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OXIDATIVE STRESS, APOPTOSIS, AND STRESS PROTEINS

Reaktywne formy tlenu są stale wytwarzane w organizmie w procesach przemian o charakterze tlenowym. Początkowo ich obecność w tkankach była traktowana jako niekorzystne zjawisko, łączone z różnymi schorzeniami, mutagenezą, procesami degeneracyjnymi i biologicznym starzeniem się organizmu. Z czasem poznano jednak ich fizjologiczne znaczenie w zakresie regulacji różnych procesów wewnątrzustrojowych. Stres oksydacyjny może powstać w wyniku wpływu różnych czynników zewnątrz- i wewnątrzpochodnych, zwłaszcza mających charakter stresora. Czynniki występujące w nurkowaniu ten charakter zazwyczaj mają. Obok nich, wykonywanie pracy fizycznej prowadzi także do zwiększenia ilości powstających reaktywnych form tlenu. W odpowiedzi na stres organizm wytwarza szereg białek stresowych, które regulują homeostazę wewnątrzkomórkową, ułatwiają przeżywanie komórki i odgrywają zasadniczą rolę w ochronie komórki. W razie niedostatecznej ochrony powstaję uszkodzenie komórki i jej śmierć poprzez apoptozę lub nekrozę. Oba procesy są ściśle ze sobą związane. Zmiany wywołane stresem oksydacyjnym i pojawienie się apoptozy nie zawsze prowadzą do śmierci komórki. W mniej intensywnym wymiarze służą przebudowie komórki, mającej znaczenie adaptacyjne. Proces ten jest pod nadzorem białek stresowych.

It is generally accepted that due to the aerobic metabolism small amounts of reactive oxygen species are constantly generated in living organism. At the beginning these species, discovered in biological materials, were treated as hazardous factors that may account for cellular damage, mutagenesis, cancer, degenerative processes and biological aging. With time there were discovered some defensive mechanisms against damaging oxidative effects, including enzymatic and non-enzymatic scavengers. Soon it was also clear that reactive oxygen species, formed as by-products of enzymatic reactions, must have physiological significance. It was discovered they are involved in smooth muscle relaxation, control of ventilation, signal transduction from various membrane receptors, enhancement of immunological functions, maintenance of redox homeostasis, control of erythropoietin production and other hypoxia-inducible functions (more in 1 and 2).

The most important reactive oxygen species are superoxide anion ($O_2^{\bullet -}$) and nitric oxide ($NO^{\bullet}$). The later was usually analyzed as a special group of radicals. It is formed by the oxidation of L-arginine and the process is catalyzed by the enzyme nitric oxide synthase (NOS). Superoxide is formed in a process mediated enzymatically by enzymes NAD(P)H oxidase and xanthine oxidase or nonenzymatically by redox-reactive compounds such as semi-ubiquinone. In biological tissues superoxide is converted into other reactive oxygen species having different physiological functions.

Table 1

Physiological significance of reactive oxygen species

- support of phagocytosis by macrophages ("oxidative burst")
- stimulation of guanylate cyclase activity → cGMP
- relaxation of smooth muscles
- inhibition of platelet adhesion
- stimulation of interleukin-2 synthesis in lymphocytes T
- induction of heme oxygenase gene expression
- sensing of oxygen tension
- activation of nuclear factor κB (NF-κB)
- amplification of immune responses
- induction and execution of apoptosis
- degradation of damaged proteins and structures

Antioxidant enzymes and compounds are responsible for clearance of reactive species and in physiological conditions there is a balance between production and clearance. When this balance is disrupted there appears a condition of excessive augmentation of reactive oxygen species referred to as oxidative stress. In this condition oxidative damage of numerous substances appears. It has been observed various local injuries in cells and in intercellular matrix, such as induction of lipid peroxidation, oxidative damage of proteins and antiproteases inhibition, change in DNA conformation and chemical changes in bases of DNA chain.

Oxidative stress can appear as a consequence of several external and internal influences. Basic conditions of its appearance are enhancement of oxidative substances apply, intensification of metabolic pathways where a leakage of oxidative radicals is possible and inhibition of antioxidant defense. During diving all these circumstances are possible. The factors acting on divers during diving exposure include the influence of pressure per se, hyperoxia, decompression and stress associated with the formation of gas microemboli, temperature of water, physical work and psychophysical (emotional) stress. All these factors can induce oxidative stress, but it seems that toxic effects of oxygen are mainly responsible for generation of excessive reactive oxygen species. Some experimental results indicate that during deep saturation diving exposure to hyperoxia results in oxidative damage in tissues of divers (3)

Another factor inducing the reactive oxygen species generation during diving is physical work. It is well known that reactive oxygen species production is enhanced during intensive muscular exercise (4, 5). This production is likely to come from different sources. Increased oxygen due to metabolism intensification generates increased absolute quantities of superoxide in mitochondrial respiratory chain. The enhanced purine metabolism leads also to increased radical formation. Additionally there is an augmented formation of ATP derivates during its breakdown with the end-points such as hypoxanthine, uric acid and reactive oxygen species. Phagocytes are further source being activated in response to ischemia-reoxygenation state in muscles and other tissues. In response there are several disturbances and damages (6, 7). All these events can result in enhancement of decompression sickness risk which is generally accepted since the classic investigations of W.D. McElroy and co-workers in fortieth last century.

The character of diving environment and the factors acting in divers create a situation that can be assumed as stress condition. Indeed, many of the reactions in the

organism of divers can be related to stress. Diving should therefore be viewed as providing stimuli that stress various systems of the diver's body. These actions are different according to the type, duration, breathing gas mixture, kind of physical work performed and other characteristics of diving. Nevertheless as the general reaction to stress, there are numerous repairing processes to withstand all negative events related to stress. There are scarce experimental informations on the nature of these processes. Among others the induction of stress proteins synthesis seems to be of special interest.

Table 2

Main stress proteins

HSP8-3 HSP10 Small HSP	HSP10, HSP17 HSP22, αA- and αB-crystallin, HSP27, HSP28
HSP40-60 HSP40 HSP60	HSP32, HSP40, HSP47 HSP58, HSP60, HSP65, GRP58
HSP70	HSP68, HSC70, HSP72, HSC73, GRP75, GRP78
HSP90	HSP83, HSP87, GRP94, HSP90-α, HSP90-ß
HSP110	HSP94, HSP104, HSP105, HSP110, GRP170

Mammalian cells are known to synthesize a variety of highly conservative proteins as a response to stress exposure. These substances, known as stress proteins or heat shock proteins (HSP) are highly conserved and act mainly as molecular chaperones in mediating intracellular protein translocation and subsequent folding and assembly. They regulate cell homeostasis, promote cell survival and play a major role in cytoprotection. In mammalian cells there are different stress proteins and their classification is related to their molecular weight. Many stress proteins are constitutive, but some of them are inducible and their concentrations in the cell can rapidly increase during the influence of various stressors.

In humans, particularly during the mechanical and metabolic stress, HSP27, HSP60, HSP70 and GRP 75 are the most important. Their protective role against disturbances due to stressor actions are discovered in liver, neutrophiles and NK cells, muscle fibers, spleen, extracellular milieu and other parts of the body. As regards abnormalities in lipid metabolism, structural alterations or post-translational

modifications resulting from oxidation or metabolic disturbances, the induction of stress proteins is going on by means of several mechanisms. The transmission of external mechanical influences into the muscle fiber is realized by activation of mitogen-activated protein kinase (MAPK) cascade. According to the type of exercise three of the MAPK pathways appear to be activated: e-jun NH_2-terminal kinase (JNK), extracellular-signal regulated kinase (ERK) and protein p35 (8). It is very likely that the above mentioned activation is a consequence of oxidative stress In other tissues stressors can act via the activation of the Janus kinase (JAK) / signal transducers and activators of transcription (STAT), S-nitrosylation of numerous proteins or by direct influence of superoxide on heat shock factor (HSF) in cytoplasm (HSF1, HSF2) or nucleus (HSF4) (9, 10, 11).

The cell protection by means of stress proteins synthesis induction can be insufficient in cases of very intensive stress influences. In that cases there appear a cell damage leading to cell death. There are two forms of cell death - the programmed death called apoptosis and the immediate damage referred to as necrosis. Both processes are possibly interrelated and depend on energy availability in the cell. Apoptosis is an active process requiring maintenance of sufficient intracellular energy level, it is ATP amount, and a redox state compatible with caspases activation. Necrosis as a process does not require energy. So the level of available energy seems to be a check-point turning the disturbances leading to cell death in the direction of apoptosis or necrosis.

Numerous experimental data have indicated that reactive oxygen species are essential mediators of apoptosis. As regards diving conditions it is to point out the importance of oxygen-sensing mechanisms. It is clear that changes in oxygen tension are sensed by changes in reactive oxygen species production (12). This sensing, mediated by transcription factor HIF-1 (hypoxia-inducible factor-1) is mainly investigated in relation to hypoxia and normoxia. The action or reactive oxygen species change the structure of HIF-1 by inducing the degradation of the subunit HIF-1α what is dependent on oxygen tension (13). Unfortunately, this mechanism was not investigated according to hyperoxia but one cannot exclude this sensing during diving. Bubble formation, cell adhesion due to changes in hemostasis (14) and other disturbances of blood flow can result in local ischemia and reperfusion with elevation of reactive oxygen species production and impairment of oxygen sensing.

The two forms of cell death, apoptosis and necrosis, are probably close interrelated. Both necrosis and apoptosis can be initiated by the same stressors, can be prevented by antiapoptotic mechanisms and can be transformed, at least in the early phase, from one form to other. Caspases, specific signaling molecules for apoptosis, also participate in necrosis (15). However in contrary to necrosis, apoptosis is used to eradicate impaired cells, to enhance the appropriate homeostasis of tissues and whole organism and to maintenance the integrity of the organism.

Table 3

Induction factors of apoptosis

- *specific ligand-receptor interactions*
- *cytolytics secreted by cytolytic lymphocytes*
- *disruption of cell-cell interactions*
- *disruption of cell matrix interaction*
- *changes in concentration of specific growth factors*
- *changes in concentration or action of specific hormones*
- *nonphysiological external factors*

There are two basic signaling pathways inducing and executing apoptosis: mediated by death receptors on the cell surface (extrinsic pathway) and mediated by mitochondria (mitochondrial or intrinsic pathway). Stressors inducing apoptosis act in concert through a perturbation of mitochondrial membrane integrity. Inside mitochondria there are numerous potential pro-apoptotic proteins and their release requires a change in permeabilization of their outer membrane. The precise mechanism of the changes in membrane permeabilization is not clear. Currently two mechanisms are recognized as leading to release of mitochondrial pro-apoptotic proteins: the opening of the permeability transition pore (PTP) or change in regulation of permeabilization by members of B-cell lymphoma-2 (Bcl-2) family of proteins. As a result there are released cytochrome c, apoptosis-inducing factor (AIF), second mitochondrial-derived activator of caspases (Smac/DIABLO), endonucleases G and other proteins inducing the caspases cascade or a caspases-independent apoptosis (16, 17).

Many apoptosis inducers also activate the production or reactive oxygen species. Radical forms, as described above, stimulate a reaction in form of enhanced stress protein synthesis. Stress proteins are thought to protect against oxidative stress and apoptosis but the final outcome varies from cell to cell in the same cell population or tissue. There are experimental results showing a depletion of oxidative stress with the signs of enhanced apoptotic events. Various experimental results show also that stress proteins-induced cell protection can be attributed, at least in part, to the suppression of apoptosis. Unfortunately the exact mechanism is not clear. Stress proteins act in very complex fashion during the regulation of apoptosis. Due to their cytoprotective role they inhibit several key points of apoptotic cascade and protect as chaperones some proteins involved in cell homeostasis. However there are also results indicating that stress proteins can act as chaperons for apoptotic signaling proteins. In that case stress proteins will promote apoptosis.

Among various stress proteins forms in particular HSP72 and HSP27 can inhibit some apoptotic pathways. This action is probably related to the mitochondrial pathway. It was shown that some of the stressors inducing HSP72 accumulation inhibited H_2O_2-induced loss of mitochondrial osmotic potential ($\Delta\psi$).This change can result in cytochrome c release, a process leading to apoptosis. HSP27 and αB-crystallin maintain the redox equilibrium of the cell and inhibit oxidant-induced cellular damage. In cytosol HSP90, a compound being indispensable for folding and activation competence of numerous kinases, also was demonstrated to counteract, being in over-expression, the kinase cascade induction.

Interaction between reactive oxygen species, stress protein induction and induction of caspases cascade (apoptosis) suggest a distinct role of these events in adaptation processes. There are evidences showing that apoptosis does not always bring cell death. Some features, especially in multinuclear cells such as muscle fibers, indicate that apoptosis leads to destruction only some parts of intracellular milieu. These destroyed substances and structures are then replaced by new forms, more adapted to changed environmental load (22, 23). The same can be said about oxidative stress, where there are strong evidences on the role of reactive oxygen species in regulation of numerous physiological processes. Stress proteins act in concert with oxidative stress and apoptosis, modifying the way for renovation of cell with the aim to achieve most useful adaptation to given circumstances (24).

WYKAZ LITERATURY

1. Dröge W. Free radicals in the physiological control of cell function. *Physiol.Rev.* 2002; 82:47-95
2. Murawska E, Jethon Z. Oxidative stress and antioxidant defense in physiological and pathological conditions of the organism (in Polish). In Zaton M, Jethon Z. *Aktywność ruchowa w świetle badań fizjologicznych i promocji zdrowia.* Wrocław: Wyd.AWF, 1998: 7-26
3. Ikeda M, Nakabayashi K, Shinkai M AT al. Supplementation of antioxidants prevents oxidative stress during a deep saturation dive. *Tohoku J.Exp.Med*. 2004; 203:353-357
4. McArdle A, van der Meulen JH, Catapano M, Symons MCR, Faulkner JA, Jackson MJ. Free radical activity following contraction-induced injury to the extensor digitorum longus muscles of rats. *Free Radical Biol.Med.* 1999; 26:1085-1091
5. Reid MB. Nitric oxide, reactive oxygen species, and skeletal muscle contraction. *Med.Sci.Sports Exerc.* 2001; 33:371-376
6. Fehrenbach E, Northoff H. Free radicals, exercise, apoptosis and heat shock proteins. *Exerc.Immunol.Rev.* 2001; 7:66-899
7. Somani SM, Husain K. Influence of exercise-induced oxidative stress on the central nervous system. In Sen CK, Packer L, Hänninen O. *Handbook of oxidants and antioxidants in exercise*. Amsterdam: Elsevier, 2000: 713-751
8. Thomson HS, Maynard EB, Morales ER, Scordilis SP. Exercise-induced HSP27, HSP70 and MAPK responses in human skeletal muscle. *Acta Physiol.Scand.* 2003; 178:61-72
9. Ignarro LJ, Napoli C, Loscalzo J. Nitric oxide donors and cardiovascular agents modulating the bioactivity of nitric oxide: an overview. *Circ.Res*. 2002; 90:21-28
10. Madamanachi NR, Li S, Patterson C, Runge MS. Reactive oxygen species regulate heat shock protein 70 via JAK/STAT pathway. *Arterioscler Thromb.Vasc.Biol.* 2001; 21:321-326
11. Morimoto RI. Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. *Genes Develop.* 1998; 12:3788-3796
12. 12 Neumcke I, Schneider B, Fandrey J, Pagel H. Effects of pro- and antioxidative compounds on renal production of erythropoietin. *Endocrinology,* 1999; 140:641-645
13. Semenza GL. HIF-1: mediator of physiological and pathophysiological responses to hypoxia. *J.Appl.Physiol.* 2000; 88:1474-1480
14. Olszański R. Estimation of divers' safety in hyperbaria by evaluated decompression procedures (in Polish).Łódź: Wojsk.Akad.Med., 2003
15. Proskuriakov SY, Konoplyannikov AG, Gabai VL. Necrosis: a specific form of programmed cell death? *Exp.Cell Res*. 2003; 283: 1-16
16. Orrenius S. Mitochondrial regulation of apoptotic cell death. *Toxicol.Lett.* 2004; 149:19-23
17. Sreedhar AS, Csemerly P. Heat shock proteins in the regulation of apoptosis: new strategies in tumor therapy. A comprehensive review. *Pharmacol.Therap.* 2004; 101:227-257
18. Podhorska-Okołów M, Murawska-Ciałowicz E, Dzięgiel P, Jethon Z, Zabel M The structural changes of a acute physical exercise in rat's muscles and kidney. *XXXIX Symposium PTHC (Abstracts)*. Wroclaw, 2003: 36-37
19. Podhorska-Okołów M, Dzięgiel P, Gomułkiewicz A et al. The role of AT1 and AT2 angiotensin receptors In the mechanism of apoptosis In renal tubular Wells after physical exercise. *Ann.Acad.Med.Bialostocensis*, 2004; 48/Suppl.1:8-10

20. Piatelli MJ, Doughty C, Chiles TC. Requirement for a hsp90 chaperone-dependent MEK1/2-ERK pathway for B cell antigen receptor-induced cyclin D2 expression in mature B lymphocytes. *J.Biol.Chem.* 2002; 277:12144-12150
21. Samali A, Orrenius S. Heat shock proteins: regulators of stress response and apoptosis. *Cell Stress Chaperones*, 1998; 3:228-236
22. Sandri M, Carraro U. Apoptosis of skeletal muscles during development and disease. *Int.J.Biochem.Cell Biol.* 1999; 31:1373-1390
23. Siu PM, Bryner RW, Martyn JK, Always SE. Apoptotic adaptations from exercise training in skeletal and cardiac muscles. *FASEB J.* 2004; express article 10.1096/ fj.031291 fje
24. Jethon Z, Murawska-Ciałowicz E, Dzięgiel P, Podhorska-Okołów M. Role of stress proteins in exercise adaptation (in Polish). *Post.Biol.Komórki* 2005 (in press)

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