ОГЛЯДИ

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A possible role for the mitochondrial permeability transition pore (MPTP) in bronchial asthma

Бронхіальна астма - хронічна хвороба дихальних шляхів, яка включає їх запалення з гіперреактивністю бронхіальних гладеньких м'язів та зі звуженням бронхів. Клітини з вогнища запалення здатні продукувати велику кількість вільних радикалів кисню, які є вирішальним фактором розвитку та підтримання запалення. Мітохондрії є головним внутрішньоклітинним джерелом вільних радикалів кисню, особливо після відкриття мітохондріальної пори. В огляді висвітлюються сучасні погляди на природу мітохондріальної пори та можливі механізми її залучення до розвитку скорочень бронхіальних гладеньких м'язів. Наголошується, що використання блокаторів мітохондріальної пори а також деяких антиоксидантів може бути корисним для корекції астматичних станів.

Bronchial asthma is one of the main disorders of the respiratory system. Its prevalence is increasing worldwide. While asthma affects all age groups, various studies indicate an increase in the incidence of asthma among children and young adults [1].

Asthma is a chronic disease of the airways symptomatically characterised by wheezing, dyspnoea, cough and chest tightness. The pathological processes in the development of asthma include severe inflammatory changes in the airways associated with bronchial hyperreactivity. The bronchial hyperreactivity or hyperresponsiveness refers to increased sensitivity to different stimuli, including allergens, pollutants, cold air, drugs, and bacterial infection, all of which can result in bronchoconstriction.

INFLAMMATION IS AN IMPORTANT STEP IN THE DEVELOPMENT OF ALLERGIC ASTHMA

The immune system plays a key role in the development of allergic asthma. After sensitisation, antigen exposure leads to recruitment and activation of different cell types including mast cells, eosinophils, neutrophils, lymphocytes, leukocytes, macrophages and platelets [2], which produce and release a variety of proinflammatory factors and substances. Under such conditions, lymphocytes produce interleukins (IL). They promote generation and activation of eosinophils and induce the expression of IgE receptors both on mast cells and eosinophils. Interaction of antigen with mast cell-fixed IgE leads to the release of histamine, eicosanoids, pre-formed tumour necrosis factor (TNF), cytokines, tryptases, other proteases, and chemokines. Histamine, eicosanoids and tryptases cause vasodilatation and extravasation of fluid, being responsible for the oedema of the airway wall.

Neutrophils, macrophages, leukocytes and eosinophils produce various chemotactic and growth factors, such as IL-1, IL-2, IL-3, IL-5, IL-10, TNF-α, transforming growth factor-β (TGF-β), matrix metalloprotease-9 (MMP-9), prostaglandins (PGs), tromboxane A_2 (TX) and leukotrienes (LT) [2,3]. LTs, such as LTD_4 and LTC_4, are known to mediate bronchoconstriction [4]. PGs also have the ability to induce broncho-

constriction, enhance airway responsiveness and to increase mucus secretion from the bronchial wall [4]. TX has also been implicated in airway hyperresponsiveness [5].

REACTIVE OXYGEN SPECIES (ROS) ARE A CRUCIAL FACTOR OF INFLAMMATION DEVELOPMENT

The synthesis of PGs and TXA_2 from arachidonic acid by cyclooxygenase (COX) enzyme is accompanied by the production of superoxide anions (O_2^-) [6]. The conversion of arachidonic acid to LT by 5-lipoxyginase (5-LO) is an another source of O_2^-. The superoxide anion is a negatively charged free radical belonging to the group of reactive oxygen species (ROS). In addition, ROS includes a range of toxic species, such as hydrogen peroxide, hypochlorous acid, and the hydroxyl radical [7]. The superoxide anion is the basic radical for the generation of different sour radicals. Hydrogen peroxide is generated as a result of the dismutation of O_2^- by superoxide dismutase (SOD). Myeloperoxidase (MPO) or eosinophil peroxidase metabolizes H_2O_2 to hypochlorous acid (HOCL). The hydroxyl radical (OH) is formed during the reaction of H_2O_2 with O_2^- in the presence of iron or other metal ions. The upregulation of inducible NOS (iNOS) during inflammation strongly stimulates the production of nitric oxide (NO). The reaction of superoxide anions with NO leads to formation of peroxynitrite ($ONOO^-$). Cytokines are able to stimulate the generation of peroxynitrite [8]. $ONOO^-$, a very powerful oxidant, belongs to the reactive nitrogen species (RNS) group. Nitrogen dioxide, another strong oxidising agent produced in significant amount from $ONOO^-$, also belongs to RNS.

Inflammatory cells of asthmatics have an increased capability to generate free radicals [9]. The release of large quantities of O_2^-, H_2O_2, OH^-, $ONOO^-$ results in increased quantities of free radicals in the airway tissue. The excess quantities of sour radicals overcome the antioxidant defenses and cause oxidative stress [10]. Oxidative stress contributes to the enhancement of the inflammatory reaction leading to an increase in airway smooth muscle contraction [11], airway hyperresponsiveness [12], mucus hypersecretion and vascular exudation [13].

SOURCES OF OXIDANTS SPECIES PRODUCTION

The produced reactive oxygen as well as reactive nitrogen species damage and oxidise proteins, lipids and DNA, leading to their impaired function. The main sources of free radical generation, additional to the 5-LO and COX pathways, include NAD(P)H oxidase, xantine oxidase [14] and mitochondria.

NADPH oxidase, a membrane-bound flavohemoprotein, is responsible for the production and release of superoxide (O_2^-). NADPH oxidase consists of the membrane and cytosolic components. Membrane-bound subunits, gp91phox and p22phox, are involved in the formation of the electron transfer component of the oxidase. p47phox, p67phox and G-protein rac-2 are the cytosolic components, which modulate oxidase activity. Phagocytic cells produce only minimal amounts of O_2^- until the p47, p67 and rac-2 bind to the gp91 and p22 subunits [15]. When NADPH oxidase is activated, huge amounts of O_2^- are generated on the external surface of the plasma membrane. Under asthma conditions, the phagocytic cells are abundant and thus the amount of O_2^- produced by NADPH oxidase is large [16]. Membrane oxidase (Mox), which is similar to the phagocytic NADPH oxidase, was identified in endothelium and smooth muscle cells [17]. The activity of this oxidase is upregulated by cytokines, growth factors, e.g. TNF-alpha and and platelet-derived growth factor (PDGR), which contribute to extracellular O_2^- production. Cytochrome P-450, due to its NADPH oxidase activity, is also a source of O_2^- production, contributing to intracellular superoxide anion production.

Xanthine oxidase is a molybdoenzyme, which exists in two forms, either as xanthine oxidase or xanthine dehydrogenase. Xantine oxidase is responsible for the metabolization of hypoxan-

thine, xantine and NADH, leading to the production of both O_2^- and H_2O_2. It was reported that under conditions of hypoxia these substances accumulate and xanthine oxidase activity increases [15]. Under pathophysiological conditions, xantine oxidase appears to be a potential source of ROS production.

Under oxidative stress conditions, the increased amount of released ROS induces the constant production of free radicals by mitochondria. The electron transport chain of mitochondria plays a key role in ROS production.

Under normal conditions, the respiratory chain, inserted into the inner mitochondrial membrane, constantly produces superoxide radicals (O_2^-). These radicals are converted into H_2O_2 by Mn-superoxide dismutase (MnSOD). Glutathione peroxidase (GPx) and thioredoxin peroxidase (TPx) then metabolize H_2O_2 to water by reducing it with electons derived from the oxidation of glutathione (GSH) and thioredoxin (TSH) to their disulfide forms (GSSG, TSST). Oxidized glutathion and thioredoxin are recovered by glutathione (GR) – and thioredoxin reductases (TR), which use NADPH as an electron donor. NADH then reduces NADP+, using the NADP transhydrogenase (TH). NADPH-dependent glutathione reductases seem to be the major system that maintains GSH in its reduced form [18]. Thus, the ability of mitochondria to maintain the normal redox status of a redox system is an important factor in the control of the oxidant signalling system.

Under conditions of oxidative stress, the mitochondria generate more O_2^- and H_2O_2 than under normal conditions. This leads to H_2O_2 accumulation in large quantities. In the presence of Fe_2+, highly toxic OH is generated from hydrogen peroxide. There exists no inhibiting OH enzyme, and therefore, via a direct effect of both H_2O_2 and OH, the mitochondria are damaged. The capacity of the redox system is often limited by the level of GSH and NADPH and therefore is not able to disactivate sour radicals. The significant damage of mitochondria and its consequences are described by the term “the mitochondrial disease”. Since mitochondria are a major source of ROS, this organelle often suffers damage through ROS. Free radicals attack polyunsaturated lipids, proteins and also mitochondrial DNA, which lacks some of the defense and repair mechanisms. The mitochondrial membrane, which contains important factors and enzymes for electron transport, oxidative phosphorylation and ATP generation, is getting damaged easily. The oxidation of protein thiol groups of inner mitochondrial membrane leads to conformational changes that result in a large non-selective pore, the mitochondrial permeability transition pore (MPTP) [19].

MITOCHONDRIAL PERMEABILITY TRANSITION PORE

The mitochondrial pore consists of a potential dependent anion channel, located on the outer mitochondrial membrane, ATP-ADP translocase on the inner mitochondrial membrane and cyclophilin D in the matrix. The diameter of the pore on the inner mitochondrial membrane is 2-2,5 nm and on the outer membrane it is 2,5-3 nm.

A rise in the matrix Ca concentration plays a pivotal role in the opening of MPTP [20-22]. External divalent cations such as Sr, Mn, Ba and Mg as well as Ca lead to a decreased probability of pore opening [23, 24]. Low pH (< 7.0) inhibits MPTP opening due to competition between protons and Ca for binding at the trigger site [23-25]. The accumulation of excessive quantities of Ca^{2+} ions in the intramitochondrial Ca^{2+} stores leads to a large decrease in transmembrane electrical potential, stimulating MPTP opening [26-28]. On the other hand, high membrane potentials are accompanied by slower respiratory rates contributing to ROS generation [29].

Opening of the MPTP allows water to enter the mitochondria, causing the swelling of the matrix and consequent rupture of the outer mitochondrial membrane. Increasing matrix volume further induces collapse of the mitochondrial membrane potential. The production of ATP by oxidative phosphorylation in mitochondria is inhibited by hydrogen peroxide even before the mitochondrial potential is changed. In experiments with

adult rat heart cells, it was found that ATP synthase activity was inhibited by hydrogen peroxide to 67% of the control value [30, 31]. The adenine nucleotide translocase (ANT) activity was also inhibited in a concentration and time-dependent manner. ANT is an integral inner membrane protein, which normally is responsible for the translocation of ATP and ADP across the inner membrane. When matrix Ca^{2+} is elevated, pyrophosphate (PPi) accumulates in the matrix and displaces adenine nucleotides from ANT molecules [32]. This leads to ATP depletion. The increase in PPi, which is caused by the inhibition of pyrophosphatase by micromolar Ca^{2+}, can increase the mitochondrial matrix volume [32]. Ca^{2+} and inorganic phosphate (Pi) are able to lead to a condition of oxidative stress in mitochondria in the absence of NAD(P)H oxidants [33]. It was shown that under conditions of decreased matrix free Ca^{2+}, even Pi alone is able to increase ROS generation and to stimulate MPTP [34]. Thus, oxidative stress, adenine nucleotide (ATP) depletion, increased Pi concentration and mitochondrial depolarisation [35] greatly enhance the sensitivity of the pore to Ca^{2+} and contribute to its opening. As discovered a couple of years ago, arachidonic acid, the substrate for the 5-LO and COX, is able to take influence on the opening of the mitochondrial pore [36].

After opening of the pore, a massive release of ROS takes place. It leads to the global oxidation and damage of the whole cell. This damage causes dysfunction and depletion of important enzymes in mitochondria. Oxidative peroxidation of lipids and reduction in ATP production are important factors of progression of mitochondrial dysfunction [37]. It contributes to the irreversible MPTP [38]. Irreversibility of the pore opening leads to the cell death. Cytochrome C, a caspase activator, is released through the opened mitochondrial pore, as are second mitochondria-derived activator of caspase (Smac) and apoptosis-inducing factor (AIF). Cytochrome C activates caspase 9. Further, caspase 9 activates caspase 3, 6 and 7, which are able to cause cell apoptosis [39, 40].

CONSEQUENCES OF MPTP AND THEIR POSSIBLE CORRECTION

As a result of oxidative stress, damage and apoptosis of the airway epithelium occur, and consequently, the endothelial nitric oxide syntase (eNOS) is not able to generate enough nitric oxide (NO). The small amount of the produced NO will be rapidly inactivated with superoxide. NO is a key molecule responsible for the relaxation of the smooth muscle cells. Diminished NO production contributes to airway dysfunction [41]. Because of the lack of NO, the airway smooth muscle cells ability to relax reduces and subsequently, the maintainance of bronchodilatation is impaired. Reduced synthesis or activity of NO as well as the apoptosis of the airway epithelium may contribute also to the development of airway hyperresponsiveness. In studies on isolated cardiomyocytes it was shown that during MPTP the elevated mitochondrial Ca^{2+} efflux as well as increasing myofibrillar Ca^{2+} sensitivity can induce the hypercontraction of the muscle [42]. The reduced production of ATP as a result of mitochondrial damage and mitochondrial pore opening contributes to the hypercontraction of the muscle. The ATP depletion impedes the activity of two main cation exchangers, namely Ca^{2+} ATPase of the sarcoplasmic reticulum (SR) and Na+/K+ ATPase of the sarcolemma [43]. This leads to the dramatic enhancement of Ca^{2+} in the cytosol and to an excess of Na+ in the interior of the cell together with lower levels of K+. Under these conditions, the end result is a highly contracted muscle without ATP to resequest Ca^{2+} into the SR and to break actomyosin cross-bridges. Hypercontraction contributes to the severe constriction of the airways. The following respiratory distress can be life-threatening.

In such a pathological state, where mitochondria, the major intracellular source of ROS, produce high levels of oxidants and the ability of the mitochondrial antioxidant defence is insufficient, the use of antioxidants in order to prevent or diminish cell damage may be a therapeutic option. Blockers of MPTP would be expected to alleviate the consequences of oxidative stress. As

shown in experimental traumatic brain injury, cyclosporin A (CsA) inhibits the mitochondrial pore, thus leading to the maintenance of the mitochondrial membrane potential as well as to calcium homeostasis in mitochondria. Such a treatment is accompanied by lower levels of ROS production by mitochondria. Therefore, CsA has shown a neuroprotective influence because of the MPTP modulation [44]. CsA is known as an immunosuppressive agent, with immunological effects such as inhibition of T-cell activation and blocking transcription of cytokines in activated T-cells. Allergic bronchial asthma patients showed reduced airway inflammation by means of decreased bronchial eosinophils and downregulation of eosinophil-associated cytokines and chemokines after CsA treatment [45]. Also, it was reported, CsA may improve severe asthma in patients through inhibition of T lymphocyte activation and reduction of the degree of airway hyperresponsiveness [46]. In experiments on guinea pigs, the usage of CsA aerosol led to decreased airway hyperresponsiveness. As it was presented on pulmonary histological studies, the eosinophil infiltration in the epithelium and subepithelial connective tissue of bronchi after CsA treatment also was inhibited [47]. Arimas et al showed in a guinea pig model that inhalation of CsA improves the allergen-induced late asthmatic response and inhibits airway hyperreactivity [48]. It can be resumed that CsA has a positive effect on the allergic airway inflammation. Taking into consideration the ability of CsA to block the mitochondrial pore, it is possible to propose that MPTP is able to play an important role in the bronchial asthma.

Melatonin, which is a scavenger of both ROS and RNS, is able to prevent mitochondrial injury induced by hepatic ischemia and reperfusion. Mitochondrial lipid peroxidation, glutathion peroxidase activity and the pH change connected with mitochondrial energy transfer were reduced after melatonin treatment [49]. Even the mitochondrial structure was restored to near normal in comparison to disorganized mitochondria because of ischemia/reperfusion. Melatonin protects mitochondria not only by reducing oxidative stress but also by stimulating electron transport and ATP production [50]. Until now, no detailed information about the role of melatonin in bronchial asthma is available. It would be interesting to investigate the role of MPTP and melatonin under bronchial asthma conditions.

The release of mitochondrial cytochrome C can be inhibited by another MPTP blocker, the water-soluble derivative of vitamin E, trolox [51-53]. Various reports point to the protective properties of trolox in the maintenance of mitochondrial homeostasis. The positive influence of MPTP inhibitors on organelle and tissue damage indicates possible therapeutical approaches.

Extracellular fluids and biological tissues contain a range of antioxidants contributing to the protection against oxidants. Interstitial fluid and lymph contain ascorbate and urate. Human blood has vitamin E, C, beta-carotene, catalase and SOD. Respiratory tract lining fluid contains mucin, uric acid, reduced GSH and vitamin C [54]. Patients with mild asthma have an altered lung antioxidants status. The analysis of bronchoalveolar lavage (BAL) fluid showed reduced levels of vitamins C and E and increased level of oxidised GSH [55]. Also, it is known that reduced antioxidant intake can lead to the increase in asthma severity [56]. Thus, the supplementation of the diet with vitamin C [57] and Se [58], which is important for the normal activity of glutation peroxidase, leads to improvement in asthma symptoms. Consumption of fish has resulted in lower airway hyperreactivity [59].

CONCLUSION

Bronchial asthma is a chronic disease of the airways which includes severe inflammatory changes associated with bronchial hyperreactivity and bronchoconstriction. Inflammatory cells of asthmatics have an increased capability to generate reactive oxygen species, which are crucial factor of inflammation development. Mitochondria are the major intracellular sources of ROS production especially after mitochondrial per-

meability transition pore opening. After opening of the pore, a massive release of ROS leads to the global oxidation and damage of the whole cell. Oxidative peroxidation of lipids, reduction of ATP production and cytochrome C release from mitochondria lead to cells apoptosis, the airway epithelial dysfunction, reduced synthesis NO and the airway smooth muscles hypercontraction. The use of blockers of mitochondrial pore as well as some antioxidants may be useful for correction of asthmatic disorders.

REFERENCES

1. Downs S.H., Marks G.B., Sporik R. et al. Continued increase in the prevalence of asthma and atopy // *Arch Dis Child*. – 2001. – **84**. – P. 20 – 23.
2. Nathan C. Points of control in inflammation // *Nature*. – 2002 – 420. – P. 846 – 852.
3. Sampson A.P. The role of eosinophils and neutrophils in inflammation // *Clin. Exp. Allergy*. – 2000, **30**, Suppl 1. – P. 22 – 27.
4. McKay S, Sharma HS. Autocrine regulation of asthmatic airway inflammation: role of airway smooth muscle. *Respir Res*. 2002;3:11.
5. Jongejan R.C., de Jongste J.C., Raatgeep R.C. et al. Effects of inflammatory mediators on the responsiveness of isolated human airways to methacholine // *Amer. Rev Resp. Dis*. – 1990. – **142**. – P. 1129 – 1132.
6. Kukreja R.C., Kontos H.A., Hess M.L. et al. PGH synthase and lipoxygenase generate superoxide in the presence of NADH or NADPH // *Circulat. Res*. – 1986. – **59**. – P. 612 – 619.
7. Bowler R.P., Crapo J.D. Oxidative stress in airways: is there a role for extracellular superoxide dismutase? // *Amer. J. Resp. Crit Care Med*. – 2002 – **166**. – S38 – S43.
8. Radi R., Beckman J.S., Bush K.M. et al. Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide // *Arch Biochem and Biophys*. – 1991. – **288**. – P. 481 – 487.
9. Jarjour N.N., Calhoun W.J. Enhanced production of oxygen radicals in asthma // *J. Lab. Clin. Med*. – 1994. – **123**. – P. 131 – 136.
10. Bowler R.P., Crapo J.D. Oxidative stress in allergic respiratory diseases // *J. Allergy Clin. Immunol*. – 2002. – **110**. – P. 349 – 356.
11. Rhoden K.J., Barnes P.J. Effect of hydrogen peroxide on guinea-pig tracheal smooth muscle in vitro: role of cyclooxygenase and airway epithelium // *Brit. J. Pharmacol*. 1989. – **98**. – P. 325 – 330.
12. Katsumata U., Miura M., Ichinose M. et al. Oxygen radicals produce airway constriction and hyperresponsiveness in anesthetized cats // *Amer. Rev. Resp. Dis*. – 1990. – **141**. – P. 1158 – 1161.
13. Del Maestro R.F., Bjork J., Arfors K.E. Increase in microvascular permeability induced by enzymatically generated free radicals. II. Role of superoxide anion radical, hydrogen peroxide, and hydroxyl radical // *Microvasc. Res*. – 1981. – **22**. – P. 255 – 270.
14. Quinlan G.J., Lamb N.J., Tilley R. et al. Plasma hypoxanthine levels in ARDS: implications for oxidative stress, morbidity, and mortality // *Amer. J. Resp. Crit Care Med*. – 1997. – **155**. – P. 479 – 484.
15. Wolin M.S. Interactions of oxidants with vascular signaling systems // *Arterioscler Thromb. Vasc. Biol*. – 2000. – **20**. – P. 1430 – 1442.
16. Delbosc S, Cristol JP, Descomps B et al. Oxygen and the superoxide anion. Modulation of NADPH oxidase?// *J Soc Biol*. 2001.– **195**.– P. 401-411.
17. Griendling KK, Sorescu D, Ushio-Fukai M. NAD(P)H oxidase: role in cardiovascular biology and disease// *Circ Res*.– 2000.–**86**.– P. 494-501.
18. Hoek J.B., Rydstrom J. Physiological roles of nicotinamide nucleotide transhydrogenase // *Biochem J*. – 1988. – **254**. – P. 1 – 10.
19. Kowaltowski A.J., Castilho R.F., Vercesi A.E. Mitochondrial permeability transition and oxidative stress // *FEBS Lett*. – 2001 – **495**. – P. 12 – 15.
20. Halestrap A.P. The mitochondrial permeability transition: its molecular mechanism and role in reperfusion injury // *Biochem. Soc. Symp*. – 1999. – **66**. – P. 181 – 203.
21. Gunter T.E., Pfeiffer D.R. Mechanisms by which mitochondria transport calcium // *Amer. J. Physiol*. – 1990. – **258**. – P. C755 – C786.
22. Bernardi P. Mitochondrial transport of cations: channels, exchangers, and permeability transition // *Physiol Rev*. – 1999. – **79**. – P. 1127 – 1155.
23. Haworth R.A., Hunter D.R. The Ca2+-induced membrane transition in mitochondria. II. Nature of the Ca2+ trigger site // *Arch Biochem. and Biophys*. – 1979. – **195**. – P. 460 – 467.
24. Bernardi P., Vassanelli S., Veronese P. et al. Modulation of the mitochondrial permeability transition pore. Effect of protons and divalent cations // *J. Biol Chem*. – 1992. – **267**. – P. 2934 – 2939.
25. Halestrap A.P. Calcium-dependent opening of a non-specific pore in the mitochondrial inner membrane is inhibited at pH values below 7. Implications for the protective effect of low pH against chemical and hypoxic cell damage // *Biochem J*. – 1991. – **278** (Pt 3). – P. 715 – 719.
26. Jurkowitz MS, Geisbuhler T, Jung DW et al. Ruthenium red-sensitive and -insensitive release of Ca2+ from uncoupled heart mitochondria // *Arch Biochem Biophys*. 1983;**223**:120-128.
27. Zago EB, Castilho RF, Vercesi AE. The redox state of endogenous pyridine nucleotides can determine both the degree of mitochondrial oxidative stress and the solute selectivity of the permeability transition pore// *FEBS Lett*. 2000.–**478**.– P. 29-33.
28. Catisti R, Vercesi AE. The participation of pyridine nucleotides redox state and reactive oxygen in the fatty acid-induced permeability transition in rat liver mitochondria// *FEBS Lett*.– 1999.– **464**.– P. 97-101.
29. Korshunov S.S., Skulachev V.P., Starkov A.A. High protonic potential actuates a mechanism of production of

reactive oxygen species in mitochondria // *FEBS Lett*. – 1997. – **416**. – P. 15 – 18.
30. Tatsumi T, Kako KJ. Effects of hydrogen peroxide on mitochondrial enzyme function studied in situ in rat heart myocytes// *Basic Res Cardiol*.– 1993.– **88**.– P. 199-211.
31. Zhang Y., Aberg F., Appelkvist E.L. et al. Uptake of dietary coenzyme Q supplement is limited in rats // *J. Nutr*. – 1995. – **125**. – P. 446 – 453.
32. Halestrap A.P. The regulation of the matrix volume of mammalian mitochondria in vivo and in vitro and its role in the control of mitochondrial metabolism // *Biochim. and Biophys Acta*. – 1989. – **973**. – P. 355 – 382.
33. Kowaltowski A.J., Netto L.E., Vercesi A.E. The thiol-specific antioxidant enzyme prevents mitochondrial permeability transition. Evidence for the participation of reactive oxygen species in this mechanism // *J. Biol. Chem*. – 1998. – **273**. – P. 12766 – 12769.
34. Kowaltowski A.J., Castilho R.F., Vercesi A.E. Opening of the mitochondrial permeability transition pore by uncoupling or inorganic phosphate in the presence of Ca2+ is dependent on mitochondria-generated reactive oxygen species // *FEBS Lett*. – 1996. – **378**. – P. 150 – 152.
35. Crompton M., Costi A. Kinetic evidence for a heart mitochondrial pore activated by Ca2+, inorganic phosphate and oxidative stress. A potential mechanism for mitochondrial dysfunction during cellular Ca2+ overload // *Eur J. Biochem*. – 1988. – **178**. – P. 489 – 501.
36. Scorrano L., Penzo D., Petronilli V. et al. Arachidonic acid causes cell death through the mitochondrial permeability transition. Implications for tumor necrosis factor-alpha aopototic signaling // *J. Biol. Chem*. – 2001. – **276**. – P. 12035 – 12040.
37. Halestrap A.P., McStay G.P., Clarke S.J. The permeability transition pore complex: another view // *Biochimie*. – 2002. – **84**. – P. 153 – 166.
38. Yang J.C., Cortopassi G.A. Induction of the mitochondrial permeability transition causes release of the apoptogenic factor cytochrome c // *Free Radic. Biol. Med*. – 1998. – **24**. – P. 624 – 631.
39. Srinivasula S.M., Ahmad M., Fernandes-Alnemri T. et al. Autoactivation of procaspase-9 by Apaf-1-mediated oligomerization // *Mol. Cell*. – 1998. – **1**. – P. 949 – 957.
40. Hu Y., Benedict M.A., Ding L. et al. Role of cytochrome c and dATP/ATP hydrolysis in Apaf-1-mediated caspase-9 activation and apoptosis // *EMBO J*. – 1999. – **18**. – P. 3586 – 3595.
41. Shaul P.W. Regulation of endothelial nitric oxide synthase: location, location, location // *Annu Rev Physiol*. – 2002. – **64**. – P. 749 – 774.
42. Korge P., Goldhaber J.I., Weiss J.N. Phenylarsine oxide induces mitochondrial permeability transition, hypercontracture, and cardiac cell death // *Amer. J. Physiol. Heart. Circ. Physiol*. – 2001. – **280**. – P. H2203 – H2213.
43. Piper H.M., Garcia – Dorado D., Ovize M. A fresh look at reperfusion injury // *Cardiovasc Res*. – 1998. – **38**. – P. 291 – 300.
44. Sullivan P.G., Thompson M.B., Scheff S.W. Cyclosporin A attenuates acute mitochondrial dysfunction following traumatic brain injury // *Exp. Neurol*. – 1999. – **160**. – P. 226 – 234.
45. Khan LN, Kon OM, Macfarlane AJ et al. Attenuation of the allergen-induced late asthmatic reaction by cyclosporin A is associated with inhibition of bronchial eosinophils, interleukin-5, granulocyte macrophage colony-stimulating factor, and eotaxin //*Am J Respir Crit Care Med*.– 2000.– **162**.– P. 1377-1382.
46. Fukuda T, Asakawa J, Motojima S et al. Cyclosporine A reduces T lymphocyte activity and improves airway hyperresponsiveness in corticosteroid-dependent chronic severe asthma// *Ann Allergy Asthma Immunol*. –1995.–**75**.–P. 65-72.
47. Xie QM, Chen JQ, Shen WH et al. Effects of cyclosporin A by aerosol on airway hyperresponsiveness and inflammation in guinea pigs // *Acta Pharmacol Sin*.– 2002.– **23**.–P. 243-247.
48. Arima M, Yukawa T, Terashi Y et al. Effect of inhaled cyclosporin A on the allergen-induced late asthmatic response and increased in airway hyperresponsiveness in a guinea pig model of asthma // *Arerugi*.– 1994.–**43**.– P. 1316-1325.
49. Okatani Y., Wakatsuki A., Reiter R.J. et al. Protective effect of melatonin against mitochondrial injury induced by ischemia and reperfusion of rat liver // *Eur. J. Pharmacol*. – 2003. – **469**. – P. 145 – 152.
50. Reiter R.J., Tan D.X., Manchester L.C. et al. Melatonin reduces oxidant damage and promotes mitochondrial respiration: implications for aging // *Ann N. Y. Acad. Sci*. – 2002..– **959**. – P. 238 – 250.
51. Zorov D.B., Filburn C.R., Klotz L.O. et al. Reactive oxygen species (ROS)-induced ROS release: a new phenomenon accompanying induction of the mitochondrial permeability transition in cardiac myocytes // *J. Exp. Med*. – 2000. – **192**. – P. 1001 – 1014.
52. Sagach V.F., Scrosati M., Fielding J. et al. The water-soluble vitamin E analogue Trolox protects against ischaemia/reperfusion damage in vitro and ex vivo. A comparison with vitamin E // *Pharmacol Res*. – 2002. – **45**. – P. 435 – 439.
53. Wu D., Cederbaum A.I. Cyclosporine A protects against arachidonic acid toxicity in rat hepatocytes: role of CYP2E1 and mitochondria // *Hepatology*. – 2002. – **35**. – P. 1420 – 1430.
54. Cross C.E., van d, V, O'Neill C.A. et al. Oxidants, antioxidants, and respiratory tract lining fluids // *Environ Health Perspect*. – 1994. – **102**. – Suppl 10. – P. 185 – 191.
55. Kelly F.J., Mudway I., Blomberg A. et al. Altered lung antioxidant status in patients with mild asthma // *Lancet*. – 1999. – **354**. – P. 482 – 483.
56. Fogarty A., Britton J. The role of diet in the aetiology of asthma // *Clin. Exp. Allergy*. – 2000. – **30**. – P. 615 – 627.
57. Anah C.O., Jarike L.N., Baig H.A. High dose ascorbic acid in Nigerian asthmatics // *Trop Geogr Med*. – 1980. – **32**. – P. 132 – 137.
58. Hasselmark L., Malmgren R., Zetterstrom O. et al. Selenium supplementation in intrinsic asthma // *Allergy*. – 1993. – **48**. – P. 30 – 36.
59. Romieu I., Trenga C. Diet and obstructive lung diseases // *Epidemiol Rev*. – 2001. – **23**. – P. 268 – 287.

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