu, hepatocytes may enter in a hibernation-like state into which they reduce their metabolism by decreasing activities non-essential for cell survival, even though they sustain indispensable enzymatic reactions.

Cardiomyocytes may enter in a down regulation state as well. The myocardial hibernation after ischemia is a known process, although the mechanism is far from being completely revealed. It is defined as a state of left ventricular dysfunction in chronic ischemia due to reduced blood flow and is considered as an adaptive response to maintain cardiomyocyte viability in this setting [28]. During hibernation, perfusion and contractile function seem to be matched due to a downregulation in energy requirements and contractility after ischemia. Thus, the transient increases in myocardial lactate, the dysfunction in substrate utilization and the reductions in creatine phosphate and ATP at the onset of ischemia gradually return towards normal over a period of hours [28, 29]. Myocardial characteristics of hibernation include increased storage of glycogen and upregulation of specific myocardial glucose transporters. Interestingly, similar changes have been reported in rats submitted to sepsis by cecal ligation and puncture. These animals developed diminished cardiac performance and increased myocardial glucose uptake in the absence of alterations in arterial oxygen pressure and myocardial perfusion [30]. These findings suggest that myocardial dysfunction of sepsis may be a cellular metabolic downregulation secondary to early microcirculatory dysfunction amplified by late mitochondrial impairment and resembling the events associated with ischemic hibernating myocardium.

Inflammatory cells may also present characteristics of metabolic shutdown during inflammatory episodes. Macrophages, for instance, when exposed to endotoxin undergo an initial increase followed by a progressive reduction in ATP consumption. Through a mechanism resembling hibernation, they transiently manage to maintain stable ATP levels despite an inhibition of cellular respiration [31, 32]. Moreover, under tissue hypoxia states (like microcirculatory disturbances in underesuscitated septic shock), macrophages can impair their mitochondrial oxygen consumption by a NO-mediated mechanism [33]. This downregulation mechanism of inflammatory cells may have important consequences to the infection control and posterior evolution to MOF. A similar mechanism of "hibernation" has been demonstrated in endothelial cells submitted to lack of substrate [34]. Under absence of glucose, porcine aortic endothelial cells reduce 80% of their protein synthesis, start using endogenous triglycerides as an alternative energy fuel and upregulate their purine de novo synthesis. These findings suggest that endothelial cells, which are in the frontline for substrate deprivation, have developed a hierarchy of metabolic reactions in order to cope with metabolic stress [34].

Although the comparison of sepsis-induced metabolic down regulation with similar mechanisms of hibernation and estivation in animals seems attractive, a real link between these phenomena has not been shown. However, some characteristics of hibernation mechanisms in animals deserve further consideration. Hibernating and aestivating animals depress their metabolic rate when the climate changes and food and water provision become restricted. Some of these animals have impressive anoxia-resisting abilities. Neurons from fresh water turtles, for instance, tolerate anoxia 100 to 1000 times longer than mammals and they do so by reducing their metabolism through decreasing ion fluxes, so that cell membrane polarization and ATP levels are maintained at least 80% of normal [35]. This hibernation-like state may also be stimulated exogenously. When a suspended animation-like state was induced in mice by administration of hydrogen sulfide (a complex IV inhibitor), they experienced a dramatically decrease in their metabolic rate which was fully reversed without any permanent behavioral or functional damage [36]. Moreover, this suspended animation has been shown to be protective against more than 6 hours of 5% oxygen hypoxia, which otherwise is claimed to be lethal after just 20 minutes of exposure [37]. Interestingly, hydrogen sulfide production has been demonstrated in animal models of sepsis, although in these studies this compound seems to induce inflammation [38].

Finally, some mention to the hormonal pathways related to cellular shutdown of sepsis has to be done. The endocrine response to stress is well recognized and involves massive releases of stress hormones, catecholamines and vasopressin. In an earlier moment, these and other hormones induce an increase in tissue oxygen consumption with cellular metabolism augmented by up to 200% [26]. However, in a later phase, there are substantial changes in the hormonal profile with reduced adrenal response to adrenocorticotropic hormone and onset of sick euthyroid syndrome. These endocrine changes may include possible modulation of mitochondrial function by thyroid hormones, leptin, sex hormones, insulin and glucocorticoids [39]. A reduced hormonal stimulation associated with decreased metabolic feedback from decreased metabolic demands is consistent with the late hypodynamic phase of septic shock where, unlike the initial phase, reductions in oxygen consumption have been reported [40].

REACTIVE OXYGEN SPECIES PRODUCTION IN SEPTIC MITOCHONDRIA

The mitochondrial respiratory chain is the major source of ROS in most mammalian cells. In average, 1-4 % of molecular oxygen in the mitochondria is incompletely reduced to superoxide, which can generate other ROS *via* enzymatic and nonenzymatic reactions. This ROS production by mitochondria could seriously damage membranes, proteins or DNA if these organelles did not dispose of very high concentrations of antioxidants.

Fig. (**2**) indicates the main sites of superoxide production in mitochondria. The suggested mechanism of superoxide production by complex I is reverse electron transfer from complex II upon succinate oxidation in the absence of NADH substrates [41]. Mitochondrial complex I can only

release superoxide in the matrix. Conversely, complex III-derived superoxide production can release this radical in the matrix and inter-membrane space [42]. Complex III produces superoxide by autoxidation of the ubisemiquinone radical intermediate formed during the Q cycle in the complex. The location where superoxide will be released after complex III production will depend on which portion of the complex is activated. This ROS generation seems to be regulated by several mechanisms, like activation of uncoupling proteins (UCPs) that control ROS production by suppressing membrane potential through proton leak across the inner membrane [43]. Other ROS and RNS such as NO and peroxynitrite can also regulate mitochondrial superoxide production.

During sepsis, the loss of efficiency of mitochondrial oxidative phosphorylation may divert electrons from the electron transport chain to the Q cycle, generating augmented quantities of superoxide. Indeed, increased superoxide generation has been demonstrated in septic shock and this phenomenon, associated with low levels of antioxidant enzymes, may increase potentially mitochondrial and cellular deleterious effects of superoxide or its toxic by-products [44].

Besides mitochondrial dysfunction, ROS production during sepsis may be associated to vascular dysfunction and thus contribute to sepsis-induced MOF as well [45]. Recent data have demonstrated that the absence of response to infusion of exogenous vasoconstrictors such as norepinephrine and dopamine in septic patients may be due to inactivation of catecholamines by superoxide [46]. It has been also demonstrated that superoxide may induce adhesion of platelets to endothelial cells in venules during endotoxemia [47]. In addition, superoxide produced by microparticles derived from platelets of septic patients may induce apoptosis of endothelial and vascular smooth muscle cells in culture [48]. A link between coagulopathy and ROS has also been described, since oxygen radicals may induce an increase in expression of tissue factor in vascular cells [49], therefore contributing to sepsis-induced microvascular dysfunction.

As a charged molecule, superoxide is not freely diffusible across cell or mitochondrial membranes and is generally restricted to the cell compartment in which it is produced. Thus, the mitochondrial permeability transition pore (through the voltage-dependent mitochondrial anion channel) can serve as a way to the passage of superoxide to the cytosol (Fig. (**2**)) [50]. Indeed, the opening of this pore during sepsis has been described, which could therefore facilitate the propagation of the harmful effects of superoxide on cells [51].

Although superoxide is not freely diffusible, hydrogen peroxide, which is the product of its dismutation reaction by superoxide dismutases (SOD), can more easily move across mitochondrial membrane [50]. In order to avoid the reaction of hydrogen peroxide with transition metals (copper, iron)

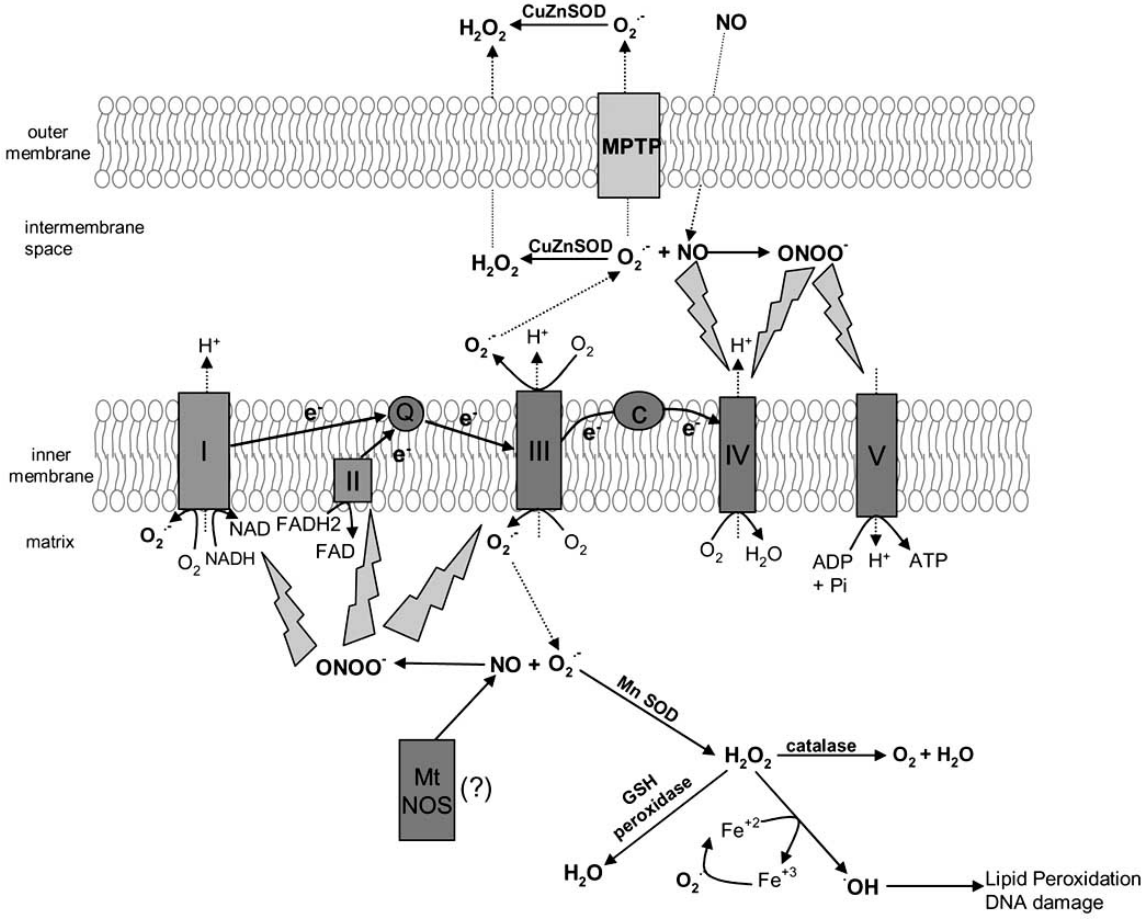

Fig. (2). Main pathways related to ROS/RNS production in mitochondria and free radical scavengers.

with the subsequent production of highly reactive hydroxyl radicals, mitochondria display several antioxidant enzymes such as glutathione peroxidase, periredoxin and catalase (present only in heart mitochondria), which can convert H_2O_2 into water (Fig. (**2**)) [52]. Hydrogen peroxide has been reported as another regulator of vascular tone and mitochondrial production of this ROS has been associated with alterations in vascular tone under flow conditions [53]. Moreover, excessive hydrogen peroxide has been reported in the intact rat pulmonary microcirculation during endotoxemia [54]. However, the role of hydrogen peroxide in sepsis has been addressed in very few studies and remains an open issue.

IS NO THE KEY ELEMENT BEHIND MITOCHONDRIAL DYSFUNCTION IN SEPSIS?

Nitric oxide is indeed a very good candidate for being the most important mediator in sepsis induced-mitochondrial dysfunction. NO is a small molecule with a short half-life that is very diffusible through cell membranes. It is synthesized in biological tissues by specific nitric oxide synthases (NOS), which metabolize arginine to citrulline with NO formation through a five electron oxidative reaction. Among several others, the known physiological processes related to NO are immune regulation, inhibition of platelet aggregation, blood pressure and vascular tone regulation [55]. In addition, NO can react readily with superoxide to form peroxynitrite ($ONOO^-$) (Fig. (**2**)), which is an oxidizing agent capable of inducing DNA fragmentation, membrane damage and lipid peroxidation. This condition seems to occur especially in inflammatory and immune cells during the oxidative burst related to inflammatory processes. Due to its shorten half-life, it is probable that most of the effects related to NO in biological systems are more appropriately carried out by its derivatives, such as nitrosothiol compounds, which retain NO characteristics and are more stable molecules.

The primordial participation of NO during sepsis, despite far from being completely clarified, has been increasingly demonstrated in the last years. From a physiological standpoint, NO production by constitutive NOS in vascular cells is necessary for regulation of vascular tone and maintenance of vascular homeostasis. Nevertheless, one of the hallmarks of septic shock is a progressive vasodilation with subsequent lack of vascular contractile response (vasoplegia). Evidences relating this vasoplegia to excessive NO production during sepsis are considerable *via* upregulation of NOS2 stimulated by excessive cytokine production during sepsis [56, 57].

Due to its lipophilic nature and high diffusion capacity, it is very possible that this excessive amount of NO produced in microvasculature during sepsis can reach mitochondria from adjacent cells [58]. A variety of cells including macrophages, microglia, endothelial cells and astrocytes during inflammatory states have been reported to produce sufficient NO not only to inhibit their own respiration but also that of surrounding cells [59]. Intrinsic mitochondrial production of NO has also been reported *via* several mechanisms including mitochondrial NOS (which existence has been a matter of debate), reductase activity of xanthine oxidoreductase or nonenzymatic conversion of nitrite or nitrate [60]. This adjacent and intrinsic cellular excessive NO production may inactivate mitochondria.

The effects of NO on mitochondrial function are related to the concentration of available NO. In low oxygen concentrations, NO is a fast, physiological and reversible inhibitor of cytochrome c oxidase (complex IV). In high concentrations, however, NO may affect several of the respiratory chain complexes, *via* its reaction with superoxide to produce peroxynitrite or by S-nitrosation or oxidation of protein thiols. Peroxynitrite generation can affect irreversibly mitochondrial respiration and oxidize several mitochondrial components, as depicted in Fig. (**2**). In addition, formation of S-nitrosothiols induces direct permeabilization of mitochondrial membranes *in vitro* and may also irreversibly inhibit complex I [59]. Other possible mechanisms of mitochondrial derangements by NO or its derivatives include regulation of mitochondrial permeability transition pore, accumulation of mitochondrial calcium and activation of soluble guanilate cyclase [61].

Tissue hypoxia is another important feature of sepsis, mainly in the early phases when microcirculation abnormalities are present and there may be associated low circulating blood volume. Tissue hypoxia may actually increase NO-induced mitochondrial dysfunction, as demonstrated in the experiments where hypoxia sensitizes mitochondria to induce necrosis of inflamed aortas [62] or apoptosis of endothelial cells *via* mitochondrial alterations related to peroxynitrite production [63]. As discussed previously, a similar mechanism of hibernation can occur with macrophages exposed to endotoxin and hypoxia [32, 33]. If lower oxygen tensions are sustained for long periods, direct competitive inhibition of respiration by NO will be increased with ROS generation and peroxynitrite production that will furthermore depress mitochondrial function. Under these conditions, ATP levels may decrease to an extent that does not allow cell survival.

Myocardial dysfunction is another frequent hallmark in sepsis. Nitric oxide has also been related to this derangement, either by itself or by toxic effects of peroxynitrite generated after reaction with superoxide [64]. Indeed, there are reports of enhanced generation of superoxide, NO and peroxynitrite in dysfunctional hearts from endotoxemic animals [65]. Among the possible mechanisms of myocardial dysfunction is inhibition of mitochondrial activity in cardiomyocytes, as demonstrated by studies depicting decreased activities of mitochondrial electron transport chain complexes in experimental sepsis [66]. In addition, there has been reported increased mitochondrial NOS activity in hearts after endotoxemia, as well as an augmented production of mitochondrial NO, superoxide and peroxynitrite [67]. Moreover, treatment of septic myocardial dysfunction with polyethylene glycol-superoxide dismutase decreased ROS formation and preserved cardiac contractility [68], thus demonstrating a clear link between sepsis, myocardial dysfunction, mitochondria and ROS production.

One remarkable demonstration of the duality of NO action in mitochondrial function is related to its effect in mitochondrial recovery. Sepsis has been reported as an inducer of mitochondrial dysfunction and, at the same time, an activator of mitochondrial biogenesis by an increase in mitochondrial genes related to recovery [67]. Interestingly, NO has been linked to mitochondrial biogenesis since cell treatment with

NO increases mitochondrial DNA, a marker of mitochondrial biogenesis, in a mechanism mediated mainly by increased expression of peroxisomal proliferator activator receptor-gamma coactivator-1alpha (PGC-1α) the main regulator of mitochondrial biogenesis [69]. A very recent study demonstrated that NOS2 regulates mitochondrial Hsp60 chaperone function, which is a regulator of mitochondrial DNA transcription and replication [70]. Taken together, these data may suggest that the increased NO production may reduce mitochondrial function during the acute phase of sepsis for the organism to cope with an overwhelming disorder and, in this meanwhile, accelerates the functional recovery by stimulating mitochondrial biogenesis.

STRATEGIES TO PREVENT OR HASTEN RECOVERY FROM MITOCHONDRIAL DYSFUNCTION IN SEPSIS

Although far from being completely clarified, the increasingly recognition of the importance of mitochondrial dysfunction in sepsis enabled the physicians to elaborate treatment proposals to reduce vascular and cellular functional damage and therefore decrease the morbidity and mortality of sepsis. However, so far most of these strategies were not associated with benefits in clinical trials.

As discussed previously, hormonal changes may undoubtedly contribute to mitochondrial dysfunction in inflammatory disorders. For example, anabolic hormones are activated in the early moments of sepsis and subsequently downregulated as sepsis evolves, in accordance with the reduction on energy consumption in this phase. Thus, any attempt to increase metabolic demands in this scenario may be detrimental, as demonstrated by studies where thyroid hormones or growth hormone were administered to critically ill patients [71, 72]. On the other hand, the beneficial effect of insulin supplementation on mitochondrial function has been discussed in the last years. Insulin administration to achieve normoglycemia in intensive care patients has been associated with reduced damage to mitochondrial ultrastructure and improved mitochondrial enzymatic activity [73] as well as microvascular protection by reducing iNOS expression and NO levels [74]. However, there has been some controversy if these results may be transposed to clinical practice [75-77].

As described before, ROS production by mitochondria is considered as an extremely relevant pathway for sepsis-induced mitochondrial dysfunction. Thus, attempts to blockade free radical generation during sepsis may ultimately reverse in clinical benefits. As a key regulator of metabolism in sepsis, it would be expected that NO and therefore NOS should be targets of several experimental and clinical studies. Indeed, the blockade of NO production in sepsis has been addressed several times, with disappointing clinical results. The increased mortality evidenced in the clinical trials of sepsis patients receiving non-selective NOS inhibitors could be due to complete blockade of NO activity. As described before, NO has several physiologic activities useful for maintenance of organic homeostasis, including possibly mitochondrial biogenesis after insult [69]. Thus, the complete inhibition of NO-associated pathways could result in an imbalance of normal functions and therefore harmful effects.

Increasing SOD activity may also be a logical choice for reducing ROS production during sepsis. SOD mimetics, small molecules containing a manganese group that possess SOD function have been tested in experimental endotoxemia and sepsis and their protective effects against oxidant injury have been demonstrated [46]. In addition, SOD mimetics reduced mitochondrial staurosporine-induced cytochrome C release in the cytosol from cultured neurons [78], thus evidencing that these drugs may become an interesting therapeutic approach.

One difficulty associated with antioxidant treatment in sepsis is the relative inaccessibility of the compounds to the interior of the mitochondria. Thus, some molecules were synthesized which use the high potential gradient across the mitochondrial inner membrane to accumulate into the matrix. Some of the antioxidants that are merged with cations to facilitate their access to the mitochondrion are vitamin E (MitoVit E), ubiquinone and ubiquinol (Mito Q). Some of these cationic antioxidants have already demonstrated to be efficacious in scavenging ROS *in vitro and ex vivo* [79]. Other recently discovered compounds designed to accumulate in the mitochondria are the hemigramicidin–TEMPO conjugates, which take advantage of the correspondence of mitochondria with bacteria to carry out their antioxidant activity, since gramicidin is a fragment of antibiotic with high selectivity for bacterial membranes [80]. However, data regarding administration of these compounds in sepsis are still scarce.

Vitamin C may also be considered in sepsis treatment, with experimental data reporting improvement in microcirculatory function after ascorbate administration in sepsis [81] as well as experimental evidence of improvement in mitochondrial function with ascorbate in cultured fibroblasts [82]. However, the clinical results with Vitamin C administration in critically ill patients have been disappointing as well.

Other plausible approach to reduce mitochondrial dysfunction in sepsis could be providing the mitochondria with increased amounts of substrates in order to facilitate mitochondrial reactions. This optimization of the residual cellular ability to produce energy could prevent the ATP level to drop below the cellular death threshold. As in sepsis-induced mitochondrial dysfunction one of the most important cellular metabolic blockades are at complex I of mitochondrial respiratory chain, the administration of substrates to complex II could potentially increase the electron flow through respiratory chain, since complex II is not normally depressed during sepsis. For example, succinate administration has been associated with improvements in liver ATP content during sepsis [83] and, more recently, with improvements in oxygen consumption in a long term rat septic model [84]. Another substrate possibly useful to treat sepsis-induced mitochondrial dysfunction would be cytochrome c. As the electron transfer from complex III to complex IV in normal electron transport chain, cytochrome c could provide additional substrate for mitochondrial complex IV, an enzymatic system known to be dysfunctional on sepsis in a mechanism probably mediated by NO and/or peroxynitrite [85]. Indeed, a recent study demonstrated an increase in complex IV activity in septic rats treated with cytochrome c 24 hours after sepsis [86].

More importantly, treatment with this molecule improved myocardial contractility in septic animals. These results have two important consequences. First, this is another demonstration that mitochondrial dysfunction could contribute for sepsis-induced myocardial depression. Secondly, although these data require reproducibility, it may be a more concrete evidence that mitochondrial dysfunction is really connected to the pathogenesis of sepsis and not just an epiphenomenon. However, some drawbacks have to be considered in this study, since they could not demonstrate a quantitative incorporation of cytochrome c into the mitochondria and some other properties of cytochrome c may have accounted for this effect, such as its general antioxidant capacity.

Finally, treatments that induce mitochondrial biogenesis could also improve mitochondrial function after sepsis. This process seems to be regulated at a transcriptional level, and messengers such as PGC-1α could be upregulated and therefore increase biogenesis. Resveratrol, for example, is an antioxidant compound isolated from the skin of grapes whose chronic administration has been demonstrated to increase mitochondrial activity by a PGC-1α-dependent mechanism [87]. Another antioxidant which has been depicted to stimulate mitochondria is piruvate, though so far the mechanism seems to be PGC-1α independent [88]. Likewise, roziglitazone, a peroxisomal proliferator activator receptor agonist and insulin sensitizer used to treat diabetes, has been demonstrated to improve mitochondrial biogenesis in mouse brain [89] and mRNA of PGC-1α as well as increase muscle oxidative enzyme activity in obese diabetic patients [90].

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