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Oxidative stress in respiratory tract diseases in babies

Stres oksydacyjny w chorobach układu oddechowego u niemowląt

Oxygen, despite the benefits derived from its metabolism, may be potentially toxic for organisms due to forming free oxygen radicals. The name "free radical" indicates an atom or molecule which is able to exist independently, usually very reactive with one or more unpaired electrons on its orbital. The term reactive oxygen form (ROF) is used more broadly and it comprises also active oxygen forms without unpaired electrons, however (1).

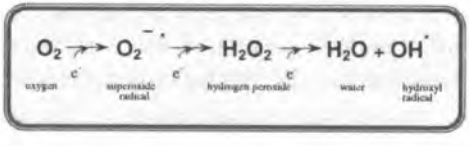

Fig.1. The generation of reactive oxygen forms

The examples of ROFs are the products of oxygen reduction by three electrons: superoxide radical ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$) (Fig.1).

The important source of free radicals is cell metabolism and, above all, oxidative phosphorylation in mitochondria. Other examples of endogenous generation of ROFs are: cytoplasm (xantine oxidase, haemoglobin, flavin, catecholamines, metals – e.g. iron), cell membrane (lipooxygenase, cyclooxygenase), endoplasmic reticulum and nuclear membrane (cytochrome P_{450} and b_5), peroxisomes and phagocytosis processes. Free radicals can be produced in biological systems by a number of exogenous agents: xenobiotics such as paraquat and doxorubicin, heavy metals such as chrome (Cr), lead (Pb), nickel (Ni), cigarette smoke, ionizing radiation (2). Intracellular ROFs play a very important

role in many physiological processes, e.g. phagocytosis, platelets' aggregation, regulation of muscle and vascular tone or intercellular signalling (1).

ROFs are potentially toxic for the majority of cell structures, such as proteins, nucleic acids, lipids, carbohydrates, or micromolecullar compounds (glutathione, ascorbic acid, NADPH) and may lead to their damage. The hydroxyl radical is most reactive species, because it is particularly unstable, and it may be a major mediator of injury (3, 4). Because the lipid component of the cell or organelle membrane is commonly the first structure encountered by these highly reactive species, it is most frequently damaged by toxic oxidants, resulting in lipid peroxidation. The main mechanisms of cell injury by oxidative stress are: DNA damage, GSH depletion, cytoskeletal damage, direct damage to proteins, rise in intracellular free Ca^{++}, membrane blebbing, inhibition of ATP synthesis, NAD(H) depletion, poly(ADP)ribose synthetase activation, rise in intracellular free iron, membrane peroxidation and destruction (1).

Antioxidant mechanisms have been developed by aerobic organisms. There are three main protection lines with respect to their mechanisms of effect (3). The first line consists of antioxidant enzymes: superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX) and proteins transporting and storing metal ions of transitory groups. They include transferin, ferritin, lactoferrin, ceruloplasmin. Micromolecullar antioxidants form the second line. Lipid-phase antioxidants include β-carotene (vitamin A), α-tocopherol (vitamin E), bilirubin, biliverdin, cholesterol and others. Ascorbic acid (vitamin C), uric acid, glutathione (GSH), creatinine are aqueous-phase compounds. The last protection line leads to reparation or removing of molecules damaged by ROFs.

Shifting the balance between prooxidative and antioxidative mechanisms into oxidant reaction is called oxidative stress (5). One of the most important causes of the oxidative stress in the organism is inflammation. Activated phagocytes and above all neutrophils are most often endogenic, enzymatic sources of ROFs in the respiratory system. The generation of ROFs in these cells is possible due to the activity of NADPH-oxidase and myeloperoxidase. Among the antioxidative mechanisms in the respiratory tract the most important is glutathione system, connected with the enzymes: glutathione peroxidase, transferase and reductase (6).

Due to high frequency of acute respiratory tract diseases in children, learning their etiopathogenesis is very interesting. This study was inspired by a small number of publications focused on the influence of ROFs and antioxidant mechanisms in these disorders.

MATERIAL AND METHODS

Seventy four 1 – 23-month-old children, with the average age 7.08 ± 4.09 month, were examined in The Department of Newborns' and Infants' Pathology at Medical Uni-

versity of Lublin. The patients were treated for pneumonia or bronchitis. The control group consisted of twenty-one children aged 2 – 24 months (mean age 10.09 ± 6.90 month). On the basis of routine laboratory examinations the infection in this group was excluded.

In all the children the concentrations of malonylodialdehyde (MDA) and vitamin E (VIT E) were estimated using fluometric method, and the activities of superoxide dismutase (SOD), glutathione peroxidase (GPX) and complete antioxidant potential (TAS) - using Randox reagents. Tests were set twice: in the severe period of the disease and during the remission period.

The acquired results were statistically analysed using non-parametrical tests.

RESULTS

The imbalance between prooxidative and antioxidative processes in the acute respiratory diseases in infants was found on the basis of the obtained results. Statistically lower activity of glutathione peroxidase (GPX) and higher concentration of micromolecular antioxidants (vitamin E, complete antioxidant potential - TAS) was observed in the patients in comparison to the control group.

The dependence between oxygen stress parameters and the period of disease was found. In the severe period of the disease the concentration of malonylodialdehyde (MDA) was significantly lower, whereas the value of complete antioxidant potential was higher in comparison to the remission period.

The obtained results are presented in Table 1:

Table 1. Obtained results in respiratory tract diseases in children and in control group

Parameter tested	Function	MDA nmol/ml	SOD U/ml	GPX U/ml	VIT E μg/ml	TAS mmol/l
Acute phase	mean	2.05*	89.84	4.02**	17.31**	1.48**
	SD	0.81	22.74	2.35	6.77	0.51
Remission phase	mean	2.33 *	91.9	4.03**	17.88**	1.34*
	SD	1.05	25.34	1.67	8.02	0.47
Control group	mean	2.12	93.1	5.36**	13.24**	1.22**
	SD	0.68	19.81	2.53	4.64	0.19

* $p<0.05$ in Wilcoxon's test

** $p<0.05$ in U Mann-Whitney's test

DISCUSSION

The respiratory tract fluid containing numerous antioxidative compounds is the first protective barrier reacting with inhaled oxidants. It is not easy to obtain sufficient for the test quantities of that fluid (7). The composition of the respiratory tract fluid reflects in a great degree plasma composition, at least if it concerns plasma protein content including ceruroplasmin and transferin. It also pertains to antioxidants such as ascorbic acid or vitamin E (8). Glutathione derived from pneumocytes type II and Clar's cells mainly is also found in the blood serum (9). Therefore the serum may be a model for *in vitro* examinations.

Oxygen-reactive forms produced by both primary and secondary effector cells (first of all by eosinophils, neutrophils and blood platelets) are counted among the mediators of allergic and inflammatory reactions, which have an important impact in asthmatic "eosinophilic" bronchitis (6). According to Barnes (10) cytotoxic influence of ROFs upon the bronchial epithelium results in its damage and leakiness, which facilitates receptor I and fibre C irritability indirectly cause bronchial contraction. Other authors underline lipid peroxidation products influence upon the course of bronchitis. They claim that membranous lipid peroxidation stimulates phospholipase activation, which on one hand intensifies metabolism of arachidonic acid on cyclo- and lipooxygenase way (5-lipooxygenase in neutrophils and 12-lipooxygenase in blood platelets), on the other hand however it leads to β-receptors dysfunction. The stimulation of leukotrien production enhances bronchial overreactivity, however β-adrenergic receptors dysfunction caused by sulphohydrolic SH group blockage leads to the prevalence of muscarin receptors, which is another cause of bronchial narrowing (6).

Intensified oxidative stress due to inflammatory factors in the respiratory tract is expressed by increased concentration of lipid peroxidation products such as malonylodialdehyde (MDA) (11). According to Ledwożyw (12), increased lipid peroxidation products are parallelled by increased antioxidative enzyme activity. The author hypothesised that the phenomenon described reflected the induction of adaptive mechanisms in the respiratory system that protected the tissue against destructive effects of free radicals.

According to Repine et al. (13) the response to oxidative stress occurs only in few patients and is manifested by increased antioxidative enzyme activity. The causes of the presence or absence of a reaction to stress in that group of patients also consider the role of genetic factors. Additionally glutathione peroxidase activity may be connected with the influence of serum selenium concentration, thus nutritional factors upon that enzyme activity (14). According to Zachara (15), the changes in selenium concentration in newborns and infants are related to food selenium content. In his research, the author found that mean selenium consumption by newborns and infants was significantly increased in the group of breast-fed babies compared to the group receiving artificial food.

Also the change in vitamin E concentration may be explained by the influence of life style, diet and living conditions (13). In our own research, because the patient group consisted of infants, the changes in vitamin E concentration were additionally affected by mother's diet and life style during pregnancy (14).

CONCLUSIONS

On the basis of our examinations the hypothesis concerning the significant role of free radicals in pneumonia and bronchitis in infants has been put forward. The use of antioxidants in the therapy can help immature protective mechanisms in small children and shorten the time of necessary hospitalisation.

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STRESZCZENIE

Reaktywne formy tlenu odgrywają znaczącą rolę w etiopatogenezie wielu chorób. Na przebieg kliniczny oraz możliwość wystąpienia powikłań u dzieci mają wpływ niedojrzałe mechanizmy obronne. Autorzy wykazali występowanie zaburzeń równowagi oksydacyjno-antyoksydacyjnej w przebiegu ostrych zakażeń dróg oddechowych u niemowląt. Stwierdzili statystycznie istotnie niższą aktywność peroksydazy glutationowej (GPX) oraz wyższe stężenie przeciwutleniaczy niskocząsteczkowych (witamina E, całkowity potencjał antyoksydacyjny - TAS) w grupie dzieci chorych w porównaniu z grupą kontrolną. Zaobserwowali także występowanie zależności pomiędzy parametrami stresu oksydacyjnego a fazą choroby: w ostrym okresie zakażenia znacząco niższe było stężenie malonylodialdehydu (MDA), natomiast wyższy całkowity potencjał antyoksydacyjny (TAS) w stosunku do okresu remisji choroby.