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Larycheva O.M.,
Tsebrzhynskyi O.I.,
Cherno V.S.
V.O. Sukhomlynskyi Mykolaiv National University, Mykolaiv, Ukraine, laricheva72@mail.ru

PROOXIDANT-ANTIOXIDANT BALANCE OF THE LUNGS IN CARRAGEENAN PLEURISY AND SHORT-TERM PINEAL GLAND HYPERFUNCTION

Abstract. *We have studied free radical and antioxidant processes in the lungs of rats with carrageenan pleurisy under10-day epiphysis hyperfunction. It was established that melatonin did not cause significant changes in the formation of superoxide in animals with carrageenan pleurisy but it promoted a reduction of peroxidation processes in the experimental organs. In this case there were no significant changes in the activity of antioxidant enzymes.*
Key words: *melatonine, epiphysis, carrageenan pleurisy, prooxidant-antioxidant system.*

Introduction. Lipid peroxidation (LPO) is an essential element of vital activity of any cell and it is a variant of free radical oxidation by its chemical nature. [5, 6, 9, 16]. Normally, all tissues of living organisms undergo a constant generation of reactive oxygen species(ROS), which, as signaling molecules, ensure the preservation of normal metabolic background necessary for functional activity of cells and are a component of nonspecific host defense against pathogens, microorganisms, tumor cells. As ROS represent a serious threat to the functioning of cells, there is quite a complex multilevel system of protection against them. Physiological prooxidant-antioxidant system (PAS) has a protective (effector) and regulatory functions.

Disorders in prooxidant-antioxidant balance in favor of the first one, lead to a potential damage, commonly called oxidative stress [20]. Oxidative stress occurs in the normal course of life too, being a kind of obligatory byproduct, caused by the function of oxidoreductases (cyclooxygenases, lipoxygenases, xanthine oxidases) multienzymatic ensembles of mitochondria, microsomes, resulting from immune responses (phagocytosis, immune observation) and non-enzymatic auto-oxidation reactions that occur due to a contact of gaseous (lungs, skin) and dissolved oxygen with the lipid membrane structures that are easily oxidized.

LPO and its regulation are of particular importance for the respiratory system, due to the great intensity of lipid metabolism in the lungs and close dependence of aero-hematic barrier function on the structure of alveolar phospholipids [15].

Neurohormone melatonin (MT) is a compound, possessing an antioxidant activity. Its protective effect in lipid oxidation is carried out by two mechanisms, which include direct inactivation of free radicals and / or inhibition of their generation in the cell and regulation of the activity of antioxidant enzymes by the impact on the genetic apparatus of cells, meaning it acts both as a straight and as a secondary antioxidant. [3]. But the impact of the MT on PAS of the lungs remains poorly studied.

Objective: to study free radical and antioxidant processes in the lungs of rats with carrageenan pleurisy under 10-day epiphysis hyperfunction.

Materials and methods. We used Wistar rats weighing 240-260 g, which were kept under standard vivarium conditions. We selected males for the study because melatonin levels in blood plasma of females depends on the menstrual cycle phase [12]. The animals were randomized into four groups with 7 animals in each one: an intact group, 10 day epiphysis hyperfunction, carrageenan pleurisy, 10 day epiphysis hyperfunction against the background of carrageenan pleurisy.

Pineal gland hyperfunction was modeled by

keeping animals in conditions of constant darkness and intragastric administration of melatonin solution (Sigma, USA) at a dose of 1.0 mg / kg [18].

To create a non-immune model of acute inflammation, we used 1% solution of carrageenan (Sigma, USA) [13, 19]. The experimental pleurisy was induced in anesthetized animals by intrapleural injection of 0.1 ml of carrageenan. The solution was injected on the 8th day of the experiment, and 48 hours later euthanasia of the animals was carried out.

Euthanasia of the rats was conducted under the rules of bioethics in accordance with the "European Convention for the Protection of vertebrate animals used for scientific experiments and other scientific purposes" (Strasbourg, 1986) and "General ethics of animal experimentation," approved by the first national bioethics congress (Kyiv, 2001).

The ROS products were evaluated by superoxide content. Superoxide products in tissue homogenates were determined by the reaction with nitro blue tetrazolium under the influence of NADH, NADP and pirogenal [17].

To evaluate the intensity of peroxidation in homogenates of organs, we determined the contents of primary and secondary products: diene conjugates (DC) oxidienes, trienes and TBA-active products. The effectiveness of antioxidant activity was assessed by lung superoxide dismutase (SOD), catalase (CT), glutathione peroxidase (GPO) and the concentrations of vitamins A, α-tocopherol and β-carotene.

The concentration of diene conjugates was determined by I.D. Stalna technique (1977) [14]. The concentration of TBA-active products was determined by I.D. Stalna, T. Harishvili method (1977) by reaction with 2- thiobarbituric acid [14]. The catalase activity was determined by M.O. Koroliuk with co-authors technique (1988 [7]. Superoxide dismutase activity was determined kinetically by the reaction of adrenaline autooxidation in the alkaline environment with the generation of superoxide-anion (radicale) [4]. Glutathione peroxidase activity was determined by the method of co-V.A. Pakhomova using tert-Butyl hydroperoxide as a substrate [11].

The total proteolytic activity was determined by hydrolysis of casein [8]. The concentration of trienes, oxidienes, α-tocopherol, vitamin A and β-carotene was determined by a modified method based on the molar extinction coefficient [10].

Statistical analysis was performed using Microsoft Office Excel 2003.

The test for the normal distribution was performed using Shapiro-W Wilkie criterion. Evaluation of reliability differences between groups with normal distribution of characteristics was performed using Student's t-test. When comparing the two groups with the free distribution of signs, we used nonparametric Wilcoxon (Mann-Whitney) U-test. The difference was considered statistically significant at $p < 0.05$ [1, 2].

Results and discussion. In assessing superoxide generation sources we found out that short-term light deprivation and exogenous MT contributed to the activation of generation of superoxide-anion (radicale) of mitochondrial ETL by 16% ($p < 0.01$) (Table. 1).

The rats with pleurisy had a significant increase of superoxide in their lungs from all sources of its generation. For instance, its content increased by 63% ($p < 0.001$) due to the mitochondrial electron transport chain by 50% ($p < 0.001$) – from microsomal electron transport chain and NO-synthase and by 26% ($p < 0.001$) – from phagocytes.

The rats with pleurisy that were in conditions of light deprivation and received MT when compared with normal active sources of superoxide radicals had both the mitochondrial chain (73%, $p < 0.001$) and microsomal one (66%, $p < 0.001$) as well as tissue phagocytes (26%, $p < 0.001$). When compared to the rats with epiphysis hyperfunction, the growth of superoxide generation turned out to be from two sources - mitochondrial (49%, $p < 0.001$) and microsomal (48%, $p < 0.001$) respiratory chains. The generation of superoxide anion-radicale in the lungs of experimental rats remained at the level of values, typical for pleurisy control.

The study of influence of MT on PAS in the lungs of rats (Table. 2) showed a slight decrease of peroxidation processes in animals with epiphysis hyperfunction compared with the norm which was illustrated by a reduced trienes

Table 1

The content and sources of superoxide-anion (radicale) generation in rats' lung tissue homogenate (M ± m, n = 7)

Group / Value	Intact	10-day epiphysis hyperfunction	carrageenan pleurisy	10dayepiphysis hyperfunction combined with carrageenan pleurisy
$\bullet O_2^-$ from mitochondrial electron-transport chain (nmol $\bullet O_2^-$/g·sec.), induced with NADH	19,900±0,224	23,110±0,102 p_1<0,01	32,382±0,752 p_1<0,001	34,525±1,086 p_1<0,001 p_2<0,001
$\bullet O_2^-$ from microsomal electron-transport chain and NO-synthase (nmol $\bullet O_2^-$/g·sec.), induced with NADP	21,000±0,845	23,530±0,967	31,525±1,846 p_1<0,001	34,906±0,777 p_1<0,001 p_2<0,001
$\bullet O_2^-$ from tissue phagocytes (nmol $\bullet O_2^-$ /g·sec.), induced with pirogenal	4,018±0,090	4,743±0,260	5,044±0,360 p_1<0,001	5,047±0,209 p_1<0,001

Notes: it is statistically reliable compared to p_1 – intact group; p_2 – epiphysis hyperfunction.

Table 2

Biochemical parameters of PAS in rats' lungs (M±m, n=7)

Group / Value	Intact	10-day epiphysis hyperfunction	carrageenan pleurisy	10-day epiphysis hyperfunction combined with carrageenan pleurisy
Diene conjugates (mmol/kg)	10,140±0,810	9,816±0,308	13,370±1,160 p_1<0,05	11,350±0,699
Trienes (mcmol/kg)	216,757±43,374	78,517±28,016 p_1<0,05	238,787±23,902	99,894±24,881 p_1<0,05 p_3<0,001
Oxidienes (mcmol/kg)	531,231±71,307	175,714±42,295 p_1<0,01	628,453±51,621	343,766±59,224 p_2<0,05 p_3<0,01
TBA-active products (mcmol/g)	8,423±0,354	10,700±1,345	10,220±0,170 p_1<0,001	10,070±0,155 p_1<0,01
Catalase activity (mAbs / kg)	4,691±0,017	3,931±0,142 p_1<0,01	6,374±0,333 p_1<0,001	6,407±0,431 p_1<0,01 p_2<0,001
SOD activity (st.un./g)	0,091±0,021	0,182±0,023	0,255±0,019	0,291±0,043

		p_1<0,05	p_1<0,001	p_1<0,01 p_2<0,05
GPO activity (mAbs / kg)	5,500±0,431	5,756±0,396	4,307±0,453	5,924±0,167 p_3<0,01
Total proteolytic activity (mAbs / kg))	57,320±10,160	59,390±11,640	68,210±6,180	85,330±4,512 p_1<0,05
VitaminA (mcmol/kg)	337,578±22,139	257,735±42,906	364,437±55,042	373,598±79,363
β-carotene (mcmol/kg)	73,439±13,187	90,058±15,963	84,384±17,532	134,696±35,382
α-tocopherol (mcmol/kg)	525,685±40,563	305,592±67,051 p_1<0,05	600,154±56,119	367,125±62,779 p_3<0,05

Notes: it is statistically reliable compared to p_1 – intact group; p_2 – epiphysis hyperfunction; p_3 – carrageenan pleurisy.

and oxidienes content in homogenates of the experimental organs by 3 times (p <0.05 and p <0.01). The changes in the prooxidant rate took place against the background of divergent changes in antioxidant system, namely the reduction of catalase activity by 16% (p <0.01) and rising by twice (p <0.05), SOD activity and a decrease in the concentration of α-tocopherol almost 2 fold (p <0.05).

In epiphysis hyperfunction the rats with pleurisy underwent some changes in the processes of peroxidation and antioxidant protection. MT contributed to the reliable reduction of trienes (p <0.001) and oxidienes (p <0.01) by almost 2-fold and increased GPO b activity by 38% (p <0.01) in the carrageenan rats, but at the same time the content of α-tocopherol reduced significantly (by 61%, p <0.05).

The findings show that the MT during the short epiphysis hyperfunction only inhibits the generation of superoxide radicals in carrageenan pleurisy; it also reduces the intensity of peroxidation in animals with pleurisy, and without it. In carrageenan inflammation the changes due to the activity of antioxidant enzymes were much less significant, since MT, perhaps, acted as a direct antioxidant. This may explain the fact that there was no significant reduction of antioxidant vitamins in the lungs of animals.

Conclusions. 1. Melatonin reduces the free radical processes under conditions of oxidative stress.

2. In carrageenan inflammation melatonin acts primarily as a direct antioxidant.

Perspectives of further investigations. It is planned to study free radical and antioxidant processes in the lungs of rats with carrageenan pleurisy under 10-day decrease in the carrageenan activity.

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