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ROLE OF THE OXIDATIVE STRESS IN PATHOPHYSIOLOGY OF IMMUNE SYSTEM

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ULOGA OKSIDATIVNOG STRESA U PATOFIZIOLOGIJI IMUNSKOG SISTEMA

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SAŽETAK

Slobodni radikali se stvaraju kao uzgredni produkti mnogih fizioloških procesa u ćelijama ili zadesno. Najveći biološki značaj imaju reaktivni oblici kiseonika i azota. Ti molekuli mogu izazvati lančane reakcije koje prouzrokuju oštećenje ćelijskih membrana, proteina i nukleinskih kiselina. Da bi se kontrolisali njihovi efekti i održala redox homeostaza, postoje brojni zaštitni antioksidativni mehanizmi. »Oksidativni stres« predstavlja stanje u kome je narušena redoks homeostaza, odnosno balans između pro-oksidativnih i antioksidativnih supstanci. Oksidativni stres može biti prouzrokovan aktivacijom endogenih mehanizama stvaranja slobodnih radikala ili dejstvom egzogenih faktora. Ćelijske reakcije na povećanje koncentracije reaktivnih oblika kiseonika predstavljaju »odgovor na oksidativni stres«. Ako se povećana koncentracija reaktivnih oblika kiseonika održava uprkos aktivaciji antioksidativnih mehanizama, narušava se redox homeostaza i može doći do pojave bolesti. U ovom radu su prikazani glavni mehanizmi stvaranja slobodnih radikala koji vode nastanku oksidativnog stresa uključenog u proces starenja i nastanak mnogobrojnih i raznovrsnih bolesti. S obzirom na ulogu imunskog sistema u njihovoj patogenezi, posebno je prikazan uticaj oksidativnog stresa na urođene i stečene imunske mehanizme, kao i uticaj slobodnih radikala na aktivaciju i preživljavanje T limfocita.

Ključne reči: slobodni radikali, oksidativni stres, imunski sistem, T limfociti

ABSTRACT

Free radicals are generated as the byproducts of many physiological cellular reactions or accidentally. Biologically, the most relevant free radicals are highly reactive oxygen and nitric molecules. These molecules can establish chain reactions causing damage of cell membranes, proteins and nucleic acids. Numerous antioxidative mechanisms exist in order to control their effects and maintain redox homeostasis. The situation in which the cellular redox homoeostasis is altered, i.e. the balance between pro-oxidants and antioxidants, is known as the "oxidative stress". An oxidative stress may be induced by the activation of endogenous generating systems or by conditions generated by environmental factors. The response to increased levels of ROS is known as "oxidative stress response". In cases of persistently high ROS levels the loss of homeostasis might be developed which could result in pathological conditions. In this study, we rewiev the main mechanisms that generate free radicals and lead to oxidative stress conditions included in aging and pathogenesis of many different disorders. Because of the involvement of the immune system in many of these diseases, a particular attention was focused on oxidative stress influence on both natural and acquired immunity, with the special emphasis on free radical influence on T cell activation and survival.

Key words: free radicals, oxidative stress, immune system, T cells

FREE RADICALS ORIGIN

Free radical formation is a byproduct of many normal cellular reactions in the body, including energy generation, breakdown of lipids and proteins, and inflammatory processes (1). Exogenous sources of free radicals include tobacco smoke, certain pollutants and organic solvents, hyperoxic environments, pesticides and radiation. Biologically, most relevant free radicals are reactive oxygen species (ROS) superoxide radical ($O2^{\bullet -}$) generated during autooxidation in mitochondria or by enzymes in cytoplasm (such as xantine oxidase or cytochrome P-450), hydroxyl radical ($OH^{\bullet}$) obtained by the hydrolisis of water and nitric oxide (NO) or their derivatives, peroxnitrite anion ($ONOO^-$), as well as $NO_2^{\bullet}$ and NO_3^- (2). Although the hydrogen peroxide (H_2O_2) generated by the enzyme superoxide dismutase or directly by oxidases in peroxisomes is not free radical, it is highly reactive oxygen species.

Normal energy metabolism in aerobic organisms is associated with the generation of highly reactive oxygen molecules (3). Practically, all cells and tissues convert continuously a small proportion of oxygen (approximately 2–5% of oxygen consumption) by electron transport chain (ETC) into reactive oxygen molecules in the mitochondrial matrix (4). Beside their production in oxygen biochemistry, these molecules are produced by the cell as signaling molecules in response to extracellular stimuli (5-7).

The main source of the free radical NO in mammalian cells is the enzymatic oxidation of L-arginine by NO synthases (8). NO generated by these enzymes has been established as signaling molecule in the regulation of key functions in the immune, cardiovascular and nervous systems (9, 10).

Higher organisms developed mechanisms for the advantageous use of free radicals or their derivatives as well as mechanisms that suppress their potentially dangerously interaction with biomolecules (4). Antioxidants, like enzymes (11, 12) superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx), as well as nonenzymic compounds (13, 14) such as α-tocopherol (vitamin E), β-carotene, ascorbic acid (vitamin C), and glutathione, are substances that are able, at relatively low concentrations, to significantly delay or inhibit oxidation of substrates in the presence of free radicals or their derivatives (15).

Nevertheless, at moderate concentrations, NO and reactive oxygen species (ROS) play an important role as regulatory mediators in signaling processes (16), at high concentrations these molecules are hazardous for living organisms and could damage all major cellular constituents. That is the reason why, under normal physiological conditions, these molecules are generated and maintained at a relatively high steady-state level (17, 18). This

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balance could be disturbed either by an increase in ROS concentrations or by means of the decrease in the activity of one or more antioxidant systems.

OXIDATIVE STRESS GENERATION

The term "oxidative stress" is commonly used to designate a situation in which the cellular redox homeostasis, i.e. the balance between pro-oxidants and antioxidants, is altered because of excessive production of ROS and/or impairment of cellular antioxidant mechanisms (19, 20). In analogy to oxidative stress, the term "nitrosative stress" was coined for the excessive or deregulated formation of NO and NO-derived reactive nitrogen species (RNS) (21).

An oxidative stress may be induced by the activation of endogenous generating systems or by conditions generated by environmental factors. The response to increased levels of ROS is known as "oxidative stress response." To cope with oxidative insult, the cell has developed numerous defense strategies, at the level of oxidative damage repair and ROS scavenging mechanisms (22-24). If the initial increase in ROS is relatively small, the antioxidative response may be sufficient to compensate for the increase in ROS and this response allows cells and tissues to maintain redox homeostasis. Under certain conditions, however, ROS production is increased more strongly and persistently, and the antioxidative response may not be sufficient to reset the system to the original level of redox homeostasis. In such cases, the system may still reach an equilibrium, but the resulting quasi-stable state is associated with higher ROS concentrations. In more extreme cases of persistently high ROS levels chronic shift in the level of homeostasis or loss of homeostasis may develop. In these cases, the pathological conditions may result from both the damaging effects of ROS and ROS- -mediated changes in gene expression.

OXIDATIVE STRESS - INDUCED MOLECULAR DAMAGE

High ROS level is hazardous because it produces extensive oxidative damage of membrane lipids (25), DNA molecules (26) and proteins (27) as well as peroxidation of lipoproteins. The macromolecular damages (4) such as oxidation and decomposition of lipids, single- and double DNA-strand breaks, DNA–protein and protein–protein cross-linking, and protein fragmentation, result in the formation of dangerous products such as hydroperoxides, alkyl radicals, cyclic endoperoxides, and aldehydes (28). The result of these changes could be disturbance in protein synthesis, decreased enzyme activity and progressive impairment of the functions of mitochondria and other organelles (26). In proliferating cells, oxidant--induced damage does not accumulate because the process of cell division dilutes damaged structures (29, 30), but in postmitotic cells the damage is being accumulated. Both proliferating and postmitotic cells can, however, renew themselves by degrading defective macromolecules and organelles. Mitochondria are the major intracellular source of ROS (17, 26, 31) and the main target for free radical attack. Due to the fact that mitochondria possess their own genome, the mitochondrial DNA (mtDNA) that encodes proteins essential for aerobic respiration is highly susceptible to mutagenic insults. Oxidative damage of mtDNA occurs at a frequency approximately 20 times greater than for nuclear DNA (32, 33). Mitochondria are not fully competent in DNA repairing of various types of damage caused by ROS and free radicals because nucleotide excision repair does not exists in their DNA repair mechanism.

OXIDATIVE STRESS IN AGING AND DISEASES

The widely popular free radical theory of aging (34) states that the age-related degenerative process is to a large extent the consequence of free radical damage. There are various indirect manifestations of oxidative stress in old age, including DNA oxidation, protein oxidation, lipid peroxidation and a shift in the redox states of thiol/disulfide redox couples (26, 35, 36). It is generally accepted that accumulation of mutated mtDNA is a contributory factor for the age-dependent decline of the respiratory function, especially in postmitotic cells (31, 37, 38). Mitochondria undergo gradual structural alterations associated with decreased capacity to produce energy (39-41). In the scenario of a "vicious cycle" disturbances in protein synthesis and decreased enzyme activity cause the progressive impairment of the functions of mitochondria and other organelles (26). It was shown that telomere shortening, which characterize aging process, is induced by oxidative stress conditions (42, 43).

Excessive ROS production has been implicated in the pathogenesis of atherosclerosis (44, 45), hypertension (46, 47), diabetes mellitus (48, 49), inflammatory autoimmune diseases (50, 51), sepsis (52), ischemia-reperfusion injury and carcinogenesis.

These diseases could be distributed into two major categories (4). In the first category of diseases, such as diabetes mellitus and cancer, there is a pro-oxidative shift in the systemic thiol/disulfide redox state and impaired glucose clearance, indicating that skeletal muscle mitochondria may be the major site of elevated ROS production (48). These conditions designated as "mitochondrial oxidative stress" (4) are typically associated with skeletal muscle wasting. Additionally, ROS are potential carcinogens as it is well known that they facilitate mutagenesis, tumor promotion, and progression (53-56).

The second category of disease is associated with an excessive stimulation of NAD(P)H oxidase activity by cytokines or other agents. In this case oxidative stress is the result of "inflammatory oxidative conditions" (4). Increased ROS levels or changes in intracellular glutathione levels in "inflammatory oxidative stress" could be produced by dysregulation of signal cascades and/or gene expression of cell adhesion molecules (57-60).

Ischemia and reperfusion can lead to tissue injury and serious complications in organ transplantation (61), myocardial infarction (62, 63), and stroke (64-67). A disturbed oxidative/antioxidative balance is present in human myocardial reperfusion injury (68).

Nitrosative insult may occur *in vivo* in pathologies associated with inflammatory processes, neurotoxicity and

ischemia (69, 70) as well as during neurotransmission (71).

OXIDATIVE STRESS AND THE IMMUNE SYSTEM

Oxygen-derived free radicals are important in both natural and acquired immunity (72). Phagocytosis by neutrophils or macrophages stimulates various cellular processes including the "respiratory burst" whereby increased cellular oxygen uptake results in the production of potent oxidant agents. There is evidence that the intracellular redox state modulates the immunological functions of macrophages. The balance between "reductive" and "oxidative" macrophages regulates the ratio of helper T cells of type 1 versus type 2 (Th1/Th2) (73).

The oxidative stress may influence acquired immunity by influence on T cell activation (acting on gene expression of various cytokines, chemokines, and cell adhesion molecules) and survival (by regulating apoptosis). The activation of T lymphocytes is strongly enhanced by ROS (74). Superoxide and/or physiologically relevant concentrations of H_2O_2 were shown to augment the production of interleukin-2 (75, 76). Besides, low micromolar concentrations of H_2O_2 were shown to induce the expression of the interleukin-2 receptor (76). Exposure of T lymphocytes to physiologically relevant concentrations of environmental ROS or to some moderate inducers of oxidative stress does not bypass the requirement for signaling initiated by specific cell membrane receptors, and can amplify signaling cascades after relatively weak receptor stimulation. (74). This finding supports the activation of immune responses by small concentrations of antigen (77).

On the other hand, the activation increases the amount of ROS in T cells (78-80), but it is unclear how these ROS are produced. T cells lack the conventional NADPH oxidase enzymes used by granulocytes, but other mechanisms for producing ROS might be included. Strong activation of T cells causes a significant decrease in intracellular glutathione levels and the endogenous production of hydrogen peroxide (76). Although under most circumstances the ROS are produced by the T cells themselves (78, 79, 80), the rapid increase in ROS level detected in T cells within 15 minutes after activation (80) indicates that bystander neutrophils might be the source of the increased ROS levels in activated T cells. In this instance, it is postulated that activated neutrophils produce the ROS which then diffuse into neighboring T cells (81).

Despite the fact that very little is known about the molecular events that lead to ROS production within T cells, several studies have shown that activated T cells could be killed by ROS (79, 80). Besides ROS-induced, NO-dependent apoptosis has been observed (82). There are some contradictory results indicating that influence on apoptotic process depends on NO concentrations. It was shown that the low concentrations of NO could provide protection from apoptotic cell death by inhibiting certain caspases (83, 84).

The studies performed with primary T cells indicate that the formation of intracellular ROS regulates activation-induced T-cell apoptosis (85), therefore suggesting that intracellular ROS could play a role in peripheral T-cell homeostasis (79, 86). Apoptosis of activated T cells can be inhibited by culture with the antioxidant (79). Red blood cells inhibit T-cell apoptosis (85) and protect T cells from activation-induced cell death, at least in part by reducing the pro-oxidant state. Recent studies have identified the molecular details of this apoptotic process that operate in vivo (87). It is now becoming clear that two separate pathways — activation-induced cell death (AICD) and activated T cell–autonomous death (ACAD) — control the fate of antigen-specific T cells. One of these pathways, AICD, is driven by signals delivered exogenously to the cell. The other pathway, ACAD, is driven by signals that are intrinsic to the activated T cell. Interestingly, reactive oxygen species (ROS) can control both pathways through reciprocal modulation of the main effector molecules FasL and Bcl-2. Engagement of the TCR increases FasL expression on T cells (88). Although both superoxide and H_2O_2 were produced following TCR stimulation, it was suggested that superoxide and not H_2O_2 was responsible for upregulation of FasL (80). Several experiments suggest that ACAD is controlled by various members of the Bcl-2 family of proteins. This family consists of pro- and antiapoptotic members that control the fate of cells by protection or destruction of mitochondria (89). However, some authors observed membrane changes typical of apoptosis in the absence of ROS (90, 91), indicating that pro-oxidative conditions are not a general prerequisite for apoptotic cell death. Nevertheless, high ROS concentrations induce apoptotic cell death in various cell types (92, 93), suggesting that ROS contribute to cell death whenever they are generated in the context of the apoptotic process.

In addition, an increase in cellular ROS production in apoptotic processes (4) is often observed. The ROS generation and subsequent oxidative stress are implicated as unavoidable byproduct of the apoptotic execution process (94). However, some authors found that triggering of the Fas receptor does not induce ROS production (95).

Inappropriate initiation of apoptosis has been proposed to underlie the progressive neuronal attrition associated with various neurodegenerative diseases (96) such as Alzheimer's disease (97-99), Parkinson's disease (100, 101), and other neurological disorders that are characterized by the gradual loss of specific populations of neurons (96), as well in some autoimmune neurological diseases such as Guillain-Barré syndrome, demyelinating polyneuropathy, and motoneuron disease (96). Besides, the oxidative and/or nitrosative stress, by affecting numerous molecules in different tissue could be included in the pathogenesis of many other pathological conditions or diseases.

CONCLUSIONS

ROS and NO are proving to be ubiquitous effectors of physiological function in most cell types and organ systems. New tools are rapidly evolving to measure ROS and NO in cellular systems, to assess redox effects on regulatory proteins, and to modulate ROS and NO activities experimentally. Final understanding how modulation of

the redox-sensitive signaling process may be used to specifically alter the expression of genes involved in the pathogenesis of a variety of diseases will encourage the exploration of novel treatment modalities targeting these redox-sensitive pathways.

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