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REVIEW ARTICLE

Reactive Oxygen Species (Ros) Generation in Sepsis

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Summary

Sepsis and septic shock remain as leading cause of death in adult intensive care units. It is widely accepted that sepsis and septic shock are caused predominantly by gram-negative bacteria and their endotoxins. Endotoxin or Lipopolysaccharide (LPS) have important roles as host responses and trigger the inflammatory processes, caused by gram-negative bacterial infection. Production of oxygen radicals by neutrophils and macrophages such as reactive oxygen species (ROS), NO (nitric oxide) and peroxynitrite promote gene expression of proinflammatory mediators. Enhanced generation of ROS well be responsible for tissue injury in septic shock and endotoxemia. Oxidative stress is defined as an unbalance between oxidants and antioxidants. Antioxidant capacity may be compromised in patients with severe infections and high levels of the metabolic products of free radical damage can be observed. The aim of this review is to inspect the play role of inflammatory mediators with oxidative stress is associated reactive oxygen species or reactive nitrogen species and the negative effects including DNA damage of sepsis pathogenesis.

Key Words: *Oxidative stress, Sepsis*

Sepsiste Reaktif Oksijen Türleri

Özet

Sepsis ve septik şok, erişkin yoğun bakım ünitelerindeki ölümlerin önde gelen nedenlerinden olmaya devam etmektedir. Sepsis ve septik şoka başlıca gram negatif bakteri ve endotoksininin neden olduğu kabul edilmektedir. Gram negatif bakteriyel enfeksiyonun neden olduğu endotoksin ve lipopolisakkarid, konakçı cevabında önemli rol oynar ve inflamatuar süreci tetikler. Nötrofiller ve makrofajlar tarafından üretilen reaktif oksijen türevleri, nitrik oksit ve peroksinitrit gibi proinflamatuar mediatörlerin ifadelerinde artışa yol açar. Bu nedenle, reaktif oksijen türlerinin aktivasyonu, endotoksemi ve septik şoktaki doku hasarından sorumlu olabilir. Oksidatif stress oksidanlar ve antioksidanlar arasındaki dengesizlik olarak tanımlanır. Bu durum normal antioksidan defansın kaybı ve/veya vücutta serbest radikallerin artmasından dolayı olabilir. Ciddi enfeksiyonlu hastalarda antioksidan kapasite kötüleşebilir ve serbest radikal hasarına bağlı metabolik ürünlerin yüksek seviyeleri gözlenebilir. Bu derlemenin amacı sepsis patogenezinde rol oynayan inflamatuar mediatörler ile reaktif oksijen ve reaktif nitrojen türlerinden kaynaklanan oksidatif stres ve DNA hasarını içeren negatif etkileri gözden geçirmektir.

Anahtar Kelimeler: *Oksidatif stres, Sepsis*

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Sepsis

Sepsis is among the most common reason for admission to intensive care units (ICUs) throughout the world. Sepsis originally means putrefaction, and a decomposition of organic matter by bacteria and fungi. Variety of definitions have been associated to sepsis, such as sepsis syndrome, severe sepsis, and septic shock (1, 2). As sepsis progresses, it begins to affect organ function and eventually can lead to septic shock. Young or old individuals with compromised immune systems, patients with invasive devices, such as urinary catheters or breathing tubes, and very sick hospitalized patients are the risk groups for developing sepsis.

The pathogenesis of sepsis

The pathogenesis of sepsis is exceedingly complex and involves an interaction between multiple microbial and hosts factors (3). Microbial products that activate the innate immune system include both cell wall components and secreted proteins. Lipopolysaccharide (LPS) or bacterial endotoxin forms a major portion of all Gram- negative cell wall and is the most important bacterial product (4, 5) besides peptidoglycan the gram positive bacteria, and other bacterial products such as lipoteichoic acid and fungal antigens implicated in sepsis. LPS's activity is modulated to a degree by a number of proteins including bactericidal/permeability-increasing protein (BPI). BPI is produced by neutrophils and belongs to a conserved family of lipid-transfer proteins that includes as its closest relative, the LPS-binding protein (LBP). LPS's induced signal transduction begins with CD14-mediate activation of toll-like receptors (TLRs). TLRs mediate intracellular signaling that leads to inflammatory gene expressions. In response to agonists, TLR aggregation enables the recruitment and/or activation of TLR-specific adapter molecules. In addition to the TLRs, several additional pathways have been identified by which cells recognize microbial components. Peptidoglycan-recognition proteins (PGRPs) and a family of PGRP genes have been found in humans (6, 7, 8).

The inflammatory mechanism and oxidative stress in sepsis

The inflammatory mediators best characterized as per the roles they play in sepsis are interleukin-1 (IL) -1, tumor necrosis factor alpha (TNF-α), and IL-6. Mononuclear cells play a key role, releasing the classic proinflammatory cytokines IL-1, TNF-α and IL-6, addition to an array of other cytokines including IL-8, IL-12, IL-15, IL-18, and a host of other small molecules. On the other hand anti-inflammatory mediators (IL-4, IL-10, IL-1ra) are produced to balance the proinflammatory mediators in an attempt to rid the body of the foreign antigens without damaging the host. The activation of pro- and anti-inflammatory pathways is tightly controlled and regulate. These pathways are closely linked to other homeostatic pathways including the coagulation/fibrinolytic system, lipid mediators, acute phase and heat shock proteins, neutrophil-endothelial cell activation, activation of the hypothalamic-pituitary-adrenal axis, immune and non-immune cell apoptosis, increased nitric oxide production, and the oxidant/antioxidant pathway (9, 10). Severe sepsis and septic shock are considered to result from a irregulation of these tightly integrated homeostatic mechanisms. Following the release of IL-1 and TNF-α, the anti-inflammatory cytokines IL-4, IL-10, IL-13, and transforming growth factor-I2 (TGF-I2) are released associated with a switch from TH1 to TH2 activation. The anti-inflammatory cytokines suppress the gene expression of IL-1 and TNF-α. In addition, these cytokines inhibit antigen presentation by monocytes as well as T- and B-lymphocyte function (11, 12). The role of the anti-inflammatory cytokines is to keep the inflammatory response. In most infected persons, the body is able to achieve a balance between proinflammatory and anti-inflammatory mediators and the homeostasis is restored. However, in some patients, this balance is upset, resulting in systemic inflammatory response (SIRS) and multisystem organ dysfunction if the proinflammatory process is excessive. If the compensatory anti-inflammatory response is excessive, it will manifest clinically as energy with an increased susceptibility to infection (13, 14).

These complex host-pathogen interactions lead to inflammatory mediators, as well as reactive oxygen species (ROS) and reactive nitrogen species (RNS). Neutrophils and monocyte/macrophages are the sentinel phagocytic cell primarily responsible for engulfment and destruction of pathogenic

organisms during infection of the host. ROS and RNS are antimicrobial agents produced by these leukocytes that can directly destroy microbial pathogens. During sepsis, excess production of ROS and RNS can be a detriment, including significant cytotoxicity to organs and contributing to the squeal of unresolved sepsis, multiorgan system failure (15). The inflammatory mediators, including IL-1, IL-6, TNF, as well as ROS and RNS induce cytotoxicity leading to multiple organ damages, and without resolving these activities may ultimately result in multisystem organ failure. ROS and RNS are known to directly induce cytotoxicity to organs and can also alter cell signaling pathways. Interestingly the source of ROS may be a key to the extent of cellular cytotoxicity that occurs during sepsis and may also be involved in the ultimate ability of the host to limit sepsis. It is reported that ROS derived from NADPH oxidase limited acute inflammatory responses in vivo induced by LPS administration, suggesting that the ROS derived from this enzyme source may limit the extent of cytotoxicity and alteration reduction reactions such as changes in reduced glutathione (GSH) level during sepsis (16, 17, 18). Many cellular processes, such as inflammatory host defense and energy metabolism involve redox processes, which take place all over the cell comprising simple electron transfer reactions, radical processes as well as thiol/disulfide exchanges. To ensure proper function, the living cell has to monitor, control and maintain the intracellular redox balance. However, in septic shock an imbalance between ROS and antioxidant defense mechanisms occurs, resulting in oxidative stress (19, 20). The reason for this imbalance is an overwhelming production of ROS and/or a deficit in antioxidant systems. The most important ROS/RNS are represented by the following candidates: superoxide anion, nitric oxide, hydroxyl radical, hydrogen peroxide and peroxynitrite. Among the various ROS, superoxide anion plays a key role in the pathogenesis of hemodynamic instability and organ dysfunction during septic shock. Superoxide anion is primarily produced by activated neutrophils and macrophages as part of the innate immune system (21, 22), and has been associated with the inflammatory response that accompanies tissue damage in septic shock (23). Beside non-enzymatic antioxidants, e.g., vitamins C and E, bilirubin, GSH and albumin, superoxide dismutase (SOD), catalase and glutathione peroxides are referred to as major enzymatic antioxidant systems. Under normal conditions, the formation of superoxide anion is kept under tight control by endogenous SOD enzymes. Despite their importance in innate immunity representing one important defense mechanism against invading pathogens (20), the overwhelming production of ROS threatens the integrity of various biomolecules including proteins (24), lipids as well as lipoproteins, protein oxidation and DNA (25) resulting in tissue damage, by lipid per oxidation of cell membranes, protein oxidation and DNA strand breaks. These mechanisms contribute to multi organ failure during sepsis resulting in myocardial depression, hepatocellular dysfunction, endothelial dysfunction, and vascular catecholamine hypo responsiveness.

It must be underscored that, beside the negative effect associated with oxidative stress, ROS exert several important and vital beneficial physiological cellular functions which have been demonstrated in different areas including intracellular signaling and redox regulation (26, 27). First, ROS represent a defense mechanism against invading organisms by activated phagocytes (20); ROS are produced by the NADPH oxidize complex in this system. Second, ROS can directly affect the conformation and/or activities of all sulfhydryl-containing molecules, such as proteins or glutathione by oxidation of their thiol moiety (27). For example, superoxide, hydrogen peroxide, and NO are well known regulators of transcription factor activities and other determinants of gene expression (28-30). Several cytokines, growth factors, hormones and neurotransmitters use ROS as secondary messengers in the intracellular signal transduction (31). Well-known examples of redox-sensitive transcription factors are nuclear factor-kappa B (NF-KB) and activator protein-1 (AP-1) (29, 32), the nuclear factor-E2 related factor 2 (NrF2) pathways targeting the antioxidant element (33), or the ROS mediated sensing of hypoxia (34). The mechanisms for altered transcription factor control could be either via decreased binding to promoter regions via oxidative damage to the DNA or more direct by redox regulation of transcription factor activation (35)

and/or altered DNA-binding due to redox-induced modification of the transcription factor protein (36, 37). Third, in addition to their physiologic beneficial effects, ROS are, due to their high reactivity, prone to cause damage being, thereby, also potentially toxic, mutagenic or carcinogenic. Thus, the targets for ROS/RNS damage include all major groups of biomolecules as already mentioned above: proteins, lipids, DNA. The increase in neutrophil apoptosis has been detected in early sepsis cases in clinics. The proinflammatory properties of superoxide anion include endothelial cell damage and increased micro-vascular permeability (38, 39), formation of chemo tactic factors, e.g., leukotriene B_4 (40), recruitment of neutrophils at sites of inflammation, lipid per oxidation and DNA single strand damage (41), release of cytokines (42, 43), and formation of $ONOO^-$, a potent cytotoxic and pro-inflammatory molecule triggering DNA single strand breaks (44, 45, Çetin-46).

Regarding the glutathione- and thioredoxin-reduction pathways, it has become clear that there are two parallel, interdependent enzymatic systems. On the one hand, glutathione as a reducing substrate seems to be more effective in reducing small disulfide molecules and in reacting directly with ROS, whereas, on the other hand, thioredoxin is more effective in reducing the exposed disulfides of proteins. Thus, the thioredoxin system can also be seen as an antioxidant defense/repair system for (accidentally) oxidized cytokine proteins. GSH is among the most important intracellular antioxidant within the human cells. It exists in equilibrium with its disulfide from (GSSG), and the ratio of GSH to GSSG could be used as an indicator of the redox status of the cell. Several important human antioxidant-defense systems are base around glutathione, e.g. glutathione peroxides as a major cellular reducer of hydrogen peroxide (together with catalase and peroxiredoxin) (36). Oxidized proteins tend to change their tertiary structure and when the oxidation is reversed they have to be refolded by chaperones to gain their optimal structure (47).

Inflammatory responses initiated by oxidative stress occur via the activation of redox pathways for transcriptional activation, for example, increased activation of nuclear factor kB (NFkB) and increased circulating inflammatory mediators including cytokines and pentraxin-3 have been reported in patients with sepsis Sepsis-induced organ dysfunction has been suggested to be at least in part due to mitochondrial dysfunction resulting from oxidative stress and which results in failure of energy membranes. Mitochondrial oxidative damage leads to the release of cytochrome-c into the cytosol resulting in apoptosis. Increased permeability makes the inner membrane permeable to small molecules. Mitochondrial ROS are also important in cell signaling pathways which modulate several cellular functions. The pathogenesis of mitochondrial damage as a result of sepsis is probably a complex series of events. Both RNS and ROS combined with the release of a variety of exacerbating inflammatory mediators can act to directly or indirectly influence mitochondrial function and energy production. It remains unclear if the self-amplifying cycle of ROS generation and mitochondrial damage occur with mitochondrial dysfunction leading to oxidative stres and more mitochondrial impairment as the primary event, or if oxidative stress initiates mitochondrial dysfunction and further ROS release (48,49). Under sepsis, various processes, triggered by ROS/NOS contribute to oxidative stress. As a major source of ROS production, mitochondria are especially prone to ROS-mediated damage. Such damage can induce the mitochondrial permeability transition caused by opening of nonspecific high conductance permeability transition pores in the mitochondrial inner membrane. ROS themselves also provide a signal leading to the induction of autophagy, apoptosis, and necrosis. Excessive ROS production and adenosine triphosphate depletion from uncoupling of oxidative phosphorylation promote necrotic cell death. Release of cytochrome-c after mitochondrial swelling activates caspases and initiates apoptotic cell death. Redox sensors were first described in bacteria, including the redox-sensitive transcription factors. All of these redox receptors have a structure designedto sense specific ROS, oxidants, or other reactive intermediates. These ancestral redox sensors can essentially contribute to rapid mechanisms designed to deal with ROS and to make critical

adjustments allowing survival of the bacteria (49, 50, 51). On the other hand, High-mobility group (HMG) box proteins are targeted to particular DNA sites in chromatin by either protein–protein interactions or recognition of specific DNA structures. The HMG-box protein contains cytokine activity by inducing macrophage secretion of proinflammatory cytokines. HMG box1 protein is a highly conserved nuclear protein, acting as a chromatin-binding factor that bends DNA and promotes to transcriptional protein assemblies on specific DNA targets. Furthermore, the accumulation of HMGB1 protein is found at sites of oxidative DNA damage in live cells, thus defining HMGB1 as a component of an early DNA damage response (51, 52).

Sepsis and septic shock remains as leading cause of death in adult intensive care units. It is widely accepted that gram-negative bacteria and their endotoxins cause sepsis and septic shock predominantly. Enhanced generation of reactive oxygen species (ROSs) may be responsible for tissue injury in septic shock and endotoxemia.

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