REVIEW ARTICLE

Josefina Duran-Bedolla, MD¹

Marco A. Montes de Oca-Sandoval, MD²

Vianey Saldaña-Navor, MD¹

José A. Villalobos-Silva, MD³

Maria Carmen Rodriguez, PhD¹

Selva Rivas-Arancibia PhD⁴

¹ Centre for Research on Infectious Diseases, National Institute of Public Health, Cuernavaca, Mexico

² Department of Critical Medicine and Intensive Therapy, Medical Center ABC, Mexico

³ Unit of Critical Medicine, Regional High Specialty Hospital, Cd. Victoria, Mexico

⁴ Department of Physiology, School of Medicine, National Autonomous University of Mexico (UNAM), Mexico.

Sepsis, mitochondrial failure and multiple organ dysfunction

Abstract

Purpose: The purpose of this review is to consider the state of oxidative stress, failure of the antioxidant systems and mitochondrial failure as the main physiopathological mechanisms leading to multiple organ dysfunction during sepsis.

Principal findings: Sepsis is a clinical syndrome caused by a severe infection that triggers an exaggerated inflammatory response. Involved in the pathogenesis of sepsis are the activation of inflammatory, immune, hormonal, metabolic and bioenergetic responses. One of the pivotal factors in these processes is the increase of reactive species accompanied by the failure of the antioxidant systems, leading to a state of irreversible oxidative stress and mitochondrial failure.

In a physiological state, reactive species and antioxidant systems are in redox balance. The loss of this balance during both chronic and infectious diseases leads to a state of oxidative stress, which is considered to be the greatest promoter of a systemic inflammatory response. The loss of the redox balance, together with a systemic inflammatory response during sepsis, can lead to progressive and irreversible mitochondrial failure, energy depletion, hypoxia, septic shock, severe sepsis, multiple organ dysfunction and death of the patient.

Conclusion: Knowledge of the molecular processes associated with the development of oxidative stress should facilitate the development of effective therapies and better prognosis for patients with sepsis and organ dysfunction.

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Correspondence to:

Josefina Duran-Bedolla

Centro de Investigación Sobre Enfermedades Infecciosas,
Instituto Nacional de Salud Pública.

Av. Universidad #65. Col. Santa María Ahuacatitlán, Cuernavaca, Morelos, México.

E-mail: josefina.duran@insp.mx

Current Concepts in Sepsis

Sepsis is an excessive or poorly regulated systemic inflammatory response that causes intravascular damage in the host. It is diagnosed by clinical evidence of infection (suspected or documented) and at least two parameters that indicate a systemic inflammatory response [1, 2] (Inflammatory variables, Table 1). Severe sepsis is associated with the dysfunction of one or more organs, and is characterized by hyperlactatemia, oliguria, altered mental status, arterial hypoxemia, arterial hypotension and hyperglycemia [1] (Organ dysfunction variables, Table 1).

Septic shock is produced by the presence of sepsis with refractory hypotension (Figure 1). It is defined as systolic blood pressure (SBP) <90 mmHg, mean arterial pressure (MAP) <70 mmHg, or a decrease in SBP of >40 mmHg in adults or less than two standard deviation below normal according to age, despite adequate fluid resuscitation [3, 4] (Hemodynamic variables, Table 1). Septic shock can be understood as severe sepsis with cardiovascular failure.

With septic shock, there is a decrease in blood perfusion and supply of oxygen to cells. As is amply demonstrated, cellular hypoxia causes an increase of free radicals, which increases the already high state of oxidative stress and leads to the entry of calcium to the endoplasmic reticulum and mitochondria, causing the release of cytochrome c and the induction of cellular death. Finally, mitochondria failure with the consequent energy deficit does not allow for normal cellular metabolism [5]. With ischemia, the accumulation of intracellular calcium leads to an increase of AMPc and vascular endothelium injury [6]. Inflammation, manifested clinically as the systemic inflammatory response syndrome (SIRS) is a prerequisite for the development of sepsis. SIRS is a progressive systemic inflammatory response to infection or some non-infectious process. Thus there is a close relationship between oxidative stress and the inflammatory response in septic shock.

It has been shown that inflammation causes a state of oxidative stress; therefore, SIRS, together the hypoxia present in septic shock, induces a state of oxidative stress with irreversible mitochondrial failure causing organ dysfunction. This condition is characterized by the progressive dysfunction of two or more systems (Figure 1).

Finally, two major factors lead to multiple organ dysfunction during sepsis: 1) SIRS, which is characterized by an inflammatory response that produces free radicals, thus increasing the state of oxidative stress and 2) failure of cellular perfusion, which leads to hypoxia and produces a large quantity of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in cells, leading to mitochondrial failure, to multiple

TABLE 1. Diagnostic criteria of sepsis.

Diagnostic criteria of sepsis	General variables <ul style="list-style-type: none"> Fever (> 38.3 °C) Hypothermia (core temperature < 36° C) Heart rate > 90 min or more than two SD above the normal value for age Tachypnea Altered mental state Significant edema or positive fluid balance (> 20 mL/kg over 24h) Hyperglycemia (plasma glucose > 140 mg/dL or 7.7 mmol/L) in the absence of diabetes
	Organ dysfunction variables <ul style="list-style-type: none"> Arterial hypoxemia ($\text{PaO}_2/\text{FiO}_2 < 300$) Acute oliguria (urine output <0.5 mL kg/h for at least 2 h despite adequate fluid resuscitation) Creatinine increase >0.5 mg/dL or 44.2 $\mu\text{mol/L}$ Coagulation abnormalities (INR >1.5 or aPTT >60 s) Ileus (absence of bowel sounds) Thrombocytopenia (platelet count <100,000 μL)
	Inflammatory variables <ul style="list-style-type: none"> Leukocytosis (WBC count >12,000 μL) Leukopenia (WBC count <4,000 μL) Normal WBC count with greater than 10 % immature forms Plasma C-reactive protein more than two SD above the normal value Plasma procalcitonin more than two SD above the normal value
	Hemodynamic variables <ul style="list-style-type: none"> Arterial hypotension (SBP <90 mmHg, MAP <70 mmHg, or SBP decrease >40 mmHg in adults or less than two SD below normal for age)
	Tissue perfusion variables <ul style="list-style-type: none"> Hyperlactatemia (>1 mmol/L) <ul style="list-style-type: none"> Decreased capillary refill or mottling
Organ dysfunction variables <ul style="list-style-type: none"> Sepsis-induced hypotension Lactate above upper limits of laboratory normal Urine output <0.5 mL/kg/h for more than 2 h despite adequate fluid resuscitation Acute lung injury with $\text{PaO}_2/\text{FiO}_2 < 250$ in the absence of pneumonia as infection source Acute lung injury with $\text{PaO}_2/\text{FiO}_2 < 200$ in the presence of pneumonia as infection source Creatinine >2.0 mg/dL (176.8 $\mu\text{mol/L}$) Bilirubin >2 mg/dL (34.2 $\mu\text{mol/L}$) Platelet count <100,000 μL Coagulopathy (international normalized ratio >1.5)

FIGURE 1. Main physiopathological mechanisms leading to multiple organ dysfunction during sepsis.

organ dysfunction and finally to death (Figure 1).

Mitochondrial Failure and Flawed Bioenergetics in the Multiple Organ Dysfunction Syndrome

Mitochondria are a specialized organelle of eukaryotic organisms that generate most of the energy in a cell (in the form of ATP) through oxidative phosphorylation. These organelles are involved in crucial processes, such as cell death pathways, intracellular regulation of calcium, cell signaling and the sensing of O₂ levels. Mitochondria are the main producers of reactive oxygen species; however, their own antioxidant systems contribute to the maintenance of redox balance and mitochondrial function, both of which are crucial for multiple cell processes. To carry out their functions, mitochondria have an important quality control, known as mitophagy, which eliminates damaged mitochondria. This process maintains oxidative phosphorylation, as well as the integrity of the mitochondrial function, and, consequently, preserves the health of cells.

Under conditions of stress, cells can adapt in order to maintain viability. Cells try to protect themselves (defense mechanism) through a decrease in the consumption of O₂ energy use, ATP demand and basal cellular functions, as well as an

increase in the consumption of energy reserves (for example, cellular hibernation) and the activation of the damage repair mechanism.

The modulation of the oxidative state of cells is crucial to health, since an increase in the number of new mitochondria and the elimination of old and damaged mitochondria are essential processes for maintaining an adequate capacity of oxidative phosphorylation. When cells are under metabolic or environmental stress, this capacity must respond to situations of increased energy demand, promote apoptosis, compensate for a lack of nutrients and maintain a network of healthy mitochondria [7, 8].

The production of ATP in the mitochondria is a regulated process whose substrates are provided by the oxidative metabolism of glucose, lipids and amino acids. These substrates are then metabolized to pyruvate. Subsequently, through the enzyme complex of pyruvate dehydrogenase (PDH), NAD (nicotinamine adenine dinucleotide) and coenzyme A, acetyl-CoA is formed within the mitochondria. Acetyl-CoA enters the Krebs cycle to produce energy in the form of GTP, which is used to promote the synthesis of ATP [5]. A large number of reactive species are produced as a result of this energy metabo-

lism. During pathological processes, such as sepsis, these reactive species provide the potential for cell damage. Since mitochondria are the main producers of energy in the cell, they are the main site of ROS production. Accordingly, studies with different experimental models of sepsis have reported ultrastructural and functional changes in mitochondria [9, 10]. It has been reported that these changes are reversible during treatment and recovery from sepsis, leading to improvement in mitochondrial function [11]. In contrast, there are also reports of irreversible mitochondrial failure associated with oxidative stress and in the presence of multiple organ dysfunction.

Reactive Species

A free radical is a molecule characterized by the presence of one or more free electrons in the outer orbit. The presence of these electrons gives the molecule great instability, making it highly reactive and toxic [12]. Reactive species, produced under physiological conditions as a result of the metabolism of free radicals, include both free radicals and other molecules that are not free radicals. The most common are ROS and RNS.

In a redox balance, reactive species play an important role in the life cycle of cells (including development, physiology and migration) [13], the induction of cell signaling pathways [14], the activation of intra- and intercellular secondary messengers [15] and immune cell defense mechanisms during phagocytosis of pathogenic agents [16]. RNS are also involved in the regulation of blood pressure and vascular tone [12], the activation of NF κ B by TLR-4 receptors [17], the release of inflammatory cytokines and the expression of adhesion molecules [18]. Finally, they are inducers of the antioxidant response that is pivotal for maintaining redox balance. An excess of oxidant molecules and/or a decrease in antioxidant molecules causes the loss of this balance, known as oxidative stress. This condition can seriously compromise the health of a patient.

An increase in ROS and RNS has been observed in certain pathological conditions, including anoxia, re-oxygenation, ischemia/reperfusion, excessive inflammation, trauma, infection and sepsis. In the early stages of sepsis, the lipopolysaccharides (LPS) of pathogenic microorganisms induce the release of ROS. At the same time, phagocytic cells use ROS to attack and eliminate these pathogenic agents. During this battle between the invasive microorganisms and the immune response, an insufficient antioxidant system would lead to increased levels of reactive species, mitochondrial dysfunction [19] and oxidative stress. This cascade can occur during infection and is associated with sepsis [20].

Reactive Oxygen Species (ROS)

The main endogenous ROS are the super oxide anion (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH\cdot$). During the production of ATP in mitochondria, electron leakage occurs in complexes I and III of the electron transport chain, which is the main site of O_2^- production. Under physiological conditions, and as a product of mitochondrial respiration, approximately 2% of the oxygen consumed is converted into O_2^- . O_2^- -induced mitochondrial damage is prevented by the activity of the superoxide dismutase (SOD) enzyme of manganese (Mn-SOD), which is present in mitochondria [12] (Figure 2-A).

The production of O_2^- and other ROS is also caused by phagocytosis, which involves an oxidative burst with bactericidal activity (Figure 2-B). ROS can activate the nuclear factor κ B (NF κ B), induce apoptosis, increase the expression of transcription factors and prolong inflammatory processes [17]. H_2O_2 is an ROS that easily diffuses through the cell membranes. It is produced in peroxisomes and by the activity of two enzymes: xanthine dehydrogenase/xanthine oxidase (XDH/XO) [21] and NADPH oxidase (NOX) [21, 22]. ROS produced by NOX regulate multiple cellular processes, such as cellular differentiation, proliferation and migration. These ROS also help to maintain vascular tone [23]. During processes of inflammation and ischemia, XDH is converted to XO by oxidation of cysteine residues. In this process, XO uses O_2 to produce large quantities of ROS [24], which contributes to the increased severity of oxidative stress during sepsis (Figure 2-C).

Protein synthesis and the production of ROS

The endoplasmic reticulum is another cell organelle that contributes to the production of ROS during the processes of synthesis, folding, posttranslational modifications and the traffic of proteins of secretory pathway. In addition, enzymes such as cytochrome P450 and cyclooxygenase can contribute to an increased production of ROS [25]. Protein folding takes place in the highly oxidized environment of the endoplasmic reticulum, where enzymes such as protein disulfide isomerase (PDI) and oxidoreductase 1 of endoplasmic reticulum (ERO1) participate in the formation of the disulfide bridges that give the necessary stability for the anchoring of secretory proteins. PDI captures electrons released by the oxidation of the thiol group (SH) of cysteine residues during the formation of disulfide bridges. In the presence of FAD, ERO1 transfers electrons from PDI to O_2 , and this oxidative process generates a large amount of H_2O_2 (Figure 2-D).

FIGURE 2. The main processes for maintaining redox balance in cells. (A) In mitochondria, electron leakage occurs in complexes I and III of the electron transport chain, which is the main site of $O_2^{\bullet -}$ production. (B) ROS are produced during phagocytosis, inflammatory processes, ischemia and in response to LPS and cytokines. (C) ROS are produced in peroxisomes by the activity of two enzymes: xanthine dehydrogenase (XDH) and xanthine oxidase (XO). (D) Synthesis, folding and post-translational modifications of secretory proteins are oxidative processes that produce ROS in the endoplasmic reticulum (ER). (E) Antioxidant systems, including superoxide dismutase (SOD), peroxiredoxins, glutathione and thioredoxin systems, maintain the redox balance.

Reactive species cause alterations in polypeptides

ROS can break polypeptide chains; a process that produces alterations in the charge of proteins (thus affecting bonding with other proteins), oxidation of specific amino acids (such as methionine and cysteine) and an increased susceptibility to proteolytic attack. Oxidation of amino acids may cause conformational changes, misfolding and degradation of proteins. Correctly folded proteins are less susceptible than misfolded proteins to oxidation [26]. This may be a mechanism of quality control of cell functions. During sepsis, the immune and others

systems attempt to maintain and/or reestablish homeostasis by stimulating the production of peptides and proteins.

Reactive Nitrogen Species (RNS)

Nitric oxide (NO) is a toxic, reactive and unstable molecule. Nitric oxide synthase (NOS) catalyzes the formation of NO from the amino acid L-arginine. There are three types of nitric oxide synthase: (i) NOS I, also known as neuronal NOS (nNOS), (ii) NOS II, or inducible NOS (iNOS), and (iii) NOS III, or endothelial NOS (eNOS). nNOS and eNOS are continually producing small amounts of NO to regulate vascu-

lar tone, whereas iNOS is only activated during inflammation, when it can produce large amounts of NO for long periods of time. This activation can be caused by stimulation from LPS, IL-1, TNF- α and IFN- γ . NO modifies vascular contractility by favoring vasodilation. This contributes to increased microvascular permeability at the site of infection and, in the case of septic shock, to hypotension. In addition, as observed in experimental animal models, NO participates in the development of microvascular damage, vascular hyporeactivity, the dysfunction of organs [27] and the induction of apoptosis [28].

It is important to mention that neutrophils and endothelial cells are the main sources of the iNOS that increases the production of RNS during sepsis [29]. The reaction of RNS with other reactive species produces molecules that are even more toxic, including peroxynitrite. The latter, a product of the reaction between NO and O₂⁻, is a potent inducer of cell death and increases oxidative stress during sepsis. Paradoxically, NOS has a crucial protective role in the maintenance of blood flow by preventing the formation of microvascular thrombosis, adhesion molecules and platelet aggregation. NOS also is involved in the migration of leukocytes. These processes are all characteristic of acute inflammation [30, 31].

Therapy with selective and non-selective inhibitors of NOS has improved hemodynamic parameters of septic patients, but has significantly increased the rate of mortality as well [32, 33]; thus, NO has a deleterious effect as well as a protective role, the latter probably resulting from its function as an immunomodulator.

Reactive Species and Cell Damage

Reactive species and damage to DNA

ROS and RNS cause modifications or degradation of the nitrogenous bases or sugars of the DNA structure, which leads to a breakdown, deletion or mutation of the DNA strands. Damage to nitrogenous bases, mainly to guanine, resulting in the formation of 8-oxoguanin, has mutagenic and carcinogenic properties [34]. In addition, binding sites of transcription factors containing cytokine- and guanine-rich sequences are more susceptible to oxidative attack. On the other hand, ROS contribute to an inflammatory response by activating transcription factors, such as NF- κ B, which are sensitive to the redox state. NF- κ B participates in the production of inflammatory cytokines under conditions of stress.

Reactive species cause lipid oxidation

The oxidation of molecules or receptors on cell membranes may alter the selectivity or permeability of the membranes, producing changes in the osmotic balance of the cell [34]. In contrast, the oxidation of lipids can lead to a chain reaction resulting in apoptosis. This chain reaction begins with cardiolipin, a phospholipid that is associated with cytochrome c inside the mitochondria. Oxidation of cardiolipin causes the opening of mitochondrial transition pores [35] and the dissociation of cytochrome c [36, 37], causing this molecule to be released into the cytosol where it interacts with the Apaf-1 adapter molecule. This results in the activation of caspase 9, which activates caspase 3 and 7, which are responsible for the biochemical and morphological changes characteristic of apoptosis.

Due to its short half-life, detection of ROS is difficult *in vivo*. The average half-life of hydroxyl is 10⁻⁹ seconds, singlet oxygen (¹O₂) 10⁻⁶ seconds and NO 3-5 seconds. Because of the short half lives of these ROS, biomarkers are used to measure the level of oxidative stress. One such biomarker is malondialdehyde (MDA), a toxic end product of lipid peroxidation. MDA can react with nucleic acids and lipoproteins, free sulfhydryl groups (SH) and amino groups (NH), or the latter groups bound to proteins and is used as an indicator of cellular damage [38, 39]. When MDA reacts with the bases of DNA, it produces adducts of deoxyadenosine (M1A), deoxyguanosine (M1G) and other molecules. The main DNA adduct is M1G, which is both mutagenic and carcinogenic, and is also used as a biomarker of oxidative stress [40].

Paradoxically, oxidized phospholipids have a protective role against the inflammation induced by LPS in experimental animal models. As an inflammatory modulator, they reduce cell mortality [41].

Antioxidant Systems

To maintain the cellular environment in a redox balance, the accumulation of reactive species is prevented by the production of antioxidant molecules, which can delay or inhibit oxidation [12]. Antioxidant systems contain highly conserved molecules and are very important for the growth and development of eukaryotic organisms [42]. These molecules are classified as enzymatic and non-enzymatic, as well as endogenous and exogenous [34]. Enzymatic molecules include superoxide dismutase (SOD), glutathione peroxidase, catalase and thioredoxin. Among non-enzymatic molecules, usually ingested in the diet, are vitamins (A, C and E), amino acids and metals (copper and selenium). These molecules participate in the reduction of inflammatory interleukins, the improvement of mitochondrial

function and the relief of oxidative stress. Antioxidant systems show a complementarity and synchronized activity (Figure 2-E).

Superoxide dismutase (SOD)

SOD is a metallo-enzyme that catalyzes the dismutation of the superoxide anion (from oxidative phosphorylation, inflammation processes, ischemia, phagocytosis and in response to LPS and cytokines) to H_2O_2 [43]. This H_2O_2 is later metabolized into water by glutathione peroxidase and catalase. In humans, SOD takes the form of copper-zinc SOD (CuZn-SOD) and Mn-SOD, which have a molecular weight of 32 and 88 kDa and are located in the cytosol and mitochondria, respectively [42]. SOD is an antioxidant enzyme with one of the highest activity levels in organs such as the liver, adrenal gland, kidney and spleen [44]. Mutations of Mn-SOD can cause death by oxidative stress [45].

Glutathione system

Glutathione is a thiol tripeptide (L- γ -glutamic acid, L-cysteine and glycine) that is present in all cells of the body and has a critical role in the maintenance of the intracellular redox balance [42]. The glutathione system is composed of glutathione peroxidase (GP) and glutathione reductase (GR). GP catalyzes the formation of glutathione disulfide (the dimeric form) during the reduction of hydroperoxides and GR, which produces glutathione (the monomeric form). Glutathione status is dependent on the amount of reduced glutathione (GSH, the monomeric form) and oxidized glutathione (GSSG, the dimeric form) (Figure 2-E) [46]. Glutathione participates in the reduction of peroxides (including H_2O_2) (Figure 2-E), keeps proteins and other molecules in their reduced state and contributes to the regulation of protein and DNA synthesis [46]. Through the donation of hydrogen, this thiol directly inactivates free radicals and detoxifies metabolites of drugs [47]. The concentration of intracellular glutathione is 1 to 8 mM, reflecting a dynamic balance between synthesis, consumption and regeneration [47]. High glutathione levels are found in the liver, muscle and brain [47].

There are factors that increase the synthesis of glutathione during sepsis, such as increased levels of inflammatory cytokines, ROS, NOS, growth hormones (GH) and thyroid hormones (T3 and T4) [47]. In addition, sepsis increases the activity of enzymes related to the metabolism of glutathione, γ -glutamylcysteine synthetase, GR, GP and glutathione transferase (GT) [47, 48]. Tissues are able to increase glutathione levels through *de novo* synthesis in response to infection. Simi-

larly, there are factors that decrease the synthesis of glutathione during sepsis, such as anti-inflammatory cytokines, malnutrition, hyperglycemia and the administration of erythropoietin, glucocorticoids and catecholamines. Low glutathione levels are associated with higher mortality in experimental models of sepsis [49]. Glutathione is the main mechanism that protects the cell against oxidative damage [48].

Thioredoxin system

Thioredoxins, highly conserved molecules in humans and other organisms, are a family of proteins classified as thioredoxin-1 or thioredoxin-2. Thioredoxin-1 is located in the cytosol and nucleus, while thioredoxin-2 is found in the mitochondria. Mutations in thioredoxin are lethal to living organisms [50, 51]. Thioredoxins are involved as donors of hydrogen to ribonucleotide reductase in the synthesis of DNA. They also catalyze the reduction of H_2O_2 , prevent oxidative stress (Figure 2-E) and the induction of apoptosis, and regulate the activation or inhibition of transcription factors such as $\text{NF-}\kappa\text{B}$ [34]. Thioredoxins are secreted by lymphocytes, hepatocytes and fibroblasts, and their cellular levels reflect the degree of inflammation in humans.

Catalase

Catalase is one of the most abundant enzymes of mitochondria and peroxisomes in mammals, with high activity in the liver and kidneys, low activity in connective tissue and no activity in nervous tissue. When glutathione peroxidase concentrations are low, catalase catalyzes the H_2O_2 generated during cellular metabolism [12]. Transgenic mice with low levels of catalase show a normal development, but are more sensitive to oxidative damage [52].

Vitamins

Vitamin C inhibits the formation of O_2^- during digestion, inhibits endothelial dysfunction and iNOS-induced inflammation, prevents vascular reactivity and improves vascular stability induced by vasoconstrictor drugs [53]. In animal models, the administration of vitamin C reversed the microcirculatory dysfunction that appeared within 6-24 hours after the onset of sepsis [54]. This study strongly suggests that the use of high doses of vitamin C represents a therapeutic option for the treatment of shock in critical cases [55].

Vitamin E is one of the first barriers against the peroxidation of polyunsaturated fatty acids. In combination with vitamin A, it acts by decreasing the formation of free radicals [56]. The administration of 3000 mg of vitamin C, in combination

with 2000 IU of α -tocopherol per day, reduced the risk of lung disease as well as the duration of mechanical ventilation and multiple organ dysfunction [57].

Patients with sepsis show a decrease in the levels of some antioxidants, such as tocopherol, retinol, carotenoids, selenium, zinc and glutathione, an increase in the oxidation of lipids as well as an increase in the production of ROS and RNS, XO and MDA [58, 59]. *Ex vivo* studies demonstrated that enteral administration of a combination of vitamins A, C and E improved mitochondrial function, maintained redox balance, decreased the formation of free radicals and improved the resistance to oxidative stress [60].

Studies on the effects of antioxidants in experimental animal models show a decrease in proinflammatory cytokines and a 22% reduction in mortality [61]. The administration of antioxidants has been shown to benefit patients with sepsis; for example, selenium attenuates organ failure [62], and, when combined with copper and zinc, reduces nosocomial pneumonia in burn patients [63]. Administration of glutamine reduces the complications due to infection [64], and, in association with *N*-acetylcysteine, reduces the damage caused by the oxidation of lipids in patients with septic shock [65]. Finally, eicosapentaenoic acid, in combination with micronutrients, shows anti-inflammatory effects [66].

Organ Dysfunction

Sepsis is a stress condition that produces a state of oxidative stress, a decrease in antioxidant activity and a loss of regulatory mechanisms. While cell systems try to protect themselves from damage caused by oxidative stress, the activation of apoptotic pathways produces an irreversible response of cellular death that induces organ dysfunction.

As discussed above, ROS and RNS production is increased and antioxidant protection reduced under pathogenic conditions. Moreover, there is mitochondrial damage and an increase in the inflammatory response. The generation of ROS is amplified, leading to mitochondrial dysfunction and a massive release of cytokines. The latter leads to the continuous activation of macrophages, polymorphonuclear leukocytes and lymphocytes, which recruits more immune cells. In patients with sepsis, this contributes to organ dysfunction, characterized by a high level of the biochemical markers of tissue injury caused by ischemia, reperfusion and systemic inflammation [67].

Microcirculation is the main target for injury in sepsis [68]. Capillary function is decreased, which prevents oxygen uptake and increases endothelial permeability [68]. The latter changes produce edema with protein-rich fluid [69]. In the lungs, vascular epithelium injury produces interstitial and al-

veolar edema and the phagocytic cells retained in microcirculation increase the injury to alveolar membranes [69]. In the kidney, factors such as systemic hypotension, renal vasoconstriction and release of cytokines produce acute tubular necrosis and renal injury [70]. In the liver, elevated levels of markers such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and bilirubin, as well as coagulation defects, can be involved in the failure of this organ [71].

Sepsis initially induces high metabolic activity that leads to an increase in mitochondrial function; however, prolonged illness induces a metabolic dysfunction and mitochondrial damage, which, in turn, reduces cellular metabolism and energy production. The cells enter a state of hibernation, expressed as organ dysfunction [72]. Organ dysfunction in patients with sepsis can be explained by two hypotheses: the microvascular hypothesis, which suggests a defect in delivery of O_2 in the blood vessels and capillaries [70], or the cellular hypoxia hypothesis, which suggests the development of mitochondrial dysfunction and an ineffective use of O_2 [71]. In both cases, cells can adapt to these changes and maintain their viability through a reduced consumption of O_2 , lower energy requirements and less demand for ATP; for example, myocardial hibernation is characterized by a decrease in cardiac contractility and a maintenance of ATP levels, indicating that cells try to protect and maintain the integrity and function of oxidative phosphorylation [72].

Cell Hibernation And Mitochondria

During sepsis, there are various mechanisms by which the organism tries to protect itself against energy dysfunction, including a reduction in the basal functions (and therefore in the energy requirements) of cells and metabolic pathways, an increase in the consumption of energy reserves and the activation of damage repair mechanisms. Additionally, there are mechanisms that involve changing the energy metabolism of cells and avoiding the development of cytopathic hypoxia [71].

1. A decrease in the amount of pyruvate that enters the Krebs cycle

Acetyl-CoA, a compound of high energy, is a product of the metabolism of amino acids, fatty acids and carbohydrates. It is synthesized from pyruvate through oxidative decarboxylation. In this process, pyruvate is catalyzed by the PDH complex; thus, inhibition of PDH leads to a decrease in the pyruvate that enters the Krebs cycle. Since pyruvate is a vital molecule for the production of energy, this causes disorders in energy metabolism, which is manifested as low ATP production and by lactate

accumulation, which, in turn, can trigger the organ dysfunction that is characteristic of patients with sepsis [71].

2. Inhibition of mitochondrial enzymes involved in the Krebs cycle and electron transport chain

Sepsis is associated with the induction of iNOS, which increases the production of NO and peroxynitrite. NO is a signaling and effector molecule that reversibly inhibits the enzymatic activity of cytochrome *a/a3* in the electron transport chain, leading to a decrease in cellular respiration [73]. In addition, NO reacts quickly with other molecules to produce other RNS, such as peroxynitrite. Mitochondrial enzymes may be the targets of these reactive species [30] and of the cytokines that undergo increased production during sepsis. Consequently, there is damage to cellular energy metabolism, leading to multiple organ dysfunction [74].

On the other hand, cytochrome oxidase uses electrons donated by cytochrome *c* to reduce O_2 to H_2O . In sepsis-associated myocardial depression, cytochrome oxidase is competitively inhibited and produces oxidative phosphorylation dysfunction, a condition that improves with exogenous administration of cytochrome *c* [75].

3. Activation of enzymes involved in DNA damage repair: Poly (ADP-ribose) polymerase (PARP-1)

Oxidative damage to DNA caused by free radicals or infectious disease stimulates the activation of PARP-1 for repairing DNA, but at the same time leads to a decrease in levels of NAD^+ /NADH, which is involved in oxidative phosphorylation for the production of energy. An excessive stimulation of PARP-1 can lead to depletion of NAD^+ /NADH and can therefore interfere with the synthesis of ATP [76], leading to a state of cytopathic hypoxia and cell death. This has been studied in various cells, including macrophages and smooth muscle cells stimulated with oxidizing molecules [77], and enterocytes stimulated with proinflammatory cytokines and oxidizing molecules [78].

Cell death

A systemic inflammatory response and organ dysfunction are characterized by increased cell death in the affected organs [79], such as lungs, liver, spleen, heart, skeletal muscle and blood cells. Oxidative stress can induce cell death via the apoptotic or necrotic pathway. Apoptosis requires the participation of apoptotic factors, including the release of cytochrome *c*, changes in electron transport, loss of the mitochondrial membrane potential, loss of redox balance and condensation of chromatin and nuclear fragmentation. Apoptosis is character-

ized by the preservation of intact cellular organelles and the plasma membrane, and, at the same time, the condensation of chromatin, nuclear fragmentation and formation of apoptotic bodies. High concentrations of ROS induce apoptosis in different types of cells [80]; for example, T-lymphocytes exposed to moderate concentrations of H_2O_2 induce apoptosis through activation of caspase [81].

Conclusions

The main role of mitochondria is to provide energy to the cell through aerobic metabolism. However, large amounts of ROS and RNS are generated as a result of cellular metabolism, which then requires an effective antioxidant system to maintain homeostasis. When this balance is not maintained, cellular bioenergetics is compromised and so is the functioning and survival of cells. One disorder that involves an imbalance in oxidative and antioxidant mechanisms is sepsis, whose incidence has significantly increased morbidity and mortality in recent decades. Pathophysiological insights indicate that it is an extremely complex disease. Sepsis is an exaggerated inflammatory response whose main promoter and mediator is oxidative stress. This imbalance involves elevated levels of reactive species, which, in turn, cause damage to vital molecules of cells, such as DNA, lipids and proteins. The consequent energy depletion and mitochondrial dysfunction can trigger organ dysfunction and death in patients. Greater understanding of the molecular processes associated with the development of sepsis should facilitate the development of new diagnostic tests and an effective treatment for patients.

In animal models, it has been shown that managing the state of oxidative stress and maintaining the proper function of mitochondria is crucial during the treatment of sepsis. Further studies are needed on patients to confirm that these findings are applicable in the clinical setting.

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