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CHANGES IN ENERGY METABOLISM OF MITOCHONDRIA IN PULMONARY DISEASES AND THEIR IMPORTANCE IN THE PATHOGENESIS OF COMMUNITY-ACQUIRED PNEUMONIA

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Abstract

The article presents the results of studying the energy metabolism of mitochondria and the antioxidant system in patients with community-acquired pneumonia. It was established that community-acquired pneumonia is an energy-dependent process, which manifests in the form of decreasing the concentration of adenosine triphosphate, against the background of increasing the concentration of adenosine diphosphate and adenosine monophosphate, relative to the indicators of a group of practically healthy humans. It has been proved that changes in the parameters of the lipid peroxidation system serve as markers of the severity of the inflammatory process and the effectiveness of the treatment of community-acquired pneumonia. Decrease of the catalase concentration against the background of increase of the TBA-reactants content and a significant decrease in the antioxidant-prooxidant index (6.5 times) are not only energy-saving mitochondrial mechanisms, but also oxygen-dependent processes. The obtained results expand the possibilities of diagnosing community-acquired pneumonia, which must be considered in establishing the diagnosis.

All human studies were conducted in compliance with the rules of the Helsinki Declaration of the World Medical Association "Ethical principles of medical research with human participation as an object of study". Informed consent was obtained from all participants.

Keywords: *community-acquired pneumonia, energy processes, mitochondria, antioxidant protection, lipid peroxidation*

Introduction

The main functions of mitochondria are the synthesis of ATP, electron transfer from NADH to oxygen with the formation of H_2O, oxidation of acetyl-CoA with the formation of two molecules of CO_2, etc. [7].

Adenyl nucleotides are among the most important effectors. Adenosine monophosphoric (AMP) and adenosine diphosphoric (ADP) acids act as positive effectors, stimulating the speed of energy processes and increasing the yield of adenosine triphosphate (ATP). The energy status of the cell is essential for maintaining such vital functions as: contractile, thermoregulatory, transport, metabolic. The decrease in ATP content violates, first of all, the energy-dependent process of phosphorylation-dephosphorylation of membrane proteins and lipids, which ensures the structural integrity of membranes [1, 6, 11, 12].

Changes in the energy-generating function of mitochondria and their significance in the mechanisms of inflammation in community-acquired pneumonia (CAPo have not been discussed in the literature available to us.

Aim of our research – to study the changes in mitochondrial energy metabolism and their significance in the pathogenesis of community-acquired pneumonia.

Methods

We examined 104 patients with community-acquired pneumonia (63 men and 41 women) aged 20 to 80 years, who were treated in the therapeutic department of the Kharkiv Clinical Hospital No. 25.

The CAP diagnosis was verified with the obligatory detection of focal infiltration of the lung tissue according to the X-ray examination of the chest organs and the presence of at least two of the following signs:

- acute beginning of the disease with an increase in body temperature > 38°C;
- cough with sputum production;
- physical signs (dullness/dullness on percussion; weakened, hard, or bronchial breathing; focus of voiced fine moist rales and/or crepitation);
- leukocytosis (>10×10^9/l) or stab shift (> 10 %).

Exclusion criteria from the study:

- self-administration of antibiotics before hospitalization for the disease > 24 h;
- absence of cough during hospitalization;
- age < 18 years;
- prospective macroaspiration;
- immunodeficiency conditions, including glucocorticoid therapy;
- tumors of any localization;
- decompensated heart defects;
- mental illness;
- alcoholism;
- drug addiction;
- intestinal absorption disorders [2].

The control group was formed of 20 apparently healthy individuals (AHH), comparable to the patients in age and sex.

During hospitalization, all examined patients were prescribed standard antibiotic therapy according to the Recommendations of the International Society of Pulmonologists and the National Institute of Phthisiology and Pulmonology named after F. G. Yanovsky (Kiev, 2019).

The concentrations of ATP, ADP, AMP were determined by thin-layer chromatography on Silufon plates in an erythrocyte homogenate, which was obtained by centrifugation in an PS-6 centrifuge at 3000 rpm for 15 min at a temperature of +4°C. The erythron energy charge (ECE) was calculated as a ratio: ECE=ATP×(ADP+AMP) [4, 7].

Also, we studied the state of the lipid peroxidation system (LPO) and the antioxidant system (AOS): by the concentration of malondialdehyde (MDA), the activity of ceruloplasmin, catalase in the blood serum, and the antioxidant-prooxidant index (API), which was calculated from the ratio of catalase activity to concentration of malondialdehyde.

To assess the LPO system and the activity of AOS enzymes, we used spectrophotometry methods on a «Specord UV VIS» two-beam spectrophotometer. The concentration of MDA was determined by the TBA method [8]. The principle of the method is based on the formation of a colored complex upon the interaction of MDA with thiobarbituric acid [8]. The level of ceruloplasmin in blood serum was investigated using the Ravin method using paraphenylenedamine as a substrate [4]. The principle of the method is based on the oxidation of paraphenylenediamine with the participation of

ceruloplasmin. The enzymatic reaction is stopped by the addition of sodium fluoride. By the optical density of the products that were formed, the level of ceruloplasmin was obtained [4]. The activity of catalase was determined by the method of S. V. Girin by reducing the content of hydrogen peroxide in the incubation medium, since catalase breaks down hydrogen peroxide [3].

To analyze the reliability of differences between the groups, statistical processing of the research results was carried out depending on the nature of the distribution of the data as follows: if the distribution was close to normal, the analysis was performed using the methods of variation statistics, the Statistica 8.0 software package – the statistical two-way ANOVA method (Fisher LCD post -hoc test), if it was significantly different from normal, the differences between the groups were determined using the «Kruskal-Wallis ANOVA and median test» method. Correlation analysis was carried out in the same Statistica 8.0 package, using parametric and nonparametric methods, depending on the type of distribution. The reliability of the differences between the indicators of the control and experimental groups was determined by the Student's and Kruskal-Wallis's criteria) using the «Excel» program. The reliability level was taken at $p < 0.05$ [5].

Results

On the first day, in patients with CAP revealed the same type of changes in the parameters of the adenyl system, which were characterized by a decrease in the concentration of ATP and a compensatory increase in the concentration of ADP and AMP in the erythrocyte homogenate (Table 1).

The ATP content in the group of patients with CAP was, on average, in 1.6 times lower than the AHH (632.7±15.2 mmol/l) and amounted to 395.4±15.3 mmol/l ($p<0.05$); ADP – in 1.58 times higher than the AHH group (367.4±13.4 mmol/l); AMP was also increased, on average, in 1.8 times compared with the AHH group (53.5±2.6 mmol/l) and reached 96.3±9.3 mmol/l ($p<0.05$). The ECE indicator in the group of patients with CAP was 0.85±0.09 ($p<0.05$), which, on average, in 2.6 times lower than the corresponding indicator in the AHH group (2.25±0.3).

The obtained results indicate about significant decrease in the level of ATP in the erythrocyte homogenate against the background of an increase in the concentration of AMP and ADP. This is most likely associated with an increase in the body's energy requirements, since pneumonia is an energy-dependent process [9]. On the other side, such changes can be caused by the disturbance and resynthesis of ATP with di- and monophosphate forms in the human body, as a result of disturbances in energy homeostasis due to hypoxia, which develops during the inflammatory process in the lungs.

On the 10th day of our studying the biochemical parameters of the level of adenyl nucleotides significantly improved (table 2). Thus, in patients with CAP, the ATP content increased in relation to the initial level by an average in 1.2 times and amounted 469.8±14.5 mmol/l ($p<0.05$). The ADP indicator was 311.3±12.2 mmol/l, which was in 1.2 times lower than the initial indicator and did not significantly differ from the AHH indicator ($p<0.05$). The AMP content in the erythrocyte homogenate in relation to the initial indicator decreased in 1.16 times and amounted 82.7±8.4 mmol/l, which practically corresponded to the upper limit of the norm ($p<0.05$). The ECE indicator, in comparison with the initial value, increased in 1.43 times and amounted 1.22±0.06.

Our obtained data indicate that as a result of inflammation in the lungs, the processes energetically provided by mitochondria are activated.

When we are studying the state of LPO and AOS in the blood serum it were observed LPO activation and a decrease in antioxidant protection, which is confirmed by an increase in ceruloplasmin in 1.9 times and MDA in 1.6 times as compared with AHH indices. There was also a decrease in the API index in 3.4 times, which also indicated about the presence of an inflammatory process in the lungs (Table 3).

An increase of the serum ceruloplasmin concentration in patients with CAP proved, firstly, about its redistribution in the bloodstream and was a compensatory reaction aimed at maintaining the level of AOS in the body, and secondly, it confirmed the presence of inflammation in the lungs already on the first day of the examination.

After 10 days of treatment with standard antibiotic therapy, we continued to study LPO and obtained unidirectional results: depletion of AOS, as evidenced of catalase activity decrease, which is very important, since catalase is an enzyme of the oxidoreductase class that decomposes hydrogen peroxide, which is formed during biological oxidation to water and molecular oxygen, and also oxidizes low molecular weight alcohols and nitrites in the presence of hydrogen peroxide, and thus takes part in the process of cellular respiration [1, 6, 11].

The results of the study of LPO indicators on the 10th day are presented in the table 4.

A decrease in the concentration of catalase against the background of an increase in the content of TBA-reactants and a significant decrease in API (6.5 times) indicates that not only energy-saving mitochondrial mechanisms, but also oxygen-dependent processes are mainly important in the pathogenesis of community-acquired pneumonia.

Conclusions

1. It was established that community-acquired pneumonia is an energy-dependent process, which manifests in the form of decreasing the concentration of adenosine triphosphate, against the background of increasing the concentration of adenosine diphosphate and adenosine monophosphate, relative to the indicators of a group of practically healthy humans.

2. It has been proved that changes in the parameters of the lipid peroxidation system serve as markers of the severity of the inflammatory process and the effectiveness of the treatment of community-acquired pneumonia.

3. Decrease of the catalase concentration against the background of increase of the TBA-reactants content and a significant decrease in the antioxidant-prooxidant index (6.5 times) are not only energy-saving mitochondrial mechanisms, but also oxygen-dependent processes.

4. The obtained results expand the possibilities of diagnosing community-acquired pneumonia, which must be considered in establishing the diagnosis.

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The authors declare that there are no conflicts of interest.

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Table 1. The level of adenyl nucleotides in patients with community-acquired pneumonia ($X \pm S_X$) – at the first day of observation

Indicators	Apparently healthy humans (n=20)	Patients with community-acquired pneumonia (n=104)
ATP, mmol/l	632,7±15,2	395,4±15,3*
ADP, mmol/l	232,1±6,2	367,4±13,4*
AMP, mmol/l	53,5±2,6	96,3±9,3*
ECE	2,25±0,3	0,85±0,09*

Note: * - p <0.05 in comparison with the indices of the AHH group.

Table 2. The level of adenyl nucleotides in patients with community-acquired pneumonia ($X \pm S_X$) – at the 10^{th} day of observation

Indicators	Apparently healthy individuals (n=20)	Patients with community-acquired pneumonia (n=104)
ATP, mmol/l	643,2±17,2	469,8±14,5*
ADP, mmol/l	232,0±6,2	311,3±12,2*
AMP, mmol/l	53,5±2,6	82,7±8,4*
ECE	2,23±0,11	1,22±0,06*

Note: * - p <0.05 in comparison with the indices of the AHH group.

Table 3. LPO and AOS indicators in blood serum of patients with community-acquired pneumonia ($X \pm S_X$, n=124)

Groups	Indicators			
	Ceruloplasmin, mg%	Catalase, mkkat/g	Content of TBA-reactants, µmol/g	API, conv.units
Apparently healthy individuals (n=20)	27,5±0,9	0,55±0,06	1,30±0,08	0,42±0,07
Patients with community-acquired pneumonia (n=104)	52,6±0,9*	0,36±0,08*	2,1±0,07*	0,17±0,03*

Note: * - p <0.05 in comparison with the indices of the AHH group.

Table 4. LPO and AOS indicators in blood serum of patients with community-acquired pneumonia at 10th day of observation ($X \pm S_X$, n=124)

Groups	Indicators		
	Catalase, mkkat/g	Content of TBA-reactants, μmol/g	API, conv.units
Apparently healthy individuals (n=20)	1,72±0,09	0,64±0,09	2,69
Patients with community-acquired pneumonia (n=104)	0,98±0,01*	2,4±0,02*	0,41

Note: * - p <0.05 in comparison with the indices of the AHH group.