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Зміст

X Anniversary Ukrainian - Polish - Belorussian Conference “Physiology and Pathology of Respiration: Advances in Basic Research and Clinical Applications” Kiev, 10-13 October, 2013

ABSTRACTS	3
PROCEEDINGS OF THE CONFERENCE	
<i>Kowalski Janusz, Gutkowski Piotr, Serebrovskaya Tatyana</i> Decade of the cooperation between Polish, Ukrainian and Belorussian physiologists and pulmonologists (2004 - 2013)	38
<i>Malieieva G.V., Lushnikova I.V., Skibo G.G.</i> Mitochondrial dynamics in oxygen-glucose deprivation on a model of cultivated hippocampal slices	44
<i>Serebrovskaya T.V.</i> Lessons from twenty years' investigations of intermittent hypoxia: principles and practices	48
<i>Levashov M.I., Berezovskii V.A., Chaka E.G., Yanko R.V.</i> Effect of intermittent normobaric hypoxia on total oxygen consumption and efficiency of cardio-respiratory mechanisms of oxygen supply in patients with a high risk of chronic obstructive pulmonary disease	57
<i>Gustavo R. Zubieta-Calleja, Gustavo Ardaya, Natalia Zubieta-De Urioste, Poul-Erik Paulev and Gustavo Zubieta-Castillo</i> Tolerance to hypoxia	65
<i>Сегізбаєва М.О., Александрова Н.П.</i> Сравнительная оценка устойчивости инспираторных мышц к утомлению при моделируемой обструкции дыхательных путей	73
<i>Avdonina L.I., Pryima N.V., Avdonina O.V., Vaintrub V.F.I., Khrystian G.Ye.I., Arlindo Valerio Arao de Oliveira</i> Tuberculous periodontitis: clinical-laboratory and epidemiological aspects	79
<i>Gumeniuk SA, Barkhina TG, Polner SA, Gushchin M.Yu.</i> Clinical , epidemiological and morpho-functional features of bronchial asthma with combined pathology.	88
<i>Gushchin M.Yu., Barkhina T.G., Polner S.A., Gumeniuk S.A.</i> Bronchial asthma and allergic rhinitis in different age groups	93
<i>Kislyakova L.P., Kislyakov Yu.Ya., Zaiceva A.Yu., Gulyaev V.I.</i> Multisensory educational system «electronic tongue» for the diagnosis of the functional state of the human body on the characteristics of exhaled breath condensate	99
<i>Kislyakov Yu.Ya., Kislyakova L.P., Zaiceva A.Yu., Gulyaev V.I.</i> Universal mobile analytical complex for research of respiration and gas exchange in man and animals	103
<i>Дорофеев А.Е., Хоростовска-Винімко Й., Коваленко С.В., Хорунжа В.С., Василенко І.В., Давиденко І.С., Струнявски Р.</i> Зміни клітинного інфільтрату в слизовій оболонці бронхів у хворих на хронічне обструктивне захворювання легень залежно від рівня антипротеолітичного захисту	107
<i>Донина Ж.А.</i> Влияние антиортостатического положения на регуляцию дыхания и компенсаторные реакции дыхательной системы	113

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Ukrainian-Polish-Belorussian Working Group of Polish Respiratory Society

From the Lab to the Bedside

X Anniversary Ukrainian - Polish - Belorussian Conference “Physiology and Pathology of Respiration: Advances in Basic Research and Clinical Applications”

Kiev, 10-13 October, 2013



The main idea of this forum is to give our colleagues from different countries a platform for the introduction of advanced achievements in basic research of lung functions (from molecular to systemic level) directly into clinical practice.

Under support:



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ABSTRACTS

DECADE OF THE COOPERATION BETWEEN POLISH, UKRAINIAN AND BELORUSSIAN PHYSIOLOGISTS AND PULMONOLOGISTS (2004 - 2013)

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The initiative to organize the Polish-Ukrainian Respiratory Working Group has arisen in October 2003 during the official visit of Prof. Janusz Kowalski to the Bogomoletz Institute of Physiology, Kiev, Ukraine. The driving motion of this initiative was the great wish to establish closer collaboration between Polish and Ukrainian researchers and physicians, to inspire the exchange of mutual experiences in research work and clinical practice and finally to help our patients who suffer the same pain and diseases on both sides of Polish-Ukrainian border. This concept has been approved by Prof. Jerzy Kozielski (President of the Polish Respiratory Society, Silesia Medical Academy, Poland), Prof. Platon Kostyuk (Director of the Bogomoletz Institute of Physiology, Ukrainian National Academy of Sciences, Kiev) and National Delegates of the European Respiratory Society Prof. Joanna Chorostowska (National Research Institute of Tuberculosis and Lung Diseases, Warsaw, Poland) and Prof. Yuri Feshchenko (Institute of Phthisiology and Pulmonology AMSU, Kiev, Ukraine). Next year Ukrainian scientists and doctors were invited by the Organizing Committee of 28th Congress of the Polish Respiratory Society to participate in this meeting in Lodz. During the Congress the Polish-Ukrainian Working Group of Clinical and Experimental Pathophysiology of Respiration was organized. Prof. Janusz Kowalski (Institute of Tuberculosis and Lung Diseases in Warsaw) and Prof. Tatiana Serebrovskaya (principal researcher of the Bogomoletz Institute of Physiology in Kiev) have been elected as Coordinators of this group. From 2012 Prof. Piotr Gutkowski headed the Polish section of the working group. During following decade the annual meetings in Kiev (2005), Opole (2006), Donetsk (2007), Katowice (2008), Ternopil (2009), Mikolaiki (2010), Chernivtsi (2011), and Wisla (2012) took place. In 2008 Belorussian colleagues from Grodno and Minsk joined working group. Besides scientific conferences, exchange of therapeutics and students was organized for training courses both in Polish and Ukrainian pulmonological clinics. The fundamental monograph «Lung function in lung diseases» (Eds. J.Kowalski, A. Kozerowski, L.Radwan) written by prominent Polish specialists in physiology and pathophysiology of respiration was translated from Polish to Russian by Dr. Eugenia Kolesnikova. The monograph was published in Poland, transported to Ukraine and distributed among main Ukrainian and Belorussian medical universities and libraries. A number of joint researches were provided, and international scientific papers were published in peer-reviewed journals. Many other initiatives have focused on the exchange of scientific and medical information between the Polish, Ukrainian and Belorussian colleagues to the benefit of the health of our populations. In conclusion, despite many organizational and financial difficulties in cooperation between Polish, Belorussian and Ukrainian universities, this initiative should be considered as significant and fruitful, integrating students, physicians and scientists of Central and Eastern Europe. The history of our Polish-Ukrainian-Belorussian Respiratory Working Group is going on. We ought to write it together.

THE RESPIRING BRAIN – THE REGULATION OF MITOCHONDRIAL FUNCTION AND SIGNALING BY HYPOXIA

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Mitochondria play an essential role in the regulation of cellular energy metabolism, intracellular signaling, apoptotic and degenerative processes. Mammalian organs with high energy requirements, such as the brain, essentially depend on oxidative mitochondrial energy metabolism and thereby the availability of oxygen representing the energy substrate for cytochrome c oxidase (COX). COX is the terminal, often rate-limiting and highly regulated enzyme complex of the mitochondrial respiratory chain. We could

show that the catalytic activity of the mammalian enzyme is regulated by the intracellular energy level (ATP/ADP ratio). Binding of ATP to one out of thirteen subunits of COX (COX subunit IV-1) causes an allosteric inhibition of the enzyme at high energy level and thus plays an important role in adjusting energy production to cellular energy requirements. However, in the brain under hypoxic conditions, a second isoform of this subunit, COX IV-2, is induced specifically in astrocytes and causes a change in the kinetic behavior of the enzyme, a suppression of COX sensitivity to the cellular energy level, and an increased mitochondrial peroxide production. In turn, this affects not only astrocytic but also neuronal survival during and after hypoxia and suggests a pivotal role of COX as an oxygen sensor for mitochondrial signaling and brain function. Moreover, this indicates a similar regulatory mechanism for other organs with high energy demand.

METHODS OF THE BREATHING RESTORATION AFTER THE RESPIRATORY ARREST UNDER DEEP HYPOTHERMIA IN RATS

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The purpose of this study is to compare the effects of injections of Na₂EDTA and polyglucinum on the breathing and cardiovascular system in the terminal stage of deep hypothermia and the breathing arrest. The experiments were performed on white male Wistar rats, which were cooled in water (9-10°C) up to respiratory arrest (rectal temperature is 15-16°C, the brain temperature is 16.5-18°C). After removal from the water, the first group of rats was injected intravenously by Na₂EDTA, the second group - by polyglucinum, and the third group – by the two drugs. After removal from the water, control animals (without pharmacological support) have not breathing restored. It is shown that the breathing is recovered in rats with Na₂EDTA injection. Respiratory rate in these rats increased to higher values (25-30 cycles/min) compared to rats who received polyglucinum (5-12 cycles/min) only. The polyglucinum introduction led to high blood pressure maintenance (60-80 mm Hg) which was observed in experiments both with polyglucinum introduction and additional introduction of Na₂EDTA. It is concluded that the main factor that contributes to the restoration of respiration after the cold paralysis is the concentration of calcium ions reduction in the blood by intravenous injection of Na₂EDTA. It is shown that the combined use of Na₂EDTA and polyglucinum provides faster recovery after respiratory arrest and the maintenance of high blood pressure under deep hypothermia.

AGE-RELATED CHARACTERISTICS OF MICROCIRCULATION AT BREATHING WITH POSITIVE EXPIRATORY PRESSURE

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The term Positive End Expiratory Pressure (PEEP) is widely used in clinical medicine and refers to breathing with the positive pressure at the end of expiration. Effect of PEEP on the microcirculatory part of the vascular system in aged people is still poorly investigated. Meanwhile, the microcirculatory system plays an important role in the mechanisms of adequate oxygen supply to the organs and tissues of the body and hypoxic tissue disorders compensation. *Material and methods:* A study was conducted on 16 aged (60-74 years old) and 12 young people (20-35 years old). The state of skin microcirculation was estimated by volume speed of blood flow at the inner side of forearm. Test with reactive hyperemia (by the method of O. V. Korkushko et al., 2002) was used to evaluate functional state of endothelium at the microcirculatory system level. Reactive hyperemia was caused by cross-clamping of the shoulder vessels for 3 min with help of a sphygmomanometer cuff with a pressure which exceeds systolic BP of a patient at 50 mmHg. The level of maximum volume speed of blood flow and time of blood stream renewal to normal were estimated. This test characterizes the ability of endothelium to synthesize endothelial factors of relaxation and reveals functional state of endothelium. Investigation was performed at the initial state (without resistance to exhale) and after 15 min of PEEP breathing with different settings of resistance for the exhale: 5 mm H₂O, 10 mm H₂O, 15 mm H₂O. PEEP breathing was achieved with help of breathing device "Threshold PEP" (Germany). *Results:* Investigation showed that skin micro-

circulation in young people does not change with PEEP breathing neither at 5 mm H₂O or 10 mm H₂O nor at 15 mm H₂O. Microcirculation in aged people does not also change with PEEP breathing at 5 mm H₂O, but at 10 and 15 mm H₂O a decrease in the volume speed of skin blood flow in the forearm was shown. Breathing with resistance to exhale causes a decrease in cardiac output of aged people. So, the decrease of the skin microcirculation as a result of breathing with resistance to exhale can be considered as compensation reaction of the circulatory system and particularly of the microcirculation which was caused by rearrangement of the blood stream and its sustaining at the needed level in the vitally important organs (brain, heart). In aged people, PEEP breathing at 10 and 15 mm H₂O causes a decrease in the cerebral hemodynamics. *Conclusions:* Breathing with resistance to exhale leads to the decrease in the microcirculation and function of endothelium in aged people. In addition, reduction of skin blood circulation and dysfunction of endothelium appears with PEEP breathing at 10 and 15 mm H₂O.

PROGNOSTIC FEATURES OF THE EPIDEMIC SITUATION OF TUBERCULOSIS AND ITS CONCOMITANCE WITH HIV INFECTION IN UKRAINE AND IN THE REPUBLIC OF BELARUS

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Aim: To monitor HIV-associated tuberculosis in Ukraine and Belarus for the last 10 years. *Results:* It was shown that the epidemiological indicators of tuberculosis in Ukraine over the last years are swiftly worsening. A negative tendency to increase in destructive forms of tuberculosis incidence is noted; a high percentage of patients with multidrug-resistant forms of tuberculosis is revealed; a number of patients with co-infection TB/ HIV increases. There was a significant exponential increase in HIV-associated tuberculosis in Belarus ($p < 0.001$) with a concentration of co-infections in the Gomel region— $63.0 \pm 5.6\%$ (95% CI, 58.3%–67.7%). In Belarus, one of the main opportunistic infection leading to death among HIV-infected persons is tuberculosis: there was a significant increase in the proportion of TB in the structure of AIDS mortality (from 16.7% in 2001 to 43.2% in 2011). Whether a mathematical simulation technique might be used to predict new cases of tuberculosis and its concomitance in HIV-infected patients was studied. There was a stable trend for increasing number of cases of tuberculosis concurrent with HIV infection in the Dnipropetrovsk region. Results of the study indicate that the application of mathematical simulation technique allow TB doctors and organizers of health to develop specialized care to patients with comorbidity, plan financial costs to take measures that will lead to enhanced TB control in Ukraine and in the Republic of Belarus based on prognostic features of epidemic situation in the regions.

TUBERCULOUS PERIODONTITIS: CLINICAL-LABORATORY AND EPIDEMIOLOGICAL ASPECTS

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To the present day, tuberculous periodontitis is not diagnosed and not present in the classification of tuberculosis. Its microbiological diagnostics is the most complicated as it is performed by investigating small quantities of paucibacillary material. The purpose of work was defining the significance of mycobacterial population variability forms in the clinical picture and epidemiology of tuberculous periodontitis. Comparative analysis of clinical and epidemiological features of tuberculous periodontitis was performed in two groups of patients aged 20 to 70 years over different observation periods from 1980 till 2012. Group A was composed of 258 patients who were observed from 1980 till 1990, and Group B consisted of 250 patients who were examined from 2005 till 2012. Periodontal focal points of infection in chronic forms of tuberculous periodontitis were used as the material for the advanced microbiologi-

cal and pathohistological examination. It has been established that in Group A, peridental focal points of infection serve as a reservoir for persisting mycobacteria. The main forms of such mycobacteria are unstable L-phase variants which were found in 50% of the investigated samples. We have noticed that the number of focal points of infection in which the changed mycobacteria forms vegetate tends to increase: L-forms (55,2%) and granular forms in Group B. L-form transformation of mycobacteria and emerging of granular forms is the reason of exacerbations coming up more frequently and persistent course of tuberculous periodontitis. The strains of mycobacteria in peridental focal points of infection susceptible to all antituberculous drugs that were detected 30 years ago has been supplanted by mycobacteria resistant to isoniazid and rifampicin in patients who had not taken antituberculous drugs before. At present, the fact that tuberculous periodontitis in HIV-positive patients can be a prognostic for clinical manifestations of AIDS is a point of interest. The clinical and epidemiological features of tuberculous periodontitis that have been revealed are similar to clinical pathomorphism of extrapulmonary tuberculosis; therefore tuberculous periodontitis can be included in the classification of tuberculosis as a separate nosological entity.

MAINSTREAM CAPNOGRAPHY ANALYSIS AS A TOOL FOR THE ASSESSMENT OF ALVEOLAR VENTILATION EFFECTIVENESS IN CHILDREN WITH BRONCHIAL ASTHMA UNDER INTERMITTENT HYPOXIA TRAINING (IHT)

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Capnography is a technique that provides both a waveform and a numerical value for the end-tidal carbon dioxide partial pressure ($P_{ET}CO_2$) during each respiratory cycle. In patients with bronchial asthma the lower CO_2 content in alveolar gas is often observed between attacks compared with healthy people. Clinicians see the cause of hypocapnia in hyperventilatory syndrome and offer special training programs with the use of mainstream analysis to teach patients breathing techniques that reduce the base hypocapnia. However, in clinical practice the mainstream analysis has not yet found a fairly wide use as a diagnostic test for bronchial asthma patients. This study was designed to determine whether the mainstream $P_{ET}CO_2$ measurement can accurately evaluate the effectiveness of alveolar ventilation under IHT in children with bronchial asthma (BA). Two groups of children aged 9–13 years with persistent atopic BA (moderate form, between attacks, without the signs of respiratory insufficiency) participated in the study: experimental group (Gr. I) – 15 children who underwent a two-week IHT (5 min exposures to 12% O_2 with intervening 5 min room air breathing, 4 times a day) alone with traditional medical treatment (anti-inflammatory and spasmolytic drugs), and control group (Gr. II) – 8 children who received the same medical treatment, but without IHT. Most children in both groups had a hyperventilation-induced hypocapnia. The clinical effect of treatment was assessed by physicians who did not participate in the study. The effect “improvement” was registered in 4 (Gr. I) and 3 (Gr. II) patients; the effect “significant improvement” was recorded in 11 (73.3%, Gr. I) and 5 (62.5%, Gr. II) children. IHT program resulted in a significant decline in breath shortness and feelings of chest congestion, the cough was diminished or disappeared, and the amount of sputum was reduced and passed more easily. The attacks of asphyxia disappeared or became more occasional. Exercise tolerance was significantly improved. A decrease in hyperventilation by 26 % and an increase in basal $P_{ET}CO_2$ from hypocapnic ($32,3 \pm 0,8$ mm Hg) to normocapnic range ($35,6 \pm 0,7$ mm Hg, $p < 0,05$) was registered in 11 children of Gr. I. The proportion of dead space in the alveolar ventilation diminished by 11, 4 %. Thus, capnography can better assess the IHT effectiveness in children with bronchial asthma.

QUALITY OF LIFE IN PATIENTS WITH PULMONARY SARCOIDOSIS

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Aim: to investigate the quality of life (QL) in pulmonary sarcoidosis patients compared with QL in patients with tuberculosis and evaluate the influence of good clinical monitoring. We examined 50 patients with sarcoidosis and 40 patients with tuberculosis by “Quality of life - 100”. All sarcoidosis patients have

low QL in the following scales: physical health, social relations, independence and emotional status. Total score in these patients was significantly lower than in healthy persons (60.5 ± 4.7 , $p < 0.01$). Dispnoea, fever, arthritic syndrome have the main influence on QL in sarcoidosis. Total score (42.7 ± 4.5 , $p < 0.01$) and especially score in physical health scale (33.4 ± 3.8 and 75.8 ± 9.3 , $p < 0.01$), independence (36.7 ± 4.1 and 56.4 ± 5.8 , $p < 0.01$), and social relations were significantly lower in tuberculosis than in sarcoidosis. But QL in sarcoidosis compared to tuberculosis was lower in emotions scale (51.7 ± 4.9 and 67.2 ± 6.3 , $p < 0.01$). All sarcoidosis patients were divided into two groups: 1st group received only basal treatment, 2nd group has additional clinical monitoring by physician during 20 months. Total score of QL in the 1st group increased slightly after treatment but not statistically significant. Total score in the patients of 2nd group has reached 72.4 ± 4.9 and in the emotional status scale - 80.6 ± 7.3 . The level of clinical improvement was significantly higher in 2nd group (improving of lung function, small number of relapses). **Conclusion:** In spite of better physical health, sarcoidosis patients have much more psychological problems than in tuberculosis, and physicians must take this circumstance into consideration in the every day activity. Clinical monitoring and good compliance can positively impact on the QL in sarcoidosis.

IS THERE CORRELATION BETWEEN LUNG FUNCTION PARAMETERS AND THE LENGTH OF INDUSTRIAL WORK RECORD IN POTASH MINE?

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Exposition to industrial factors in coal mine is firmly correlated with lung function parameters in miners. The impact of the length of industrial work record of service in potash mine on the lung function has not been so far well investigated. **Aim:** To evaluate the correlation between the lung function characteristics and the industrial work record of service in potash mine. **Materials and methods:** We examined 111 potash miners of Soligorsk (average age 36.1 yrs) by spirometry. I group included 21 miners with the record of service less than 10 yrs; II group ($n=52$) has the record of service from 10 to 20 yrs, and III group consisted of 38 miners with the record of service more than 20 yrs. **Results:** In average, lung function parameters were absolutely normal. There are no significant differences between groups. We found only a slight tendency to increase of lung function disturbance rate with the length of industrial work record.

Table. Lung function parameters of miners with different exposition to industrial factors.

Groups of miners	VC,%	FVC,%	FEV ₁ , %	MEV ₇₅ , %
Group I	98.1±14.6	111.9±15.9	107.5±14.2	99.1±10.4
Group II	97.1±11.6	99.3±12.8	101.4±12.6	100.7±10.2
Group III	93.9±13.6	95.9±14.8	99.3±13.6	93.2±9.8

Conclusion: Despite of influence of industrial aerosols the lung function of miners remained normal for a long time. This is, probably, the result of broncho- and mucolytic effects of potassium salt and absence of microbial contamination of the mine air. Positive effect of the potassium mine microclimate neutralizes the negative impact of industrial aerosols.

CHANGES IN EPITHELIAL BARRIER OF AIRWAYS IN ALLERGY

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Aim: To compare clinical and morphological data indicating the damage of the mucous membranes of the nose and bronchi in patients with bronchial asthma (BA) and allergic rhinitis (AR). **Materials and methods:** We investigated the scrapings of the mucous membrane of the nasal cavity and bronchobiotates in patients of different ages with AR and BA. Scanning (SEM) and transmission electron microscopy (TEM) were performed. **Results:** The surface of the epithelial cells of the upper and lower respiratory tract of patients with AR and BA tend to have the same changes that, along with clinical data on hyperreactivity of these parts as determined by provocative tests, indicates a common mechanism of epithelium damage. The greatest damage to the exposed ciliated cells (CC) was detected by SEM, and intracellular destruc-

tive processes - by TEM. Significant changes in goblet cells (GC) can enhance its main function. This reaction was more pronounced in the nasal mucosa. Clinical effect after allergen specific immunotherapy (ASIT) in adolescents was morphologically confirmed by demonstration of endocellular regeneration processes in epithelium. *Conclusion.* The intensity of changes in epithelial barrier of airways is directly related to the severity, early onset, dynamics and duration of BA or AR. This whole complex of changes suggests major modifications in the morphology of the airway in response to allergic aggression. Timely basic therapy and ASIT contribute to a better clinical effect, helps to prevent potential complications, and to restore a damaged nose and bronchial mucosa.

THE ALPHA-1-ANTITRYPSIN (AAT) DEFICIENCY IN RESPIRATORY PATIENTS – TARGETED SCREENING PROGRAMS IN POLAND AND CENTRAL EASTERN EUROPE (CEE AAT NETWORK).

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Inherited alpha-1 antitrypsin deficiency (AATD) is listed among the three most common genetic disorders in Caucasians. It considerably increases the risk of progressive obstructive lung diseases, mostly chronic obstructive pulmonary disease. Data concerning the prevalence of AAT deficiency in general population or respiratory patients in CEE countries are limited or non-existent. The overwhelming majority of individuals with AAT deficiency remains undiagnosed. In 2009 the targeted screening program for Poland has been established offering free access for high-tech AAT diagnostic for all respiratory patients. AAT serum concentration in DBS is measured by nephelometry and PI-phenotype identified by real-time PCR. The PI*S and PI*Z alleles are confirmed by isoelectrofocusing; rare phenotypes identified by direct sequencing. Between late 2009 and 2013 841 samples from respiratory patients were referred for diagnostics. 113 (13.6%) patients demonstrated AAT deficiency phenotypes. Calculated frequencies for main deficiency alleles PI*Z and PI*S were respectively 46.6 per 1000 (95% CI: 32.3-60.8) and PI*S 20.3 per 1000 (95% CI: 10.8-29.8), significantly higher than in general Polish population. Recently, as a result of the LPP Leonardo da Vinci EU program (2011-1-PL-LEO04-197151) “Introducing standards of the best medical practice for the patients with inherited Alpha-1 Antitrypsin Deficiency in Central Eastern Europe” the CEE AAT NETWORK has been established. Between October 2012-January 2013 samples were collected from 328 COPD patients in Poland (n=198), Bulgaria (n=44), Romania (n=26) and Slovakia (n=60). PI*S or PI*Z deficiency alleles were observed respectively in 14 (4,27%) and 30 (9,14 %) patients. There were 6 patients with rare AAT mutations: FM-3, IM-2, Mzbristol-1. In total, any AAT mutation was diagnosed in 48 COPD patients (14,63%). The calculated frequency for PI*Z and PI*S alleles in overall tested population was 46,1 (95% CI: 31,1-60,9) and 15,8 (95% CI: 6,92-24,6), respectively. The AAT gene prevalence calculated by Hardy-Weinberg equilibrium was: 1/1.15 for MM, 1/34 for MS, 1/4011 for SS, 1/12 for MZ, 1/688 for SZ and 1/471 for ZZ. *Conclusion:* Our results confirm the relatively high frequency of AAT deficiency in COPD patients from CEE. This initiative is the very first attempted to establish the diagnostic and scientific collaboration for AATD in CEE.

SOME INDICATORS OF OXIDANT-ANTIOXIDANT HOMEOSTASIS IN PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE COMBINED WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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In different countries 3 to 24% of the population suffers from nonalcoholic fatty liver disease (NAFLD). NAFLD is often diagnosed in people aged 40-60 years. It is also known that in this age there is a peak in the incidence of chronic obstructive pulmonary disease (COPD). In this regard, the actual problem

is a combined course of this pathology. *Aim:* To examine the state of oxidant-antioxidant homeostasis in patients with NAFLD combined with COPD. *Material and methods:* The study involved 23 patients with NAFLD combined with COPD (first group) and 18 patients with NAFLD without concomitant COPD (second group). The control group consisted of 20 healthy individuals. It was studied the content of malondialdehyde (MDA), the content of reduced glutathione (RH), glutathione peroxidase (GP) and glutathione -S-transferase (GT) activities in the blood. *Results:* In the first group of patients, the content of MDA was higher than in patients of second group (by 39.6%,) and healthy individuals (by 2.3 times). In addition, there was a significant decrease in RH level in the blood of patients compared with healthy individuals (by 1.8 times - in group 1 and by 1.5 times - in group 2, $p < 0.05$). These changes were accompanied by a compensatory increase in GP and GT activities in both groups of patients. *Conclusion:* Thus, the results indicate the presence of more severe disturbance of oxidant-antioxidant homeostasis in patients with NAFLD combined with COPD, which requires appropriate correction of treatment.

TREATMENT OF THE ADVANCED CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

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COPD is a chronic progressive disease. In the end-stage of the disease, when respiratory failure is developed, a prolonged home oxygen therapy is used. Indications to the prolonged home oxygen therapy in patients with COPD include: $PO_2 \leq 55$ mm Hg or $PO_2 < 60$ mm Hg with accompanied cor pulmonale or heart failure, or polyglobulia ($Hct > 55$). Home oxygen therapy aims at prolonging patient's life. To achieve this, it is necessary to use oxygen therapy for at least 15 hours per 24 hours.

Another therapy used in the chronic respiratory failure is non-invasive ventilation. Indication to such a treatment in the exacerbations of the chronic respiratory failure is:

- persisting, severe hypoxemia despite oxygen therapy ($Pa_{O_2}/O_2 < 50$ mm Hg);
- increasing hyperkapnia ($PaCO_2 > 60$ mm Hg);
- respiratory decompensated acidosis with $pH < 7.30$.

Noninvasive mechanical positive pressure ventilation (e.g. respirator of BiPAP type) in the treatment of the acute or exacerbated respiratory failure in the course of COPD brings about several advantages:

- decreases the number of patients requiring intubation;
- decreases the rate of complications resulting from the treatment with invasive mechanical ventilation (e.g. pneumonia);
- decreases in-hospital mortality.

EBUS/TBNA – A CHALLENGE FOR PATHOLOGIST

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Endobronchial ultrasound-guided transbronchial needle biopsy (EBUS/TBNA) is a new diagnostic tool useful in hilar or mediastinal lymphadenopathy diagnosis. The alternative method is Endoscopic (EUS) /TBNA. These procedures are performed in the cases of CT-confirmed mediastinal or hilaradenopathy (the diameter of short axis > 1 cm). Two main clinical indications for EBUS/TBNA are: lung cancer staging and clinically suspected sarcoidosis. However, many other pathological processes may involve lymph nodes. For aspiration the thin needle, less than 1 mm is used. The cell smears are alcohol fixed and hematoxylin- eosin stained. The rapid on site evaluation (ROSE) is possible with immediate diagnosis during bronchoscopy procedure, but in practice the final cytological diagnosis is available after 1-2 days. The wide spectrum of pathological changes in enlarged lymph nodes is recognized by cytopathology: normal cells- lymphocytes and their young forms, reactive inflammatory reaction, unspecific inflammation, granulomatous diseases and the presence of metastatic malignant cells. EBUS/TBNA is useful in lung cancer diagnosis often being the only available material. The best results are obtained in the cases of small cell lung cancer. The distinguishing main types of non small cell lung cancer: squamous cell type from adenocarcinoma is possible in about 60% in classical hematoxylin-eosin staining, but the use

of immunocytochemistry improves the effectiveness of diagnostic. If lymphoproliferative disorder is suspected the flow cytometry analysis of cells is indicated. Tuberculosis remains the common cause of lymphadenopathy. The use of molecular biology in the analysis of TBNA allows for a proper diagnosis of TBC infection. We conclude that EUBS-TBNA may have applications in both benign and malignant lung diseases as a routine examination by the experienced cytopathologist.

EFFECTS OF HEAD-DOWN TILT ON RESPIRATORY CONTROL AND LOAD COMPENSATORY RESPONSES IN RATS

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Aim: To examine the role of vagal afferents in the control of ventilation and compensatory capacity of the respiratory system during short-term head-down tilt (HDT) exposure. The experiments were performed in vagally intact or vagotomized, anesthetized rats. The response of tidal volume (VT), breathing frequency (f), minute lung ventilation (VE), esophageal pressure (Poes), inspiratory occlusion pressure ($P_{0.1}$) were examined at supine position (baseline) and after 30 min HDT-30° exposure. **Results:** There was a significant decrease in VT by 18% and VE by 14% after 30min HDT-30° exposure compared to supine. An increase both resistive by 50% ($p < 0.05$) and elastic component by 90% ($p < 0.05$) of respiratory resistance was found. On the other hand, it was seen the Poes rising by 116%, which reflects an increase in inspiratory muscle output occurring when respiratory load increases. After bilateral vagotomy, Poes rose only by 65% in comparison to vagal intact animals. Inspiratory occlusion pressure evoked an increase of $P_{0.1}$ in both supine and HDT. In supine, this value rose by 536% ($p < 0.01$), whereas in HDT - by 320% ($p < 0.05$). **Conclusions:** HDT in anesthetized animals induces ventilatory augmentation accompanied with the rise of the airway resistance due to the increased intrathoracic blood volume. A compensatory capacity of the respiratory system is diminished during short-term exposure at HDT-30°. One of the possible mechanism of such respiratory system compensatory response is the vagal afferent, presumably originating from the slowly adapting lung stretch receptors due to the decreased lung volume under HDT.

ROLE OF HYPOXIA-INDUCIBLE FACTOR 3A (HIF-3 α) IN PHYSICAL ENDURANCE OF RATS

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RNA-interference is the novel perspective method for knockdown of the genes involved in different mechanisms of adaptation to physical exercises. One of the crucial factors in this process is hypoxia inducible factor (HIF) that regulates transcription of many target genes encoding proteins that are involved in molecular adaptation to hypoxia. Based on previously data, HIF-3 α is considered as negative regulator of HIF-1 α and HIF-2 α , thereby gene encoding HIF-3 α appears to be an attractive target for RNA interference application. The purpose of this study is to examine the effect of HIF-3 α silencing on physical endurance of rats. **Methods:** Experiments were conducted on adult male Fisher rats. All animals were divided into three groups: (1) control (intact animals); (2) endurance training (ET) + scrambled RNA injection; (3) ET + specific HIF-3 α siRNA injection. Real-time PCR analysis was performed for quantitative evaluation of HIF-3 α , IGF1, GLUT-4 and PDK-1 in m. gastrocnemius and m. soleus. Mitochondrial respiratory function was measured by the polarographic method of Chance and Williams. **Results:** Knockdown of HIF-3 α (expression of mRNA is decreased by 1.7 times in m. soleus and by 2.6 times in m. gastrocnemius) using siRNA increases time of swimming to exhaustion in 1.5 times, but level of NAD- and FAD-dependent oxidative pathways is decreased. Efficiency of phosphorylation and the respiratory control ratio are significantly increased after HIF-3 α siRNA treatment. Expression of HIF target genes IGF-1 and GLUT-4 was not changed significantly, but PDK-1 expression was increased in m. soleus by 2.1 times. So, HIF-3 α silencing increases exercise endurance of rats, efficiency of phosphorylation and the respiratory control ratio. These results might represent a potential therapeutic approach for increase efficacy of athletes endurance training.

ROLE OF ENDOGENOUS INTOXICATION AND NEUTROPHILS IN MECHANISMS OF ACUTE LUNG INJURY IN CASE OF EXPERIMENTAL PERITONITIS

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The study was carried out on 78 albino Wistar male rats (180-230 g) divided at random into 3 groups. Group 1 consisted of 58 rats with experimental acute diffusive peritonitis (ADP), 2nd group – control group with sterile 0,9% NaCl at equilibrium (n=10) and 3rd intact group (n=10). ADP was induced by intraperitoneal injection of 10% suspension of feces. We analyzed total WBC count, Wet/Dry lung indexes, data of the oxidative and antioxidative systems and endogenous intoxication indexes, light and electron microscopy. Blood samples and lung tissue were obtained in 1, 12, 24 and 48 h after ADP induction. It has been established that after ADP induction was noticed progressive endogenous intoxication with lipid and protein peroxidation and inhibition of antioxidant protection for 24 hours with further exhaustion after the first day of experiment. The most informative and sensitive biomarker of acute lung injury is a coefficient of leukocyte lung regulation. The latter suggests enhanced leukocyte sequestration in respiratory system even on the background of leucopenia, which implies lung injury. Furthermore, lung injury has been confirmed by the analysis of morphological alterations of laboratory rats' lung tissue micro- and ultrastructure.

ADAPTIVE EFFECTS OF INTERVAL HYPOXIA AND HYPEROXIA ON EXERCISE TOLERANCE IN PROFESSIONAL ATHLETES: ROLE OF ROS AND REDOX PATHWAYS

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The pilot study has been performed to demonstrate protective effects of adaptation to interval hypoxia-hyperoxia in eliminating the overtraining syndrome features and enhancing physical capacity in professional athletes. On the first, experimental study on Wistar rats was established that combination of adaptation to physical exercise with adaptation to hypoxia-hyperoxia sessions, not to hypoxia-normoxia, improves tolerance under conditions of acute physical stress. Analysis of free radical processes, antioxidant enzymes and HSP proteins levels revealed that adaptation to hypoxia-hyperoxia provides optimization of both - hypoxic and stress components in systemic response to physical load, that's why in applied studies in 15 young over-trained professional athletes the efficacy of adaptation to 15 hypoxia-hyperoxia sessions with low-intensity sport exercises has been tested. The study results demonstrate improving the functional status, autonomic balance, physical endurance and aerobic performance in athletes with overtraining syndrome. Adaptation to physical loads and its combination with adaptation to variable oxygen levels increases the resistance of membrane structures to free radical oxidation at the expense of excessive activation of antioxidant defense enzymes in the course of physical training, which is partly compensated by adaptation to hypoxia/normoxia and is fully prevented by adaptation to hypoxia/hyperoxia. The combination of two adaptation forms (i.e. direct adaptation to physical loads and cross adaptation to variable oxygen levels) compensates markedly elevated content of HSP proteins in the course of physical training, which is especially well-pronounced during adaptation to hypoxia/hyperoxia. This novel technique is apparently less "physiologically demanding" and more beneficial for the organism.

MORPHOFUNCTIONAL STUDY OF BRONCHIAL ASTHMA WITH EXTRAHOSPITAL PNEUMONIA

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Aim: Study of Bronchial Asthma (BA) in conjunction with the extrahospital pneumonias (EP) of different etiopathogenesis and cell-humoral mechanisms. *Materials and methods:* 1241 patients were divided into 3 groups: BA (1), EP (2), BA with EP (3). Morphology: cytological and electron-microscopic methods. *Results:* 7% (86 P) with BA had EP. The patients were divided by severity: mild BA - 19%, medium level - at 55.8%, and severe degree of BA - at 24.4%. A coccal flora was found in sputum. In nasal

secretions we identified different number of lympho- and granulocytes. The most pronounced changes in the cells were observed in group 3. The surface of the epithelial cells of the upper and lower airways had the identical changes and match clinical data. Intensity changes of ciliated cells correlated with the severity of the diseases. The changes were accompanied by a sharp extrusion of goblet cells, which sometimes formed lacuna. These changes led to severe hypoxic and anoxic amendments to the mucous membranes of the respiratory tract. *Conclusion:* Epidemiology, clinical and morphological data of EP with combined pathology of inflammatory disorders have more severe cytological and morphological changes in the upper and lower airways. The group 3 of patients needs an individual approach to the treatment and prevention.

BRONCHIAL ASTHMA AND ALLERGIC RHINITIS IN PATIENTS OF DIFFERENT AGES

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Aim: to study the epidemiological, clinical, and morphological features of allergic rhinitis (AR) and bronchial asthma (BA). *Materials and methods:* common clinical, allergological, morphological (cytological and electron-microscopic methods) examination of patients with AR and BA. *Results:* the study was conducted among 685 adolescents with AR and BA between the ages of 14 to 18 years and 870 adults aged 18 to 65 years who were divided into groups. Divided by severity of adults: light level - 23%, medium - at 72%, and severe degree - at 5%. Divided by severity of teenagers: light level - 33,1%, medium - at 42,1%, and severe degree - at 24,8%. In the group of adolescents onset of the diseases occurred mostly from 5 to 15 years, and also a hormonal imbalance was detected that increased the severity of illness at this age. The results of allergological examination in the spectrum of allergens in patients with more severe BA revealed sensibilization to multiple allergen groups. In adolescents with AR and BA, there was found unique clinical and morphological changes in the mucous membranes of the respiratory tract. It was installed expressed morphological changes that have a direct relationship to the severity of the diseases. *Conclusion:* revealed similarities and differences in the clinical course, allergy data and morphological changes in patients of all ages with AR and BA.

SPROMETRY LIMITATIONS – A FEW EXAMPLES

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Spirometry is the basic method of respiratory function assessment. Several examples of spirometric and plethysmographic measurements performed in adults and children are presented in the paper. The comparison of these measurements indicate that: 1. Spirometry is unable to measure TLC and therefore to diagnose restrictive dysfunction of respiration. 2. In some cases spirometry does not show the airway obstruction. 3. Spirometry may not be sensitive enough to assess bronchodilatation response to beta-agonists nor to assess the bronchocontriction response in bronchoprovocation test. Nevertheless spirometry remains the primary diagnostic tool in pulmonary medicine.

SLEEP DISORDERED BREATHING (SDB) IN CHILDHOOD

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The natural history and classification of SDB in childhood are to be described in the presentation. The pathomechanisms of SDB and its effects on cardiac and central nervous system are shortly discusses as well as the risk factors of obstructive sleep apnea (OSA) in childhood. In the paper are also given the recent literature examples of OSA impact on the course of bronchial asthma in children on one hand and on impaired behavioral and neurocognitive functions on the other.

THE ROLE OF PULMONARY EDEMA AND SURFACTANT INACTIVATION IN THE PATHOGENESIS OF HCL-INDUCED ACUTE LUNG INJURY

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Acute lung injury (ALI) is the major cause of the acute respiratory failure. The aim of this study was to estimate the degree of pulmonary edema and the condition of surfactant metabolism in the dynamics of hydrochloric acid (HCl)-induced ALI. Nonlinear male rats (weight range 200–220 g) were used in all experiments. Were determined the oxygenation index pO_2/FiO_2 of arterial blood, level of total protein in bronchoalveolar lavage (BAL), Wet-to-Dry ratio and coefficient of stability by R. Pattle. The development of lungs damage in the case of intratracheal instillation of HCl was confirmed by three times increased concentration of protein in BAL after 24 hours of experiment. Elevated calculated ratios of pulmonary edema indicated a significant increasing in the lung tissue edema and pulmonary exudate in all research groups ($p < 0, 01$). It was found the progressive decrease in the level of surface activity of pulmonary surfactant system during the first day of observation: significant increase in the level of maximum surface tension and simultaneous decrease in the stability ratio by 45.8%.

Thus, presented data indicate the formation of ALI as a result of lung tissue's edema progression, the accumulation of exudate fluid and hence, impaired oxygenation with the development of hypoxemia. Accumulation of protein-rich fluid in the alveoli is the factor of surfactant inactivation that leads to a decrease in the stability ratio.

HOW TO TREAT SARCOIDOSIS.

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Sarcoidosis is a granulomatous disease of unknown cause, ranging from limited to multisystem disorder. About 2/3 patients develop an acute form strongly associated with spontaneous remission, others evolve into chronic course. Persistent sarcoidosis is likely to involve lungs but also other, in practice, any organs. Therefore, with the diffuse and especially with progressive onset, it could be potentially life-threatening. Due to ATS/ERS/WASOG consensus statement- the diagnosis of this disease is based on clinico-radiological findings, supported by histological evidence of noncaseating granulomas obtained by biopsy from involved organs. However, there are not unequivocal recommendations for treatment. Because of the heterogeneous course of the disease, it is not clear when, if at all, to apply therapy. Despite spontaneous remissions some patients have mild forms of this disease and adverse effects of drugs could be potentially worse than expected benefits. Therefore, a specific score of severity of sarcoidosis has just been created to help provide appropriate treatment. An acute form- Lofgren's syndrome, should not be cured with any immunosuppressive agents, as well as many stages of pulmonary or extrapulmonary sarcoidosis with no potentially lethal course. The involvement of heart and nervous system is an indication for immunosuppressive drugs application. Severe renal involvement leading to renal failure (with or without hypercalcemia) is also an indication for treatment. Very rare, but serious complication is hepatic localization of granulomas combined with cholestasis. Usually, liver and spleen localizations are asymptomatic and require no treatment, similarly to bone, cutaneous and other localizations that do not severely impair their function. Taking into account the serious course of some cases of pulmonary and systemic sarcoidosis over the past years, an increasing number of prospective controlled treatment trials have been completed.

EPIGENETIC REGULATION OF OXYGEN SENSING AND ITS IMPACT ON AUTONOMIC FUNCTIONS

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Recurrent apnea with intermittent hypoxia (IH) is a major clinical problem in preterm infants. Adults who are born preterm exhibit increased incidence of sleep-disordered breathing and hypertension suggesting

that apnea of prematurity predisposes to autonomic dysfunction in adulthood. Here, we demonstrate that adult rats exposed to IH for 10 days in the neonatal period exhibit remarkable cardio-respiratory abnormalities in adulthood manifested as irregular breathing, spontaneous apneas and hypertension. The enhanced hypoxic sensitivity was associated with elevated oxidative stress, downregulation of genes encoding anti-oxidant enzymes, and upregulation of pro-oxidant enzymes in the carotid body and adrenal medulla, which are known to be major regulators of cardio respiratory functions. We examined how perturbations in O₂ levels in neonatal period produce long lasting autonomic abnormalities in adulthood. Emerging evidence suggests that epigenetic mechanisms underlie long-lasting changes in gene expression. Epigenetic changes are heritable modifications of DNA that does not involve changes in DNA primary sequence. Of the different epigenetic mechanisms DNA methylation has been shown to mediate neonatal programming of adult diseases. In general, DNA hypermethylation leads to repression of gene transcription and hypomethylation causes transcriptional activation. Our results showed marked DNA hypermethylation in adult rats exposed to neonatal intermittent hypoxia, which was associated with persistent down regulation of anti-oxidant enzyme genes. Further analysis of the superoxide dismutase 2 (*Sod2*) gene, which encodes the anti-oxidant enzyme Sod2 showed DNA hypermethylation of a single CpG dinucleotide close to the transcription start site. Decitabine, a DNA hypomethylating agent, prevented DNA hypermethylation, oxidative stress, enhanced hypoxic sensitivity, and autonomic dysfunction. These findings implicate a hitherto uncharacterized role for epigenetic modulation of oxygen sensing in mediating neonatal programming of hypoxic sensitivity and the ensuing autonomic dysfunction in adulthood. The current findings are of considerable relevance for understanding the early onset of autonomic dysfunction in adults that were born preterm. Supported by NIH-HLBI grants HL-76537, HL-90554, and HL-86493.

COMPLEX THERAPY OF RECURRENT OBSTRUCTIVE BRONCHITIS COMBINED WITH FOOD ALLERGY IN CHILDREN AGED 1 TO 3 YEARS

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Aim: to prove the usefulness of pharmacological correction in babies' recurrent obstructive bronchitis that occurs on a background of food allergy. *Materials and methods:* There were 76 children aged from 1 to 3 years under the supervision. All of them suffered from recurrent obstructive bronchitis that occurs on a background of food allergy (to cow's milk protein). These children were cured by usual therapy: antibiotics, short-acting β -adrenergic agonists, theophyllines, H1- histamine blockers, antitussives. It was additionally assigned the probiotic-antagonist "Enterogermina" to 48 children during the course of antibiotic therapy (experimental group). The control group was consisted of 28 children. It was provided physical examination, determination of total immunoglobulin E, calculation of the reactive-protective potential of organism such as: phagocytic index of cell protection, allergic disposition index, lymphocyte-monocyte ratio. *Results:* (1) Diarrhea was registered in 6.4% of cases within the experimental group, and 27% of cases within the control group ($p < 0,05$). (2) The frequency of detection of neutral fat and fatty acids in coprogram of experimental group is lower in comparison with control group by 1,5 and 2,3 times respectively, ($p < 0,05$). (3) The level of total Ig E had a downward trend within the experimental group; it has grown by 23 % within control group. (4) Children of experimental group had a real decrease of allergic disposition index and lymphocyte-monocyte ratio in comparison with control group. *Conclusions.* Results of clinical supervision verified by positive shifts in coprogram, blood count, reflecting the state of immunobiological resistance and allergic disposition of organism, allow recommending probiotic "Enterogermina" as accompanying therapy of bronchial obstructive bronchitis in babies.

UNIVERSAL MOBILE ANALYTICAL COMPLEX FOR RESEARCH OF RESPIRATION AND GAS EXCHANGE IN MAN AND ANIMALS

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Experimental model of universal mobile and compact analytical complex for continuous monitoring of O₂ consumption and CO₂ emissions in breathing humans and laboratory animals has been developed. These

gas exchange parameters are calculated based on the simultaneous recording of expiratory flow rate and partial pressures of oxygen (pO_2) and carbon dioxide (pCO_2) in the exhaled air during each respiratory cycle (for a man), and the dynamics of the partial pressures of O_2 and CO_2 in a metabolic chamber (for animals and other biological objects). An analytical complex, in contrast to existing systems for the control of breathing and gas exchange in humans (EOS-Sprint, ER 800, ER 900, Ergo-line, Oxycon 5, Meta Max 3B, Rapidlab 840, Synthesis 15, Omni 3), has a small size, light weight and measure human respiration and gas exchange immediately during each breathing cycle. It consists of two parts - mobile and stationary. The mobile part includes a sensor unit, electronic converters, microcontroller unit, wireless transmitter and a miniature compact battery. Stationary part includes a wireless receiver and a computer control module. The sensor unit consists of the O_2 sensor (amperometric membrane electrode), the CO_2 sensor (the method of infrared correlation spectroscopy) and expiratory flow rate sensor (differential strain gauge pressure). Wireless transmission of information from the analyzer to the computer provides a radio modem. Computer control module with microcontroller provides calibration, measurement and calculation studied parameters, performance monitoring of all modules of the complex. The computer calculates indicators of the intensity of the gas exchange. The experimental results showed that the mobile analytical complex allows to measure with high precision volumetric expiratory flow rate and the partial pressure of O_2 and CO_2 in the breath of man, as well as the partial pressures of O_2 and CO_2 in a metabolic chamber with the animal (Table 1).

Table 1. Technical characteristics of sensors.

Registered parameters	Range	Error limit of measurement
pO_2 , mmHg	0–800	± 0.2 (0–200)
pCO_2 , mmHg	0–100	± 0.3
Expiratory flow rate, l / s	0 – 16	$\pm 2\%$

The complex has been tested in human studies during exercise and in studies on laboratory animals in surgical simulation of myocardial infarction and bleeding.

The project was supported by grants of the Program of the Presidium Russian Academy of Sciences “Fundamental Sciences - Medicine”.

MULTISENSORY EDUCATIONAL SYSTEM “ELECTRONIC TONGUE” FOR THE DIAGNOSIS OF THE FUNCTIONAL STATE OF THE HUMAN BODY ON THE CHARACTERISTICS OF EXHALED BREATH CONDENSATE

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The structure of a learning diagnostic system produced on basis of a new concept of multi-parameter electrochemical analysis, simulating the organization and functioning of biological sensory systems is developed. System features: 1) the use of an array of cross-selective sensors with sensitivity to various chemical components of the medium, 2) evaluation of the measurement results using the methods of handling large volumes of multidimensional data and pattern recognition. Such analysis systems are called “electronic tongue”. They have been successfully used for the diagnosis of patients on integrated electrochemical indicators of urine. The purpose of this study is the development of a learning multisensory analytical system for the diagnosis of the functional state of the human body using indicators of exhaled breath condensate. The diagnostic system is composed of four modules: sample preparation, sensory, measuring and information. Sample preparation module provides a sampling of condensate. The sensor module consists of 6 potentiometric electrodes, each of which has the basic sensitivity of one of H^+ , Na^+ , K^+ , Ca^{++} , NO_3^+ , NH_4^+ and the additional cross-sensitivity to other ions and organic components of the condensate. He formed an “image” investigated medium in the form of the composition of the electrode potentials. The measuring module is a precision electronic microprocessor meter output potential sensors (error limit of measurement ± 0.002 mV, input impedance 10^{13} Ohm). Information module contains a set of data processing techniques derived from the metering unit via telemetry. They realize the functions of the system learning and recognition of multi-dimensional “images” generated by

the sensor module. Studies performed on the subjects showed that each of them has its own individual reproduced “image” of exhaled breath condensate and can be identified on the “image”. Changes in the functional state of the subjects are shown in the characteristic changes of the “image”. The results indicate efficiency of the described software and application possibilities of the proposed method for the diagnosis of the functional condition of the parameters exhaled breath condensate. The project was supported by grants of the Program of the Presidium Russian Academy of Sciences “Fundamental Sciences - Medicine”.

EXPRESSION OF PYRUVATE DEHYDROGENASE KINASE-1 (PDK-1) GENE IN BRAIN-STEM MITOCHONDRIAL DYSFUNCTION DURING ADAPTATION TO INTERMITTENT HYPOXIC AND HYPOXIC-HYPEROXIC TRAINING

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It's well known that brain stem as respiration-supporting structure is relatively stable under different physiological conditions. At the same time, deviations of mitochondrial (MT) function at the brainstem nuclei as non-specific reaction at early stage of different disorders could be a cause for the drop in ATP production, enhancement of reactive oxygen species (ROS) production and hence significant shifts in Glu/GABA balance at respiratory rhythm generation. During hypoxia, the induction of pyruvate dehydrogenase kinase-1 (PDK-1), a glycolytic gene, is considered critical for metabolic adaptation to hypoxia through increased conversion of glucose to pyruvate and subsequently to lactate. Simultaneously, forced PDK-1 expression increases ATP levels, attenuates hypoxic ROS generation. We examined the expression of PDK-1 gene in Wistar rats with brainstem MT dysfunction (EMBD, single rotenone injection, 3 mg/kg s.c.) during adaptation to 14-days intermittent hypoxic training (IHT, 15 min hypoxia (12% O₂) with 15 min room air breathing, 5 times a day) and hypoxic-hyperoxic training (IHHT, 5 min hypoxia (12% O₂) with 5 min hyperoxia (30% O₂), 12 times a day). EMBD was accompanied by the drop in PDK-1 gene expression (by 29%). At the same time, 14-days course of IHT and IHHT enhanced PDK-1 gene expression (by 34% and 40%, respectively, $p < 0.05$). After IHT and IHHT, EMBD rats were characterized by significant rise of PDK-1 gene expression (by 35% and 200%, respectively). Our observations suggest that adaptation to IHT and IHHT promotes the expression PDK-1 to maintain ATP production and to prevent toxic ROS production in the brain stem of EMBD rats.

EFFECT OF INTERMITTENT HYPOXIA TRAINING (IHT) ON OXYGEN CONSUMPTION IN ELDERLY PEOPLE WITH AND WITHOUT OBESITY

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Obesity is accompanied by a decrease in physical performance due to glycogenolysis disorder that leads to a deficiency of energy substrates and more rapid fatigability during exercise. Many investigations showed cardiovascular chronic hypoxia progression and carbohydrate metabolism disorders in obese humans. Short episodes of breathing with hypoxic gas mixture (5 min) with intervening 5 min room air breathing, 4 times a day (IHT) improve cardiovascular system efficiency, normalize lipid and carbohydrate metabolism, and increase tissue insulin sensitivity. *Aim:* To investigate oxygen consumption in elderly people with and without obesity under IHT. *Methods.* Two groups of elderly people were examined: Gr. 1 - 15 subjects without obesity (body mass index, BMI = 22.6 ± 0.5 kg/m²), and Gr. 2 - 20 subjects with obesity (BMI = 32.6 ± 0.5 kg/m², $p < 0.01$). IHT was conducted during 10 days. With the subject seated, normobaric hypoxia (12% O₂) was administered using a device ‘Hypotron’ (Ukraine). Oxygen consumption at rest and at 55 W load was studied during bicycle ergometer test by Oxycon-4 (Netherlands) before and after IHT. Exercise power at lactate threshold was also estimated. *Results.* Before IHT, oxygen consumption at rest was higher by 13% in Gr. 1 (5.3 ± 0.2 ml/kg) compared to Gr. 2 (4.6 ± 0.1 , $p < 0.05$). The same pattern was observed at 55 W load: 18.3 ± 0.8 ml/kg in Gr. 1 against 14.2 ± 0.4 ml/kg in Gr. 2 ($p < 0.05$). Exercise power at lactate threshold was 70.0 ± 5.0 W in Gr. 1 and 62.5 ± 3.2 W in Gr. 2 ($p > 0.05$). After the IHT course, oxygen consumption did not change in Gr. 1 (5.2 ± 0.2 ml/kg at rest and 17.8 ± 0.9 ml/kg at 55 W load) as well as lactate threshold. In Gr. 2 the tendency to increase the oxygen consumption at rest (4.8 ± 0.1 ml/kg) and at 55 W load (14.7 ± 0.5 ml/kg) was observed.

Exercise power at lactate threshold increased significantly (66.8 ± 2.4 W, $p < 0.05$). **Conclusions.** IHT improves tissue oxygen consumption in elderly obese people. This leads to the inclusion of anaerobic mechanism of energy supply during physical load. The results suggest the potential use of IHT in the complex treatment of obese people.

H₂S PROTECTS HEART FROM DEFEROXAMINE-INDUCED CARDIOTOXICITY PREVENTING ROS AND RNS PRODUCTION

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Objective. Hydrogen sulfide (H₂S) has been shown to protect against oxidative stress injury and inflammation in various hypoxia-induced insult models. However, it remains unknown whether H₂S protects rat cardiomyocytes against chemical hypoxia-induced damage. We hypothesized that deferoxamine (DFO), an iron chelator and activator of the hypoxia-inducible factor-1 α (HIF-1 α), would provide cardiotoxicity by oxidative (ROS) and nitrosative (RNS) stress activation in an in vivo model of ischemia in part through inhibition of the cardiac H₂S availability. **Model.** Deferoxamine mesylate salt (Sigma Cat # D9533) was injected intraperitoneally 15mg/100g for 2 days to adult rats. **Main outcome measures.** Content of H₂S, H₂O₂, NO₂⁻, NO₃⁻ and urea, as well as ROS (*O₂⁻ and *OH) and RNS (NO) generation rate were determined in hearts tissue homogenate of control and DFO-pretreated rats. NO generation rate was calculated by cNOS (eNOS+nNOS) and iNOS activity of NO de novo synthesis pathways. Oxygenation index ([nitrite]1000/([nitrate]+[urea]), RNS/ROS ratio and % cNOS in total NOS (cNOS+iNOS) activity were calculated in both groups. **Results.** H₂S content in DFO-pretreated heart homogenates was dramatically decreased (from 12.01 ± 1.71 nmol/mg protein to 1.15 ± 0.14 nmol/mg protein) simultaneously with dramatic increasing of ROS (*O₂⁻ from 4.02 ± 0.97 r.u. to 13.09 ± 2.34 r.u., *OH from 0.92 ± 0.15 r.u. to 16.85 ± 3.78 r.u.) and RNS (cNOS from 6.38 ± 3.76 pmol/min/mg protein to 35.71 ± 3.76 pmol/min/mg protein, iNOS from 1.28 ± 0.36 pmol/min/mg protein to 13.71 ± 1.56 pmol/min/mg protein, nitrite from 359.01 ± 22.6 to 1906.83 ± 440.4 pmol/mg protein, nitrate from 12.96 ± 0.75 to 45.28 ± 9.05 nmol/mg protein. Total NO generative activity of NOS raised from 7.63 ± 1.5 pmol/min/mg protein to 49.42 ± 5.32 pmol/min/mg protein. In addition, oxygenation index in DFO pretreated hearts was markedly increased (from 3.49 ± 0.53 r.u. to 13.51 ± 2.17 r.u.). H₂O₂ pool, % cNOS and RNS/ROS ratio were not affected. **Conclusions.** Our findings showed that exposure of rats to hypoxia mimetic agent DFO for 48 h induces both dramatic decreases in H₂S level and nitrosamine and oxidative stress in heart tissues, suggesting protective effect of endogenously generated H₂S in normoxic heart condition against stress and inflammatory factors. Taken together, our findings suggest for the first time that H₂S may protect cardiomyocytes against ROS- and RNS-induced injuries and inflammatory responses through inhibition of ROS- and RNS-activated pathways, mainly by peroxynitrite (under excess NO and *O₂⁻) formation.

CHANGES OF THE PARAMETERS OF THE PLASMATIC COMPONENT OF THE HEMOSTATIC SYSTEM IN PATIENTS WITH BRONCHIAL ASTHMA

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Introduction. An indisputable role in the development of the chronic inflammatory process in the bronchopulmonary apparatus is played by disturbances in the system of hemostasis. **The object of the research.** To study the specific characteristics of the hemocoagulating component of the system of hemostasis in patients with bronchial asthma (BA). **Material and methods.** Have been estimated the total anticoagulating blood potential according to the level of plasma fibrinogen; the activity of antithrombin III (ATIII), Hageman-dependent fibrinolysis, total (TFA), nonenzymatic (NFA) and enzymatic (EFA) fibrinolytic activity of the blood, the potential activity of plasminogen (PAPG); a quantitative evaluation of the activity of factor XIII (fibrinases) in 50 patients with asthma. **The experimental findings.** It has been found an increased level of fibrinogen. While investigating the NFA level increase was registered as a result of an elevation of its share within the TFA pattern. The potential activity of plasminogen did not increase significantly compared with healthy persons. The activity of antiplasmin was reliably decreased

in BA patients. There occurred an activation of the clotting blood system in case of a BA exacerbation, as a consequence – an increase of the ATIII level, decrease of the level Hageman-dependent factor, a reliable increase of factor XIII. *Conclusions.* The signs of marked blood hypercoagulation are observed in patients with BA at the time of an exacerbation.

NICOTINE DEPENDENCE AND MOTIVATION TO QUIT SMOKING IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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Introduction: The percentage of smokers in Poland is 33.5% among adult men and 21.0% of adult women, which is, respectively, 5.2 and 3.5 million people. Diseases of tobacco are the cause of many deaths. These include patients with acute myocardial infarction. *Objectives:* The aim of this study was to assess the degree of dependence and the strength of motivation to quit smoking of patients hospitalized with acute myocardial infarction (AMI) as compared to asymptomatic smokers and analysis of the results with regard to demographic data and disease severity. *Method:* The surveys: motivation test to quit smoking and Fagerström test (tF) was performed in 32 patients-current smokers hospitalized for AMI. The control group consisted of 32 "healthy" cigarette smokers. In addition to the clinical examination and questionnaire regarding addiction, each patient was carried out anti-smoking advice by the rules. *Results:* The majority of patients reported the first episode of the AMI, dominated transmural infarction. Fagerström test mean score in the group of men did not differ between patients and healthy controls and ranged from 5.84 to 5.72. In the women patients group with AMI, Fagerstrom test average was 2.6 and 6.1 in the group of healthy subjects. In the particular case of the patients with AMI observed a strong dependence of the simultaneous strong motivation in patients with a first myocardial infarction with coexisting hypertension. *Conclusions:* Patients with AMI (especially women) have an average degree of dependence and high motivation to quit smoking. Strong biological and psychological dependence was observed in men. These observations point to the need to include patients with AMI to specialist care.

PECULIARITIES OF TUBERCULOSIS EPIDEMIOLOGY IN RURAL POPULATION

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Aim. To study peculiarities of tuberculosis epidemiology of rural population in region of Minsk. *Methods.* We used report forms of the official statistics and made comparative analysis of tuberculosis cases in rural and urban population of Minsk region during 2007-20011. *Results.* We revealed common conformities of tuberculosis prevalence in urban and rural population. Tuberculosis morbidity of rural population exceeded analogous data of urban population 1,7 – 1,8 times and corresponded to average data in the country. Morbidity of female tuberculosis in rural population was 1,6 times higher as compared with urban population; male tuberculosis morbidity 1,5-2 times accordingly. We revealed reliable differences in morbidity data of some age groups. The highest tuberculosis morbidity was registered in males of 35-55 years, in females of 25-40 years. Risk to fall ill with tuberculosis decreases with age in urban population and increases in rural population. Incidence rate of tuberculosis in urban males and females of 65 years age are the same. But rural females older than 65 years fall ill with tuberculosis 1,7 times more offer as compared with males. *Conclusion.* Modern unfortunate tuberculosis situation as in Minsk region also in the country depends high morbidity in rural population. Revealed differences of some age groups of rural population require following study.

BACILLI SHEDDING SIGNIFICANCE IN DIFFERENTIAL DIAGNOSTICS OF LUNG TUBERCULOSIS AND CANCER

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Aim. To study bacilli shedding character in patients with lung tuberculosis and cancer. **Methods.** We analysed the results of complex examination of 80 patients with new detected lung cancer in clinic of Scientific Practical Centre for Pulmonology and Phthisiology in 2007-2011 years. **Results.** We detected mycobacterium tuberculosis in sputum of 13 (16.1%) patients, 7 of them had tuberculosis formerly – 2-10 years ago. 10 patients had central cancer and 3 patients - peripheral lung cancer. Most of patients (11 persons) had advanced (3-4 stage) lung cancer forms. Lung cancer diagnosis was confirmed with bronchoscopy and morphological studies of biopsies in all the cases. 9 patients had localized lung cancer in zone of post tuberculosis changes. Only 1 patient had profuse and frequent bacilli shedding; 7 patients had single or dual bacilli shedding; 5 patients had second bacilli shedding in 5-10 months. 12 patients had drug resistance. 3 patients had active tuberculosis and lung cancer simultaneously; 3 patients had cancer on background of chronic tuberculosis; 2 patients had tuberculosis reactivation thanks to tumor. **Summary.** Physicians interpret MBT detection in sputum or post tuberculosis changes in tuberculosis favour during the differential diagnosis process. The further examination is conducted only in aggravation of clinical and roentgen data of illness.

STATE OF RESPIRATORY MUSCLES (RM) IN MEN WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

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Background. It is necessary to introduce new methods of assessment of RM. **Aim:** to investigate the RM status by echography and compare results with histological data. **Material and Methods:** We obtained the indices (ultrasonic): homogeneity (H), structural density (SD), echogenicity (E) in units. We carried out histological research of biopsies of intOAM. Research was made in 20 pts: 1st group (10 – 1st COPD stage; mean age – 55 yrs; FEV₁ – 79%; BMI – 24kg/m²); 2nd group (10 – 2nd COPD stage, age – 59 yrs; FEV₁ – 59%; BMI – 24kg/m²); control group - 12 pts. **Results:** There have been areas of myolysis, in which we found cell proliferation of perimysium, “ingrowth” of fatty tissue between myofibrils (MF). There is interstitial sclerosis around the individual MF and bundles, sections “contractions” of MF. Among the relatively preserved MF was found deep dystrophic one with protein granules of different sizes in the appearance of the sarcoplasm. The atrophy and crimp of muscular fibrils with the phenomena of “contractions” of MF was observed predominantly in the 2nd group. Increasing of COPD severity was associated with significant enhancing of “contractions” (r=0.72), destruction of MF (r=0.69) and proliferation of fibroblasts (r=0.52). We detected the presence of negative correlations between H, SD and intensity of sclerosis manifestations (r=-0.39 and r=-0.51 respectively; p<0.05). Meanwhile the E was higher in these pts and correlated directly (r=0.48; p<0.05). The E of intOAM correlated in the 2nd group with fat mass index (r=0.62). This may indicate the accumulation of intramuscular fat in the second stage. **Conclusion:** The proposed echodensitometric parameters reflect the degenerative processes occurring in the RM.

IMPACT OF DIFFERENT FACTORS ON BONE MINERAL DENSITY IN MEN WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE.

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Background: COPD is often associated with the systemic extra pulmonary effects such as osteoporosis. **Aim:** to study the likelihood of low bone mineral density in male patients with COPD. **Material and Methods:** We examined 92 COPD male patients aged 40 – 70 years. BMD was measured in spine (L₁-

L₄) and femoral necks (FN) using DEXA. *Results:* The index of “odds ratio” (OR) was 4.14 with the confidence interval (CI) (95%CI, 3.12-5.51) in patients with the presence of exacerbations in the year 3 and more. The likelihood of reduction in BMD with COPD significantly increase in patients with disease duration 10 years or more (OR, 7.34; 95% CI, 5.41-9.95); work related to hazardous occupational factors – OR, 2.30 (95% CI, 1.63-3.24); smoking – OR, 5.25 (95% CI, 3.20-8.35); the presence of emphysema in the X-ray – OR, 3.11 (95% CI, 2.37-4.06). Age 57 years and over - the probability increases 5.25 times (95% CI, 3.90-7.07). The likelihood of lower BMD in the 2nd stages COPD is 2.18 (95% CI, 1.36-3.50) and in the 3rd stage is greatly increase – OR, 10.91 (95% CI, 6.98-17.05). The presence of hypoxia (SpO₂%) significantly increases the risk of secondary reduction of BMD in COPD - OR 15, (95% CI 10.05-22.38). *Conclusion:* Factors that increase the likelihood of reducing the BMD in male patients in age 40-70 years are the frequency and type of exacerbations, age, duration and severity of disease, the presence of occupational factors, emphysema and hypoxia.

EFFECT OF INTERMITTENT NORMOBARIC HYPOXIA ON TOTAL OXYGEN CONSUMPTION AND EFFICIENCY OF CARDIO-RESPIRATORY MECHANISMS OF OXYGEN SUPPLY IN PATIENTS WITH CHRONIC PULMONARY DISEASES

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Intermittent normobaric hypoxic training (INGT) is one of the effective non-pharmacological methods for care and rehabilitation of patients with chronic pulmonary diseases. The total oxygen consumption (OC) and main indices characterizing the efficiency of cardio-respiratory mechanisms of oxygen supply were studied in 103 adult high-risk chronic obstructive pulmonary diseases patients. The 1st group of patients received only conventional treatment. The 2nd group received the same treatment in combination with INGT. INGT comprised of a daily 90 minute sessions in chamber “Orotron” for 2 weeks under such environmental parameters: PO₂ - 147-160 hPa, relative humidity - 60-70%, ambient temperature - 16-18°C and content of light negative ions - up to 6000 per cm³. The heterogeneity of OC dynamic of both the amplitude and direction was observed in 2nd group of patients after INGT. The pattern and degree of changes were dependent on the initial level of OC. In this regard the 2nd group of patients was divided into three subgroups: 2a - 33% of patients had a normal value of OC, 2b - 53% of patients had a lower value and 2c - 14% of patients had a higher value of OC. INGT did not change OC but improved the efficiency of hemodynamic mechanisms of oxygen supply in patients subgroup 2a. The total OC and efficiency of respiratory mechanisms of oxygen supply moderately increased in patients subgroup 2b. The value of OC decreased to normal level in patients subgroup 2c, but respiratory and hemodynamic mechanisms of oxygen transport remained highly effective. It was concluded that INGT normalized OC in patients with chronic pulmonary diseases.

THE MODERN SPIROMETRY: CHALLENGES AND RESPONSES

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The aim of study was to discuss the spirometry problems and offer their solutions. Spirometry is a medical technology that combined methodological, metrological and informational aspects. Modern microprocessor spirometers have reached their limit - the requirements of ATS/ERS standard and have a reserve to improve their performance. This, however, doesn't solve all problems of spirometry. They are designated mainly in methodological and informational support: (1) Algorithms for interpreting the results of spirometric tests based only on the parameters of FVC, FEV1 and Tiffeneau' index, whereas flow rates parameters of the forced expiratory “flow-volume” loop don't participate in the diagnostic conclusion formation. (2) Too great a role of subjective factors (operator training and patient cooperation) in the conduct of forced spirometry. (3) Spirometric testing software isn't standardized. For these problems solving, we have developed:

- the prospective requirements for spirometers;
- algorithm for interpreting the results of spirometric tests, taking into account the values of MEF25, MEF50, MEF75 and FEF25-75 of forced expiration;
- the requirements for the unified spirometry software;

- scenes of animation programs to encourage patients with forced expiratory maneuvers. For the further development of the spirometry it's necessary to: (1) put into practice a patient incentive programs; (2) develop the interpretation algorithms that take into account parameters of forced expiration flow rates for deeper and more objective assessment of breathing function as a result of spirometry; (3) create the dynamics standards of methodological, metrological and informational support for spirometry with a mandatory upgrade every 3 years.

DYNAMICS OF RESPIRATORY MUSCLE CHANGES DURING CHRONIC OBSTRUCTIVE PULMONARY DISEASE PROGRESSION

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Background The pathological changes of respiratory muscles (RM) during chronic obstructive pulmonary disease (COPD) progresses have not been studied in details yet. *The aim* was to assess RM status in COPD patients with different stages and detect the relationship between echodensitometric and morphological changes in RM. **Methods.** All 152 COPD male patients were divided according to the severity: COPD₁ (mild: age - 55 yrs, FEV₁ - 85%) – COPD₂ (moderate: age - 57 yrs, FEV₁ - 55%) - COPD₃ (severe: age – 60 yrs, FEV₁ - 33%). We studied the following muscles of inspiration (sternocleidomastoid, scalenus anterior, external intercostals) and expiration (abdominal internal oblique, abdominal external oblique, rectus abdominis, transversus abdominis and internal intercostals). The status of RM was detected in these groups both by using the echodensitometry indices (echogenicity - IE; homogeneity - H and structure density - ISD) and morphological material of abdominal internal oblique muscle (which was received during inguinal herniotomy). **Results.** In COPD, the tendency to increase of the indices of homogeneity (IH) and) was detected (due to hypertrophy of RM), while IE reacted differently. In moderate COPD IH and ISD were decreased, while IE was increased (due to fatty infiltration and sclerosis of RM according to our parallel morphologic data). In severe COPD IH and ISD rose against the background of IE decrease (due to reduction of fatty mass and intensification of RM sclerosis). **Conclusion** The proposed echodensitometric parameters reflect in a complex way the dynamics of the degenerative processes occurring in RM during COPD progression.

MITOCHONDRIAL DYNAMICS IN OXYGEN-GLUCOSE DEPRIVATION ON A MODEL OF CULTIVATED HIPPOCAMPAL SLICES

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Ischemia injury is one of the most common death reasons in Ukraine and in a whole world. At the cells level stroke characterized by oxygen and glucose supply loose that cause energy failure and neurdegeneration. Mitochondra is the key organelles of cell energy homeostasis. In order to discover dynamics of energy metabolism in ischemia damage we have been focused on investigation of mitochondrial functioning after 30-min oxygen-glucose deprivation (OGD) and reoxygenation 1 and 4 hours. We have done a comparison analyze of active mitochondrial activity of hippocampal pyramidal neurons and glial cells of CA1 and CA3 areas using fluorescent dye MitoTracker Orange. In the present study it was determined that pyramidal neurons and glial cells have different dynamics of mitochondrial activity. Hippocampal pyramidal neurons have demonstrated the increasing of activity during first hour and the decreasing for four hours after OGD. Glial cells increased their mitochondrial activity fourth hours after start of reoxygenation, comparing with first hour. This indicates a specific activation of pyramidal neurons in response to the OGD, which, however, can not be maintained for a long time. For glial cells is characteristic of a more prolonged and sustained increase in mitochondrial activity. Thus, it can be assumed that the more resistant glial cells are able to some extent, to modulate the function of neurons in a lack of oxygen and glucose.

INTERMITTENT HYPOXIA-HYPEROXIA, A NEW MODE OF ADAPTIVE TRAINING IN CORRECTION OF LUNG MITOCHONDRIAL DYSFUNCTION

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Hypoxic and hyperoxic therapy has a various application in traditional and alternative medicine for the treatment and prophylaxis of various pathological syndromes. Glutathione (GSH), GSH-related enzymes as well as MnSOD play an important role in preventing of lung mitochondrial dysfunction following oxidative stress injury. However, its role in forming of compensatory-adaptive responses to hypoxia/hyperoxia is unclear. It was explored a new mode of adaptive training, which combines periods of hypoxia and hyperoxia (H/H) and is characterized by upregulation of adaptive ROS signals compared to classical intermittent hypoxic training. The purpose of this study was to determine the influence of repetitive moderate sessions of hypoxia and hyperoxia on pro-/antioxidant homeostasis in lung mitochondria of rats exposed to acute severe hypoxia. It was shown that H/H pretreatment [5 cycles of 5 min hypoxia (10% O₂ in N₂) alternated with 5 min hyperoxia (30% O₂ in N₂) daily for two weeks] reduced the acute hypoxia-induced basal and stimulated *in vitro* lipid peroxidation, increased the GSH/GSSG ratio, and decreased the GSSG content. The enhancement in the level of GSH and activities of MnSOD, glutathione peroxidase and glutathione reductase in comparison with acute hypoxia as well as the maintenance of glutathione-S-transferase activity at control level confirm that mitochondrial protection during H/H may be mediated through the modulation of mitochondrial antioxidant levels. In lung H/H training caused the increase in MnSOD protein synthesis, at the same time, no changes in mRNA MnSOD expression was registered. This study supports the viewpoint that moderate periodic generation of free radical signal during changes in the oxygen level causes the induction of antioxidant enzyme protein synthesis that may be an important trigger for specific adaptations.

CONTENT OF ANTIMICROBIAL PEPTIDES IN OROPHARYNGEAL SECRETIONS OF CHILDREN SUFFERING FROM ACUTE RESPIRATORY DISEASES

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Today the studying of children local immunity of oropharyngeal secretions that often suffer from acute respiratory diseases is a matter of current interest. Antimicrobial peptides, especially defensins, are the first link of antiinfectious defense. Their level in children is almost not studied. The aim of the work: to study features of acute respiratory diseases (ARD) course and human β -defensins level in oropharyngeal secretions (OS) in children of different age suffering from chronic tonsillitis. 30 children that suffered from ARD and chronic tonsillitis aged from 4 to 12 were examined. The level of human β -defensins in OS during non acute period was investigated. Clinical, immunological and statistical methods were used. The level of human β -defensins in OS of children that suffered from ARD aged from 4 to 7 years is much smaller than in OS of the children with the same diseases aged from 8 to 12 years. It is 0,85 (0,12-2,25) mkg/ml and 4,8 (0,26-9,88) mkg/ml. The level of defenses in children aged from 4 to 7 is smaller than minimal level, when human β -defensins can demonstrate their antimicrobial activity. Clinically, the group of children that suffer from ARD aged from 4 to 7 is characterized by higher frequency of ARD and exacerbation of chronic tonsillitis, 11 children (73,3%) aged from 4 to 7 had bacteriologically confirmed bowels dysbiosis. During bacteriological studying of tonsils swab *St. aureus* was found in 9 children (60%) aged from 4 to 7 and in 7 children aged from 8 to 12 (46,7%). They showed smallest levels of human β -defensins: 0,46 (0,12-0,59) mkg/ml and 2,61 (0,26-5,94) mkg/ml relatively. Thus, we determined that children who suffer from ARD and chronic tonsillitis have low level of human β -defensins in OS. That is more pronounced in the younger age group (from 4 to 7 years). It can explain high frequency of ARD of children under school age.

FUNGAL CONTAMINATION OF PREMISES AS A RISK FACTOR FOR RESPIRATORY ALLERGY

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Background: Currently, moulds are among the unconditional risk of allergic rhinitis and asthma, in closed rooms. The inhabitants of modern cities, especially children, spend a significant part of their time indoors. Our objective was to determine the structure of mycobiota indoor and its impact on respiratory allergy development. **Methods:** questionnaire to assess the living conditions of patients, suggest fungal infection, visual examination of lesions of microscopic fungi, mycological examination of premises, sampling locations of the alleged mold damage, the selection of fungi in culture. Evaluation of 174 patients living in the fungal contamination included a medical history of life and disease, allergic history, physical examination, skin allergotesting, blood samples for determination of specific IgE to the major inhalation allergens. **Results:** Mycological analysis of the isolates showed a high degree of microscopic fungi spores sample contamination, dominated genera *Penicillium*, *Cladosporium*, *Aspergillus*, *Ulocladium*. A total of 174 people living in the areas affected by fungi, 59 of them (33.9%) have a sensitization to fungal allergens, including thresholds specific Ig E. Almost half of the sensitized patients (16.7%) were sensitized to several species of fungi. 174 residents of the surveyed areas were affected by the fungus, and 33 people (19%) had a diagnosis of asthma. Asthma fungal installed in 21 patients, 6 of whom had isolated fungal sensitization, 15 - sensitization to fungal allergens combined with sensitization to house dust mite allergens. **Conclusion:** Among the patients living in a residential area where the growth of fungi took place, there were increased sensitivity to fungal allergens in isolation or in combination with other types of sensitization. There is a need of residential patients with fungal sensitization inspection, the development of measures for the indoor fungi elimination. When moulds induce the early type of hypersensitivity specific immunotherapy should be used as the primary method of pathogenetic therapy.

SYMPATHETIC-ADRENAL SYSTEM AT PSYCHO-EMOTIONAL STRESS UNDER HYPOXIA IN AGING

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The onset of CNS changes in aging creates the prerequisites for reducing of the resistance to psycho-emotional stress influences. Age changes of the sympathetic-adrenal system present the key mechanism of age-dependent disturbances in adaptation to various factors including hypoxia and psycho-emotional stress. Our earlier investigations have shown that during aging there develops a more marked reaction of the sympathetic-adrenal system to the hypoxic stress. In literature there is lack of data about the peculiarities of adaptation to psycho-emotional stress under hypoxia in old age. We have tried to elucidate the reaction of the sympathetic-adrenal system to psycho-emotional stress under hypoxia in the old age. The study included 15 young and 25 essentially healthy elderly people in the age range from 60 to 74 years. Normobaric hypoxia was induced by the inhalation of gas mixture with low oxygen content (12% of O₂ and 88% of N₂) during 20 min. Blood plasma catecholamine concentrations were measured at the basal state and at 20th min of hypoxia. To reproduce the state of psycho-emotional stress, we used the laboratory model of psycho-emotional strain developed by A.A. Polyakov, N.F. Prokopenko and A.V. Pizaruk. Psycho-emotional stress at normoxic conditions caused a typical universal reaction to stress which is mediated by activation of sympathetic-adrenal system in all study groups. The increase in blood adrenalin content was observed in young subjects and both catecholamines (adrenalin and noradrenalin) - in old persons. Under hypoxic conditions the psycho-emotional load did not lead to further activation of the sympathetic-adrenal system in young subjects. On the contrary, in elderly subjects the combined action of psycho-emotional and hypoxic stress led to more marked reactions of the sympathetic-adrenal system in comparison with their young counterparts. **Conclusions.** In aging there develops a more pronounced reaction of the sympathetic-adrenal system to psycho-emotional stress. Combination of psycho-emotional and hypoxic stresses leads to more pronounced activation of sympathetic-adrenal system in elderly versus young people.

PULMONARY ALVEOLAR MICROLITHIASIS: REPORT OF 2 CASES**Patil P.V.¹, Gaude G.S.², Paramjyothi G.¹, Jain R.¹, Kaur K.¹***1Navodaya Medical College, Raichur -584103. 2J.N. Medical College, Belgaum - 590010. India. docpvpatil@gmail.com*

Pulmonary Alveolar Microlithiasis (PAM) is a rare pulmonary disease of unknown aetiology and pathogenesis, characterized by diffuse microliths in the alveoli. We report 2 cases of PAM encountered by us in last 16 years. Case 1: A 40 years male presented with cough and expectoration and progressive exertion dyspnoea since 6 months. He gave history of occasionally bringing sand like particles in the sputum. Sputum AFB was repeatedly negative. The RA test, LE cell phenomenon and ANA tests were also negative. The sputum examination revealed sand like particles to be microliths. Fiberoptic transbronchial lung biopsy showed calcified spherules (microliths) in alveoli. Case 2: A 22 years female presented with cough with scanty expectoration and dyspnoea since 3 years. The patient was treated in rural area as a case of pulmonary tuberculosis (PTB) based on chest X ray (CXR). However sputum was negative for AFB on repeated examination. Later the patient was referred to our hospital where the CXR and high resolution computed tomography (HRCT) –findings were suggestive of PAM. The above 2 cases are being presented for their rarity. Such cases are often under-diagnosed and treated as PTB in rural setup. Facilities for genetic studies to detect the mutation in SCL34A2 gene, which encodes a type IIb sodium dependent phosphate transporter, are not available in our setup.

THE ROLE OF POLY(ADP-RIBOSE) POLYMERASE-1 (PARP-1) IN CONCAVALIN A (CONA) INDUCED MICROCIRCULATION DISTURBANCES IN MOUSE LIVER**Pavlovych S.I., Martynova T.V., Makogon N.V., Grushka N.G., Sukhina V.S., Bryzgina T.M., Yanchii R.I.***Bogomoletz Institute of Physiology, Kyiv, Ukraine, spavl@biph.kiev.ua*

Experimental hepatitis induced by polyclonal T lymphocyte activator ConA closely mimics pathological features of immune-mediated liver diseases, such as microcirculatory disturbances leading to tissue hypoxia. This is a good and convenient model for studying the mechanisms of autoimmune hepatitis, particularly mediated by nuclear enzyme PARP-1. It participates in DNA repair and regulates transcription factors. Excessive activation of PARP-1 has been shown to contribute to some inflammatory disorders. Here we examined the effect of PARP-1 inhibitor 4-hydroxyquinazoline (4-HQN) on ConA-induced liver microcirculation disturbances to estimate the involvement of PARP-1 in the development of hepatic tissue hypoxia. ConA administration resulted in hepatitis with pathological changes in liver vascular system which caused acute tissue hypoxia. Hypervolemia, microvessel enlargement, stasis, hemolysis, thrombosis, vessel wall damage and perivascular edema were observed. The vascular injury was accompanied with the enhanced neutrophilic infiltration in the liver and with intensified superoxide production by activated neutrophils. These changes caused oxidative stress and increased lipid peroxidation (estimated by the level of malondialdehyde in the liver). The treatment of mice with 4-HQN (2h before ConA) decreased the total score of liver vascular injury (sum of scores for microvessel enlargement, hypervolemia, thrombosis, vessel wall damage and perivascular edema), $P < 0.001$. 4-HQN administration significantly reduced diffuse and focal neutrophilic infiltration in Con A-induced hepatitis and attenuated hepatic oxidative stress. These results suggest that PARP-1 plays an important role in pathogenesis of immune-mediated hepatic vascular damage and support the potential therapeutic application of PARP inhibitors in the treatment of hypoxic liver injury.

SYSTEMIC CONDITION OF SOME INFLAMMATORY MEDIATORS IN PATIENTS WITH BRONCHIAL ASTHMA AND WAYS OF THEIR CORRECTION**Pobedjonna G.P., Shuper V.O.***Lugansk state medical university, Lugansk, Ukraine, g-pobeda@ukr.net*

Cytokines (CK), in particular, interleukins (IL) play a significant role in the development of chronic inflammation in bronchi of patients with Bronchial Asthma (BA). The purpose of work: to study IL-1 β , IL-4, IL-8, IL-10, TNF- α contents in blood serum (BS) and condensate of moisture of expiratory air (CMEA) in patients with BA of different severity with exacerbation; to define the directions of their

correction. Materials and methods. 222 patients with BA were studied including those with mild degree - 107 (group I), moderate degree - 74 (group II), severe degree - 41 (group III). IL-1 β , IL-4, IL-8, IL-10, TNF- α were studied in CMEA and BS by enzyme immunoassay method.

Results. In group I IL-1 β , IL-4 were increased in BS and CMEA. In group II IL-1 β , TNF- α , IL-8, IL-4 were increased almost doubled of the norm in BS and CMEA. IL-10 in BS was around the norm, and in CMEA - significantly higher. In group III IL-1 β , IL-4 TNF- α in BS and CMEA were significantly higher than norm; IL-8 in CMEA was higher than in BS, IL-10 in BS and CMEA was close to norm.

Conclusions. After basic treatment the studied cytokines in patients of II and III groups didn't become normal in BS and CMEA. Medications with pleotropic effects which aren't contraindicated in BA should be added for correction of cytokines.

SENSING CHANGES IN OXYGEN CONTENT: THE ROLE IN HEALTH AND DISEASE

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Oxygen is indispensable for life, as its molecule participates in energy metabolism in mitochondrial electron transport chain. Sensing changes in oxygen is thus a vital homeostatic function of the organism. Oxygen is basically sensed by the sensory organ of the carotid body located at the bifurcation of the common carotid artery. The organ produces a powerful defensive chemoreflex consisting of lung hyperventilation in response to reductions in O₂. The innate mechanisms of carotid body function are still unknown and are subject of intensive research; in particular the role of a spate of neurotransmitters and/or ionic channels in the organ's receptor neurons, called chemoreceptor cells or Type I cells, is unsettled. The assessment of carotid body function consists of taking the ventilatory responses to hypoxia (HVR) and hyperoxia. Clinical usefulness of these tests is subject to debate. In health, the HVR may be used to predict the ability to adaptively respond to hypoxia, e.g., during strenuous exercise, which comes down to the prediction of safety of hypoxic episodes which someone may encounter. In disease, the HVR may be used to predict, e.g., if supplemental oxygen given to patients in case of emergency or in chronic respiratory disease would diminish ventilation. The hypoxic ventilatory response, and thus the ability to deliver oxygen through the aged lungs, also is crucially important during the aging process. Interestingly, despite severe structural and ultrastructural changes in the aged lungs, the response to hypoxia is well preserved. Therefore, in advanced, but healthy, old age the central and peripheral neural respiratory drive is able to compensate for the morphological lung tissue decline. The lecture will end up with the hints on the newest theories concerning the detection of oxygen changes along the hyperoxia-hypoxia continuum, having to do with the transient receptor potential channels.

PHASE CHANGES IN ENERGY METABOLISM DURING RECOVERY AFTER HYPOXIA OR STRESS

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Patterns of energy metabolism changes during hypoxia or stress are poorly characterized and understood. Male Wistar rats were exposed to periodic hypobaric hypoxia (PHH, 5600 m, 1 h, 6 séances every 3 days), acute hypoxia (5600 m, 3 h), or stress (6 h immobilization daily, 2 weeks). During recovery period, oxygen consumption (VO₂), pattern of respiration, body temperature (Tm), mitochondrial respiration by Chance, HIF-1 α and HIF-3 α gene expression were determined. Four phases of metabolic changes were identified. The first phase, hypometabolic (up to 7 days), is characterized by decrease in VO₂ and Tm, induction of HIF-1 α and HIF-3 α with delayed transitory stimulation of metabolism in response to each séance of hypoxia. It was found that during above-mentioned phase the mitochondrial complex II was significantly activated. During the second (transitional) phase the metabolic shifts were developed. In the third (hypermetabolic, 7-12 day or more) phase, metabolism was intensified that accompanied by VO₂ enhancement and the mitochondrial complex I activation. The fourth phase of primary adaptation (after 5 séances of PHH or 2 week of stress) was characterized by the normalization of VO₂ and Tm, expression of HIF-1 α and HIF-3 α , mitochondrial respiration, the increase in NAD-dependent oxidation of carbohydrate and lipid substrates. Thus, we identified four general patterns of the rebuilding in energy metabolism during periodic, acute hypoxic episodes and stress.

CELLULAR AND MOLECULAR BASIS OF ACUTE O₂ SENSING AND ITS PHYSIOLOGICAL CONSEQUENCES

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The carotid body is a sensory organ for detecting arterial blood O₂ levels and reflexly mediates systemic cardiac, vascular and respiratory responses to hypoxia. Sensory discharge of the carotid body is low under normoxia and dramatically increases in response to even modest hypoxia. Type I (also called glomus) cells, the site of O₂ sensing in the carotid body, express haem oxygenase-2 and cystathionine- γ -lyase, the enzymes which catalyze the generation of gaseous messengers carbon monoxide (CO) and hydrogen sulfide (H₂S), respectively. Physiological studies have shown that CO is an inhibitory gas messenger, which contributes to the low sensory activity during normoxia, whereas H₂S is excitatory and critical for sensory stimulation by hypoxia. Hypoxia increases H₂S generation in the carotid body, which requires redox-dependent interaction of cystathionine- γ -lyase with haem oxygenase-2, which generates CO. The transcriptional activators, hypoxia-inducible factors 1 and 2 (HIF-1 and HIF-2) constitute important molecular underpinning of hypoxic sensing by the carotid body. Recent studies demonstrate that redox balance determined by mutual antagonism between HIF-1 α and HIF-2 α isoforms defines the *set point* for hypoxic sensing by the carotid body. Emerging evidence suggest that developmental programming of the carotid body response to hypoxia involves epigenetic changes, e.g., DNAmethylation of genes encoding redox-regulating enzymes. Available evidence implicates heightened carotid body chemo-reflex in the progression of autonomic morbidities associated with cardiorespiratory diseases, such as sleep-disordered breathing with apnoea, which causes intermittent hypoxia (IH). Exposing rodents to chronic IH leads to sensitization of the carotid body response to acute hypoxia and induces sensory long-term facilitation and these effects are mediated by altering the redox state. Analysis of the systemic responses revealed that rodents exposed to chronic IH exhibit: a) more number of spontaneous apneas (cessation of breathing greater than 2-3 breaths), b) elevated baseline and augmented sympathetic nerve response to hypoxia, c) augmented catecholamine secretion from adrenal medulla, d) hypertension and e) elevated ROS levels in central and peripheral nervous system. Remarkably, selective ablation of the carotid body, while preserving the carotid baroreceptor function, prevents IH-evoked systemic and cellular responses. Supported by grants from NIH-HL- 090554; HL-086493.

THE INFLUENCE OF HYPERINFLATION AND SYSTEMIC INFLAMMATION ON THE 6 MINUTE WALKING DISTANCE IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Dyspnea and impaired exercise tolerance are frequent in patients with chronic obstructive pulmonary disease (COPD). Hyperinflation contributes to exercise intolerance by its negative impact on lung mechanics. The aim of the study was to evaluate the influence of hyperinflation on the 6 minute walking distance and the markers of systemic inflammation in patients with COPD. The study group consisted of 149 patients with stable COPD (61F, 88M) aged 68 \pm 8.8 yrs in all stages of severity. All the patients underwent spirometry and bodyplethysmography with bronchial reversibility testing. Hyperinflation was defined as RV%TLC > 48% and > 126% predicted. The 6 minute walking test (6MWT) was performed in accordance with the ATS recommendations. The following serum inflammatory markers were evaluated: C-reactive protein, IL-6, IL-8, TNF alpha, CC16, adiponectin and resistin. The patients were divided into group A (patients without hyperinflation, n=53) and group B (patients with hyperinflation, n=96). Table 1 presents significant differences in the results of the 6 MWT between the two groups.

	Group A	Group B	p
Distance (m)	472.2±102.6	401.5±108.9	0.0003
Distance (% pred.)	98.0±20.3	84.3±20.5	0.0001
HR at rest (min ⁻¹)	74.2±10.9	80.4±10.5	0.0008
Baseline SpO ₂ (%)	94.8±2.3	93.8±2.7	0.01
SpO ₂ 6 min (%)	90.5±5.8	88.5±6.5	0.01
Post-6MWT dyspnea (Borg scale)	1.5±1.5	2.3±1.9	0.01

We found significant positive correlations between 6 MWD and serum IL-8 ($R=-0.2, p=0.02$), IL-6 ($R=-0.3, p=0.002$), TNF alpha ($R=-0.2, p=0.04$) and C-reactive protein ($R=-0.2, p=0.03$); SpO₂ 6 min and resistive ($R=0.2, p=0.006$), CC16 ($R=0.2, p=0.03$); RV%TLC and IL6 ($R=0.2, p=0.007$) in the group as a whole. Conclusions: (1) Hyperinflation has a negative influence on exercise tolerance in COPD patients. (2) Systemic inflammation may also deteriorate exercise tolerance in COPD patients.

GUINEA-PIG BLOOD SERUM INTERLEUKIN-6 ACTIVITY IN THE DYNAMICS OF EXPERIMENTAL BRONCHIAL ASTHMA DEVELOPMENT

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Bronchial asthma is a common respiratory illness which affects people of all ages at any given time. Cytokines play a critical role in the orchestration of chronic inflammation in all diseases, including asthma and chronic obstructive pulmonary disease.

The aim of our research is to investigate some changes in activity of anti-inflammatory cytokine - Interleukin 6 (IL-6) in the blood serum of guinea-pigs in the dynamics of experimental bronchial asthma (BA) development. *Research materials and methods.* Researches were carried out on 60 guinea-pigs (males). The weight of each one was 180-220g. They were divided into 5 groups for 12 animals in each of them. Intact guinea-pigs formed the first group (control). Animals with an experimental BA (5th day) were among the second group. Among the third group there were guinea-pigs with an experimental BA (19th day). The fourth group consisted of animals with an experimental BA (26th day) and the last fifth group included guinea-pigs with an experimental BA (33rd day). Experimental model of bronchial asthma was restored on guinea-pigs by the V.I. Babych method (1979). Euthanasia of animals was carried out by the way of decapitation followed the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (1985). The IL-6 concentration in the blood serum was defined for all groups of guinea-pigs. It was made using hard-phase immune-enzyme analysis (ELISA), by means of the test-system "Diacclone" (France). Numerical results were adapted with static method using Student's criteria. *Research results and discussing.* During the experimental researches some changes in activity of Interleukin-6 in the dynamics of BA development was observed. So, after 5 days guinea-pigs had increased their index by 39,7% ($p<=0,05$) in comparison with control indexes. Then, on 19th day of experiment, the increasing of such index was noticed on 88,2% ($p<=0,05$) concerning the first group. The same index was noticed concerning the intact animals (by 88,2% ($p<=0,05$)). In the latest term, on 33rd day, the highest increasing of this anti-inflammatory factor was observed. It was higher by 122 % ($p<=0,05$) in regarding to healthy animals. Consequently, the research of anti-inflammatory cytokine IL-6 in the blood serum of guinea-pigs during BA showed its great increasing. It is evidence of considerable activity of immune inflammation and its direct participation in pathogenesis on the assumption of such bronchial-pulmonary disease.

SOME POSSIBILITIES OF ELIMINATION OF THE ULTRASTRUCTURAL PREREQUISITES UNDER EXPERIMENTAL PNEUMOSCLEROSIS DEVELOPMENT

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The development of pneumosclerosis under experimental pneumonia and some possibilities of its prevention were studied on adult Wistar rates. Due to peculiar structure of lung tissue, the chronisation of

acute pneumonia and the development of pneumosclerosis accompanied not only with collagen fibers germination in connective lung tissue, but to an even greater degree with disturbances of air-blood barrier with epithelium desquamation, significant cell reaction with destruction of capillaries wall endothelium and pronounced mitochondrial dysfunction. All these processes lead to hyperhydration of lung tissue and/or intra-alveolar edema origin even during 4 and 6 weeks after pneumonia simulation. Different modification of traditional therapy not always could prevent of pneumosclerosis development. In our investigations it was shown that exogenously entered phospholipids in liposomal form proved a pronounced positive effect on ultrastructure of lung tissue under acute pneumonia, consists, first of all, in a substantial decrease of edema symptoms. We have also shown that the same positive effect may reveal in the case of pneumonia chronisation. It was argued that employment of natural phosphatidylcholin (lecithin) in liposomal form in combined therapy of experimental pneumonia lead to normalization of ultrastructure of lung tissue: decreasing of destructive processes, hyperhydration and intra-alveolar edema, mitochondrial dysfunction. Simultaneously, the number of sections with collagen fibers spreading and hyalinosis in lung tissue decreased after 4 weeks of pneumonia modeling on 35% and after 6 weeks - on 75%. Thus, the effectiveness of treatment of chronic experimental pneumonia essentially increased with employment of phospholipids in liposomal form.

INFLUENCE OF PSYCHOLOGICAL FEATURES OF CHILDREN ON THE PARAMETERS OF EXTERNAL BREATHING

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The act of breathing is considered to be a means of expression in the sphere of interpersonal relations as well as a means of evidence of the inner state of an individual. *The aim* of our research was to study the influence of the psychological peculiarities of a child's organism on the parameters of the function of the external breathing. *Material and methods.* The psychological investigation and the study of the function of the external breathing of 148 practically healthy children with the help of the spirometer were carried out. The children were $12,05 \pm 2,51$ years of age in average. *Results.* The children are divided into the following groups according to the type of the temperament: phlegmatic type – 34,2%, sanguinic type – 24,4%, intermediate – 30,5%, choleric type – 7,3%, melancholic type – 3,7%. The decreasing of FVC at the children with melancholic type ($F=2,561$, $p<0,05$) was revealed. It was also revealed that there is a negative correlation dependence between schizoid dissocial type and the value FEV_1 ($r=-0,283$, $p<0,000$ and $r=-0,264$, $p<0,001$, accordingly), proneness to conflict and FVC ($r=-0,270$, $p<0,001$), positive - between conformism, stress level and IT ($r=0,279$, $p<0,001$ and $r=0,211$, $p<0,01$, accordingly). *Conclusion.* The schizoid and dissocial type of the child's temperament can be accompanied by the obstructive violation of the function of external breathing. Conformism and stress states are accompanied by the restrictive violation of the external breathing.

UNDERGROUND WORKING ENVIRONMENT PROVOKES LUNG DISEASES DEVELOPMENT

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Background: Morbidity with temporary disability is the main parameter, characterizing health status of workers with occupational aerosol exposure. Unhealthy and dangerous working conditions, their long-term exposure upon workers promote risk factors of occupational lung diseases development. We analyzed morbidity with temporary disability (MTD) of the workers with lung diseases working in underground conditions within the period of 7 years. *Materials and methods:* Study included 2 groups: 1st group – workers with underground working environment ($n=30383$), 2nd group – workers without underground working conditions ($n=5536$). Groups were divided into 5 age subgroups. Length of service of the workers with respiratory diseases was calculated on the basis of morbidity with temporary disability. *Results:* Upper respiratory tract infections developed in 1st group workers at mean age 36,1 years old and length of service in underground conditions - 11,6 years. Control group developed upper respiratory tract infections in older age - 39,6 years old ($p<0,05$). We didn't reveal statistical difference

in comparison of morbidity of workers with influenza and pneumonia. Mean age of the workers with temporary disability due to the upper respiratory tract diseases - 38,1 years old. Length of service in underground conditions – 13,7 years. 2nd group workers data demonstrated older age – 43,2 years old ($t=3,09$; $p<0,05$). **Conclusion:** Medical prophylaxis measures should be provided first of all in workers with length of service in underground conditions 5-15 years.

THE INFLUENCE OF HYPERINFLATION ON BODY COMPOSITION AND CYTOKINE PROFILE IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Body composition is an important prognostic factor in patients with COPD. The decrease in fat free mass (FFM), muscle mass (MM) and increase in visceral fat is associated with an elevated secretion of cytokines which promote systemic inflammation. The aim of the study was to evaluate body composition and the cytokine profile in patients with COPD in relation with the presence of hyperinflation. The study group consisted of 149 patients (61F, 88M) with stable COPD in all stages of severity aged 68 ± 8.8 yrs with a mean FEV1 55.9 ± 18.9 % predicted. All the patients underwent spirometry and bodyplethysmography with bronchial reversibility testing. Hyperinflation was defined as $RV\%TLC > 48\%$ and $> 126\%$ predicted. Body composition was analyzed by bioimpedance. The following serum inflammatory markers were evaluated: C-reactive protein, IL-6, IL-8, TNF alpha, CC16, adiponectin and resistin. Hyperinflation was found in 96 patients (group A) and it was more frequent in women than men (49/61 vs. 47/88, $p<0.001$). BMI and age in this group were comparable to those in patients without hyperinflation (group B). Table presents significant differences in body composition between both groups.

	Hyperinflation (+) group A	Hyperinflation (-) group B	P
Total body water (kg)	36.5 ± 8.0	40.9 ± 7.8	0.0006
Fat free mass (kg)	49.4 ± 10.2	55.9 ± 10.7	0.0003
Body fat (%)	32.5 ± 7.9	28.8 ± 8.1	0.008
Muscle mass (kg)	47.6 ± 10.6	52.4 ± 10.3	0.0005
Height (cm)	163.9 ± 8.9	168.8 ± 8.2	0.0007

We found significantly higher serum concentrations of inflammatory markers in group A: IL-6 – 6.4 ± 10.9 vs. 3.6 ± 4.2 pg/ml, $p=0.004$; resistin – 9.3 ± 4.2 vs. 7.6 ± 2.4 ng/ml, $p=0.02$, CRP 4.1 ± 2.3 vs. 2.9 ± 2.1 mg/l, $p=0.04$, respectively. **Conclusions:** (1) Although BMI in COPD patients with lung hyperinflation is not different when compared to non-hyperinflated patients, patients with hyperinflation have a lower FFM, TBW, MM and a higher proportion of fat tissue. (2) Hyperinflation is associated with systemic inflammation and could therefore be a negative prognostic factor in COPD patients

INHIBITION OF H₂S-SYNTHESING ENZYME CYSTATHIONINE-GAMMA-LYASE PROTECTS HEART FROM REPERFUSION INJURY

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Purpose: Hydrogen sulfide (H₂S) is an endogenously generated gaseous transmitter. It is produced in cardiovascular system from amino acid L-cysteine by cystathionine-gamma-lyase (CSE) enzyme. Recent studies demonstrate controversial results of exogenously applied H₂S donors. Among others H₂S exerts toxic effect at mitochondrial respiration and cardioprotective properties. Thus, mechanism of H₂S action is not fully understandable. The aim of the present study was to investigate the modulation of H₂S synthesis in heart ischemia-reperfusion model. **Methods:** Rat hearts of control group was perfused by Langendorf preparation and submitted to 20 min of non-flow ischemia followed by 40 min of reperfusion (I/R protocol). Second group of animals was pretreated with precursor of H₂S synthesis L-cysteine in dose of 121 mg per kg (intraperitoneal injection 30 min before I/R protocol). The third group was

treated with inhibitor of CSE DL-propargyl glycine (PAG) in dose of 11.3 mg per kg intraperitoneally 10 min before L-cysteine injection and underwent I/R protocol. Registration of left ventricular developed pressure (LVDP), end-diastolic pressure (EDP), dP/dt and coronary flow was performed. Oxygen cost of myocardial work (OCMW) was expressed as the ratio of the oxygen consumption and the heart work (the product of the LVDP and the heart rate). *Results:* L-cysteine protected hearts from reperfusion injury improving cardiodynamic parameters restoration after ischemia. At the 10th min of reperfusion LVDP averaged 52% comparing to 42% in control. L-cysteine prevented appearance of postischemic contraction and dramatic coronary flow reduction in early period of reperfusion: EDP averaged 34 mmHg comparing to 43 mmHg in control. This protective action of L-cysteine was not attenuated by the CSE inhibitor PAG. In third group recovering of cardiac contractile activity was even greater then in second group. At the 10th min of reperfusion LVDP averaged 105% and EDP averaged 3 mmHg comparing to 43 mmHg in control group. Other parameters showed the same pattern of recovery including OCMW which at 10th min of reperfusion averaged 216% in second group and 134% in third group comparing to 239% in control group. These data indicate more effective oxygen utilization by ischemic myocardium under L-cysteine and PAG pretreatment. *Conclusion:* Our data demonstrate that L-cysteine improves heart contractile activity recovery after prolonged ischemia. L-cysteine in combination with PAG protects hearts from ischemia-reperfusion injury and prevents non-effective oxygen utilization by ischemic myocardium.

PROTEASOME ACTIVITY CHANGES AFTER FOCAL ISCHEMIA

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Stroke is the third leading cause of mortality and a major cause of functional disability among the older people worldwide. The aim of our study was to investigate proteasomal activity in rats with focal cerebral ischemia in the locus of damage and in risk zone. *Materials and methods.* Experiments were performed on 42 adult male Wistar rats, weighing 280-320 g, animals were divided in 6 groups – №1 control (sham operated, 7 rats) and №2 experimental (7 rats) with 6 hours of reperfusion, №3 control (7 rats) and №4 experimental (7 rats) with 24 hours of reperfusion, №5 control (7 rats) and №6 experimental (7 rats) with 72 hours of reperfusion. Ischemic Stroke was modeled by transient occlusion of the middle cerebral artery according to the method of Koizumi J., et al. (1986), which lasted 60 minutes. Evaluation of ischemic damage was performed 6, 24 and 72 hours after restoration of blood flow. To confirm the specificity of proteasomal hydrolysis we added to the samples selective inhibitors of the proteasome -laktatcystyn-beta-lactone or Mg-132 at a concentration of 5 mM. *Results.* Three types of proteasomal proteolytic activity were measured in brain tissue: chemotrypsin-like (CTL), trypsin-like (TL) and peptidylglutamyl peptide-hydrolase (PGPH). Shifts in proteasomal activity in the result of ischemia were found ambiguous. The TL activity of proteasome in ischemic brain regions decreased by 2 times compared with controls ($P = 0.045$) in groups №2, 6, but increased by 11 times compared with controls ($P > 0.05$) in group №4, but increased by 6 times in group №3. CTL activity decreased by 1.2 times compared to the control ($P = 0.037$) in groups № 2, 6, but increased by 2 times compared with controls ($P > 0.05$) in group №4. PGPH activity decreased by 3 times compared to the control ($P = 0.037$) in groups № 2, 4, this type of activity was not significantly changed with either the control or with the risk zone in groups №1, 3, 5, 6. *Conclusions.* Proteasomal proteolysis is significantly reduced in a result of focal brain ischemia during the first 6 hr of reperfusion with subsequent increase observed starting from 24 hr.

FEATURES OF THE KIDNEY INFLAMMATION AND THE LEVEL OF ENDOGENOUS INTOXICATION IN ACUTE LUNGS INJURY IN EXPERIMENT

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Purpose – to determine features of the kidney inflammation progression during acute lungs injury (ALI). Simulated acute lung injury experiments were performed on white rats by introducing to the trachea HCl, pH 1,2 at a dose of 1.0 ml·kg⁻¹ at inhalation. Animals of the experimental group withdrew from the

experiment at 2, 6, 12 and 24 hours. In kidney tissues there was determined the content of TBA-active lipid peroxidation processes products, in serum – endogenous intoxication level using data on middle molecular weight of different fractions (MMW 254-280). Studies have shown that the lipid free radical oxidation is significantly intensified, what is evidenced by a significant increase in TBA-active products of lipid peroxidation, which was increased at 1.79 times ($p < 0.001$) in 6 hours. After 12 h, these figures declined by 12.6% ($p < 0.05$), but did not reach the control level. After 24 h they exceeded the level of control by 76.7% ($p < 0.001$). Content of MMW also increased up to 6 hours of observation, somewhat decreased to 12 hours, and increased again to 24 hours. Thus, during ALI the inflammation is significantly increased in the kidneys. The content MMW varies in proportion to it and shows derangement of secretory function. Reducing the content of these indexes to 12 h indicates obviously the development of adaptive-compensatory processes in the body. However, after 24 h the depletion occurs.

INSPIRATORY MUSCLE RESISTANCE TO FATIGUE DURING SIMULATED AIRWAY OBSTRUCTION

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Respiratory muscle fatigue can develop during simulated airway obstruction. The aim of this study was to characterize the pattern of inspiratory muscle fatigue and to assess the resistance to fatigue of diaphragm (D), parasternal (PS), sternocleidomastoid (SM) and scalene (SC). 6 healthy untrained subjects participated in this study. To identify signs of inspiratory muscles fatigue development electromyographic activity of D, PS, SCM and SC was recorded during 5-min exercise with loaded breathing ($40 \text{ cm H}_2\text{O} \cdot \text{s} \cdot \text{l}^{-1}$). The before-to-after exercise measurements of maximal inspiratory pressure (MIP) and EMG power spectrum changes were performed. Maximal inspiratory pressure declined about 12 % after exercise test compared with control, whereas the peak magnitude of integrated electrical activity of D, PS, SCM and SC during post-exercise Muller's maneuver was significantly greater than in pre-exercise test in all subjects. The extent of inspiratory muscles fatigue was evaluated by analysis of shift in centroid frequency (f_c) of EMG power spectrum. All subjects demonstrated a significant reduction in f_c of PS, SCM and SC. Frequency f_c of D was not changed. Diaphragm is more resistant to fatigue during obstructive breathing compared with PS, SCM and SC. The data suggest that the reduction of maximum inspiratory pressure in chronic obstructive pulmonary disease also caused primarily by the weakening of the accessory muscles, while the weakness of the diaphragm may occur in the later stages of the disease. The functional failure of accessory muscles is an additional factor, which, along with the additional breathing resistance increases the load on the diaphragm, promoting its fatigue and reduced respiratory reserve.

TUBERCULOSIS IN MEDICAL WORKERS

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According to the Belorussian Center of Medical Technologies, Computer Systems, Administration and Management of Health (BelCMT), the respiratory tuberculosis (RT) in healthcare providers takes up third place yearly in Republic of Belarus. During last 10 years 152 professional patients were revealed among medical workers. 125 of them (82%) have respiratory tuberculosis. They were occupied in TB dispensary and deal with patients, suffered from tuberculosis, which also include persons discharging bacteria, or contaminated substance. Respiratory tuberculosis (RT) was frequently diagnosed in hospital aide – 42%, nurses – 35%. Doctors morbidity was about 17% and medical technologists ones – 6%. We studied features of RT development (according to medical documentation) in 32 patients. Selection was made using casual selection method. In all cases overload at work was associated with unfavorable accommodation facilities: one-room apartment with other family members, a hostel room with 6-8 people or a communicating room. Short length of service before RT was diagnosed attracts attention: 25 people (78%) felt ill in the range from half a year till 4 years of work, 9 (28%) of them - in the range of half a year till one and half a year. The occupational disease was established in most cases in 1-2 years after

the treatment onset (90,6%), and in 9,4% of cases in 6 and more years. Some patients were not inspected by Medical Rehabilitation Expert Board(MREB), since after treatment they came back to former work. Percent of disability were established in cases of disease recurrence. The II-III group of disability and 60-80% of disability were established when an infiltrative form of tuberculosis with a disintegration and BK+ phase, a resection of part of a lung took place. Thus, it is necessary to employ injured medics in due time and to direct them on MREB for drawing up PRP (Patients Rehabilitation Program) and activity restriction extent definition.

LESSONS FROM TWENTY YEARS' INVESTIGATION OF INTERMITTENT HYPOXIA: PRINCIPLES AND PRACTICES

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Widespread use of the intermittent hypoxic training/treatment (IHT) methods in sports, military and medical practice during recent decades has provoked a discussion: "What is 'intermittent hypoxia'? Does it play pathogenic roles in disease states, such as sleep-disordered breathing, chronic pulmonary disease, cardiovascular disease, and cancer? Or, quite to the contrary, does exposure to intermittent hypoxia induce protective responses?" (Semenza, 2011). In contrast to studies from the former Soviet Union countries that emphasized mainly the beneficial effects of IHT on an organism, intermittent hypoxia research in Western Europe and North America was primarily focused on the detrimental effects associated with sleep apnea. However, during the past decade, such a gap of division between East and West is progressively shrinking, and mutual understanding on what "intermittent hypoxia" means, becomes clearer. Potential mechanisms underlying both beneficial and adverse effects of IHT have been described. Basic investigations led to the proliferation of various methods of IHT exposure, the development of different medical equipments – hypoxicators – for its implementation in sport practice, military operations and also for clinical application. However, wide array of different protocols and measurements makes the results difficult to harmonize. The questions that arise are, what are the key mechanisms determining the adaptive versus maladaptive nature of different paradigms of intermittent hypoxia, and, what molecular pathways are mediating the observed pathological or physiological response (Prabhakar, 2012)? Until now there is no exact evidence about the precise mechanism for switching adaptive or maladaptive responses to hypoxic impact. Besides, there is no exact answer on the practical question of what dose and regimen of hypoxic impact could be mostly beneficial for animals and humans. Wide spectrum of protocols for IHT is represented now in literature showing both effects. Meanwhile, the mode of hypoxic influence (depth, duration, and intermittence) appeared to be critical for the determination of healing or harmful result. Therefore, special purposeful investigations are needed to elucidate basic mechanisms of different IHT effects depending on the modality of hypoxic stimuli and elaborate most effective and safe regimen for the introduction in human practice.

MODERN TRENDS IN THE TREATMENT OF COPD, COMBINED WITH OSTEOARTHRISIS

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COPD is one of the most common human diseases, which, according to the WHO data, affects 0,8% of the population of the planet, mainly persons over the age of 40, with the excess of incidence rate among men (0,9%) than among women (0,7%). In recent years, the importance of such factors of COPD pathogenesis, as hypoxemia and hypercapnia, increases and leads to limitation of physical activity, increasing of body mass index as unfavorable prognostic factor for life, and in the future – to development of systemic effects of disease, such as progressive cachexia, secondary anemia, systemic inflammation, systemic osteoporosis as a basis for the osteoarthritis (OA) development. In patients older than 40 years the comorbidity of COPD and OA occurs quite often. In turn, OA is a common pathology, especially in people of middle and elderly age, which significantly reduces the quality of life of the patients, thus makes its important medico-social value. The degree of severity, functional changes and progression

of disease in patients with COPD and OA are at least partly connected with level of chronic inflammation. The coexistence of COPD, which is accompanied by a systemic inflammation, with OA, in the progression of which the influence of the mediators of inflammation was also revealed, requires careful consideration to search for ways of rational treatment of COPD and OA combination. At the present time the physical rehabilitation has a significant role in the treatment of COPD; it should increase the tolerance of the patient for physical loads, slow down the fall of the respiratory volumes and improve the social adaptation of the patient. Accompanying of OA with COPD, especially with the defeat of the large joints and pain syndrome, can significantly limit the recovery capability of the patient and degrade the quality of his life. That is why the determination of ways of pharmacological correction of the revealed pathogenic factors and directions of medical rehabilitation of patients with a combination of COPD and OA has a considerable practical importance.

APPLICATION OF INTERMITTENT HYPOXIC TRAINING FOR THE AMELIORATION OF BALNEOTHERAPEUTIC COMPLEX SPA TRUSKAVETS EFFECTS ON IMMUNE SYSTEM

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Children with chronic pyelonephritis and concomitant chronic bronchitis being at balneotherapeutic complex spa Truskavets were subjected to additional IHT course using the apparatus Hypoxytron- Simplex (Kiev). Such treatment prevented in some cases the reduction of Stange test and the concomitant reduction of CD8-T cells, caused the increase in blood serum immunoglobulins G, A, M content and serum and saliva lysozyme content with normalizing of circulating immune complexes. IHT also potentiated the stimulatory effect of balneotherapy on the activity, the intensity and completeness of phagocytosis by neutrophils in blood culture Staph. aureus. IHT did not significantly affect other parameters of immunity.

HYDROGEN SULFIDE AS ENDOGENOUS MODULATOR OF Ca^{2+} -SENSITIVE MITOCHONDRIAL PERMEABILITY TRANSITION PORE IN ADULT AND OLD RAT HEART

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Introduction. Hydrogen sulfide (H_2S) is an endogenous gaseous mediator, produced by de novo synthesis in mammalian tissues during cysteine metabolism. It is known three H_2S synthesized enzymes – cystathionine β -synthase (CBS), cystathionine γ -lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (MPST). H_2S and NO are key regulators of several cell processes and organ functions under both normal and pathological conditions. Mitochondrial permeability transition pore (mPTP) opening causes mitochondrial membrane potential collapse that leads to mitochondrial dysfunction and apoptosis in aging heart. The role of H_2S in mitochondrial function regulation is now receiving increasing attention. The present study aims to clarify the mechanisms underlying the cardioprotective effects of H_2S in aging rats. **Methods.** In the isolated from the adult and old rat hearts mitochondria, we studied the intramitochondrial H_2S pools simultaneously with production of ROS ($*O_2^-$ and $*OH$ -radicals generation, H_2O_2 , uric acid, MDA and DK pools) production of RNS (activity of cNOS and iNOS, NO_2^- , GSNO and protein nitrosothiol pools) and arginase II activity. In experiments *in vivo* and *in vitro* we studied the effects of both H_2S donor (NaHS) and precursor of de novo synthesis (L-cysteine) on the sensitivity of isolated from the adult and old rat hearts mitochondria mPTP opening to its natural inductor, Ca^{2+} by mitochondria swelling. **Results.** For production of H_2S in physiological concentrations we used donor H_2S - NaHS in concentration 10^{-6} - 10^{-5} mol/l. NaHS used in these concentrations exerted the inhibiting effect on the Ca^{2+} -induced mPTP opening in adult hearts (corresponding values of such effect were 31 and 77%, respectively), while in old hearts the protector effect of NaHS was observed only at its concentration of 10^{-5} mol/l. A specific inhibitor of K_{ATP} -channels, 5-hydroxydecanoate (5-HD; 10^{-4} mol/l) decreased the both Ca^{2+} - independent and Ca^{2+} -induced mitochondrial swelling in the presence of NaHS (10^{-10} and 10^{-5} mol/l), indicating the contribution of these channels to the H_2S -dependent inhibition of mPTP transition in both adult and old rat hearts mitochondria. In experiments *in vivo*, single intraperitoneal injections of

NaHS (10^{-4} mol/kg) or L-cysteine (10^{-3} mol/kg) resulted in a decrease in the sensitivity of mPTP of its Ca^{2+} induced opening in both adult and old rat hearts. In experiments *in vivo* propargylglycine (10^{-4} mol/kg), specific blocker of H_2S de novo synthesis by cystathionine- γ -lyase, increase the sensitivity of Ca^{2+} induced mPTP opening in old hearts. The results showed that in heart mitochondria isolated from the old rat H_2S pool was decreased to 47% of control value, as well as NO_2^- (31%) and GSNO (56%) pools and cNOS activity (51%). In contrast, iNOS activity was increased to 168% of control value, as well as ROS generation (O_2^- – 308%, OH^\bullet – 288%) and pools of H_2O_2 (197%), uric acid (1042%), MDA (284%), DK (1059%) and protein nitrosothiol (697%). *Conclusion.* We demonstrated that endogenous H_2S may be include in the control of the Ca^{2+} -induced mPTP opening in heart mitochondria isolated from the adult and old rat. Concentration of H_2S in mitochondria of old rat hearts was dramatically decreased thereby resulted in increasing of sensitivity of mPTP to Ca^{2+} . These changes were accompanied by oxidative (ROS) and nitrosative (RNS) stress activation. Thus, endogenous H_2S may exerted the protective effect on the Ca^{2+} -induced mPTP opening by increasing Ca^{2+} -dependent constitutive NO de novo synthesis by cNOS and by inhibition of ROS and RNS formation.

ANTITOXIC EFFECT OF ADAPTATION TO HYPOXIA AND HYPEROXIA: REDOX SIGNALING AND PHYSICAL ENDURANCE IN THE MODEL OF LOW DOSE INTOXICATION BY CHROMIUM AND BENZENE

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The aim of this study was to evaluate the possibility of non-medicamentous prevention of toxicant-induced damage. We used the rat model of intoxication by potassium bichromate and benzene developed on the basis of data obtained with employees exposed to low doses of toxicants or inhabitants from environmentally neglected zones. New model of the adaptation to hypoxia and hyperoxia is capable of antioxidant system and energy metabolism activation. We investigated the effect of chronic intoxication with a mixture of benzene and potassium bichromate on physical endurance, behavior parameters, the intensity of oxidation processes, the activity of antioxidant enzymes, level of heat shock proteins (HSPs) and hypoxia induced factor (HIF-1 α) on male Wistar rats. It was found that preliminary adaptation to hypoxia and hyperoxia (alternately by 3-5 min, in total 1 hour daily during 7 days) can prevent disorders caused by intoxication: decrease of physical endurance, animal weight reduction, increased oxidative stress and concentration of transcription factor HIF-1 α and HSPs. *Conclusion:* preliminary respiration with hypoxia and hyperoxia can be used as preventive method of toxic damage.

DETERMINATION OF ENDOTHELIAL DYSFUNCTION IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE WITH AND WITHOUT DIABETES MELLITUS TYPE II BY THE CONTENT OF ENDOTHELIAL CELLS IN BLOOD

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In recent years, chronic obstructive pulmonary disease (COPD) is regarded as systemic inflammation with the presence of comorbidities that significantly affects the course of the underlying disease. We studied the role of the functional state of endothelium in systemic effects of COPD with the presence of comorbidities, in particular cardiovascular diseases and diabetes mellitus type II (DM II). *The aim* of the work: to estimate the content of blood circulating endothelial cells as an indicator of endothelial dysfunction in COPD patients with and without concomitant DM II. *Materials and methods.* The study involved 20 patients with COPD, combined with DM II, and 25 COPD patients without concomitant diabetes. The control group consisted of 20 healthy individuals. Number of circulating endothelial cells was determined by the method of Hladovec J., that assumes the isolation of endothelial cells with platelets with subsequent deposition of platelet adenosine diphosphate. *Results.* In the group of patients with COPD combined with type II DM, the content of circulating endothelial cells in blood was higher ($12,5 \pm 2,1 \times 10^4/\text{l}$) than in patients without concomitant diabetes ($6,0 \pm 0,9 \times 10^4/\text{l}$, $p < 0.05$) and healthy individuals ($3,5 \pm 0,9 \times 10^4/\text{l}$, $p < 0.05$). *Conclusion.* Thus, higher level of endothelial cells desquamation in blood indicated the presence of endothelial dysfunction, particularly with concomitant DM type II.

CLINICAL COURSE AND TREATMENT OF BRONCHIAL ASTHMA IN THE ELDERLY

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Bronchial asthma is a common disease in the elderly. According to epidemiological data, the prevalence of asthma in elderly patients (EP) is about 5 to 15%. In this study we examined 186 patients with bronchial asthma aged over 60 years. Allergens from focal infections (chronic bronchitis, pneumonia) played leading role in hyperresponsiveness formation and asthma development in EP. Genetic predisposition was less common, EP mainly had infectious-allergic form of asthma due to inflammatory lung diseases. The most important features of asthma clinical course in EP were comorbidity, nonspecific manifestations of the disease, age-related changes in respiratory organs, depletion of adaptive mechanisms, rapid deterioration at late start of treatment, frequent development of complications caused both by the disease and treatment.

Based on our observations and literature data, principles of asthma treatment in EP are:

- immunotherapy in most EP is not used;
- to prescribe drugs with optimal effect and the lowest probability of adverse events;
- to give priority to inhalation of anti-inflammatory drugs and bronchodilators;
- to select the optimal inhaler (without reference to the asthma clinical course severity) accordingly to lung function ventilation parameters;
- to optimize methods of drugs administration (basic therapy includes use of inhalers, spacers; nebulizers are used at exacerbation of disease);
- to follow the rules of possible complications prevention (oral hygiene, etc.);
- to educate patients to implement the treatment recommendations;
- to treat comorbidities, exclude drugs that can affect the course of asthma.

THE CHILDREN'S INCIDENCE OF ASTHMA IN GRODNO ACCORDING TO A LONG-TERM MONITORING

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According to epidemiological studies the incidence of bronchial asthma among children is 5-10%, but, according to statistical reports, this index is much lower. The purpose of the work is to assess the dynamics of asthma incidence in Grodno in 2000 to 2012. General and primary asthma incidence was studied among children in Grodno at the age of 0-17 years in 2000 to 2012 according to the statistics. It has been found the reduction in overall asthma incidence from 10,1 in 2000 to 6,1 in 2012 per 1000 children ($p < 0,001$) in the background of chronic allergic respiratory diseases during the same period. It is noted the decline of the indicator of primary incidence from 1,6 in 2000 to 0,5 in 2012 per 1000 children ($p < 0,005$). It has been found an increase in the overall asthma incidence of children aged 15-17 from 12,1 in 2000 to 37,1 in 2012 per 1000 children, $p < 0,001$. In addition, this group of children marked the increase in the number of boys suffering from asthma from 14,2 in 2000 to 49,4 in 2012 per 1000 children, $p < 0,001$. Conclusions: It was established the decrease in general and primary asthma incidence in children in the city of Grodno. The older the child, the higher the frequency of this pathology is observed. Among teenagers bronchial asthma is more often found in boys.

RAT RESPIRATORY FUNCTION AFTER HYPEROXIC GAS MIXTURE IMPACT

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Hyperoxic gas mixture (HGM) is widely used in the clinic for the treatment of diseases. However, it is known that with increasing levels of partial pressure of oxygen and duration of its effects may develop various pathological processes. Our purpose is to examine the changes of morphological and biochemical indicators of lung respiratory section of adult rats after exposure to HGM. The investigation of HGM (40% oxygen) influence was performed within 1 hour during 14 days to 24 male Wistar rats age of 12 months. Histological preparations from the lung tissue were made by the standard method. The concentra-

tion of total oxyproline was determined photometrically. In rats exposed to HGM, there is occurred the tendency to reduce the area of transversal cut to alveolus (7%). The average width and depth to alveolus remained at the control level. Width of the entrance to the alveolus and the total width of respiratory bronchioles, alveolar ducts and sacs in the experimental group of animals significantly increased by 21 and 25% accordingly. So there is an increase of respiratory bronchioles airiness and alveolar ducts, but decrease of alveolar surface area and alveolus airiness, after the exposure to HGM. Experimental rats revealed a significant increase in the number of collagen fibers, interalveolar septum thickness (41%) and the concentration of total oxyproline (29%) in the lungs. Therefore, breathing the gas mixture of 40% oxygen reduces the total alveolar surface area, increases the mass of connective tissue in lungs which leads to the reduction of gas exchange rate and ventilation in adult rats.

CHOLESTEROL LEVEL AS A MARKER OF COMMUNITY-ACQUIRED PNEUMONIA (CAP) SEVERITY

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Background: The knowledge regarding lipid biomarkers in CAP still remains limited. *The aim:* We investigated the level of cholesterol in immunocompromised CAP patients. *Methods:* 365 hospitalized CAP patients were retrospectively enrolled to the study and assigned to one of the two groups. The 1st group included 140 patients under 45 years of age with moderate and severe CAP (mean age: 32±8; 82% severe CAP and 18% moderate CAP). The 2nd group - 225 patients over 45 years of age with moderate and severe CAP (mean age: 63±1; 90% severe CAP and 10% moderate CAP). *Results:* Cholesterol level in patients with severe pneumonia appeared to be lower than those in patients with moderate pneumonia (3.8±1.0 mmol/L in patients under 45 years old and 4.3±0.9 mmol/L in patients over 45 years old) vs (4.7±0.8 mmol/L and 5.09±1.0 mmol/L respectively). We found the different cholesterol levels in patient with moderate and severe CAP. The hypo- and normocholesterolemia in patients of the 1st group was detected in 73% of those with moderate CAP and in 92% with severe CAP, in patients of the 2nd group – in 56.6% and 86% respectively (p<0.05). We analyzed 22 medical records of the deceased patients. Cholesterol level was considerably decreased in non-survivors - 2.3±0.5 mmol/L (p<0.05). Probably this could be related to the increased cholesterol use for the leukocytes membrane building. *Conclusions:* Cholesterol level was significantly decreased in patients over 45 years old versus patients under 45 years old with the similar CAP severity. Cholesterol value was significantly lower in severe CAP patients in comparison with the moderate CAP patients. Thus, cholesterol level could be a simple marker of CAP severity.

OCCUPATIONAL TUBERCULOSIS (TB) AS THE DISEASE OF MEDICAL PERSONALS

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Background. Pulmonary TB takes a leading place among all occupational diseases of medical staff in the Grodno region of Belarus. 32 patients with TB are registered in the regional center of Pathology. This is 85% of the total number of occupational diseases in medical staff. *The aim* of study was the analysis of the incidence of occupational TB cases. *Material and Methods.* All the 30 observed patients were women, age varied from 25 till 35 years (in 70% of cases) as well as the length of work varied from 6 till 15 years. There were 15 nurses, 13 physicians, 2 laboratory assistants and 2 nurses among these patients. 30 patients worked in TB hospitals and only 2 persons were from other hospitals. *Results.* Focal pulmonary TB was established in most of the patients (86%). Surgical treatment was performed in four patients. The disability group was established in 8 patients. Two patients died from pulmonary TB. It should be noticed that 3-4 of new cases of TB were diagnosed annually in the Grodno region. The exogenous and endogenous risk factors of occupational TB were: a high virulence and drug resistance of mycobacteria TB, unfavorable work conditions with high level of chronic psychoemotional or physical stress, nonphysiological conditions (night duties, uncomfortable psychological climate) and other factors. *Conclusions.* The important measures for

the prevention of occupational TB among medical personals are the following: regular periodic medical examinations, following the strict measures of infectious control and improvement of work conditions.

THE IN VIVO AND IN VITRO BLOOD OXYGEN-BINDING PROPERTIES MODIFICATION BY DIFFERENT NITRIC OXIDE DONORS

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It is known, that the donors of nitric oxide (NO) – sodium nitroprusside (SNP), nitroglycerine (NG), can protect liver against ischemia/reperfusion injury decreasing lipid peroxidation, improving antioxidant status and transaminase activity. Including the role of NO in the hemoglobin oxygen affinity regulation we aimed to investigate in vivo and in vitro the possibilities of modification of blood oxygen-binding properties by different NO donors. Infusion of SNP (2, 5 mg/kg) or NG (1, 5 microg/kg) was performed in rabbit hepatic ischemia(30min)/reperfusion(120min) model in vivo. The in vitro experiments were performed with rabbit venous blood, which was incubated during 30 or 60 min with different NO donors: SNP, NG, molsidomine (Mls), S-nitroso cysteine (CysSNO), S-nitroso-N-acetylpenicillamine (SNAP), S-nitrosoalbumin (AlbSNO). Indices of blood oxygen transport ($p50_{act}$, $p50_{stand}$, pCO_2 , pH, etc.) were measured. There is determined, that NG infusion in vivo experiments leads to decrease of hepatic venous blood $p50_{act}$ on 5 mmHg ($p<0,05$) at the end of reperfusion concerning rabbits without NG. SNP administration for animals reduces $p50_{stand}$ in the blood on 4,5 mm Hg ($p<0,05$) on the 120 min of reperfusion. The in vitro investigation has shown, that none of the NO donors changed the $p50_{act}$, but $p50_{stand}$ was reduced by CysSNO and SNAP on 2,8 ($p<0,05$) and 3,3 ($p<0,05$) mm Hg, respectively. Thus modification of blood oxygen-binding properties depends of NO donor's structure, metabolism, peroxynitrite formation and environment of experiment.

TOLERANCE TO HYPOXIA

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People living at sea level have poor tolerance to hypoxia. In striking contrast, humans experiencing hypoxia at high altitude live very well. How is it possible for man to tolerate extreme hypoxia at high altitude? In this article we propose a hypothesis that potentially explains the tolerance to hypoxia at high altitude. Close examination of values of hemoglobin and $PaCO_2$ for an altitude of 3510 m demonstrate that an increase in hemoglobin (Hb) and a decrease in arterial carbon dioxide tension ($PaCO_2$) are two essential changes that occur on high altitude exposure. We propose a formula:

$$Tolerance\ to\ Hypoxia = \frac{Hb}{PaCO_2} \times 3.01$$

We present evidence that the relationship between Hb and $PaCO_2$ explains the tolerance to hypoxia at high altitude. (Presented in part at the Leh Symposium and the Global Hypoxia Summit in New Delhi India Aug3-9, 2012).

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Decade of the cooperation between Polish, Ukrainian and Belorussian physiologists and pulmonologists (2004 - 2013)

The initiative to organize the Polish-Ukrainian Respiratory Working Group has arisen in October 2003 during the official visit of Prof. Janusz Kowalski to the Bogomoletz Institute of Physiology, Kiev, Ukraine. The driving motion of this initiative was the great wish to establish closer collaboration between Polish and Ukrainian researchers and physicians, to inspire the exchange of mutual experiences in research work and clinical practice and finally to help our patients who suffer the same pain and diseases on both sides of Polish-Ukrainian border. This concept has been approved by Prof. Jerzy Kozielski (President of the Polish Respiratory Society, Silesia Medical Academy, Poland), Prof. Platon Kostyuk (Director of the Bogomoletz Institute of Physiology, Ukrainian National Academy of Sciences, Kiev) and National Delegates of the European Respiratory Society Prof. Joanna Chorostowska (National Research Institute of Tuberculosis and Lung Diseases in Warsaw, Poland) and Prof. Yuri Feshchenko (Institute of Phthysiology and Pulmonology AMSU, Kiev, Ukraine). Next year Ukrainian scientists and doctors were invited by the Organizing Committee of 28th Congress of the Polish Respiratory Society to participate in this meeting in Lodz. During the Congress the Polish-Ukrainian Working Group of Clinical and Experimental Pathophysiology of Respiration was established. Prof. Janusz Kowalski (Institute of Tuberculosis and Lung Diseases in Warsaw) and Prof. Tatiana Serebrovskaya (principal researcher of the Bogomoletz Institute of Physiology in Kiev) have been elected as Coordinators of this group. Since 2012 Prof. Piotr Gutkowski leads the working group. During following decade the annual meetings in Kiev (2005), Opole (2006), Donetsk (2007), Katowice (2008), Ternopil (2009), Mikołajki (2010), Chernivtsi (2011), and Wisła (2012) took place. In 2008 Belorussian colleagues from Grodno and Minsk joined working group. Besides scientific conferences, exchange of therapeutics and students was organized for training courses both in Polish and Ukrainian pulmonological clinics. The fundamental monograph «Lung function in lung diseases» (Eds. J.Kowalski, A. Kozirowski, L.Radwan) written by prominent Polish specialists in physiology and pathophysiology of respiration was translated from Polish to Russian by Dr. Eugenia Kolesnikova. The monograph was published in Poland, transported to Ukraine and distributed among main Ukrainian and Belorussian medical universities and libraries. A number of joint researches were provided, and international scientific papers were published in peer-reviewed journals. Many other initiatives have focused on the exchange of scientific and medical information between the Polish, Ukrainian and Belorussian colleagues to the benefit of the health of our populations. In conclusion, despite many organizational and financial difficulties in cooperation between Polish, Belorussian and Ukrainian universities, this initiative should be considered as significant and fruitful, integrating students, physicians and scientists of Central and Eastern Europe. The history of the Polish-Ukrainian-Belorussian Respiratory Working Group is going on. We ought to write it together.

INTRODUCTION

Due to the initiative of Polish and Ukrainian pulmonologists, lung physiologists and scientists, the Polish-Ukrainian Respiratory Working Group has been formed in 2004. During the decade 2004-2013, the five joint scientific meetings
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in Poland were organized within the program of the biennial congresses of Polish Society of Lung Diseases in Lodz (2004), Opole (2006), Katowice (2008), Mikołajki (2010) and Wisła (2012). Besides that, there were four conferences in Ukraine: Kiev (2005), Donetsk (2007), Ternopil (2009) and Chernivtsy (2011). After

ten years of the successful cooperation, the next anniversary conference “Physiology and Pathology of Respiration: Advances in basic research and clinical applications” has to be held in Kiev, October 2013. There were also multiple meetings in Grodno and Minsk (Belarus). From the beginning, the Belorussian participation is very important in all common activities. Therefore in 2012 the name of the Working Group has been modified from Polish-Ukrainian into Polish-Belorussian-Ukrainian. The main purpose of the cooperation is to create a platform for the introduction of advanced achievements in basic research of lung function from molecular to systemic level. In this mini-review we summarize the main topics of scientific meetings as well as the results of other common activity.

BASIC RESEARCH

Every scientific meeting included exchange of views on the most significant achievements in modern basic research. One of such topics is the problem of intermittent hypoxia which was being intensively developed during the last decades. Many experimental and clinical data of Ukrainian scientists have demonstrated that intermittent hypoxia training (IHT) is an effective mean for increasing nonspecific body resistance to harsh or toxic environments and pathogenic agents. There were attempts to apply IHT for treatment of respiratory and cardiovascular diseases, endocrine disorders, and immunodeficits (Lei Xi & Serebrovskaya, 2009, 2012; Drevytska et al., 2009; Rozova & Mankovska, 2012; Mankovska et al., 2011; Serebrovskaya et al., 2013). It has been proved that IHT induces an increase in ventilatory sensitivity to hypoxia and various hypoxia-related physiological changes, including raised alveolar ventilation, lung diffusion capacity and heightened hematopoiesis. Intermittent hypoxia also transiently increases the release of reactive oxygen species which then induce a compensatory increase of antioxidant defense. These data could be considered as the theoretical base for the including of IHT in therapy of various diseases in which the

patogenesis of oxidative stress is involved.

IHT procedure seems to be very attractive for sportsmen and alpinists training. Considering COPD and asthma patients who suffer from hypoxia due to bronchial obstruction, the special strict requirements for IHT application should be elaborated.

Prof. Iryna Mankovska's group from Bogomoletz Institute of Physiology is focused on the latest advances in the study of mitochondrial function under different types of hypoxia. Basic mechanisms of mitochondrial dysfunction associated with impaired oxygen delivery to mitochondria, intensification of free radical processes, reduced stability of mitochondrial membrane, etc was elucidated. The methods of non-pharmacological and pharmacological correction of mitochondrial dysfunction were vividly discussed (Drevytska et al., 2009; Mankovska et al., 2011).

Dr. Evgenia Kolsnikova from Bogomoletz Institute of Physiology (Kiev) presented her results about changes in respiratory control of Parkinson's diseases patients. Her experimental research of brainstem mitochondrial function under hypoxic conditions reveals the mechanisms of non-specific reactions at early stage of different disorders. The last observations on rats suggest that adaptation to IHT promotes the expression of a glycolytic gene - pyruvate dehydrogenase kinase-1 to maintain ATP production and to prevent toxic ROS production in the brain stem normalizing mitochondrial dysfunction (Kolesnikova, 2013).

Investigations of Dr. K.V. Rozova from Kiev about structural and functional changes in biological barriers and mitochondrial apparatus of the lung and heart during hypoxia and stress were of great interest at all meetings. Particularly, it was shown that the ultrastructural reorganization in lung and heart mitochondria during hypoxic hypoxia can be attributed to the compensatory- adaptive reactions of mitochondrial apparatus. IHT mode with more severe but shorter hypoxic bursts led to the prooxidant/antioxidant imbalance in the myocardial and lung tissues which was

accompanied by the significant disorders in their morphology and function. Moderate hypoxic exposures of different duration promoted the maintenance of optimal prooxidant-antioxidant homeostasis and development of compensatory adaptive changes in tissue structure (Rozova & Mankovska, 2012).

Scientists from Ukrainian State Institute of Gerontology (Department of clinical physiology and pathology of the internal organs, Kiev) presented the results of fundamental investigations devoted to age-related peculiarities of adaptation to hypoxia and new methods for the correction of age pathology. One of such methods is non-pharmacological method of IHT which was developed for the usage in geriatric clinics in close cooperation with other members of the Polish-Ukrainian working group. As a result, the guideline for IHT implementation in elderly healthy people and patients with coronary artery disease, arterial hypertension and chronic obstructive pulmonary diseases was elaborated (Korkushko et al., 2009; 2010; Shatilo et al., 2008).

CLINICAL STUDY

Among discussed topics there was a predomination of papers devoted to chronic obstructive pulmonary diseases (COPD), asthma, interstitial lung fibrosis and tuberculosis. The progress in the bacteriological and radiological investigations, lung function tests and molecular diagnostic was very often discussed. The usefulness of modern lung function tests in differentiating COPD and bronchial asthma was presented by J. Kowalski (2008) and P. Gutkowski who also spoke about the identification and clinical relevance of the increased bronchial reactivity in the bronchial asthma diagnostics.

The very important lecture was presented by A. Dorofiejev from Medical University in Donetsk which was concerned with the impact of changes in performance barriers lining of the colon on the course of COPD (Donetsk 2007). The natural history of COPD was discussed

by J. Kozielski (Opole). Very original was also the lecture of Prof. A. Swiencicki (Medical University in Kiev) who in perfect Polish presented his observations and thoughts on the impact of gastro esophageal reflux on the course of bronchial asthma (Katowice). Prof. J. Zielinski from the Medical University in Warsaw discussed the current rules for early detection of bronchial obstruction as a very important element of COPD diagnosis and therapy. The physiological and anatomical peculiarities of the respiratory system in children were referred by P. Gutkowski. The epidemiological situations of tuberculosis in Europe were presented by T.M. Zielonka from the Medical University in Warsaw. An interesting original report by E. Korzh from Donetsk demonstrated the epidemiological situation of tuberculosis in the Donetsk region. S. Lepishyna (Kiev) drew attention to the current state of drug-resistant tuberculosis in Ukraine. A.H. Hryshchuk from Ternopil University had presented his experience about spontaneous pneumothorax in the tuberculosis clinic. Prof. Z. Zwolska (Institute of Tuberculosis in Warsaw) demonstrated the rules of modern microbiological diagnosis of tuberculosis bacilli. In her original talks, Dr. Z. Zwolska discussed the situation caused by *Mycobacterium tuberculosis* Beijing in Europe.

Interesting paper about thyroid function in community-acquired pneumonia (CAP) patients was presented by Dr. Oxana Omelyanenko and Prof. Alexandr Makarevich from Medical University in Minsk (Belarus).

Colleagues from the Department of Pulmonary Diseases in Grodno (H. Alexo, L. Pigalkowa, O. Zhernosek) in a number of reports focused on various aspects of lung tuberculosis in students, including the treatment of patients with tuberculosis and abusing alcohol.

YOUNG SCIENTISTS' SESSIONS

The Scientific Circle of students «Alveolus» at the Department of Pulmonology, Warsaw Medical University, led by Prof. J. Domagala-

Kulawik presented three reports: (1) Smoking among students.

WUM - A. Leskov, K. Orska, A. Mąka; (2) Newly detected COPD in patients with severe asthma - Śleszycka J. et al; (3) Nicotine addiction is a disease - K. Królikowski, E. Jopowicz. Since 2007 at all conferences the special young scientist sessions were organized. The student of Medical University in Donetsk A.V. Tereshkova presented preliminary observations on the use of "Coraxan" in patients with COPD and coexisting coronary artery disease, and at the same time the student O. Hryshchuk from University in Ternopil summarized the results of the treatment of liver disease in patients with pulmonary tuberculosis.

HOSPITAL PRACTICE FOR YOUNG PHYSICIANS AND STUDENTS IN POLAND AND UKRAINE

The arrangement of a two week professional visits for young physicians from Ukraine and Belarus to leading pulmonary centers in Poland demonstrates the important activity of our group. The internships in Warsaw for doctors from Kiev (2 persons), Donetsk (2), Kharkiv (2), Ternopil (2), Chernivtsi (2), Grodno (5), Minsk (3) were organized with the support of the Board of Warsaw-Otwock Branch PTChP. This assistance included the organization of the stay and financial support (hotel, meals, public transport ticket). Outside Warsaw, Wrocław Center was also very actively involved in the process of organizing of internships for young physicians from Ukraine and Belarus. Moreover the Department of Pulmonary Diseases in Zabrze organized a visit for two physicians from Donetsk.

JOINT SCIENTIFIC PROGRAMS

Scientific contacts between Poland, Belarus and Ukraine resulted in joint research programs. These included the study of asthma prevalence in Poland, Ukraine and Belarus (coordinator Professor J. Zejda, Medical University of Silesia in Katowice), alpha-1- antytrypsin deficiency

in patients with chronic bronchial obstruction (Coordinator Prof. J. Chorostowska-Wynimko, Institute of Tuberculosis and Lung Diseases in Warsaw). The program «Children of Chernobyl» in cooperation with pediatricians from Grodno Medical University, coordinated by Dr. P. Gutkowski, was focused on long-term effects of Chernobyl disaster on the respiratory system in children and young people living in areas of high contamination with radioactive isotopes.

FINAL NOTES

In conclusion, it must be underlined that scientific cooperation between the clinics and medical universities in Poland, Belarus and Ukraine should be considered as the fruitful and significant activity, integrating students and physicians of our countries. We are still convinced of the need to continue the collaboration. Our assumption is that patient suffering from asthma, chronic bronchitis, pulmonary tuberculosis and other diseases is identical regardless the native language or place of residence.

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Many thanks for Dr Krzysztof Królikowski for his great work during the organization of bilateral meetings. His perfect Russian and his boundless enthusiasm in creation of Polish and Ukrainian cooperation were very helpful in the integration process of our group and realization of our plans.

**Ковальський Я., Гутковський П.,
Серебровська Т.В.**

ДЕСЯТИРІЧЧЯ СПІВРОБІТНИЦТВА МІЖ ПОЛЬСЬКИМИ, УКРАЇНСЬКИМИ ТА БІЛОРУСЬКИМИ ФІЗІОЛОГАМИ І ПУЛЬМОНОЛОГАМИ (2004 – 2013)

Ідея заснування Польсько-української робочої групи з патофізіології дихання виникла в жовтні 2003 року під час візиту професора Інституту туберкульозу та легеневих захворювань у Варшаві Януша Ковальського до Інституту фізіології ім. О.О.Богомольця у Києві. Головною рушійною силою цієї ініціативи було велике бажання

польських та українських вчених і лікарів вільно обмінюватись досвідом з фундаментальних досліджень і клінічної практики задля кращої допомоги пацієнтам, що страждають від одних і тих самих недугів по обидва боки польсько-українського кордону. Наступного року під час 28-го конгресу Польського респіраторного товариства в Лодзі (Польща) ця ідея була втілена у життя. Пізніше до цього руху приєдналися фахівці з Білорусії. Впродовж десятиріччя були проведені щорічні наукові конференції в Києві (2005), Ополі (2006), Донецьку (2007), Катовіцах (2008), Тернополі (2009), Миколайках (2010), Чернівцях (2011) та Віслі (2012). Крім наукового обміну новітніми досягненнями з патофізіології дихання та пульмонології на конференціях і виконання спільних науково-практичних проектів, було організовано обмін молодими спеціалістами, що стажувалися на робочих місцях в провідних клініках Польщі та України. Перекладена на російську мову фундаментальна польська монографія «Функція легенів при легневих захворюваннях» (ред. J.Kowalski, A. Koziorowski, L.Radwan, переклад Є.Е. Колеснікової), що коштом Польського респіраторного товариства була видана та розповсюджена серед провідних медичних університетів та бібліотек України та Білорусії. Започатковано багато інших ініціатив по обміну досвідом між провідними вченими та лікарями країн-сусідів, що має велике значення для плідної роботи фахівців центральної та східної Європи.

Ключові слова: патофізіологія дихання, пульмонологія, обмін досвідом.

**Ковальський Я., Гутковский П.,
Серебровская Т.В.**

ДЕКАДА СОТРУДНИЧЕСТВА МЕЖДУ ПОЛЬСКИМИ, УКРАИНСКИМИ И БЕЛО- РУССКИМИ ФИЗИОЛОГАМИ И ПУЛЬМОНОЛОГАМИ (2004 – 2013)

Идея создания Польско-украинской рабочей группы по патофизиологии дыхания возникла в октябре 2003 года во время визита профессора Института туберкулеза и легочных заболеваний в Варшаве Януша Ковальского в Институт физиологии им. А.А.Богомольца в Киеве. Главной движущей силой этой инициативы было желание польских и украинских ученых и врачей свободно обмениваться опытом в области фундаментальных исследований и клинической практики для лучшей помощи пациентам, страдающим от одних и тех же недугов по обе стороны польско-украинской границы. В следующем году во время 28-го конгресса Польского респираторного общества в Лодзе (Польша) эта идея была воплощена в жизнь. Позже к этому движению присоединились специалисты из Белоруссии. На протяжении десятилетия были проведены ежегодные научные конференции в Киеве (2005), Ополе (2006), Донецке (2007), Катовицах (2008), Тернополе (2009), Миколайках (2010), Черновцах (2011) и Висле

(2012). Кроме научного обмена новейшими достижениями по патофизиологии дыхания и пульмонологии на конференциях и выполнения совместных научно-практических проектов, был организован обмен молодыми специалистами, которые стажировались на рабочих местах в ведущих клиниках Польши и Украины. Переведена на русский язык фундаментальная польская монография «Функция легких при легочных заболеваниях» (ред. J.Kowalski, A. Koziorowski, L.Radwan, перевод Е.Э. Колесниковой). Монография была издана на средства Польского респираторного общества и распространена среди ведущих медицинских университетов и библиотек Украины и Белоруссии. Воплощены в жизнь многие другие инициативы по обмену опытом между ведущими учеными и врачами стран-соседей, что имеет большое значение для плодотворной работы специалистов центральной и восточной Европы.

Ключевые слова: патофизиология дыхания, пульмонология, обмен опытом

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Mitochondrial dynamics in oxygen-glucose deprivation on a model of cultivated hippocampal slices

Ischemia injury is one of the most common death reasons in Ukraine and in a whole world. At the cells level stroke characterized by oxygen and glucose supply loose that cause energy failure and neurdegeneration. Mitochondrais are the key organelles of cell energy homeostasis. In order to discover dynamics of energy metabolism in ischemia damage we have been focused on investigation of mitochondrial functioning after 30-min oxygen-glucose deprivation (OGD) and reoxygenation 1 and 4 hours. We have done a comparison analyze of active mitochondrial activity of hippocampal pyramidal neurons and glial cells of CA1 and CA3 areas using fluorescent dyer MitoTracker Orange. In the present study it was determined that pyramidal neurons and glial cells have different dynamics of mitochondrial activity. Hippocampal pyramidal neurons have demonstarated the increasing of activity during first hour and the decreasing for four hours after OGD. Glial cells increased their mitochondrial activity fourth hours after start of reoxygenation, comparing with first hour. This indicates a specific activation of pyramidal neurons in response to the OGD, which, however, can not be maintained for a long time. For glial cells is characteristic of a more prolonged and sustained increase in mitochondrial activity. Thus, it can be assumed that the more resistant glial cells are able to some extent, to modulate the function of neurons in a lack of oxygen and glucose.

INTRODUCTION

Mitochondria are important for functioning of eukaryotic cells. It is main determinants of cellular respiration, energy homeostasis. Lots of pathological states (anoxia, ischemia, hemorrhagic shock) are accompanied by disfunction of the mitochondria and its inability to keep electrochemical proton gradient on membrane that result in cell degeneration and death. As consequence, oxide phosphorylation, ATP producing and Ca^{2+} homeostasis can not be maintained at native level. Brain ischemia is one of the most widespread pathology in Ukraine and in the whole world. The study of the cellular mechanisms of neurodegeneration is of great importance. Hippocampal organotypic culture is effective and useful model to investigate the mechanisms of ischemic damage of the brain. The natural tissue cytoarchitectonic and cell connections are preserved into cultivated slices. In present work we have been focused on mitochondria of neuronal and glial cells in CA1 and CA3 area under

oxygen-glucose deprivation (OGD) on a model of cultivated hippocampal slices.

METHODS

Hippocampal slices were obtained from the brains of 7-day-old rats and cultured according to the technique by Stoppini [1]. The procedure of oxygen-glucose deprivation was performed as described previously [2, 3]. The duration of OGD was 30 min; then, the slices were returned to normal conditions of culturing for 1h or 4 h (period of normoxic reoxygenation).

One and four hours after OGD hippocampal cultivated slices were stained with MitoTracker Orange CMTMRos (Cat. no.M7510). Next operations were performed: 1 – slices were washed with serum free medium; 2 – incubation with MitoTracker Orange (final concentration – 50 nM) was lasting 30 min at temperature 37°C; 3 – slices were washed with PBS (2 times, 5 min); 4 – staining was controlled with inverted microscope; 5 – fixation with 4% paraformaldehyde in

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PBS (30 min); 6 – slices were washed with PBS (2 times, 15 min), mounted on slides, embedded in Dako fluorescent mounting medium (Dako, Denmark). Mitochondrial activity of CA1 and CA3 hippocampal cells was analyzed at 1 and 4 hours after OGD. Pyramidal neurons and glial cells were estimated separately. The slices and sections were analyzed under a confocal microscope FV1000-BX61WI (“Olympus”, Japan, magnification – $\times 600$) using the corresponding software. Statistical analysis was performed using Statistica software, version 5 (StatSoft, USA). Numerical data are presented below as means \pm S.E.M. Intergroup differences were estimated using Student’s t-test; these differences were considered to be significant at $P < 0.05$ (marked by an asterisk in the figures).

RESULTS AND DISCUSSION

The aim of investigation was to analyze mitochondrial dynamics of CA1 and CA3 hippocampal cells after 30-min OGD and normoxic reoxygenation 1 and 4 hours. Pyramidal neurons and glial cells were estimated separately, as previously was reported that they have different susceptibility to deficiency of oxygen and glucose [4].

For estimation mitochondrial activity was used fluorescent dye MitoTracker Orange. Fluorescent probes aloud to uncover changes in membrane lipoprotein composition and to explore molecular mechanisms of cell pathogenesis. The membrane surface heterogeneity, charges accumulation, membrane ion binding and changes in membrane potential during mitochondrial functioning can be estimated [5]. The intensity of fluorescent probe luminescence was influenced by membrane dynamics and changes in trans-membrane potential.

The cell-permeant fluorescent MitoTracker probes contain a mildly thiol-reactive chromethyl moiety for labeling mitochondria. To label mitochondria, cells are incubated with MitoTracker probes, which passively diffuse across the plasma membrane and accumulate

in active mitochondria forming thiol conjugates with mitochondrial proteins. MitoTracker Orange determine amount of functionally active mitochondrias.

Electrochemical proton gradient was used as criteria of mitochondrial functional state. Mitochondrial membrane potential provided by respiratory chain electron transport and can reach 200mV [6]. Its decrease is an evidence of respiratory chain disruption and ATP synthesis failure. Mitochondrial membrane potential decreasing can provoke release of mitochondrial factors that can trigger cell death pathways. It is well known that changes in cell conditions directly connected with changes in mitochondrial membrane potential [7].

In control mitochondrial activity of pyramidal neurons in CA3 hippocampal area was $2,4 \pm 0,1$ (a.u.) and $1,7 \pm 0,2$ in CA1 area. That confirms previously observed differences in metabolic and energy state of this brain area [8]. In present investigation in control we have not observed any significant difference in mitochondrial activity between CA1 and CA3 glial cells. Mitochondrial activity of pyramidal neurons of CA1 area is lower ($1,7 \pm 0,2$) than activity of glial cells of the same zone ($2,7 \pm 0,3$). This phenomenon was not typical for CA3 hippocampal area.

The level of mitochondrial activity was higher in both pyramidal and glial cells of CA1 and CA3 areas one hour after OGD. Most prominent activation was noticed in pyramidal neurons ($3,1 \pm 0,1$) and glial cells ($3,6 \pm 0,2$) of CA3 hippocampal area. That could be an evidence of cell defense mechanisms switch and employment of mitochondrial reserve energy sources.

Mitochondrial activity level of pyramidal neurons in CA1 and CA3 areas was decreased ($2,2 \pm 0,3$ and $2,7 \pm 0,2$ respectively) four hours after OGD, but did not reach control meaning. We suppose that damaged mitochondria amount increase is responsible for that. In contrast, glial cells demonstrated a tendency to activation even four hours after OGD, both in CA1 and CA3 areas ($3,5 \pm 0,2$ and $3,8 \pm 0,2$ respectively). That lets us

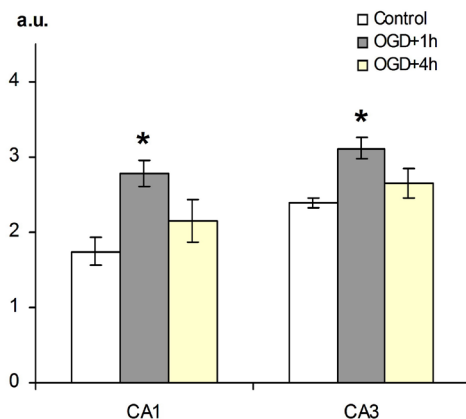
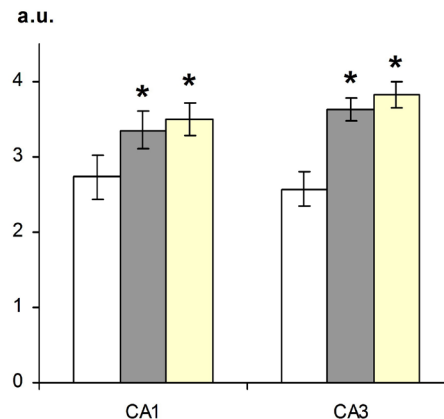
A - Pyramidal neurons**B - Glia**

Fig. 1 Level of mitochondrial activity of pyramidal neurons (A) and glial cells (B) of CA1 and CA3 hippocampal areas

to conclude that in our experimental conditions the glial cells are significant activated.

Thus, it was shown the differences of mitochondrial dynamics in hippocampal pyramidal neurons and glial cells after OGD. Mitochondrial activity of pyramidal neurons was increased during first hour and decreased after four hours of reoxygenation. Glial cells demonstrated continuing increase in their mitochondrial activity with time (one and four hours after reoxygenation). This is evidence of the specific features of the energy metabolism in the pyramidal and glial cells as well as the greater sensitivity and vulnerabilities of neurons in oxygen-glucose deficiency.

Малєєва Г. В., Лушнікова І. В., Скибо Г. Г.

МИТОХОНДРИАЛЬНА ДИНАМІКА ПРИ КИСНЕВО-ГЛЮКОЗНІЙ ДЕПРИВАЦІЇ НА МОДЕЛІ КУЛЬТИВОВАНИХ ЗРІЗІВ ГІПОКАМПА

Ішемічне ураження мозку є однією із найчастіших причин нервових патологій. Ці захворювання обумовлені порушеннями кисневого та глюкозного постачання на клітинному рівні, що призводить до падіння рівня енергетичного метаболізму і, як результат, до нейродегенерації. Ключовими клітинними органелами, які забезпечують гомеостаз клітин є мітохондрії. З метою дослідження динаміки енергетичного метаболізму в умовах розвитку ішемічного ушкодження, нами було проведено серію експериментів,

спрямованих на виявлення особливостей функціонування мітохондрій після 30-хв киснево-глюкозної депривації (КГД) та нормоксичної реоксигенації 1 та 4 години на моделі культивованих зрізів гіпокампа. Із використанням флуоресцентного барвника (MitoTracker Orange), було проведено порівняльний аналіз активації мітохондрій у пірамідних нейронах та гліальних клітинах CA1 та CA3 зон гіпокампа. Раніше було показано, що гліальні клітини більш резистентні до ішемічного ураження, ніж нейрони. Встановлено, що, в наших експериментальних умовах, для пірамідних нейронів та гліальних клітин характерна різна динаміка мітохондріальної активності. В пірамідних нейронах гіпокампа спостерігалось підвищення активності мітохондрій через одну годину після КГД та зниження через чотири години. У гліальних клітинах активність мітохондрій підвищувалася на протязі всього періоду спостереження. Це вказує на певну активацію пірамідних нейронів у відповідь на КГД, яка однак не може підтримуватись довгий час. Для гліальних клітин характерним є більш тривале підвищення мітохондріальної активності. Таким чином, можна припустити, що більш стійкі до ішемічного впливу гліальні клітини, залишаються здатними, в певній мірі, модулювати функції нейронів за умов нестачі кисню та глюкози.

Ключові слова: мітохондріальна активність, флуоресцентний зонд, киснево-глюкозна депривація

Малєєва Г. В., Лушнікова І. В., Скибо Г. Г.

МИТОХОНДРИАЛЬНАЯ ДИНАМИКА ПРИ КИСЛОРОДНО-ГЛЮКОЗНОЙ ДЕПРИВАЦИИ НА МОДЕЛИ КУЛЬТИВИРОВАННЫХ СРЕЗОВ ГИПОКАМПА

Ишемическое поражение мозга является одной из наиболее частых причин нервных патологий. Эти заболевания

обусловлены нарушениями кислородного и глюкозного обеспечения на клеточном уровне, которые приводят к падению уровня энергетического метаболизма и, как результат, к нейродегенерации. Ключевыми органеллами, обеспечивающими гомеостаз клеток, являются митохондрии. С целью исследования динамики энергетического метаболизма в условиях ишемического повреждения, нами была проведена серия экспериментов, направленных на выявление особенностей функционирования митохондрий после 30-мин кислород-глюкозной депривации (КГД) и нормоксической реоксигенации в течение 1 и 4 часов на модели культивируемых клеток гиппокампа. С использованием флуоресцентного зонда (MitoTracker Orange), был проведен сравнительный анализ активации митохондрий в пирамидных нейронах и клетках глии CA1 и CA3 зон гиппокампа. Ранее было показано, что глиальные клетки более резистентны к ишемическому повреждению, чем нейроны. Установлено, что, в наших экспериментальных условиях, для пирамидных нейронов и глиальных клеток характерна различная динамика митохондриальной активности. В пирамидных нейронах гиппокампа наблюдалось увеличение активности митохондрий через один час после КГД и снижение через четыре часа. В глиальных клетках активность митохондрий повышалась в течение всего периода наблюдения. Это указывает на определенную активацию митохондрий пирамидных нейронов в ответ на КГД, которая, однако, не может поддерживаться длительное время. Для глиальных клеток характерным является более длительное повышение митохондриальной активности. Таким образом, можно предположить, что, более стойкие к ишемическому воздействию глиальные клетки, остаются способными, в определенной мере, модулировать функции нейронов в условиях недостатка кислорода и глюкозы.

Ключевые слова: митохондриальная активность, флуоресцентный зонд, кислород-глюкозная депривация

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Lessons from twenty years' investigations of intermittent hypoxia: principles and practices

Widespread use of the intermittent hypoxic training/treatment (IHT) methods in sports, military and medical practice during recent decades has provoked a discussion: "What is 'intermittent hypoxia'? In contrast to studies from the former Soviet Union countries, that emphasized mainly the beneficial effects of IHT on an organism, intermittent hypoxia research in Western Europe and North America was primarily focused on the detrimental effects associated with sleep apnea. However, during the past decade, such a gap of division between East and West is progressively shrinking, and mutual understanding on what "intermittent hypoxia" means, becomes clearer. Potential mechanisms underlying both beneficial and adverse effects of IHT have been described. Basic investigations led to the proliferation of various methods of IHT exposure, the development of different medical equipment – hypoxicators – for its implementation in sport practice, military operations and also for clinical application. However, wide array of different protocols and measurements makes the results difficult to harmonize. Meanwhile, the mode of hypoxic influence (depth, duration, and intermittence) appeared to be critical for the determination of healing or harmful result. Therefore, special purposeful investigations are needed to elucidate basic mechanisms of different IHT effects depending on the modality of hypoxic stimuli and elaborate most effective and safe regimen for the introduction in human practice.

INTRODUCTION

Intermittent hypoxia (periodic hypoxia, interval hypoxia, hypoxic preconditioning etc) became today "the talk of the town" among physiologists and clinicians who deal with hypoxic problems. Although the roots of this topic go deep into Middle Ages, sharply intensifying in 30th years of XX century in Soviet Union due to military needs, most fundamental investigations were made during last two decades. The number of publications indexed in PUBMED under the key-word "Intermittent Hypoxia" increased from 49 in 1993 to 520 during the first half-year of 2013. Several monographs have been published [Kolb, 2004; Gozal et al., 2011; Xi & Serebrovskaya, 2009; 2012].

Many types of protocol with different numbers of hypoxia episodes, severity, and total exposure duration, have been used by investigators and these combinations may have resulted in various physiological responses. Principals of IHT application for cell cultures, animal experi-

ments (mice, dogs, cats, rabbits, pigs, horses and even insects) have been elaborated. A variety of technical implementations for treatment of animals and humans has been tested.

Widespread use of the intermittent hypoxic training/treatment (IHT) methods in sports, military and medical practice during recent decades has provoked a discussion: "What is 'intermittent hypoxia'?" [Semenza, 2012]. All papers using this term should be divided into the four main classes: (1) hypoxic hypoxia (intermittent hypoxic training using gas mixtures or barochambers, recurrent sojourn at high altitudes, hypoxic preconditioning in stem cell transplantation therapy); (2) ischemic preconditioning (cardiac, cerebral etc); (3) hypoxia induced by breath holding (divers, yogic technique Pranayama, training with extra dead space); (4) obstructive sleep apnea syndrome (OSAS) and other diseases associated with brainstem disorders.

Three first classes are generally considered as beneficially influencing on an organism,

whereas the fourth one (which is characterized by the similar pattern of hypoxic and normoxic episodes) is an example of the pathological process. Rats exposed to chronic intermittent hypoxia (CIH) simulating recurrent apnea in OSAS patients demonstrate autonomic morbidities and hypertension similar to those described in recurrent apnea patients [Fletcher, 2001; Prabhakar et al., 2005, and many others]. Meanwhile, such comparison seems to be rather mechanistic because does not take into account several significant differences between other factors accompanying hypoxia in these four paradigms.

For example, most researchers do not take into account that IHT methods in the vast majority of cases use eucapnic hypoxia which results in hyperventilation and hence hypocapnia. At the same time, ischemic preconditioning which was proved to activate endogenous defense mechanisms and shows marked protective effects is accompanied by hypercapnia, acidosis and the accumulation of metabolites absent during IHT. In experiments on rats, only hypoxic component is modulated, whereas inspired CO_2 is maintained at normal level. Meanwhile, pCO_2 and pH play one of the main regulative roles in respiration and metabolism and could affect the organism very differently from hypoxia per se. Intracellular acidosis due to hypercapnia raises concerns about potential harmful effects. In contrast to intermittent hypoxia, the effects of intermittent hypercapnia and its cohabitation with hypoxia are the areas of research that remain to be explored. Therefore, a direct comparison of IHT, ischemia and sleep apnea effects seems inconsistent.

Although intermittent hypoxia research in Western Europe and North America was primarily focused on the detrimental effects of chronic intermittent hypoxia associated with sleep-disorder breathing, during last decade such a gap of division is progressively shrinking, and mutual understanding on what “intermittent hypoxia” means, becomes clearer.

In this mini-review we will just outline the main recent achievements in the field of inter-

mittent hypoxia focusing on recent advances in the mechanisms of IH investigation.

MECHANISMS

An impressive amount of scientific information has been gathered with regard to the responses to hypoxia, from the integrative systems level to the molecular and genomic level, such as: (1) regulation of respiration and circulation; (2) free radical production; (3) mitochondrial respiration; (4) role of genetic factors (HIF, MTF-1, NF- β κ, c-Fos, c-Jun, etc); (5) epigenetic mechanisms of adaptation to IH. Repeated exposures to hypoxia have been examined for both their beneficial and adverse effects. The following questions arise: what are the key mechanisms determining the adaptive versus maladaptive nature of different paradigms of intermittent hypoxia, and, what molecular pathways are mediating the observed pathological or physiological response? Until now there is no exact evidence about the precise mechanism for switching adaptive or maladaptive responses to hypoxic impact. The most important arguments are presented in recent papers [Prabhakar & Semenza, 2012; Raghuraman et al., 2013].

Many discoveries demonstrated that intermittent hypoxia leads to remodeling of the carotid body function manifested by augmented sensory response to hypoxia and induction of sensory long-term facilitation (LTF). More than 20 years ago we have shown that intermittent normobaric hypoxia augments hypoxic ventilatory response (HVR) and do not substantially influence hypercapnic ventilatory sensitivity (HCVR) [Serebrovskaya, 1992]. Later on John Weil and his co-workers [2003] described variations in the HVR in human subjects. There are many reviews reflected further investigations in this field [Prabhakar & Kline, 2002; Serebrovskaya, 2002; Teppema & Dahan, 2010; and oth.]. Recent studies strongly indicate that endothelin-1 takes part in this process resulting from reactive oxygen species-dependent activation of endothelin converting enzyme [Peng et al., 2013]. Role of such gasotransmitters as

nitric oxide, carbon dioxide and hydrogen sulfide (H₂S) in the regulation of respiration under intermittent hypoxia was excellently described by N. Prabhakar [2013].

It is widely known that during acute episodes of hypoxia, chemoreceptor-mediated sympathetic activity increases heart rate, cardiac output, peripheral resistance and systemic arterial pressure. Tyrosine hydroxylase (TH) is the rate-limiting enzyme for catecholamine synthesis. Several mechanisms contribute to the short- and long-term regulation of TH which are well-established. IH-mediated activation of TH leads to the increase in catecholamines level in the brainstem and adrenal medulla [Raghuraman et al., 2013]. In our lab, it was shown that two-week IHT course increased dopamine synthesis in adult and old rats and the animals with experimental Parkinson's disease (PD), especially in the right striatum, restoring partially the skewness of DA distribution between brain hemispheres which has been lost during aging [Belikova et al., 2012].

However, different IH paradigms produce remarkably divergent effects on systemic arterial pressure in the posthypoxic steady state [Serebrovskaya et al., 2008]. The hypertensive effects of OSA vs. the depressor effects of therapeutic hypoxia exemplify this divergence. Why do OSA and IHT produce such disparate effects on blood pressure? It is useful to consider the fundamental differences between the two phenomena: duration of hypoxia periods; hypercapnea and acidemia versus hypocapnea and alkalemia; hypoxic episodes occur at day- or night-time, etc. As a result, OSA ignites a crescendo of factors which activate the sympathetic nervous system and systemic inflammation, culminating in maladaptive, persistent hypertension. In contrast, therapeutic IHT activates parasympathetic system and dampens other factors.

Another IH effects on cardio-respiratory system should be only mentioned here. There are increased alveolar ventilation and lung diffusion capacity; increased haematopoiesis, increased capillary density and tissue perfusion, suppressed function of mitochondrial enzyme

respiratory complex I (MEC I) and the alternative activation of MEC II and many others (see reviews Schmidt, 2002; Serebrovskaya, 2002; Mankovska et al., 2005; Lukyanova et al., 2009; Faiss et al., 2013; Prabhakar & Semenza, 2012). Some authors [Prokopov, 2012] consider intermittent hypoxia as a multifunctional tool of a natural mitochondria-rejuvenative strategy.

Besides, hypoxic exposure significantly increases the tolerance and regenerative properties of stem cells and progenitor cells. During last decade it was shown that short-term hypoxic exposures can mobilize hematopoietic stem cells (HSC) and increase their presence in peripheral circulation [Tang et al., 2009; Viscor et al., 2009; Serebrovskaya et al., 2011; Ranera et al., 2012]. Different intensities and durations of hypoxia could have important and diverse effects on stem cell development. Special study was designed to compare the effects of intermittent versus acute hypoxia on human HSCs and some immune parameters [Serebrovskaya et al., 2010]. The effect of two-week program of cyclic 5 min exposures to 10% O₂ were: (1) decrease in circulating hematopoietic stem cells; (2) complement activation; (3) phagocytic and bactericidal activities of neutrophils stimulation while suppressing pro-inflammatory cytokines. In contrast to the 14d program, a single IHT session provoked appreciable yet transitory increase in circulating HSC which quickly subsided after hypoxic exposures. Results raise the possibility that IH induces HSC emigration from niches into the circulation, followed by homing and sequestration in target tissues during post-hypoxic recovery. The IH-induced decrease in blood TNF- α content with simultaneous increase in IFN- γ could contribute to the moderation of infectious-inflammatory processes.

One of the key mechanisms of cell damage during hypoxia and reoxygenation is an excessive production of reactive oxygen and nitrogen species (ROS and RNS) in mitochondria. ROS and RNS generation leads to mitochondrial protein, lipid and DNA oxidation which impedes normal mitochondrial physiology and initiates

cellular death pathways [Thompson et al., 2013]. On the other hand, ROS function as signaling molecules in a variety of physiological systems [Prabhakar, 2011; Wang et al., 2011]. Several attempts were undertaken to analyze this question [Sazontova & Arkhipenko, 2009; Lukyanova et al, 2009, 2012]. It was shown, that low levels of ROS production are protective and may serve as a trigger for hypoxic adaptations. At the cellular level, intermittent hypoxia leads to reprogramming of mitochondrial metabolism that ensures adequate ATP generation and prevents adverse consequences of excess mitochondrial ROS generation. These metabolic adaptations are due to hypoxia-inducible factors 1 and 2 (HIF-1 and HIF-2) transcriptional regulation of glycolytic enzymes, mitochondrial electron transport chain components, and other metabolic enzymes [Semenza & Prabhakar, 2007; Prabhakar & Semenza, 2012]. Recent studies have shown that HIF-1 and HIF-2 regulate the expression of gene products with opposing functions that regulate the redox state (Prabhakar, 2013). For instance, HIF-1 regulates the expression of pro-oxidant enzymes, including NADPH oxidases, whereas HIF-2 regulates the expression of antioxidant enzymes.

In our lab, Drevytska et al [2012] investigated the role of another subunit - HIF-3 α – in adaptation to IH and physical load. It was shown that this subunit plays a negative role in the adaptation to hypoxia. HIF-3 α mRNA expression increased sharply under acute hypoxia in the heart, lung, and kidney but did not change after 5-week IHT. Inhibition of HIF-3 α expression led to an increase in physical endurance. Thus, every of HIF-subunits plays different role in response to hypoxic load. It seems that the investigation of their ensemble functioning under different IH modes (depth, duration, and intermittence) could explain the mechanism for switching adaptive or maladaptive cellular and systemic responses to hypoxic impact.

One of the new directions in the investigation of hypoxic adaptations is epigenetics – heritable modifications of DNA that do not involve changes in the DNA primary sequence [Gluckman, 2011;

Nanduri et al., 2011; Prabhakar, 2013]. Epigenetic mechanisms can determine whether a gene is activated or silenced. These studies seem to be very promising in this rapidly emerging area.

While all above mentioned fundamental studies provided important insights into mechanisms of HIF activation by hypoxia, they can not answer as yet practical question what dose and regimen of hypoxic impact could be mostly beneficial for animals and humans.

Use in clinical practice

To the present days, intermittent hypoxic training (IHT) has been used extensively for altitude pre-acclimatization, for treatment of a variety of clinical disorders and in sports. Wide spectrum of protocols for IHT is represented now in literature showing both beneficial and detrimental effects. Beneficial results were shown for treatment and prophylaxis of numerous disorders in pulmonology (chronic obstructive diseases, bronchial asthma, chronic rhinitis etc), cardiology (ischemic heart disease, hypertension, cardiosclerosis, etc), hematology (hypoplastic and iron-deficient anemia, post-radiation hematological disturbances, etc), neurology (functional neurological disorders, Parkinson's and Alzheimer's diseases, neurosis, syndrome of autonomic dystonia, diabetic neuropathy, psychosomatic disorders), diabetes mellitus, obstetrics and gynecology (juvenile bleedings, toxicosis of expectant mothers, pathology of climacteric period etc), gastrointestinal diseases (gastroduodenitis, peptic ulcer), professional diseases (pneumoconiosis, vibration- and dust-induced pathology, acute and chronic intoxication etc), post radiation disorders of immune system and male reproductive system, and many others. In this mini-review we can not mention all spectrum of papers devoted to this problem. The interested reader is referred to several reviews and monographs [Karash et al., 1988; Berezovskii and Levashov, 1992; Fesenko and Lisyana, 1992; Xi & Serebrovskaya, 2009; 2012; and many others]. Much literature may be found on the websites www.go2altitude.com

and *www.bionova.ru*. Here we mention just some last publications.

IHT clinical applications are clearly presented by S. Basovich in his last review [2013]. Among others, he described beneficial results of IHT application for treatment of bronchial asthma and chronic obstructive pulmonary disease, hypertension, to correct abnormalities during pregnancy, in epilepsy treatment, for preparation of patients to surgery to increase nonspecific resistance, etc.

The efficacy of IHT was demonstrated for improving male subfertility and other andrological disorders [Swanson & Serebrovskaya, 2012]. Intermittent hypoxia protocols may be developed for treatment and prevention of osteopenia and osteoporosis [Berezovskii et al., 2004; Guner, 2013].

Recently, a new mode of adaptive training was explored, which combines periods of hypoxia and hyperoxia [Arkhipenko et al., 2005; Glazachev et al., 2010; Sazontova et al., 2012; Gonchar & Mankovska, 2012]. A novel principle of short-term periodic adaptive training by varying the oxygen level from hypo- to hyperoxia is substantiated both theoretically and experimentally. Studies supports the viewpoint that moderate periodic generation of free radical signal during hypoxic/hyperoxic bouts causes better induction of antioxidant enzyme protein synthesis than hypoxic/normoxic exposures, that may be an important trigger for specific adaptations.

Another new direction in IHT application is developing during last years: hypoxic post-conditioning [Leconte et al., 2009; Maslov et al., 2012; Joo et al., 2013; Xie et al., 2013]. While pre-conditioning is induced before stroke onset, experiments on animals have shown that ischemic post-conditioning performed after reperfusion attenuates brain injury. Clinical investigations testify on cardioprotective impact of postconditioning in patients with acute myocardial infarction and cardiac surgery patients.

Some works are devoted to the application of hypoxic-hypercapnic or intermittent hypercapnic treatment to clinical practice. This question

is elucidated in review of Pokorski & Serebrovskaya [2009]. The effects of hypercapnia are somewhat surprising. CO₂ is a recognized vasodilator of myocardial blood vessels, it is capable to substantially increase cerebral blood flow leading to increased tissue oxygenation. Hypercapnic acidosis may have a beneficial effect in its own right in severe respiratory conditions and may, paradoxically, be helpful in patients with organ failure due to ischemia-reperfusion-related cellular injury. That brings us to the use of "therapeutic hypercapnia", a purposefully increased inspired CO₂ concentration to achieve some beneficial health effects. Hypoxia and hypercapnia, used in tandem, may strengthen the curative effects of either. So, intermittent hypercapnia seems an obvious area of future research focusing not only on the mechanisms of long-term potentiation and synaptic plasticity in the brain stem respiratory network, but also on the health-related applicability of this kind of respiratory strategy. The controversies that surround the use of therapeutic hypercapnia uphold research interest. The potential of intermittent hypercapnia is just starting to be realized and hopefully will be further explored.

During the past few years numerous debates about the ethical evaluation of diagnostic and therapeutic use of hypoxia in humans are raised. Although the works devoted to this problem obtained the approval from the Human Investigation Ethics Committees, there is the lack of evidences about strong evaluation of risk/benefit ratio. The analysis of such ratio and the creation of standardized guide-lines for hypoxic treatment/training application are complicated due to the differences in criteria for individual dosage and utilized methods. One of the attempts to solve this problem was made by applying a new mathematician method - "Method of Expert Assessing Scales" (MEAS) - for the estimation of IHT application safety in human practice [Serebrovsky & Serebrovskaya, 2009]. MEAS dilates capabilities of traditional probabilistic safety assessment and allows determining the danger degree at the most early stage of its de-

velopment and fulfilling well-timed actions for danger prevention. It includes the description of: a) hazard causal factors; b) situations as a set of values of causal factors; c) influences of separate factors on the origin of basic events; d) joint influence of factors on basic events probability. The methodology provides the forming of the system of indexes characterizing the risk of IHT negative effects and determination of legitimate value scopes for basic physiological parameters; creation of the classification system allowing to set human individual cardio-respiratory reactivity; development of proper IHT regimen for every class of reactivity.

But there is just one of the first steps which is far from the elaboration of concrete methodic recommendations. Mode of hypoxic influence (depth, duration, and intermittence) appeared to be critical for the determination of beneficial or detrimental effects of IHT. Low doses of hypoxia might not be sufficient stimuli to mobilize adaptive mechanisms, whilst severe or prolonged hypoxia may provoke dangerous pathological processes.

Meanwhile, in practice hypoxic regimens which are used for the study of hypoxic adaptations vary broadly from 3-12 short hypoxic sessions (2-10 min) with 2-20 min normoxic breaks during 7-30 days to hypoxic influences lasting from 1 - 12 hours during 2-90 days. In our lab, V.Nosar compared the effects of five most spread modes of IHT on rat gastrocnemius muscle PO_2 and heart and liver mitochondrial respiration [Serebrovskaya et al., 2013]. Min of hypoxia, % O_2 and recovery min on air in each mode were: 1) 5, 12%, 5; 2) 15, 12%, 15; 3) 5, 12%, 15; 4) 5, 7%, 5; 5) 5, 7%, 15. Our experimental data indicated that among 5 tested modes of IHT, optimal hypoxic dose for muscle oxygen supply is 5-min breathing with 12 % O_2 gas mixture and 5-min breaks (Mode 1), 5-6 times a day during two or three weeks. Under such mode, PmO_2 dropped minimally to the end of every hypoxic period and recovered quickly after every hypoxic set to initial level or even exceeded it. Two-week training with

this mode raised basal tissue oxygenation during normoxia and provided higher PmO_2 level during acute hypoxia. Such mode caused the substrate dependent reorganization of liver and heart mitochondrial energy metabolism favoring NADH-dependent oxidation and improving the efficiency of oxidative phosphorylation.

However, we must take into account that all these beneficial results were obtained on rat models. Are we ready to propose this as a clinical therapeutic method? More rigorous studies need to be provided in the near future on patients with several diseases. Besides, in actual human practice including sports and military applications of hypoxic training [Musa, 2007], the IHT regimen (the degree of hypoxia, exposure duration and number of sessions) could be also titrated to the mission requirements, such as the operational target altitude, risk of developing acute mountain sickness, or anticipated physical activity levels.

Basic investigations led to the proliferation of various methods of IHT exposure, the development of different medical equipment – hypoxicators – for its implementation in sport practice, military operations and also for clinical application [Lopata & Serebrovskaya, 2012].

In conclusion, intermittent hypoxic treatment/training represents a promising field of study in prevention and treatment of many diseases. The proper choice of the hypoxic dosage depending on individual's reactivity must be titrated for each patient to avoid negative effects of hypoxia and to augment the favorable properties. We can envisage a bright future for individualized IHT, which may play a significant role in the fast developing field of personalized preventive medicine against various human diseases.

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УРОКИ ДВАДЦЯТИРІЧНОГО ВИВЧЕННЯ ІНТЕРВАЛЬНОЇ ГІПОКСІЇ: ПРИНЦИПИ І ПРАКТИКА

Широке застосування методів інтервального гіпоксичного тренування (ІГТ) в спорті, військовій та медичній практиці протягом останніх десятиліть викликало широку

дискусію: що таке «інтервальна гіпоксія»? На відміну від досліджень, проведених у країнах колишнього Радянського Союзу, в яких підкреслювалися в основному позитивні ефекти ІГТ на організм, дослідження інтервальної гіпоксії в Західній Європі і Північній Америці були зосереджені головним чином на негативних наслідках, пов'язаних з сонним апное. Тим не менш, протягом останнього десятиліття такий розрив у поглядах між Сходом і Заходом поступово скорочується, і взаємне розуміння того, що означає цей термін, стає ясніше. Були описані потенційні механізми, що лежать в основі як позитивних, так і негативних наслідків ІГТ, розроблені різні методи застосування ІГТ та обладнання для їх реалізації в спортивній практиці, військових операціях, а також для клінічного застосування. Тим не менш, широкий спектр різних протоколів і вимірювань робить результати важко узгодженими. Проте, саме режим гіпоксичного впливу (сила, тривалість і уривчастість) є критичним для отримання позитивного або негативного результату. Тому мають бути проведені спеціальні цілеспрямовані дослідження для з'ясування основних механізмів різних ефектів ІГТ залежно від модальності гіпоксичного стимулу і розробки найбільш ефективних і безпечних режимів впливу для впровадження в практичну медицину.

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УРОКИ ДВАДЦАТИЛЕТНЕГО ИЗУЧЕНИЯ ИНТЕРВАЛЬНОЙ ГИПОКСИИ: ПРИНЦИПЫ И ПРАКТИКА

Широкое применение методов интервальной гипоксической тренировки (ИГТ) в спорте, военной и медицинской практике в течение последних десятилетий вызвало широкую дискуссию: Что такое «интервальная гипоксия»? В отличие от исследований, проведенных в странах бывшего Советского Союза, в которых подчеркивались в основном положительные эффекты ИГТ на организм, исследования интервальной гипоксии в Западной Европе и Северной Америке были сосредоточены главным образом на негативных последствиях, связанных с сонным апноэ. Тем не менее, в течение последнего десятилетия такой разрыв во взглядах между Востоком и Западом постепенно сокращается, и взаимное понимание того, что означает этот термин, становится яснее. Были описаны потенциальные механизмы, лежащие в основе как положительных, так и негативных последствий ИГТ, разработаны различные методы применения ИГТ и оборудование - гипоксикаторы - для их реализации в спортивной практике, военных операциях, а также для клинического применения. Тем не менее, широкий спектр различных протоколов и измерений делает результаты трудно согласуемыми. Между тем, именно режим гипоксического воздействия (сила, длительность и прерывистость) являются критическим для получения положительного или отрицательного результата. Поэтому должны быть проведены специальные

целенаправленные исследования для выяснения основных механизмов различных эффектов ИГТ в зависимости от модальности гипоксического стимула и разработки наиболее эффективных и безопасных режимов воздействия для внедрения в практическую медицину.

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Effect of intermittent normobaric hypoxia on total oxygen consumption and efficiency of cardio-respiratory mechanisms of oxygen supply in patients with a high risk of chronic obstructive pulmonary disease

Effect of intermittent normobaric hypoxic treatment (INHT) on total oxygen consumption (OC) and efficiency of cardio-respiratory mechanisms of oxygen supply was studied in adult patients with a high risk of chronic obstructive pulmonary disease (COPD). The 1st group (n=43) of patients received only conventional treatment. The 2nd group (n=103) received the same treatment in combination with INHT. INHT comprised of a daily 90 minute sessions in chamber "Orotron" for 2 weeks. The oxygen partial pressure was maintained in the automatic mode at 147-160 hPa. The heterogeneity of OC dynamics of both the amplitude and direction was observed in patients after INHT. The pattern and degree of changes were dependent on the initial level of OC and the functional status of the cardio-respiratory system. In this regard, the study group of patients was divided into three subgroups: 2-A – 33,1% of patients had a normal value of OC, 2-B – 52,4% of patients had a lower value of OC and 2-C – 15,5% of patients had a higher value of OC. INHT did not change the total OC but improved the efficiency of hemodynamic mechanisms of oxygen supply in patient subgroup 2-A. The total OC and efficiency of respiratory mechanisms of oxygen supply moderately increased in patient subgroup 2-B. The value of OC decreased moderately in patient subgroup 2-C, but respiratory and hemodynamic mechanisms of oxygen supply remained high enough. It was concluded that INHT optimized the oxygen consumption and improved the efficiency of cardio-respiratory mechanisms of oxygen supply in patients with a high risk of COPD.

Key words: intermittent normobaric hypoxia, chronic obstructive pulmonary disease

INTRODUCTION

Mountain climate has been used for treating patients with chronic pulmonary diseases for many years. Since the days of P.Bert (1878) and A.Mosso (1898), it has been postulated that high-altitude hypoxia is the most important factor that determines the specific nature and pattern of the physiological effects of mountain climate [17, 18]. It was found that even a relatively small decrease in oxygen partial pressure (PO_2) activated a number of compensatory and adaptive mechanisms, such as alveolar ventilation, systemic and regional hemodynamic, microcirculation, oxygen binding properties of hemoglobin, oxygen metabolism, etc. [2,7,10-13,20]. Oxygen delivery to tissue

and total oxygen consumption (OC) are changed in patients with chronic pulmonary diseases at high altitude. The nature of these changes depends on the degree and duration of exposure to hypoxia, as well as characteristics of the disease. The individual genetic and phenotypic features are another important factors that determine the resistance of patients to hypoxia and specific features of their adaptation to mountain climate. Respiratory and hemodynamic mechanisms of oxygen supply provide tissues with oxygen in an amount that corresponds to their metabolic needs. Functional efficiency of these mechanisms determines the quality of adaptation to mountain climate and severity of hypoxic disorders in patients at high altitude.

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Intermittent normobaric hypoxia (INH) is one of the effective non-pharmacological methods for care and rehabilitation of patients with different chronic pulmonary diseases, such as bronchial asthma, chronic bronchitis, etc. [1-6, 9, 13, 19]. This method is being more widely used in clinical practice now [8, 14, 15, 21-26]. But effect of INH on total oxygen consumption in patients with a high risk of chronic obstructive pulmonary disease (COPD) is not sufficiently investigated. This question is very relevant for prediction and evaluation of the therapeutic effect of INH in individual patients. In this paper we present the results of our studies regarding the INH effects on total oxygen consumption and efficiency of respiratory and hemodynamic mechanisms of oxygen supply in patients with a high risk of COPD.

MATERIALS AND METHODS

The total volume of oxygen consumption and the main characteristics of the functional efficiency of the respiratory and hemodynamic mechanisms of oxygen supply were studied in 146 adult patients. Patients ranged in age from 27 to 59 years (women – 52,1%, men – 47,9%). All of them had a cough, sputum production and under certain conditions – dyspnoea. The most of them were smokers, but they had no persistent violation of lung function. According to the Global Initiative for Chronic Obstructive Lung Disease, they were attributed to patients with high risk of COPD. All patients were divided into control (1st) and study (2nd) groups randomly. The 1st group (n=43) of patients received only conventional treatment. The 2nd group (n=103) received the same treatment in combination with INH. The intermittent normobaric hypoxic treatment (INHT) was performed in “Orotron” chamber within 2 weeks for 1,5 hours daily. Under conditions of normal atmospheric pressure, this device creates and maintains in automatic mode the following parameters: PO₂ - 147-160 kPa, relative humidity - 60-70%, ambient temperature - 16-18° C, and content of light

negative ions - up to 6000 per cm³. The total time of hypoxic exposure in intermittent regime was 21 hours. The total volume of oxygen consumption was measured using closed-system respirometer. Oxygen consumption per minute (VO₂), oxygen consumption index (VO₂I), coefficient of oxygen extraction from air (VO₂/V_e), ventilation equivalent (VE), oxygen consumption per one cycle of respiration (O₂rc), oxygen consumption per one cardiac cycle (O₂cc), hemodynamic equivalent (HE) were calculated by standard formulae. Corrections were made for barometric and vapor pressure and temperature to reduce all figures to standard conditions - STPD and BTPS. The methods of spirometry, pneumotachography, electrocardiography and rheovasography were used for investigation lung ventilation and hemodynamic' function. All parameters were recorded before and after the course of conventional treatment and the same treatment in combination with INHT. Mean values of difference between control and study groups of patients before and after treatment were calculated and compared. Students “t” test was applied for evaluating the significance of differences.

RESULTS

The studies found no significant differences in the total oxygen consumption, as well as in characteristics of the efficiency of the respiratory and hemodynamic mechanisms of oxygen supply between the control and study groups of patients before treatment (Table 1). This fact indicated that the compared groups of patients were fairly uniform in the nature of the disease manifestations.

The value of oxygen consumption was not significantly changed in the 1st group of high risk COPD patients after 2 weeks of conventional treatment. They had only a moderate increase in O₂cc and decrease a negative base excess (BE). It was found a moderate increase in VO₂, VO₂I, O₂ cc, pH, BE and decrease in VE, HR and HE in the 2nd group of patients after conventional

treatment in combination with INH. Thus the number of parameters and the degree of their changes after treatment were more pronounced in the 2nd group of patients. The functional efficiency of the respiratory and hemodynamic mechanisms of oxygen supply was evidently higher in these patients.

The heterogeneity of the OC dynamic of both the amplitude and direction was observed in the 2nd group of patients after INHT. We analyzed this fact. It was determined that the pattern and degree of changes were dependent on the initial level of OC. For this reason, we divided all patients from study group into 3 subgroups according to initial level of OC: 2-A – 32,0% of patients had a normal value of OC, 2-B – 52,4% of patients had a lower value of OC and 2-C – 15,6% of patients had a higher value of OC before INHT.

Significant differences in VO_2 could be related to various height and weight in subgroups of

patients. In this regard, we calculated the oxygen consumption index in all subgroups of patients according to their actual height and weight. The type and nature of inter-group differences in VO_2 and VO_2I remained unchanged. These results showed that established differences had a more complex mechanism of origin. We suggested that these differences were related to the individual characteristics of respiratory, hemodynamic and metabolic mechanisms of oxygen supply in individual patients. We refined this assumption.

The values of VO_2 , VO_2I and the main respiratory and hemodynamic parameters of the oxygen supply efficiency were within the physiological range in the 2-A subgroup of patients before INHT. Disturbances of acid-base balance were absent ($\text{pH}=7,36\pm0,03$; $\text{BE}=-1,22\pm0,05$ $\text{mmol}\cdot\text{l}^{-1}$) (Table 2).

The largest number of patients was in subgroup 2-B. The total volume of oxygen consump-

Table 1. Oxygen consumption and cardio-respiratory indices of oxygen supply in patients with a high risk of COPD before (I) and after (II) conventional therapy (1) and INHT in combination with conventional therapy (2)

Characteristics	Group of patients			
	1 (n=43)		2 (n=103)	
	I	II	I	II
VO_2 , $\text{ml}\cdot\text{min}^{-1}$	289,02±12,51	293,70±17,42	273,20±10,52	305,60±9,48*
VO_2I , $\text{ml}\cdot\text{min}^{-1}\cdot\text{m}^2$	155,14±15,73	162,42±12,80	149,33±12,12	164,51±11,78*
O_2 rc, ml	18,67±1,33	19,48±1,17	17,43±0,93	19,15±1,16
O_2 cc, ml	3,73±0,20	4,29±0,24*	3,45±0,13	3,98±0,14*
VO_2/V_e , $\text{ml}\cdot\text{l}^{-1}$	25,05±1,86	26,10±1,98	24,60±1,25	28,78±1,10*
V_T , ml	749,8±51,1	704,1±38,3	726,7±33,8	717,9±40,1
f , min^{-1}	16,10±1,13	16,01±1,17	16,86±0,72	16,81±0,60
V_e , $\text{l}\cdot\text{min}^{-1}$	11,54±1,42	11,21±0,73	11,74±0,49	11,52±0,43
VE	39,81±2,90	38,82±2,85	45,53±2,39	40,35±1,65*
HR, min^{-1}	78,01±1,97	76,40±2,31	79,46±1,61	75,06±1,50*
HE	13,12±0,92	12,10±1,20	14,06±0,74	12,67±0,59*
Q, ml	46,11±1,56	45,87±1,81	44,79±1,60	45,40±1,70
Q, $\text{ml}\cdot\text{min}^{-1}$	3782,51±148,45	3692,89±167,78	3652,70±156,61	3637,50±164,89
pH	7,34±0,03	7,38±0,05	7,35±0,04	7,42±0,01*
BE $\text{mmol}\cdot\text{l}^{-1}$	-2,83±0,26	-0,51±0,04*	-2,33±0,19	+1,24±0,36**

Here and below: * $p<0.05$, ** $p<0.01$ - values are significantly different from the initial state.

tion did not exceed $216,92 \pm 5,62 \text{ ml} \cdot \text{min}^{-1}$ in these patients. The low respiratory indices of oxygen supply efficiency were typical: VO_2/V_e didn't exceed $21,26 \pm 1,69 \text{ ml} \cdot \text{l}^{-1}$, $\text{O}_{2\text{rc}} = 13,88 \pm 1,04 \text{ ml}$, $\text{VE} = 52,58 \pm 3,65$. But hemodynamic parameters remained fairly stable: $\text{O}_{2\text{cc}} = 5,19 \pm 0,11 \text{ ml}$, $\text{HE} = 16,80 \pm 1,11 \text{ ml/l}$, $\text{HR} = 75,69 \pm 2,24$ beats

per minute. The low efficiency of gas exchange led to the development of metabolic acidosis: $\text{pH} = 7,32 \pm 0,02$, $\text{BE} = -5,54 \pm 0,04 \text{ mmol} \cdot \text{l}^{-1}$.

The total volume of OC was above $400 \text{ ml} \cdot \text{min}^{-1}$ in the 2-C subgroup of patients before INHT. The high intensity of OC combined with a substantial increase in expired

Table 2. Oxygen consumption and cardio-respiratory indices of oxygen supply in subgroup of patients with a high risk of COPD before (I) and after (II) INHT in combination with conventional therapy

Characteristics	Stages of research	Subgroup of patients		
		2-A (n=33)	2-B (n=54)	2-C (n=16)
$\text{VO}_2, \text{ml} \cdot \text{min}^{-1}$	I	$298,75 \pm 5,31$	$216,92 \pm 5,62$	$412,50 \pm 13,59$
	II	$309,98 \pm 19,86$	$266,15 \pm 8,93^{**}$	$362,50 \pm 18,68^*$
$\text{VO}_2\text{I}, \text{ml} \cdot \text{min}^{-1} \cdot \text{m}^2$	I	$163,19 \pm 12,80$	$119,62 \pm 10,60$	$222,91 \pm 10,12$
	II	$169,40 \pm 13,59$	$147,01 \pm 11,70^*$	$195,71 \pm 13,82^*$
$\text{O}_{2\text{rc}}, \text{ml}$	I	$20,12 \pm 1,25$	$13,88 \pm 1,04$	$23,91 \pm 2,35$
	II	$20,60 \pm 1,82$	$18,05 \pm 1,36^*$	$24,94 \pm 5,04$
$\text{O}_{2\text{cc}}, \text{ml}$	I	$3,56 \pm 0,15$	$5,19 \pm 0,11$	$4,9 \pm 0,16$
	II	$4,01 \pm 0,19$	$6,19 \pm 1,07^{**}$	$5,11 \pm 0,40$
$\text{VO}_2/\text{V}_e, \text{ml} \cdot \text{l}^{-1}$	I	$27,54 \pm 1,53$	$21,26 \pm 1,69$	$28,89 \pm 3,38$
	II	$27,14 \pm 1,65$	$25,98 \pm 1,59^{**}$	$29,02 \pm 3,41$
V_T, ml	I	$729,87 \pm 46,38$	$691,54 \pm 55,70$	$860,01 \pm 57,69$
	II	$757,44 \pm 75,89$	$653,85 \pm 51,45$	$835,00 \pm 89,90$
f, min^{-1}	I	$15,12 \pm 0,91$	$17,62 \pm 0,19$	$18,00 \pm 1,24$
	II	$15,66 \pm 0,90$	$17,46 \pm 0,85$	$17,00 \pm 1,84$
$\text{V}_e, \text{l} \cdot \text{min}^{-1}$	I	$10,77 \pm 0,57$	$10,97 \pm 0,67$	$15,37 \pm 1,49$
	II	$11,35 \pm 0,60$	$11,05 \pm 0,64$	$13,30 \pm 1,12$
VE	I	$36,19 \pm 1,96$	$52,58 \pm 3,65$	$37,77 \pm 4,03$
	II	$37,74 \pm 2,14$	$42,28 \pm 2,62^*$	$37,82 \pm 4,25$
$\text{HR}, \text{min}^{-1}$	I	$82,94 \pm 2,58$	$75,69 \pm 2,24$	$84,75 \pm 3,48$
	II	$78,31 \pm 3,09^*$	$73,85 \pm 1,90$	$72,50 \pm 3,42^*$
HE	I	$12,41 \pm 1,10$	$16,80 \pm 1,11$	$9,80 \pm 1,12$
	II	$12,20 \pm 1,11$	$13,55 \pm 0,80^*$	$10,61 \pm 1,62$
Q, ml	I	$45,20 \pm 3,40$	$43,90 \pm 2,01$	$46,8 \pm 3,90$
	II	$48,95 \pm 4,3$	$42,41 \pm 1,80$	$49,70 \pm 3,70$
$\text{Q}, \text{ml} \cdot \text{min}^{-1}$	I	$3705,11 \pm 308,50$	$3525,01 \pm 216,49$	$3920,10 \pm 332,81$
	II	$3864,81 \pm 412,91$	$3479,00 \pm 169,90$	$3973,54 \pm 493,90$
pH	I	$7,36 \pm 0,03$	$7,32 \pm 0,02$	$7,40 \pm 0,02$
	II	$7,41 \pm 0,02$	$7,37 \pm 0,01^*$	$7,44 \pm 0,03$
$\text{BE}, \text{mmol} \cdot \text{l}^{-1}$	I	$-1,22 \pm 0,05$	$-5,54 \pm 0,04$	$+1,03 \pm 0,06$
	II	$+0,50 \pm 0,03^*$	$-0,81 \pm 0,03^*$	$+2,01 \pm 0,03^*$

volume ($V_e=15,37\pm1,49$ l·min⁻¹), tidal volume ($V_T=860,01\pm57,69$ ml) and heart rate ($HR=84,75\pm3,48$ beats per minute). A high level of oxygen consumption could be due to increasing of respiration and circulation work but their functional efficiency for oxygen supply still remained high enough. It was indicated by such characteristics as VO_2/V_e , O_{2rc} , VE , O_{2cc} and HE .

Analysis of the research data showed the specific patterns in changing of the total oxygen consumption and the efficiency indices of respiratory and hemodynamic function. These patterns were subjected to correlation analysis and calculation of the bivariate correlation coefficients (r). A positive correlation coefficients were found between VO_2 and V_e ($r=0,405$, $p<0,05$), HR ($r=0,376$, $p<0,05$), V_T ($r=0,341$, $p<0,05$), Q ($r=0,342$, $p<0,05$) in patient subgroup 2-A. Less strong positive correlation coefficients were found between VO_2 and BE ($r=0,356$, $p<0,05$), pH ($r=0,305$, $p<0,05$), Q ($r=0,302$, $p<0,05$) in patient subgroup 2-B. Different magnitudes of the correlation coefficients were found between VO_2 and V_T ($r=0,506$, $p<0,05$), HR ($r=0,438$, $p<0,05$), VE ($r=0,383$, $p<0,05$), pH ($r=0,372$, $p<0,05$) and Q ($r=-0,340$, $p<0,05$) in patient subgroup 2-C.

The volume and intensity of OC and efficiency indices of respiratory function did not significantly change in the 2-A subgroup of patients after INHT. However, the functional efficiency of hemodynamic improved mainly due to decreasing in HR from $82,94\pm2,58$ to $78,31\pm3,09$ beats per minute. The total volume of OC increased by $49,23\pm8,20$ ml·min⁻¹ ($p<0,05$) in the 2-B subgroup of patients. The metabolic acidosis severity decreased due to more effective function of respiratory and hemodynamic mechanisms of oxygen supply. The VO_2/V_e index reached up to $25,98\pm1,59$ ml·l⁻¹ ($p<0,001$) and VE decreased up to $42,28\pm2,62$ ($p<0,001$). Oxygen cost of respiratory cycle increased by $4,17\pm1,03$ ml ($p<0,001$), and cardiac cycle - by $1,00\pm0,27$ ml ($p<0,05$). Amount of oxygen, which is extracted from 1 liter of blood

reached up to $13,55\pm0,80$ ml ($p<0,05$). The total oxygen consumption decreased by $50,00\pm20,71$ ml·min⁻¹ ($p<0,05$) in the 2-C subgroup of patients after INHT. This effect may be associated with decreasing of respiratory and hemodynamic work: V_e reduced by $2,07\pm0,79$ l ($p<0,05$) and HR - by $12,25\pm3,80$ ($p<0,05$) beats per minute. The relative decreasing in OC remained within the physiological limits and did not reduce the efficiency of respiratory and hemodynamic mechanisms of oxygen supply.

It is known that respiratory and hemodynamic mechanisms of human adaptation to mountain climate are closely interrelated and interdependent. However, it does not exclude the dominant role of one of them, especially in patients with pre-existing defects in one of the oxygen supply systems. The total oxygen consumption in patients with chronic pulmonary diseases has been studied for a long time. It is found that the nature and extent of changes in gas exchange is determined by many factors. The most important of these factors are the state of the lung's ventilation and hemodynamic [11, 16]. Changes of total oxygen consumption are absent in patients with the initial stages of COPD, although the functional efficiency of oxygen supply mechanisms may be decreased. These disorders become evident when the initial symptoms of airway obstruction and respiratory failure appeared. The total oxygen consumption is significantly decreased in patients with severe degree of respiratory failure. It is accompanied by the development of tissue hypoxia and metabolic acidosis.

The results of our research showed that pattern and degree of changes in OC were caused by INHT depended on the initial level of VO_2 and functional state of respiration and hemodynamics in patients with a high risk of COPD. The total volume of oxygen consumption and oxygen consumption index were at an adequate level in the 2-A subgroup of patient before INHT. The course of INHT mobilized reserves and improved the functional efficiency of hemodynamic mechanisms of

oxygen supply in these patients. Oxygen consumption was at lower level and symptoms of metabolic acidosis were present in the 2-B subgroup of patients. But the current level of the hemodynamic mechanisms efficiency prevented the development of more serious violations. INHT improved the functional efficiency of respiratory mechanisms of oxygen supply in these patients but the basic hemodynamic parameters remained stable. INHT reduced the functional overloading of cardio-respiratory system in the 2-C subgroup of patients. The breath frequency and tidal volume were decreased up to the level of physiological norm. Optimization of the total oxygen consumption was achieved by decreasing of the energy cost for work of respiration and hemodynamics.

Thus INHT normalized the total oxygen consumption in patients with a high risk of COPD. This effect was connected with moderate activation of OC and improvement of the functional efficiency of respiratory and hemodynamic mechanisms of oxygen supply. The pattern and degree of changes in total oxygen consumption were caused by INHT depended on the initial level of OC and the functional status of the patients' cardio-respiratory system. The results of this study may be used for prediction and evaluation of the therapeutic effect of INH in patients with a high risk of COPD.

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ВПЛИВ ПЕРЕРИВЧАСТОЇ НОРМОБАРИЧНОЇ ГІПОКСІЇ НА СПОЖИВАННЯ КИСНЮ ТА ЕФЕКТИВНІСТЬ РЕСПІРАТОРНИХ ТА ГЕМОДИНАМІЧНИХ МЕХАНІЗМІВ ЗАБЕЗПЕЧЕННЯ ОРГАНІЗМУ КИСНЕМ У ХВОРИХ З ВИСОКИМ РИЗИКОМ РОЗВИТКУ ХРОНІЧНОЇ ОБСТРУКТИВНОЇ ХВОРОБИ ЛЕГЕНЬ

Вплив переривчастої нормобаричної гіпокситерапії (ПНГТ) на загальне споживання кисню (СК) та ефективність респіраторних та гемодинамічних механізмів забезпечення організму киснем вивчали у хворих з високим ризиком розвитку хронічної обструктивної хвороби легень (ХОЗЛ). 1-а група (n=43) пацієнтів отримала тільки стандартну терапію. 2-а група (n=103) отримала таке ж

лікування у поєднанні з ПНГТ. Двотижневий курс ПНГТ складався з щоденних 90-хвилинних сеансів лікування у камері "Оротрон". Протягом усього сеансу PO_2 у середині камери підтримувався на рівні 147-160 гПа. Зміни СК у пацієнтів після курсу ПНГТ були неоднорідні як по амплітуді, так і за спрямованістю. Встановлено, що характер і ступінь змін у окремих пацієнтів залежали від початкового рівня СК і функційного стану стану кардіо-респіраторної системи. У зв'язку з цим, основну групу пацієнтів було розділено на 3 підгрупи: 2-А - 33% пацієнтів мали нормальні значення СК, 2-В - 53% пацієнтів мали знижене СК і 2-С - 14% пацієнтів мали високе СК. ПНГТ суттєво не змінювала СК, але підвищувала ефективність гемодинамічних механізмів забезпечення організму киснем у пацієнтів 2-А підгрупи. СК та ефективність респіраторних механізмів доставки кисню помірно підвищувались у пацієнтів 2-В підгрупи. Величина СК наближалася до рівня фізіологічної норми у пацієнтів 2-С підгрупи, але ефективність респіраторних і гемодинамічних механізмів доставки кисню залишалася відносно високою. Таким чином, ПНГТ оптимізує споживання кисню у пацієнтів з хронічними легеневиими захворюваннями, підвищує ефективність респіраторних та гемодинамічних механізмів забезпечення організму киснем.

Ключові слова: переривчаста нормобарична гіпоксія, хронічне обструктивне захворювання легень.

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ВЛИЯНИЕ ПЕРЕРЫВИСТОЙ НОРМОБАРИЧЕСКОЙ ГИПОКСИИ НА ПОТРЕБЛЕНИЕ КИСЛОРОДА И ЭФФЕКТИВНОСТЬ РЕСПИРАТОРНЫХ И ГЕМОДИНАМИЧЕСКИХ МЕХАНИЗМОВ ОБЕСПЕЧЕНИЯ ОРГАНИЗМА КИСЛОРОДОМ У БОЛЬНЫХ С ВЫСОКИМ РИСКОМ РАЗВИТИЯ ХРОНИЧЕСКОЙ ОБСТРУКТИВНОЙ БОЛЕЗНИ ЛЕГКИХ

Влияние прерывистой нормобарической гипокситерапии (ПНГТ) на общее потребление кислорода (ПК) и эффективность респираторных и гемодинамических механизмов обеспечения организма кислородом изучали у больных с высоким риском развития хронической обструктивной болезни легких (ХОБЛ). 1-я группа (n=43) пациентов получила только стандартную терапию. 2-я группа (n=103) получила такое же лечение в сочетании с ПНГТ. Двухнедельный курс ПНГТ состоял из ежедневных 90-минутных сеансов лечения в камере "Оротрон". На протяжении всего сеанса PO_2 внутри камеры поддерживалось на уровне 147-160 гПа. Изменения ПК у пациентов после курса ПНГТ были неоднородны как по амплитуде, так и по направленности. Установлено, что характер и степень изменений у отдельных пациентов зависели от исходного уровня ПК и функционального состояния кардио-респираторной системы. В связи с этим, основная группа пациентов была

разделена на 3 подгруппы: 2-А - 33% пациентов имели нормальные значения ПК, 2-В - 53% пациентов имели сниженное ПК и 2-С - 14% пациентов имели высокое ПК. ПНГТ существенно не изменяла ПК, но повышала эффективность гемодинамических механизмов обеспечения организма кислородом у пациентов 2-А подгруппы. ПК и эффективность респираторных механизмов доставки кислорода умеренно повышались у пациентов 2-В подгруппы. Величина ПК приближалась к уровню физиологической нормы у пациентов 2-С подгруппы, но эффективность респираторных и гемодинамических механизмов доставки кислорода оставалась относительно высокой. Таким образом, ПНГТ оптимизирует потребление кислорода у пациентов с хроническими легочными заболеваниями, повышает эффективность респираторных и гемодинамических механизмов обеспечения организма кислородом.

Ключевые слова: прерывистая нормобарическая гипоксия, хроническая обструктивная болезнь легких.

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Tolerance to hypoxia

People living at sea level have poor tolerance to hypoxia. In striking contrast, humans experiencing hypoxia at high altitude live very well. How is it possible for man to tolerate extreme hypoxia at high altitude? In this article we propose a hypothesis that potentially explains the tolerance to hypoxia at high altitude. Close examination of values of hemoglobin and PaCO₂ for an altitude of 3510 m demonstrate that an increase in hemoglobin (Hb) and a decrease in arterial carbon dioxide tension (PaCO₂) are two essential changes that occur on high altitude exposure. We propose a formula :

$$\text{Tolerance to Hypoxia} = \frac{Hb}{PaCO_2} \cdot 3.01$$

and present evidence that the relationship between Hb and PaCO₂ explains the tolerance to hypoxia at high altitude.

INTRODUCTION

People living at high altitude in the city of La Paz 3600m, with diverse types of lung disease exhibit extremely low arterial oxygen tensions (PaO₂). Their arterial oxygen partial pressure of oxygen (PaO₂) can range between 30 and 40 mmHg (Normal values: PaO₂ = 60 ± 2 mmHg, arterial carbon dioxide tension (PaCO₂) = 30 ± 2 mmHg and pH = 7.40 ± 0.02, Oxyhemoglobin saturation by pulse oximetry (SpO₂) are 91% ± 1%). The SpO₂ oscillates with irregular breathing and taking a deep breath can reach even 98% (like at sea level) provided there is a normal pulmonary function, as previously described [1]. This results from a decrease of the ratio between pulmonary dead space and alveolar ventilation. When the medical reports from people in La Paz were shown to physicians at sea level, they often asked: "Were these people conscious?" This clearly shows that people at sea level can not tolerate such low arterial PO₂. A patient presenting a PaO₂ below 60 mmHg at the sea level is usually sent to an intensive care unit, as his life could be in peril.

Around one out of four subjects, arriving to La Paz, have some form of Acute Mountain Sickness. Some can present extreme hypoxia. The pathologies associated with extreme hypoxia are: pneumonia upon ascent, pulmonary

thrombo-embolism, high altitude pulmonary edema, high altitude cerebral edema and several others.

For example, a 25 years old Frenchman climbed Huayna Potosi at 6088m two days after arriving to La Paz 3600 m, from Paris. On the way down he felt very short of breath and unable to sleep overnight. He came to consultation as an ambulatory patient. Blood gas analyses revealed a PaO₂ = 35 mmHg, PaCO₂ = 29 mmHg and a pH = 7.53. This was clearly a case of severe hypoxia with respiratory alkalosis and High Altitude Pulmonary Edema (HAPE). Would he have been alive if at sea level? The purpose of this article is to present potential mechanisms underlying adaptation at high altitude.

High Altitude Adaptation Index.

Adaptation to high altitude is dependent on altitude and the duration of sojourn (time). The relation between altitude and duration as a function of adaptation to altitude can be expressed as the following equation [2]:

$$\text{Adaptation to high altitude} = \frac{\text{time}}{\text{altitude}},$$

where time at altitude is in days and altitude is in kilometers (km).

A complete and optimal hematocrit and hemoglobin adaptation is only achieved at around 40 days for a subject going from sea

level to 3,510m in La Paz. It has been estimated that the time in days required to achieve full adaptation to any altitude, ascending from sea level, can be calculated by multiplying the adaptation factor of 11.4 times the altitude in kilometers. This factor was obtained based on the time of full hematological adaptation to 3510m when arriving from sea level. Conversely, descending from high altitude in La Paz to sea level in Copenhagen (35 m above sea level), the hematocrit response is a linear fall over 18 to 23 days [2,4].

Importance of Arterial PCO_2 .

The other variable that can allow for the tolerance to extremely low PaO_2 values is the low PaCO_2 values at high altitude relative to sea level. As is well known, oxygen and carbon dioxide partial pressures descend as the altitude increases. In Fig 1, the distribution of

PaCO_2 in arterial blood gases at the city of La Paz (3510m) shows clearly that the average is around 30 mmHg. Furthermore, it can be noted that the highest PaCO_2 reached is 72 mmHg in an isolated critically terminally ill patient. The great majority hardly reach a PaCO_2 above 53 mmHg. Fig 2. shows the relationship between PaCO_2 and altitude. At high altitude, high PaCO_2 levels as those seen at sea level are not compatible with life.

Hypocapnia and the ensuing alkaline pH during high altitude exposure shifts the oxygen dissociation curve to the left, allowing more capture and transport of oxygen. The Acid-Base balance in the human body is calculated by the Van Slyke equation based on sea level measurements. The maintenance of blood pH within a fairly strict range at around pH 7.4, with due consideration of the effect of hyperventilation, is essential for cellular function at any altitude. This is

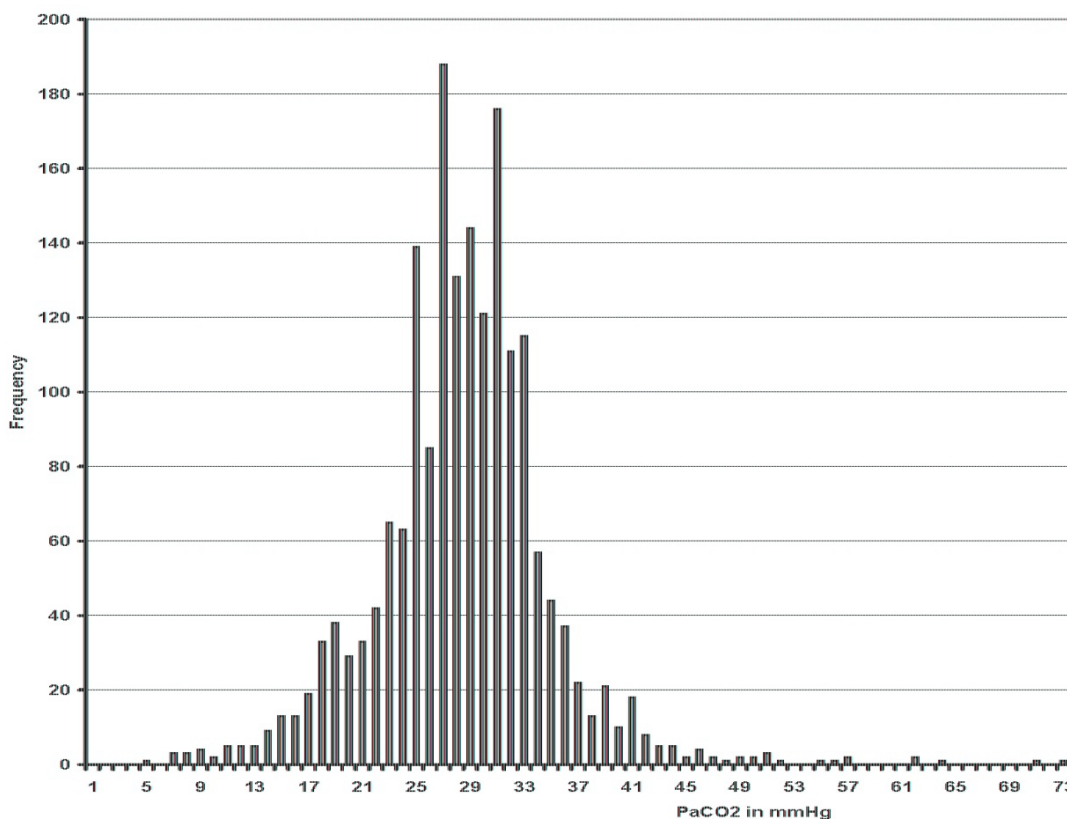


Fig 1. Distribution curve of the P_aCO_2 in 1865 patients including both sexes at 3510 m [4]

because various chemical processes occurring in the body, e.g., those involving proteins and enzymes, are pH-dependent. With chronic low levels of the arterial carbon dioxide partial pressure (PaCO_2), the acid-base balance begins to change. Mountaineering physiologists, employed the sea level equation for estimations of acid-base balance in high altitude subjects, without critical appraisal of its validity. The pH effects are inextricably linked critically with hemoglobin and oxygen status that can be crucial at high altitudes. Therefore, for a more precise recalculation of the 'Titratable Hydrogen Ion Difference' (THID), that should use a Hb and HCO_3^- values for a particular altitude, we have derived our modified Van Slyke equation: THID in eECF = $(1 - [\text{Hb}] / 43) \times (\Delta[\text{HCO}_3^-] + \beta \text{B} \times (\text{pH} - 7.4))$ [5]. An adequate Acid-Base Balance is probably the fundamental metabolic adaptation that allows for mountaineers to tolerate extreme hypoxia and even reach the summit of Mt. Everest [26].

Hemoglobin.

Hemoglobin increases with altitude as shown in Fig.3. This data is from permanent residents at different altitude cities or towns. From the trend curve one can attempt to calculate the optimal hemoglobin value upon the summit of Mt. Everest, if complete adaptation could be achieved.

The Hb values for residents at 2500m, 3600m and 4100m for the Bolivia cities of Cochabamba, La Paz and El Alto, respectively are the normal values in our labs. The Hb values of residents at 4355m, 4660m and 5500m were obtained from bibliographic data [25, 26].

It becomes evident from the observation of a longer breath holding time at high altitude in polyerythrocythemia (PEC) patients with low PaO_2 , compared to normal high altitude subjects, that they are more tolerant to hypoxia [6]. The greater oxygen content of blood in PEC, as a result of the increase in Hb, allows for a better tolerance to hypoxia. This is also demonstrated by plotting the oxygen consumption of yeast cells against time after full saturation with 100% oxygen [7]. The resulting oxygen dissociation curve of PEC patients has a much broader oxygen content area when exposed to 100% oxygen as shown in Fig 4.

The above observations on PaCO_2 and Hb prompted us to propose the hypothesis that the Tolerance to Hypoxia (TH) formula can be defined as:

$$\text{Tolerance to Hypoxia} = \frac{\text{Hb}}{\text{PaCO}_2} \cdot 3.01$$

The constant factor is obtained by using the Hb and PaCO_2 normal sea level values and equating to 1. This way, the tolerance to hypoxia

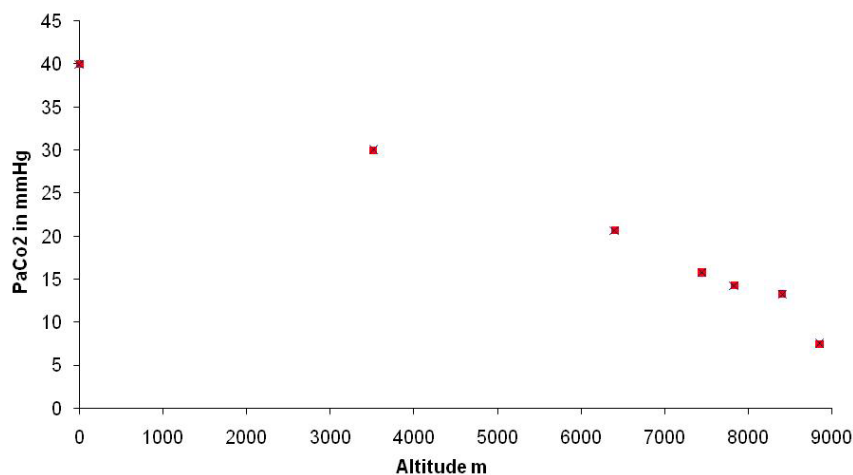


Fig. 2 PaCO_2 , in its relationship to altitude from data obtained at different altitude sites around the world ⁴. At 8400 the PaCO_2 is from ⁸. The PaCO_2 value at 8848 m is obtained from an estimated value ²⁴

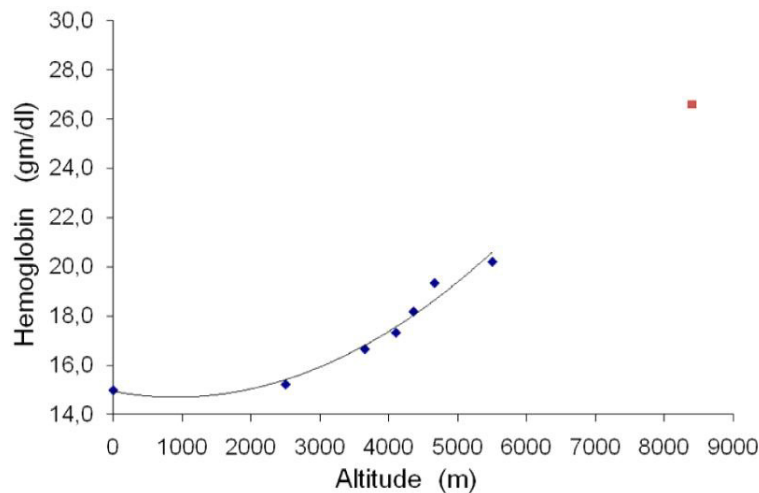


Fig. 3. Hemoglobin values found at different altitudes in resident populations. The red dot is the calculated Hb from the trend equation: $Hb = 2E-07x^2 - 0,0003x + 14,45$ with an $R = 0.9718$

for comparison purposes becomes 1 at sea level. From then on the values at different altitudes are calculated as shown in table 1.

In this table the Mt. Everest value is obtained from the paper by Grocott et.al⁸ taken on the Cauldwell Expedition to Mt. Everest. Blood gases were measured at 8400m and the Hb values were obtained from 4 subjects averaging the measurements at 5300m before and after the climb to the summit. This value should have been measured at higher altitudes and without averaging before and after climb as it reduces the true value. Only the value obtained after the ascent could be a more appropriate measure. However since the subjects were climbing and changing altitudes and the body has a fixed production of red blood cells, there was not enough time for full hematologic adaptation². An optimal hemoglobin value for the summit of Everest is not known. However,

based on Fig 2, it is estimated that the optimal hemoglobin for oxygen transport at the summit of Mt. Everest would be around 26 gm%. This roughly corresponds to a hematocrit of 78%. In our medical practice, we have seen patients with PEC even above 80%⁹⁻¹¹. Hence, although it seems that these are surprising high values, they are within biological limits for humans. It is noteworthy to mention that the maximum possible increase of Hb is double the sea level value, hence the tolerance to hypoxia formula is also ruled by this.

Our formula links Hb with $PaCO_2$, the hematological and respiratory (including acid-base parameter) responses to hypoxia, respectively. These biological responses to hypoxia in humans will also apply to animals. This is clearly demonstrated when a normal average sea level Hb in males of 13.3 gm% rises to an average of 16.6 gm% in the city of La

Table 1. Tolerance to hypoxia calculated at different altitudes from the Hypoxia Tolerance formula.
The values of Hb and $PaCO_2$ obtained from [2, 8].

	Altitude in m	Hb/ $PaCO_2$	HT
Sea Level	0	13/40 * 3.01	1
La Paz, Bolivia	3510	16.6/30 * 3.01	1.7
Mt. Everest	8842	21/13 * 3.01	4.86

Paz, Bolivia at 3510m of altitude, for example. The formula also includes the PaCO_2 where it is showing the hyperventilatory response to hypoxia. For example, a sea level normal PaCO_2 of 40 mmHg upon arrival to high altitude immediately is decreased to a PaCO_2 of 30 mmHg, again, in the city of La Paz (3510m). Although this is a tolerance to hypoxia formula, the PIO_2 is not included in the formula as it is directly related to the barometric pressure, as originally described by Paul Bert in 1878¹². The Barometric Pressure, a physical atmospheric parameter is indirectly included with the PaCO_2 which is equal to the $\text{FaCO}_2 \cdot \text{PB} - \text{PH}_2\text{O}$.

Other factors of adaptation to hypoxia, such as HIF, VEGF, increase in the density of mitochondria, increase in capillary density, increase in pulmonary artery pressure, increase in heart rate and ventilatory rate, etc are not included in this formula, as they would be automatically linked to the two biologic factors included. Adaptation to hypoxia is complex but this formula uses two essential variables

and in our criteria sufficient for the adequate interpretation on the concept.

Furthermore, upon initial ascent, the changes in the PaCO_2 are immediate, being linked to an increase in the heart rate and hyperventilation. However, as the hemoglobin gradually increases at high altitude, closely linked to the erythropoietin stimulus secondary to hypoxia, the cardio-respiratory initial responses also gradually decrease in search of a less energy consuming adaptation that allows for an efficient and effective energy-cost mechanism such as the increase in hemoglobin [13]. Noteworthy is the fact that the tolerance to hypoxia when first arriving to high altitude is not optimal until the hematocrit reaches its maximum level [2]. Hence it can be appreciated that the numerator is the factor that increases tolerance to hypoxia with time at high altitude. The denominator of the Tolerance to Hypoxia formula is fixed upon arrival to high altitude and will not change during the residence at the same altitude, provided the subject is healthy. So it is not

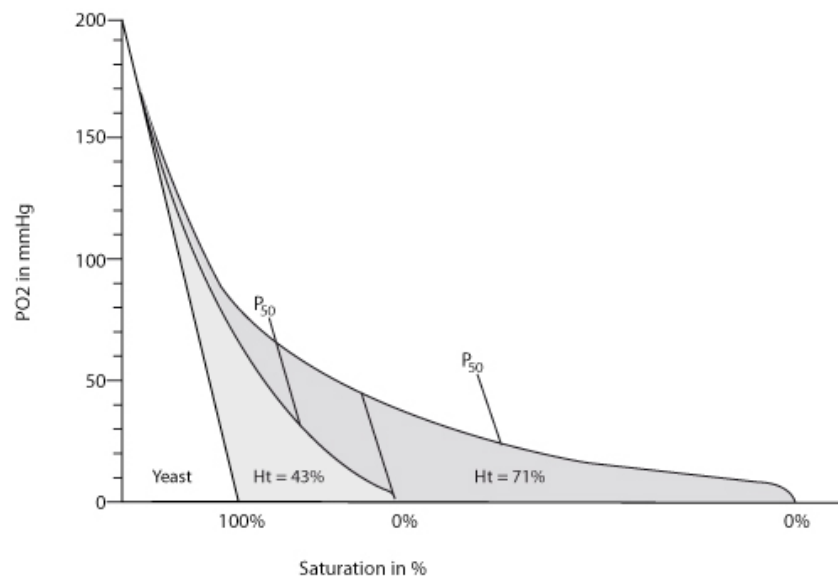


Fig 4. The oxyhemoglobin dissociation curve (ODC) of a normal high altitude resident in La Paz, Bolivia 3510m (Ht = 43%) as compared to a patient with polycythemia (Ht = 71%) using the Ryan Neville yeast cells oxygen consumption technique [7]. The ODCs can best be viewed by turning the graph 90° clockwise. The Yeast area (on the left) is the oxygen consumed by yeast cells alone. The Ht = 43% area (on the middle in light grey) corresponds to the oxygen content of a normal high altitude resident with a hematocrit of 43%. The Ht = 71% area (on the right in dark grey) plus the middle area, correspond to the total oxygen content in a polycythemic patient with a hematocrit of 71%

time dependant, but rather barometric pressure and hyperventilation dependant. The initial hyperventilation upon acute exposure to high altitude lowers the PaCO_2 however there are minor changes as time allows for the Hb increase and gradual reduction of hyperventilation.

On an autocritical side, tolerance to hypoxia cannot be oversimplified as it is very complex. In *Drosophila* flies it has been demonstrated that following long term exposure to hypoxia, adaptation through gene expression has allowed for survival even during several hours in anoxia [14]. For improving the tolerance to hypoxia, it has been found that Acetyl Salicylic Acid is neuroprotective against hypoxic hypoxia and chemical hypoxia and delays the decline of intracellular ATP content [15]. Nitrate can be converted to nitrite and nitric oxide, that can improve muscle efficiency and also dilate blood vessels allowing more O_2 to be delivered to active muscle [16]. The ability of cells to tolerate hypoxia is critical to their survival, but varies greatly among different cell types. Pulmonary arterial endothelial cells (PAEC) retain their viability and cellular integrity during hypoxic exposure, whereas renal tubular epithelial cells are extremely hypoxia sensitive and are rapidly and irreversibly damaged. The maintenance of high energy phosphates in hypoxic PAEC suggests that there exists tight regulation of ATP and GTP turnover in these cells and that preservation of these nucleotides may contribute to the tolerance of PAEC to acute and chronic hypoxia [17]. On exposure to hypoxia, biochemical behaviour of skeletal muscles was consistent with lowered reliance on glycolytic contributions to energy supply, thus improving the yield of ATP per mole of carbon fuel utilized. Using glucose in preference to fatty acids, heart adaptations also seemed to rely upon stoichiometric efficiency adjustments, improving the yield of ATP per mole of oxygen consumed [18]. Hemoglobin can also have evolutionary adjustments in Hb- O_2 affinity through alterations of the equilibrium constants of O_2 binding to deoxy- and oxyHb as shown in different vertebrates [19].

Finally, it is well known that in intensive

care units (ICU) at sea level some people tolerate hypoxia better than others. This is not clearly understood, but this tolerance to hypoxia formula may contribute significantly. Some ICU could treat selected patients by increasing the Hb level, above the normal expected for sea level. This would increase their tolerance by increasing the numerator of the formula. Of course, this requires a most careful and suitable transfusion and paying attention to the coagulation factors in order to avoid complications. Another interesting possibility would be to use hypobaric chambers in the ICU, thereby reducing the barometric pressure, but of course maintaining the hyperoxia in ventilators. Such a procedure would decrease the PaCO_2 , the denominator in the formula, further boosting tolerance to hypoxia. Following the same logic, an alternative would be to ventilate subjects trying to achieve a lower PaCO_2 , but maintaining the pH efficiently. These hypothetical proposals obviously need to be fully and extensively studied before their practical application.

CONCLUSIONS

The importance of presenting this concept of higher tolerance to hypoxia based on the PaCO_2 value rather than on the PaO_2 value allows a further understanding of the blunted respiratory drive at high altitude originally observed by S. Lahiri [20]. The gaseous messengers in oxygen sensing have recently gained importance [21]. Focusing the attention not only on the oxygen levels but essentially on the CO_2 levels, can help better understand the observed phenomena. It is also possible that it could contribute in understanding ventilatory drive during hypoxia of the complex neuronal respiratory centers [22]. Nature is wise in allowing for the sustaining of life and giving advantages in hypoxia tolerance, the higher one goes. This new concept of greater tolerance to hypoxia at high altitude further contributes to the understanding of the hypothesis of the extraordinary adaptation of man to extreme hypoxia, including the summit of Mt. Everest [23].

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СТІЙКІСТЬ ДО ГІПОКСІЇ

Люди, що проживають на рівнині, мають низьку стійкість до гіпоксії. На противагу до цього, люди, що проживають на високогір'ї, почувають себе дуже добре. Як можливо переносити екстремальну гіпоксію на великих висотах? В цій статті ми пропонуємо гіпотезу, що потенційно пояснює стійкість до гіпоксії на висотах. Реєстрація величин концентрації гемоглобіну (Hb) та PaCO_2 на висоті 3510 м демонструє, що саме підвищення Hb та зниження PaCO_2 є двома суттєвими змінами, що мають місце при підйомі на висоту. Ми пропонуємо формулу: стійкість до гіпоксії = $\text{Hb}/\text{PaCO}_2 \times 3.01$ і приводимо докази, що залежність між Hb та PaCO_2 пояснює стійкість до гіпоксії на висоті. Ключові слова: PaCO_2 , гемоглобін, високогір'я

Густаво Р. Зубиєта-Каллейя, Густаво Ардайя, Наталія Зубиєта-де Урос, Поул-Ерік Паулев и Густаво Зубиєта-Кастилло.

УСТОЙЧИВОСТЬ К ГИПОКСИИ

Люди, проживающие на равнине, имеют низкую устойчивость к гипоксии. В противоположность этому, люди, проживающие высоко в горах, чувствуют себя очень хорошо. Как можно переносить экстремальную гипоксию на больших высотах? В этой статье мы излагаем гипотезу, которая объясняет устойчивость к гипоксии на высотах. Регистрация величин концентрации гемоглобина (Hb) и PaCO_2 на высоте 3510 м показывает, что именно повышение Hb и снижение PaCO_2 являются двумя существенными изменениями, которые имеют место при подъеме на высоту. Мы предлагаем формулу: устойчивость к гипоксии = $\text{Hb}/\text{PaCO}_2 \times 3.01$ и приводим доказательства, что зависимость между Hb и PaCO_2 объясняет устойчивость к гипоксии на высоте. Ключевые слова: PaCO_2 , гемоглобин, высокогорье.

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Сравнительная оценка устойчивости инспираторных мышц к утомлению при моделируемой обструкции дыхательных путей

Проведение спектрального анализа ЭМГ разных групп дыхательных мышц показало, что после выполнения мышечной работы в сочетании с добавочным сопротивлением дыханию одновременно со снижением максимального инспираторного давления (МІР) наблюдается сдвиг центроидной частоты ЭМГ парастеральных, лестничных и грудино-ключично-сосцевидных мышц в низкую часть спектра. Изменений в спектре ЭМГ диафрагмы не отмечается. Таким образом, снижение общего силового резерва дыхательных мышц, наблюдаемое после усиленной нагрузки на дыхательную систему, обусловлено ухудшением функционального состояния не диафрагмы, а торакальных и вспомогательных инспираторных мышц. Диафрагма более устойчива к утомлению. Полученные данные позволяют предположить, что снижение максимального инспираторного давления при хронических обструктивных болезнях легких также вызвано прежде всего ослаблением вспомогательной мускулатуры, тогда как слабость диафрагмы может развиваться на более поздних стадиях заболевания. При этом функциональная недостаточность вспомогательных мышц является дополнительным фактором, который наряду с добавочным сопротивлением дыханию увеличивает нагрузку на диафрагму, способствуя развитию ее утомления и снижению резервов дыхательной системы.

Ключевые слова: инспираторные мышцы, добавочное сопротивление, электромиография, утомление, мышечная нагрузка

ВСТУПЛЕНИЕ

Современными исследованиями установлено, что существенная роль в генезе вентиляционных расстройств у больных хронической обструктивной болезнью легких (ХОБЛ) принадлежит не только внутрилегочным механизмам, но и дисфункции дыхательных мышц (Polkey, Mokham, 2001 et al.). Известно, что при ХОБЛ значительно увеличивается нагрузка на моторный аппарат респираторной системы вследствие роста бронхиального сопротивления. Для того, чтобы компенсировать увеличенную нагрузку и сохранить адекватный уровень минутной вентиляции легких инспираторные мышцы вынуждены развивать большее усилие для создания соответствующего отрицательного давления в грудной полости. Длительно действующая повышенная резистивная нагрузка на систе-

му дыхания может способствовать развитию утомления дыхательных мышц, в результате чего возможно возникновение симптомов дыхательной недостаточности, сопровождающихся нарушениями газового состава крови. Установлено, что утомление дыхательных мышц может развиваться у здоровых людей при сочетании добавочного сопротивления дыханию с мышечной работой (Сегизбаева, Александрова, 2009). В том случае, когда действие добавочного сопротивления сочетается с необходимостью выполнения физической работы, когда повышаются вентиляторные потребности организма, нагрузка на дыхательные мышцы возрастает многократно. Это может неблагоприятно отразиться на их функциональном состоянии, способствовать снижению резервных возможностей и приводить к постепенному развитию утомления. Для того, чтобы оценить функциональное

состояние инспираторных мышц при сочетании добавочного сопротивления дыханию и мышечной нагрузки, а также провести сравнительную оценку устойчивости различных инспираторных мышц человека к утомлению была выполнена данная работа.

МЕТОДИКА

В исследовании принимали участие шесть испытуемых (2 юноши и 4 девушки). Все участники были осмотрены врачом-пульмонологом, не имели в анамнезе бронхо-легочных, сердечно-сосудистых и нейро-мышечных заболеваний и не были подвержены острым респираторным вирусным инфекциям в течение предшествующих 2 месяцев. Все обследуемые были подробно проинформированы о применяемых методиках, последовательности проведения исследований и дали письменное согласие на участие в них. Эксперименты проводили в соответствии с принципами Хельсинской Декларации о проведении исследований с участием человека и с соблюдением основных норм и правил биомедицинской этики. Все приглашенные к участию в исследованиях испытуемые были одного возраста, имели сходные антропометрические данные и соответствующие росту и весу спирометрические показатели, а также примерно одинаковый уровень физической подготовленности.

В качестве функциональной пробы, позволяющей оценить силовые резервные возможности дыхательных мышц, использовался маневр Мюллера. Испытуемые выполняли максимальное инспираторное усилие при перекрытых воздухоносных путях от уровня функциональной остаточной емкости (ATS/ERS Statement on Respiratory Muscle Testing, 2002). Измерение максимального инспираторного давления (MIP) производили с помощью портативного сертифицированного прибора PowerBreath KH1 (PowerBreath, UK). Измерения производили после тщательного инструктирования и обучения испытуемого и после 5-8 ознакомительных попыток

воспроизведения инспираторного маневра. Измерения производили не менее 5 раз с перерывами в 1 минуту, в расчет принимали максимальное из достигнутых величин MIP.

Регистрацию электрической активности диафрагмы, парастернальной, грудино-ключично-сосцевидной и лестничной мышц проводили с помощью поверхностных кардиографических электродов (ARBO, Германия). Для регистрации электрической активности диафрагмы электроды устанавливали в 8 и 9 межреберьях справа по ходу передней подмышечной линии. Электрическая активность парастернальных мышц отводилась с помощью пары электродов, расположенной во 2-м межреберье справа на расстоянии 2-3 см от края грудины. Для регистрации активности грудино-ключично-сосцевидной и лестничной мышц электроды накладывались последовательно по ходу расположения волокон мышцы на расстоянии 1 см друг от друга. Электрические сигналы усиливались с помощью усилителя биопотенциалов высокой чувствительности с полосой пропускания от 10 до 1000 Гц (ГУАП, С.-Петербург). Усиленные сигналы с электромиографического усилителя подавались через аналогово-цифровой преобразователь и записывались на жестком диске компьютера с частотой дискретизации 1000 Гц для последующего анализа. Для сравнения устойчивости к утомлению различных инспираторных мышц использовался метод спектрального анализа ЭМГ. Этот метод позволяет определить силу электрического сигнала как функцию от его частотного компонента (Basmajian et al., 1975) и дает возможность выявить тонкие изменения в функциональном состоянии мышц. Для обработки ЭМГ выделялся участок нативной записи, который экспортировался в текстовый файл. После этого текстовый файл обрабатывали с помощью специальной программы, позволяющей разложить оцифрованный сигнал в дискретный ряд Фурье, а затем вычислить мощность спектральной плотности. Для того, чтобы проследить ча-

стотный сдвиг спектра ЭМГ рассчитывалась центроидная частота (f_c) до начала мышечной нагрузки и сразу же после ее прекращения. Центроидная частота является наиболее стабильным параметром, используемым при анализе спектра электрической активности мышц (Schweizer et al., 1979). Смещение центроидной частоты в ту или другую сторону отражает изменение относительного вклада низко- и высокочастотной компоненты в данную полосу спектра. Изменение частотного состава ЭМГ, с перераспределением мощности от более высоких к более низким частотам, используется в качестве критерия ухудшения функционального состояния дыхательных мышц, предшествующего развитию их утомления (Gross et al., 1979).

Каждое исследование проводилось в два этапа. В первой части исследования испытуемые выполняли мышечную нагрузку в условиях свободного дыхания, во второй – на фоне моделируемой обструкции дыхательных путей, которая создавалась путем введения добавочного резистивного сопротивления величиной 40 см вод.ст./л · с⁻¹ в инспираторный канал дыхательного контура. Мышечную нагрузку испытуемые выполняли на механическом тротуаре (угол наклона 30° относительно горизонта) – ходьба в течение 5 минут в режиме 2 шага в секунду. До и после выполнения мышечной нагрузки определяли величину максимального инспираторного давления. В покое и в процессе эксперимента производилась непрерывная регистрация пневмотахограммы (трубка Флейша №5), инспираторного внутримасочного (ротового) давления, электрической активности (ЭМГ) диафрагмы, парастернальной, грудинно-ключично-сосцевидной и лестничной мышц. Сбор экспериментальных данных производился с помощью аппаратно-программного комплекса «Biograph» (ГУАП, Санкт-Петербург) – многоканального устройства для сбора физиологических данных, соединенного с персональным компьютером через высокоскоростной USB 2.0 порт. После окончания эксперимента, полученные записи

просматривались и подвергались дальнейшей количественной обработке. Статистический анализ данных производился с использованием программ Microsoft Excel. Вычислялась средняя величина регистрируемых параметров и ошибка средней. Для сравнения данных применялся параметрический критерий Стьюдента. Различия считали статистически значимыми при $P < 0.05$.

РЕЗУЛЬТАТЫ ИССЛЕДОВАНИЯ И ИХ ОБСУЖДЕНИЕ

Максимальное инспираторное ротовое давление (MIP), развиваемое во время выполнения произвольного инспираторного усилия при перекрытых воздухоносных путях, отражает суммарную силу сокращений всех мышц, участвующих в процессе вдоха. Средняя величина MIP в группе испытуемых юношей составила 99.0 ± 3.0 см вод.ст., в группе девушек – 58.1 ± 9.2 см вод.ст. Средняя величина MIP у всех испытуемых составила 75.6 ± 11.1 см вод.ст. Эти значения MIP соответствовали должным величинам для возраста, роста и веса участников исследования. Величина MIP после мышечной нагрузки с сопротивлением снизилась на 12% по сравнению с фоновыми данными, что может служить одним из признаков снижения функциональных резервных возможностей дыхательных мышц человека и возможного развития их утомления.

Добавочное инспираторное сопротивление вызывало характерные изменения в моторном паттерне дыхания: происходило одновременное увеличение внутримасочного давления и усиление электрической активности диафрагмы, парастернальных, лестничных и грудинно-ключично-сосцевидных мышц. На фоне выполнения мышечной нагрузки как при свободном дыхании, так и, особенно, в условиях резистивного сопротивления, наблюдалась усиленная электрическая активация всех исследуемых мышц. Их инспираторные разряды были хорошо выражены и четко синхронизированы друг с

другом. Такой паттерн электрической активности поддерживался до окончания мышечной нагрузки (Рис.1).

Анализ мощности спектра показал, что в условиях покоя при свободном дыхании центроидная частота спектра ЭМГ диафрагмы располагалась в области 161.7 ± 5.9 Гц, парастеральной мышцы – 222.8 ± 9.0 Гц, грудино-ключично-сосцевидной – 137.7 ± 8.0 Гц, лестничной – 151 ± 7.0 Гц. Среднее значение центроидной частоты ЭМГ диафрагмы, измеренной сразу после выполнения мышечной нагрузки как в условиях свободного дыхания, так и на фоне добавочного сопротивления, сохранялось на уровне фоновых величин (Рис.2). После физической работы с резистивным сопротивлением значения центроидной частоты лестничной мышцы снижались на 16.6% ($P < 0.05$), парастеральной – на 11.7% ($P < 0.05$), грудино-ключично-сосцевидной – на 11.8% ($P < 0.05$) (Рис.2). Таким образом, частотно-амплитудный анализ электромиографической активности исследуемых мышц показал достоверное снижение центроидной частоты парастеральной, лестничной и грудино-ключично-сосцевидной мышц после выполнения мышечной нагрузки на фоне инспираторного резистивного сопротивления. Это отражает перераспределение

мощности спектра от более высоких частот к более низким частотам и свидетельствует об ухудшении функционального состояния мышц, предшествующего развитию утомления и снижению силы мышечного сокращения (Lindstrom et al., 1970). Эти данные позволяют предположить, что вспомогательные инспираторные мышцы человека менее устойчивы к утомлению по сравнению с основной инспираторной мышцей – диафрагмой. Одной из возможных причин сдвига спектра мощности ЭМГ в условиях нагруженного форсированного дыхания может являться уменьшение скорости проведения миопотенциалов в мышечных волокнах (Gross et al., 1979). Замедление скорости проведения является первоначальным признаком метаболических изменений в мышце, связанных с накоплением молочной кислоты, снижением внутриклеточного pH и изменением вследствие этого транспорта K^+ и Ca^{2+} .

Наблюдаемое в наших исследованиях смещение мощности спектра ЭМГ грудных и вспомогательных инспираторных мышц в сторону преобладания низких частот позволяет заключить, что эти мышцы обладают меньшей устойчивостью к утомлению, чем диафрагма. Таким образом, снижение максимальной силы сокращений инспираторной мускулатуры по-

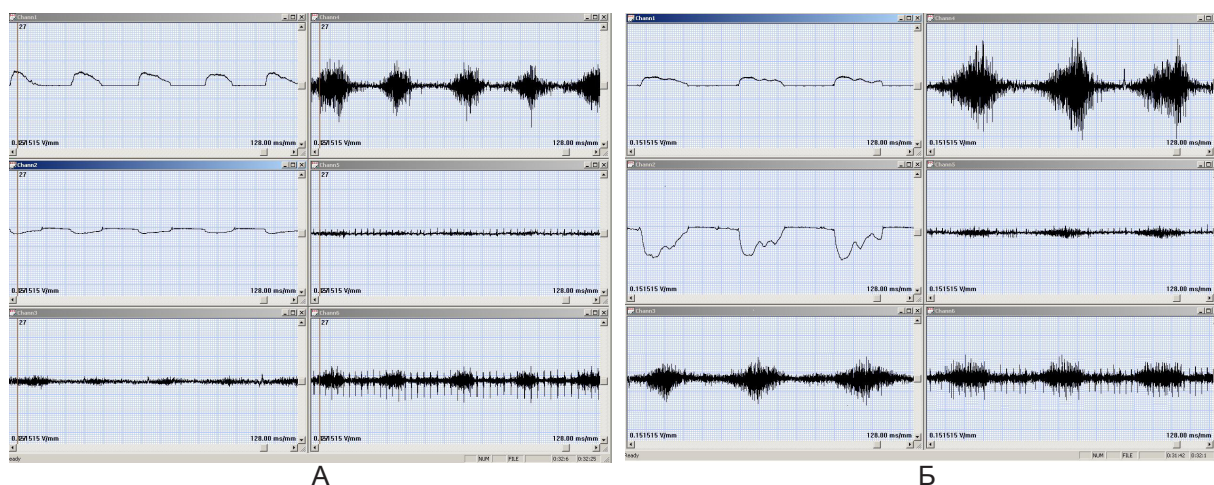


Рис.1 Нативная запись пневмотахограммы, инспираторного ротового давления, электрической активности грудино-ключично-сосцевидной, лестничной, парастеральной мышц и диафрагмы (сверху вниз) при свободном дыхании (А) и на фоне действия добавочного инспираторного сопротивления (Б) в условиях выполнения мышечной нагрузки

сле выполнения мышечной нагрузки в условиях добавочного сопротивления дыханию обусловлено ухудшением функционального состояния парастеральных, лестничных и грудино-ключично-сосцевидных мышц, но не диафрагмы. Диафрагма в меньшей степени подвержена утомлению, чем торакальные и вспомогательные инспираторные мышцы.

Результаты нашего исследования подтверждают данные, полученные ранее на основе измерения максимального эзофагального и трансдиафрагмального давлений до и после выполнения теста с резистивной нагрузкой (Hershenson et al., 1989). Авторы продемонстрировали достоверное снижение величин максимального эзофагального давления у испытуемых после «нагрузочного» теста, тогда как значения максимального трансдиафрагмального давления оставались неизменными. Величина эзофагального давления отражает силу сокращений всех инспираторных мышц, а величина трансдиафрагмального – силу сокращений исключительно диафрагмы. Поэтому снижение максимального инспираторного усилия после теста с добавочным сопротивлением может произойти за счет ослабления силы межреберных и/или иных вспомогательных инспираторных мышц вследствие развития их утомления (Hershenson et al., 1989). Кроме того, существуют данные о возможности избирательного утомления мышц грудной клетки или диафрагмы, которое может развиваться в зависимости от

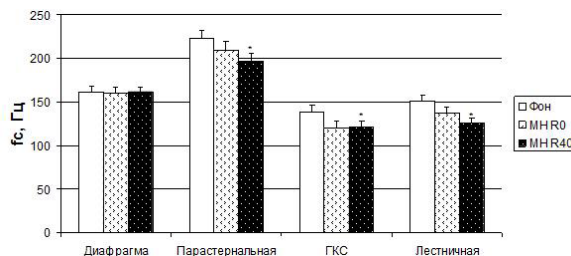


Рис.2 Динамика центроидной частоты спектра ЭМГ диафрагмы, парастеральной, грудино-ключично-сосцевидной и лестничной мышц до и после выполнения мышечной нагрузки в условиях свободного дыхания и на фоне добавочного сопротивления. * - $p < 0.05$ по сравнению с контролем

того, какой тип дыхания – торакальный или абдоминальный – произвольно выбирает испытуемый во время выполнения нагрузочного теста по преодолению резистивной нагрузки (Fitting et al., 1988).

Полученные нами данные позволяют предположить, что снижение максимальной силы сокращений инспираторных мышц при хронических обструктивных болезнях легких вызвано прежде всего ослаблением вспомогательной мускулатуры, тогда как слабость диафрагмы может развиваться на более поздних стадиях заболевания. При этом функциональная недостаточность вспомогательных мышц является дополнительным фактором, который наряду с добавочным сопротивлением дыханию увеличивает нагрузку на диафрагму, способствуя развитию ее утомления и снижению резервов дыхательной системы.

Результаты нашего исследования и литературные данные свидетельствуют о том, что выполнение мышечной нагрузки в условиях добавочного сопротивления вызывает снижение резервных силовых параметров системы дыхания. Между тем, функциональными возможностями этой системы, включая ее моторную составляющую, в значительной степени определяется способность адаптации организма к изменяющимся условиям окружающей среды. Известно, что дыхательные мышцы играют ключевую роль в приспособлении организма к увеличивающимся резистивным нагрузкам. В связи с этим прогнозируемая дисфункция дыхательных мышц при длительном воздействии резистивного сопротивления может оказывать негативное влияние на адекватность газообмена, особенно при выполнении физических нагрузок.

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ПОРІВНЯЛЬНА ОЦІНКА СТІЙКОСТІ ІНСПІРАТОРНИХ М'ЯЗІВ ДО ВТОМИ ПРИ МОДЕЛЮВАНІЙ ОБСТРУКЦІЇ ДИХАЛЬНИХ ШЛЯХІВ

Проведення спектрального аналізу ЕМГ різних груп дихальних м'язів показало, що після виконання м'язової

роботи в поєднанні з додатковим опором диханню одночасно зі зниженням максимального інспіраторного тиску (МІР) спостерігається зрушення центроїдної частоти ЕМГ парастернальних, драбинчастих і грудино-ключично-сосцевидних м'язів в низьку частину спектру. Змін у спектрі ЕМГ діафрагми не відзначається. Таким чином, зниження загального силового резерву дихальних м'язів, що спостерігається після посиленого навантаження на дихальну систему, обумовлено погіршенням функціонального стану не діафрагми, а торакальних і допоміжних інспіраторних м'язів. Діафрагма більш стійка до стомлення. Отримані дані дозволяють припустити, що зниження максимального інспіраторного тиску при хронічних обструктивних хворобах легенів також викликано насамперед ослабленням допоміжної мускулатури, тоді як слабкість діафрагми може розвиватися на більш пізніх стадіях захворювання. При цьому функціональна недостатність допоміжних м'язів є додатковим чинником, який поряд з додатковим опором диханню збільшує навантаження на діафрагму, сприяючи розвитку її втоми і зниженню резервів дихальної системи. Ключові слова: інспіраторні м'язи, додатковий опір, електроміографія, втомлення, м'язове навантаження

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INSPIRATORY MUSCLE RESISTANCE TO FATIGUE DURING SIMULATED AIRWAY OBSTRUCTION

Respiratory muscle fatigue can develop during simulated airway obstruction. The aim of this study was to characterize the pattern of inspiratory muscle fatigue and to assess the resistance to fatigue of diaphragm (D), parasternal (PS), sternocleidomastoid (SM) and scalene (SC). Six healthy untrained subjects participated in this study. To identify signs of inspiratory muscles fatigue development electromyographic activity of D, PS, SCM and SC was recorded during 5-min exercise with loaded breathing ($40 \text{ cm H}_2\text{O/l} \cdot \text{s}^{-1}$). The before-to-after exercise measurements of maximal inspiratory pressure (MIP) and EMG power spectrum changes were performed. Maximal inspiratory pressure declined about 12 % after exercise test compared with control, whereas the peak magnitude of integrated electrical activity of D, PS, SCM and SC during post-exercise Muller's maneuver was significantly greater than in pre-exercise test in all subjects. The extent of

inspiratory muscles fatigue was evaluated by analysis of shift in centroid frequency (f_c) of EMG power spectrum. All subjects demonstrated a significant reduction in f_c of PS, SCM and SC. f_c of D was not changed. Diaphragm is more resistant to fatigue during obstructive breathing compared with PS, SCM and SC. The data suggest that the reduction of maximum inspiratory pressure in chronic obstructive pulmonary disease also caused primarily by the weakening of the accessory muscles, while the weakness of the diaphragm may occur in the later stages of the disease. The functional failure of accessory muscles is an additional factor, which, along with the additional breathing resistance increases the load on the diaphragm, promoting its fatigue and reduced respiratory reserve.

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Tuberculous periodontitis: clinical-laboratory and epidemiological aspects

To the present day, tuberculous periodontitis is not diagnosed and not present in the classification of tuberculosis. Its microbiological diagnostics is the most complicated as it is performed by investigating small quantities of paucibacillary specimens. The purpose of work was defining the significance of mycobacterial population variability forms in the clinical picture and epidemiology of tuberculous periodontitis. Comparative analysis of clinical and epidemiological features of tuberculous periodontitis was performed in two groups of patients aged 20 to 70 years over different observation periods from 1980 till 2012. Group A was composed of 258 patients who were observed from 1980 till 1990, and Group B consisted of 250 patients who were examined from 2005 till 2012. Peridental focal points of infection in chronic forms of tuberculous periodontitis were used as the material for the advanced microbiological and pathohistological examination. It has been established that in Group A, peridental focal points of infection serve as a reservoir for persisting mycobacteria. The main form of such mycobacteria are unstable L-phase variants which were found in 50% of the investigated samples. We have noticed that the number of focal points of infection in which the changed mycobacteria forms vegetate tends to increase: L-forms (55,2%) and granular forms in Group B. L-form transformation of mycobacteria and emerging of granular forms is the reason of exacerbations coming up more frequently and persistent course of tuberculous periodontitis. The strains of mycobacteria in peridental focal points of infection susceptible to all antituberculous drugs that were detected 30 years ago has been supplanted by mycobacteria resistant to isoniazid and rifampicin in patients who had not taken antituberculous drugs before. At present, the fact that tuberculous periodontitis in HIV-positive patients can be a prognostic for clinical manifestations of AIDS is a point of interest. The clinical and epidemiological features of tuberculous periodontitis that have been revealed are similar to clinical pathomorphism of extrapulmonary tuberculosis, therefore tuberculous periodontitis can be included in the classification of tuberculosis as a separate nosological entity.

INTRODUCTION

This study has certainly become of current interest and taken on great significance in connection with tuberculosis pandemic. Despite the doctors' and scientists' efforts as well as numerous tuberculosis control programs, we cannot expect this dangerous disease will be defeated soon [4,10]. This is connected with aggravation of tuberculosis course concurrent with HIV/AIDS, Mycobacterium tuberculosis bacteria gaining multi-drug resistance to antituberculous drugs as well as the main diagnostics problem – detection of the pathogen in connection with mycobacterial population

diversity [8]. Microbiological diagnostics of extrapulmonary tuberculosis is the most complicated as it is performed by investigating small quantities of paucibacillary specimens. It is likely the reason that such a common form of extrapulmonary tuberculosis as tuberculous periodontitis remains undiagnosed in most observations and is not present in the classification by now.

Multiple peridental focal points of infection having tubercular origin in chronic forms of periodontitis represent one possible variation of extrapulmonary tuberculosis. Peridental focal points of infection develop when M. tuberculosis bacteria get in periodontal tissues through root

canals of destroyed teeth or by hematogenous transmission. A focal point of infection having tubercular genesis (or etiology, or origin) is formed around the tooth root apex in the form of granuloma, cystogranuloma or radiculodental cyst. Such focal points, on the one hand, have some features of tuberculosis of bones and joints and, on the other hand, some features of pulmonary tuberculosis. In apical periodontitis open root canals, like the trachea and bronchi, mediate between periodontal tissues and the ambient and improve aeration of these tissues. The infection enters periodontal tissues from oral cavity as well as vice versa, thus seeding the ambient. The first findings regarding involvement of mycobacteria in the development of specific inflammatory process in periapical tissues and their transformations from one form to another date back to the beginning of the 20th century [3].

Taking into account tuberculosis pandemic and common use of antibacterial drugs, we considered it reasonable to analyze the data about variability forms of *M. tuberculosis* bacteria and their susceptibility to antituberculous drugs in cases of tuberculous periodontitis over two periods: from 1980 till 1985 and from 2005 till 2012.

The purpose of work: to define the significance of mycobacterial population variability forms in the clinical picture and epidemiology of tuberculous periodontitis.

METHODS.

The study was performed using the capacities of dental clinics in the cities of Moscow, Dnepropetrovsk, Simferopol and Odessa. In the period from 1980 till 2010, comparative analysis of clinical and epidemiological features of tuberculous periodontitis was performed in two groups of patients aged 20 to 70 years. The analysis covered different observation periods. Group A was composed of 258 patients who were observed from 1980 till 1985, and Group B consisted of 250 patients who were

examined from 2005 till 2010. The diagnosis of tuberculous periodontitis was verified based on the medical history data and complex clinical-laboratory diagnostics.

Peridental focal points of infection (granulomas, cystogranulomas and radiculodental cysts in chronic forms of tuberculous periodontitis) were used as specimens for microbiological and pathohistological examination. To find *M. tuberculosis* bacteria, the following diagnostic techniques were used:

1) bacterioscopy for *M. tuberculosis* L-forms detection – direct microscopy of smears taken from the peridental focal points of infection specimens stained by Ziehl-Neelsen and with Auramine O as well as by Romanovsky-Giemsa;

2) culture methods with the use of different classical culture media and seeding techniques: Lowenstein-Jensen media and Finn-II, a series of semisolid media for L-form isolating;

3) seeding of the specimens using the method modified by us; it includes the following steps: the specimens – impression smears of peridental focal points of infection – are applied on a narrow microscope slide and treated sparingly with acid, and then the slide is placed in a test tube containing Finn-II medium. The test tube was filled with Shkolnikova medium by two thirds of the glass height, so that the smear would touch the inclined surface of solid medium, and was placed in a thermostat [1];

4) bioassay tests on guinea pigs;

5) immunoenzyme method; 6) biomolecular method using the TB-Biochip microarray system.

355 samples have been analyzed using microbiological (culture) methods – for *M. tuberculosis* and L-forms of *M. tuberculosis*, and 49 samples have been analyzed using biomolecular method. Microbiological and biomolecular studies were performed in the Division of Laboratory Diagnostics of Moscow Research and Clinical Center for Tuberculosis Control, Moscow Healthcare Department.

Because of complexity of mycobacteria identification, concurrent studies of isolated

cultures were performed in St. Petersburg Scientific Research Institute of Phthisiopulmonology. We used classical methods including bacteriological and biochemical identification as well as immunoenzyme biomolecular method allowing to define the species mycobacteria belong to with higher degree of reliability. Isolation of aerobic and non-sporulating anaerobic bacteria was also done. General structural changes were revealed in histologic specimens stained with hematoxylin and eosine, and distribution of *M. tuberculosis* bacteria in tissues was investigated under staining by Ziehl-Neelsen.

RESULTS AND THEIR DISCUSSION

Microbiological study of 355 impression smears made from peridental granulomas, cystogranulomas and cysts in 258 apparently healthy persons allowed to detect mycobacteria in 53.7% of cases (when bacterioscopy was used) and did not show positive results when the specimens were seeded on solid egg media. Detection of mycobacteria by bacterioscopic method and their concurrent dormancy on culture media, by analogy with the data found in references, gives evidence of the fact that mycobacteria viability is being reduced [7]. Quantitative aspects of the results of different microbiological diagnostics methods use for detecting mycobacteria in peridental focal points of infection can be found the table below.

Most frequently, mycobacteria were found in the parietal part of the cystoid cavity contents (26.6%). In half of the investigated samples, mycobacteria in such parietal parts were detected in the form of plait-like congestions. Therefore, we can make a conclusion about cord-factor presence yet in the nidus and not in the culture, and, subsequently, about *M. tuberculosis* virulency. In sporadic cases (3.3%), mycobacteria were found in the envelope of cysts or cystogranulomas. In peridental granulomas, mycobacteria were detected in the foci of decay (19%) twice as frequent as in the «quiet» parts (9%).

The maximum quantity of strains – six (12.8%) has been isolated from peridental granulomas tissue homogenate; two strains (2.9%) have been isolated from the exudate leaking out from peridental focal points of infection through the root canal, and one strain (4.5%) has been isolated from the fistulous drainage. It should be particularly noted that *M. tuberculosis* bacteria presence in the exudate leaking out from root canals and fistulous passages is epidemiologically essential as one of the ways of mycobacteria escaping into the ambient and possibly as a source of infection for the people around.

Bacterioscopy of smears in Group B indicated the presence of finely granular acid-fast forms of mycobacteria surrounded by pellucid area in 76% of peridental focal points of infection. Such forms were found 1.5 times more frequently compared to Group A. According to references data, *M. tuberculosis* granular forms are an aggravating factor in lung tuberculosis course [5].

Isolation of mycobacteria with reduced viability made it necessary to use improved bacteriological diagnostic techniques that also include isolation of the changed mycobacteria forms – L-forms.

To investigate small quantities of paucibacillary specimens from peridental focal points of infection, we have developed and introduced a modified seeding method. Under this method, the specimens in the form of smears or impression smears taken from the most infected parts of focal point of infection, i. e. cyst and cystoid cavity content as well as exudate from root canals and fistulas, was applied on narrow microscope slides. As a result of the study performed, 28 mycobacteria cultures have been isolated; in 57.8% of cases mycobacteria microcolony growth on narrow microscope slides has been noticed that allows to ascertain the presence of viable mycobacteria population in peridental focal points of infection.

Mycobacterial populations were mostly composed of L-forms found by bacterioscopy in more than 90% of investigated samples.

To identify mycobacteria, classical methods including bacteriological and biochemical identification as well as immunoenzyme method were used, and in Group B, the test system for *M. tuberculosis* detection by hybridization method with fluorescence imaging on the biological microarray system («TB-BIOCHIP») was employed [9]. These methods allowed to define what species mycobacteria belong to with higher degree of reliability. When 49 specimens taken from the peridental focal points of infection were investigated by biomolecular method, in nine cases (18.4%) *M. tuberculosis* bacteria have been identified and their drug susceptibility has been defined [6].

18 out of 28 isolated strains were analyzed in the microbiological and immunoenzyme studies from different points of view in order to identify them. The remaining 10 strains showed poor growth and died out in attempting to subculture them.

The remaining 14 out of 18 investigated strains had primarily orange pigment with different gradations; based on this characteristic, they were assigned to scotochromogenous bacteria under Runyon classification. Further identification also did not allow to assign any of the investigated strains to one or other classification group.

The results we have obtained while studying biochemical properties of the isolated mycobacteria together with the available data

provided by other researchers [2,7] suggest that these mycobacteria are revertants of *M. tuberculosis* bacteria.

The fact that the isolated mycobacteria are revertants of *M. tuberculosis* L-forms is indirectly proven by the results of defining mycobacteria drug susceptibility to 10 antituberculous drugs: all the strains of *M. tuberculosis* isolated by us show significant susceptibility to the first-line and second-line drugs, while the most nontuberculous mycobacteria are resistant to all the antituberculous drugs.

Identification of the obtained mycobacteria by immunoenzyme method allowed to find *M. tuberculosis* antigens of human type in 7 out of 18 cultures (38.9%), *M. fortuitum* antigens in 5 cultures (27.8%), and antigens of other atypical mycobacteria in 6 cultures (33.3%).

M. tuberculosis bacteria search using immunoenzyme method in 80 tissue samples from peridental granulomas and biomolecular method in 20 samples taken from peridental focal points of infection have not yielded positive results.

Productive tubercular process of the limited extent was noted in liver, spleen, kidneys and lungs of the guinea pigs and mice infected with typical acid-fast *M. tuberculosis* bacteria of human type isolated from peridental focal points of infection. The data obtained give evidence of reduced mycobacteria virulency more apparent in the changed mycobacteria.

Table. Detection rate for *M. tuberculosis* bacteria and their L-forms in the specimens taken from peridental focal points of infection in Group A using different techniques of microbiological diagnostics

Diagnostic techniques	Sample number	Cultures isolated:			
		M. tuberculosis, abs. (%)			L-forms
		typical	changed	microcolonies	
Bacterioscopy	355	84 (23.6%)	107 (30.1%)	-	321 (90.4%)
Seeding on solid media	140	No growth	No growth	No growth	-
Seeding on semisolid media	258	-	-	-	185 (71.7%)
Seeding by a modified method	417	4 (0.9%)	24 (5.7%)	241 (57.8%)	298 (71.4%)
Bioassay tests on guinea pigs and mice of the CBA strain	65	30 (46.1%)	15 (23.1%)	-	20 (30.8%)
Immunoenzyme method	18	7 (38.9%)	11 (61.1%)	-	-

Seeding of 417 samples taken from periodontal focal points of infection helped to reveal mycobacteria L-forms in the most cases (71.4%) along with mycobacteria. L-form population was heterogenous in its biological properties and varied in balancing degree. In the most observations made by us (52.2%), L-forms that had reversed into *M. tuberculosis* bacterial forms on culture media were assigned to unstable forms.

While performing bioassay test by injecting L-forms to guinea pigs the animals show tubercular inflammation of the limited extent similar to the one when *M. tuberculosis* bacteria with reduced virulency were injected, as well as paraspecific and non-specific changes like immunoprotective reaction.

The data available in references together with the results of the studies performed by the authors extend traditional assumptions about periodontal focal points of infection microflora by the established fact about mycobacteria and their changed forms involvement in periodontal focal points of infection emerging and development.

Based on cumulative results of the microbiological study performed in 1980–1990, it has been established that in periodontal focal points of infection in people apparently healthy in terms of tuberculosis and having chronic periodontitis forms, *M. tuberculosis* bacteria persist in 20% of cases [1].

The results of the performed microbiological study formed the basis for studying clinical symptoms and signs as well as course of apical periodontitis depending on the mycobacterial population structure in periodontal focal points of infection.

Group 1A included 44 (17,1%) patients; in specimens taken from their periodontal focal points of infection, bacterial forms and L-forms of mycobacteria have been detected. Group 2A included 129 (50%) persons with unstable L-forms found in periodontal focal points of infection. Group 3A was composed of 85 (32,9%) patients; in specimens taken from their periodontal focal points of infection few

stable L-forms of mycobacteria have been found.

Aggravated course with severe symptoms has been noted in 18.2% of patients in Group 1A; 5,9 times less frequently in patients from Group 2A and was not noted at all in patients from Group 3A. In Group 1A, aggravated course of the process was associated with fistula presence more frequently (Fig.1). It is known that tuberculous fistula act as a drain channel for purulence in tuberculous lesions of spine, hipbones and in other forms of tuberculosis [11]. *M. tuberculosis* bacteria discharging from fistulous passage (Fig.2) escape into the ambient and play significant part in epidemiology of the pathogen. Most patients have fistula with one external opening.

However, complex fistula with branches may have several external openings (Fig. 5). Appearance and nature of purulent fistula discharge can often help to suspect the specific process in periodontal tissues while performing initial oral exam. Specific features of this process are: 1) fluid, almost colorless purulence or yellowish exudate leaking out in a quite considerable amount; 2) slender, dryish tyroid inclusions in exudate; 3) pale, thinning mucosa with cyanotic shade in the fistula opening area. In patients from Group 1, periodontal focal points of infection of medium (34.1%) and large sizes (65.9%) have been revealed, and large periodontal focal points of infection were encountered 1.9 times more frequently compared



Fig. 1. Fistula on the gum with several external apertures

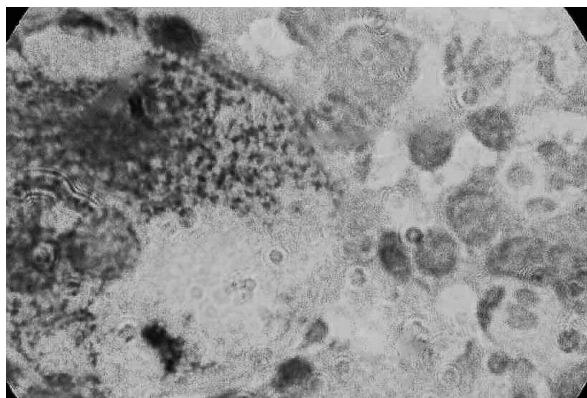


Fig .2. Microcolony of mycobacteria tuberculosis in the liquid content of fistula passage



Fig .3. Multifocal periodontal focus of infection – cystic granuloma, received in resection of apex of dental root

to medium ones. X-ray images in persons having asymptomatic course of periodontal focal points of infection showed blurred focal point contours (27.3%) that speak for latent progressing of the inflammatory process in periodontal tissues. In 15.9% of cases, osteosclerosis of bone tissue surrounding periodontal focal points of infection is caused by purulent exudate and microabscesses, as further morphologic studies have shown. In 10 out 109 (9.2%) large periodontal focal points of infection (more than 0.5 cm in diameter) multifocal points of infection were observed (Fig.3). The central focal point of infection and «growth zone» differed not only in their form, shade intensity and contour sharpness on X-ray images, but also in their histological structure: the central focal point of infection is a cystoid cavity, and the growth zone is similar to a simple granuloma in its structure (Fig.4) . In 5% of the investigated samples the central focal point of infection was similar to cavitary lesion, and histobacterioscopy helped to reveal acid-fast *M. tuberculosis* bacteria in it.

Mixed mycobacteria population vegetation has been established primarily in young persons (20–29 years old) and patients older than 50 years. According to our findings, periodontal focal points of infection with mycobacteria and L-forms in young people have been found in 17.1% of cases, and in 60-year-old and older patients – in 24.3% of cases.

Some authors who had studied mycobacterial populations in different tuberculosis forms in older age groups note a potential hazard of tuberculosis reactivation in connection with mycobacteria presence concurrent with



Fig . 4. X-ray picture of radicular cyst in tuberculosis periodontitis

lowered resistance of the macroorganism [2]. It should be marked that in periodontitis exacerbations anaerobic microorganisms were isolated from peridental focal points of infection alongside with mycobacteria. The findings of the researchers having studied associated microflora in tuberculosis show that mycobacteria in association with anaerobe bacteria cause peracute progressing of inflammatory process with extensive necrotic patches forming [12]. The results comparison of the peridental focal points of infection integrated study allowed us to make a similar conclusion.

Clinical picture of periodontitis when unstable L-forms (Group 2) were isolated from peridental focal points of infection was marked by unstable course just like the population itself. The early period of the disease was mostly hidden with undulating course and remission and exacerbation periods. Persistent progressing of peridental focal points of infection with local manifestations of different degree can be associated not only with unstable mycobacteria L-forms presence, but also with anaerobes most frequently isolated along with L-forms.

Intoxication symptoms at the time of exacerbation, especially in case of multiple peridental focal points of infection, are characterized by faintness, temperature rise, transient joint pain and headache. Face skin is pale with yellowish and grayish tints. Weight loss is observed in some patients.

In teenagers with conversion of tubercular tests, tuberculous periodontitis often progresses in intact teeth and soon exacerbates with periostitis or an abscess. A large volume of *M. tuberculosis* bacteria is found in the necrotizing pulp of such teeth.

On examination: the tooth is more often destroyed; a crown of the tooth has a changed color ranging from light grey to dark grey. On probing: the cavity of decay is opened into the dental cavity. Vertical percussion of the tooth is painful; the mucosa around the causative tooth is edematous and reddened. Vasoparesis symptom is positive.

During histological examination of peridental focal points of infection in patients of this group areas of subtle tubercular inflammation were revealed. In case of such subtle inflammation architectonics of the tubercle is preserved, but the composition of cells specific for tuberculosis is lost, i. e. signs of chronic productive nonspecific inflammation emerge.

Therefore, peridental focal points of infection serve as a reservoir for persisting mycobacteria with predominant persistence form – unstable L-phase variants of *M. tuberculosis* bacteria. Studying *M. tuberculosis* bacteria variability in tuberculous periodontitis over the period from 1980 till 2012 shows tendency to increasing the number of peridental focal points of infection in which changed mycobacteria forms persist, i. e. L-forms and granular forms.

It has been revealed that L-form transformation of *M. tuberculosis* bacteria and emerging of granular forms is the reason of exacerbations coming up more frequently and persistent course of tuberculous periodontitis. Presence of fistulas is important both from diagnostic and epidemiological points of view. Massive and diversified *M. tuberculosis* bacteria population in tooth root canals and fistula content can become a source of tuberculous infection dissemination and its propagation in the ambient.

It has been found out that the strains of mycobacteria in peridental focal points of infection susceptible to all antituberculous drugs that were detected 30 years ago has been supplanted by multi-drug resistant mycobacteria.

At present, the fact that tuberculous periodontal disease in HIV-positive patients can be a prognostic for clinical manifestations of AIDS is also a point of interest.

The clinical and epidemiological features of tuberculous periodontitis that have been revealed are similar to clinical pathomorphism of extrapulmonary tuberculosis, therefore tuberculous periodontitis should be included in the classification of tuberculosis as a separate nosological entity.

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ТУБЕРКУЛЬОЗНИЙ ПЕРІОДОНТИТ: КЛІНІКО-ЛАБОРАТОРНІ ТА ЕПІДЕМІОЛО- ГІЧНІ АСПЕКТИ

Туберкульозний періодонтит дотепер залишається не діагностованим і відсутнім в класифікації туберкульозу. Мікробіологічна діагностика його є найбільш складною, тому що здійснюється шляхом дослідження незначної кількості олігобацилярного матеріалу. Мета роботи полягала у вивченні значення змінених форм мікобактеріальної популяції в клініці та епідеміології туберкульозного періодонтиту. Був проведений порівняльний аналіз клініко-епідеміологічних особливостей туберкульозного періодонтиту в двох групах пацієнтів у віці 20-70 років у різні періоди спостереження з 1980 по 2012 р. Групу А склали 258 пацієнтів, спостереження яких проходило з 1980 по 1990 рік, група В була представлена 250 пацієнтами, обстеження яких проходило з 2005 по 2012 рік. Матеріалом розширеного мікробіологічного та патогістологічного дослідження були навколорубні вогнища інфекції (НВІ) при хронічних формах туберкульозного періодонтиту. Встановлено, що в групі А навколорубні вогнища інфекції є резервуаром персистуючих мікобактерій, основна форма яких - нестабільні Л-варіанти, виявлені в 50% спостережень. Відзначено зберігання тенденції збільшення кількості НВІ, в яких вегетують змінені форми мікобактерій: Л-форми (55,2%) і зернисті форми в групі В. Л-трансформація мікобактерій і поява зернистих форм призводить до більш частих загострень і прогресуючого перебігу туберкульозного періодонтиту. На зміну медикаментозно-чутливим до всіх протитуберкульозних препаратів штамів мікобактерій в НВІ, що були виявлені 30 років тому, прийшли резистентні до ізоніазиду і рифампіцину мікобактерії у пацієнтів, які не приймали раніше протитуберкульозні препарати. Актуальним нині є той факт, що туберкульозний періодонтит у ВІЛ-інфікованих пацієнтів може бути передвісником клінічних проявів СНІДу. Виявлені клініко-епідеміологічні особливості туберкульозного періодонтиту відображають клінічний патоморфоз позалегеневого туберкульозу, тому туберкульозний періодонтит може бути внесений в класифікацію туберкульозу як окрема нозологічна форма.

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ТУБЕРКУЛЕЗНЫЙ ПЕРИОДОНТИТ: КЛИНИКО-ЛАБОРАТОРНЫЕ И ЭПИДЕМИО- ЛОГИЧЕСКИЕ АСПЕКТЫ

Туберкулезный периодонтит до настоящего времени остается не диагностированным и отсутствует в класси-

фикации туберкулеза. Микробиологическая диагностика его является наиболее сложной, так как осуществляется путём исследования незначительного количества олигобацилярного материала. Цель работы заключалась в определении значения форм изменчивости микобактериальной популяции в клинике и эпидемиологии туберкулезного периодонтита. Был проведен сравнительный анализ клинико-эпидемиологических особенностей туберкулезного периодонтита в двух группах пациентов в возрасте 20-70 лет в различные периоды наблюдения с 1980 по 2012 г. Группу А составили 258 пациентов, наблюдение которых проходило с 1980 по 1990 год, группа В была представлена 250 пациентами, обследование которых проходило с 2005 по 2012 год. Материалом расширенного микробиологического и патогистологического исследования служили околозубные очаги инфекции (ООИ) при хронических формах туберкулезного периодонтита. Установлено, что в группе А околозубные очаги инфекции являются резервуаром персистирующих микобактерий, основная форма которых – нестабильные Л-варианты, обнаружены в 50% наблюдений. Отмечена сохраняющаяся тенденция увеличения количества ООИ, в которых вегетируют измененные формы микобактерий: Л-формы (55,2 %) и зернистые формы в группе В. Л-трансформация микобактерий и появление зернистых форм приводит к более частым обострениям и прогрессирующему течению туберкулезного периодонтита. На смену лекарственно-чувствительным ко всем противотуберкулезным препаратам штаммам микобактерий в ООИ, выявленным 30 лет тому, пришли резистентные к изониазиду и рифампицину микобактерии у пациентов, не принимавших ранее противотуберкулезные препараты. Актуальным в настоящее время является тот факт, что туберкулезный периодонтит у ВИЧ-инфицированных пациентов может являться предвестником клинических проявлений СПИДа. Выявленные клинико-эпидемиологические особенности туберкулезного периодонтита отражают клинический патоморфоз внелегочного туберкулеза, поэтому туберкулезный периодонтит может быть внесен в классификацию туберкулеза как отдельная нозологическая форма.

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Clinical, epidemiological and morpho-functional features of bronchial asthma with combined pathology

To carry out a retrospective analysis of medical records, we have developed an algorithm in which the diagnosis was recorded, reflecting the shape, weight, stage of asthma, sensitization to various groups of allergens, potential comorbidities, gender, age, the main clinical and laboratory parameters received therapy, the timing of finding patient in the hospital. In a retrospective analysis of 1241 case histories of patients were hospitalized in the department of «Asthma» Research Center of the Institute of Immunology Russia for 2007-2011 was found that about 7% (86 people) of the patients with asthma admitted to the hospital with extrahospital pneumonia (EP). A study of the ultrastructure of the surface of the mucous membranes, and in particular the different populations of cells of the nose and bronchi in the middle and severe asthma when connecting with EP. To study the scanning electron microscopy (SEM) of these divisions sparing methods used world collecting material mucous membranes of the respiratory tract.

According to official statistics, the number of asthma patients in Russia is about 7 million. However, given the epidemiological data collected in hospitals, the number of asthma sufferers is much lower, at about 3.9 million people, which is the lowest among European countries [1,3]. Of course, these data are significantly understated, in need of correction. This is due to the lack of common approaches to terminology and classification of asthma, as well as standardized methods of diagnosis and treatment of this nosology [2].

Due to the deterioration of the environmental situation in the various regions of our country, an increase in comorbidities was observed in patients with asthma [3]. Among these cases, one should note a frequent combination representing a direct threat to the life of the patient - BA with acute developing outhospital pneumonia (OHP) and the so-called «nocturnal asthma» (NA). Syndrom obstructive sleep apnea (OSA) - a temporary overnight stop respiration, life-threatening and is rarely a cause of sudden death during sleep [5]. Therefore, these combinations of pathologies are dangerous to the lives of

patients and cause serious problems during asthma therapy.

When pneumonia is exacerbation of BA, auscultation pneumonic outbreak causes significant difficulties, which generally leads to late diagnosis verification. Please note, that the existing pathology of bronchi and bronchioles, which occur in patients with asthma, pneumonia and pathology joins alveolar and interstitial lung tissue. Therefore, given a combination of two affected all parts of nosology respiratory system, leading to severe intoxication and hypoxia, increases the risk of complications from other systems, causing the need for urgent action intensive care until resuscitation. It should be borne in mind that patients with allergies often changed not only the nature of the reactivity of the bronchi, but also other organs and tissues for any additional exposure that makes significant changes in these complex immunological mechanisms. It should also be noted that previously we noted changes platelets not only in peripheral blood but also in the microvascular of the respiratory and digestive systems in BA. These changes and contributed

to increasing hypoxic and anoxic damage that we observed in patients of asthma [4]. All this has been characteristic motive in the study of clinical and immunological, allergic and morphological aspects of the study group of patients with combined pathology.

Methodology To conduct a retrospective analysis of medical records, we developed an algorithm, in which recorded the sex and age of patients, the diagnosis, reflecting the shape, weight, stage of bronchial asthma (BA); sensitization to various groups of allergens, potential comorbidities, clinical, basic and laboratory data on treatment, time to find a patient in the hospital. In a retrospective analysis of 1241 patients, who were treated at the department of «Bronchial Asthma» Institute of Immunology FMBA of Russia for 2007-2011. We found, that about 7% (86) of the patients with BA admitted to the hospital with OHP. Of the 402 patients studied in 307 people (76.4%) showed signs of obstructive sleep apnea.

For research a map of individual examination of the patient was developed which includes the application form allowing to diagnose of OSA in patients with complaints of snoring and daytime sleepiness. Total for this questionnaire surveyed 402 patients at 76.4% (307 people) found signs of OSA. Mean age 54.2 years. It was found that among these are dominated by patients with a mixed form of asthma 78.0% (288 people) and the medium-severe severity - 73.4% (271 people). Among them were associated pathology 91.8% (369 patients). The majority of patients with OSA obese 77.2% (285), type 2 diabetes, 26.3% (97 people) and hypertensive disease 51.2% (189 people).

Divided into 3 groups of patients: patients with asthma (group 1), patients with OSA without asthma (group 2), and patients with a combination of asthma and OSA (group 3). Control group consisted of healthy male volunteers, whose average age was 35 years.

Methods: physical examination, allergy testing, skin tests, Diagnostics (ERF, test with bronchodilators), polysomnography (PSG),

morphological studies of bronchoalveolar lavage (BAL) and peripheral blood cells (scanning and transmission electron microscopy (SEM, TEM).

OWN RESEARCH AND DISCUSSION

The average age of patients studied (AD and MR) was 42.5 years. The distribution by sex: women accounted for 47 people, men - 39. Experience of the disease was varied and ranged from 1 year to 20 years. The average age of the manifestation of the disease was 12 years. In 50% (43 persons) was burdened by heredity of atopy and 88.4% (38 people) of them on BA. At the same time 34.9% (15) showed a history on the mother, at 7.0% (3 people) on the father, in 58.1% (25 people) through both parents. The emergence of OHP in patients with BA in 88.4% (76) of cases occurred in a cold season. Among the analyzed group, patients were divided according to severity: mild asthma was found in 19% (17 people), medium level - at 55.8% (48 people), and severe degree of asthma - in 24.4% (21 people). Atopic asthma suffered 32.5% (28 people), infection-dependent form 14% (10 people) and a mixed form (atopic and infectious-dependent) - 55.6% (48 people). The average level of total IgE was $306,90 \pm 35,1$ IU \ ml, which is two times the upper limit of the reference values. When allergology study of patients with atopic asthma without the OHP, atopic asthma with the OHP, mixed with asthma VP spectrum was identified cause-relevant allergens. The results are consistent with a history and results of skin testing. Positive skin prick-tests with different groups of allergens were detected in all patients (Table 1).

In 91.8% (79 people) were observed other manifestations of atopy. Most of the patients suffered from perennial allergic rhinoconjunctivitis at what symptoms have been present for more than 50% of the time, suffering from seasonal allergic rhinoconjunctivitis - 76.7% (66 people), atopic dermatitis - 20.9% (18 people). By analyzing the structure of the co-morbidities of the study group, at

82.5% (71 patients) were mentioned diseases of the upper and lower respiratory tract. So comorbidities ENT (chronic rhinitis of different etiologies, sinusitis, tonsillitis, polypoid rhinosinusopathy etc.) was observed in 40.7% (35 persons), bronchopulmonary pathology (chronic bronchitis, COPD, etc.) - at 59.3 (51 people). Drug intolerance of different drug groups were 50% (43 people), NSAID-transfer 9.3% (4 people), intolerance of different groups of antibiotics (in history) were 21.6% (8). It was also noted that the average age of patients with atopic asthma form lung flow was 31.4 years old, moderate - 44.8 years; severe - these patients have been reported. BA depended- infectious form, easy flow - 36.0 years; moderate - 50.7 years; severe - 72.0 years. Mixed form of asthma, lung flow - 42.5 years; moderate - 47.2 years; severe 61.9 year. The tendency to asthma weighting with age, as well as the transition to a mixed form of atopic form of asthma. As a primary care - was held antibiotic therapy (mostly broad-spectrum antibiotics): one course of antibiotic therapy received 72.0% (62 people), a second course of antibiotics took 28.0% (24 people), and the side effects of use of antibiotic therapy were observed in 24.4% (21 people), they were mostly drugs penicillin group 90.5%

(19 patients). SCS received 61.6% of patients (n = 53) of these systemic corticosteroids - 47.6% (41 people). Mucolytics were assigned 84.9% (73 patients), methylxanthines short-acting 41.8% (36 patients), β 2-agonists, short-acting additionally used 13.9% (12 people), berodual 8.1% (7 patients). The mean number of exacerbations per year $3,59 \pm 0,45$. The mean number of hospitalizations per year $1,51 \pm 0,36$. Based on analysis of medical records, it was found that quickly started, adequate and simultaneous treatment of both diseases -OHP and asthma, proper selection of rehabilitation therapy helped to reduce the severity of clinical manifestations of the disease in question and the number of complications significantly reduced hospital stay, accelerated period convalescence and recovery efficiency. Cytology of nasal secretions and sputum found mainly coccal flora as well as the presence of a large number of lymphocytes. The study of the nasal mucous membranes by SEM [6] suggests major inflammatory changes of the surface epithelial cells and the build-up to them macrophages and lymphocytes. And the study of BAL revealed serious damage ciliated cells and increased goblet cells, mostly in the extrusion step (fig.1 a,b).

Table 1. Range of identified allergens.

Positive prick-tests with allergens	Group 1 n = 20		Group 1 n = 28		Group 1 n = 48	
	the number of persons	%	the number of persons	%	the number of persons	%
Dust mites	12	60,0	16	85,6	35	72,9
Feather pillows	3	15,0	6	21,0	8	16,7
Library dust	5	25,0	6	21,0	10	20,8
Hair cats	8	40,0	10	35,7	13	27,1
Dog hair	5	25,0	5	17,8	8	16,7
Wool sheep	0	0	3	10,7	5	10,4
Horse dander	0	0	2	7,1	0	0
The family tree	4	20,0	6	21,0	10	20,8
The family of grasses	2	10,0	5	17,8	6	12,5
The family of weeds	5	25,0	10	35,7	13	27,1

Group 1 - atopic asthma without the EP. [6] Group 2 - atopic asthma with OHP. Group 3 - mixed BA with OHP.

With the combination of BA with OSA methods were used: a standard physical examination, allergy tests (history taking, questioning of patients, skin scarification tests with atopic allergens, the determination of total and specific Ig-E, on the testimony - nasal provocation tests), Diagnostics (ERF, the sample with bronchodilators), polysomnography (PSG), consulting (ENT, cardiologist, neurologist, endocrinologist (indication)), morphological studies of peripheral blood cells by transmission (TEM) and (SEM).

In functional studies of respiratory (ERF) broncho-pulmonary system in group 3 patients have lower rates of airflow obstruction than in the other two groups, a significant reduction of the flow rate on the bronchi of all calibers.

Poliosomnografiya (PSG) is the preferred method of diagnosis and assessment of severity of OSA [7]. Derived parameters: electroencephalogram, electrooculogram, electromyogram (mentalis), traffic of lower limbs, electrocardiogram, snoring, nasal-oral airflow,

respiratory movement of the chest and abdomen, body position, blood oxygen saturation.

Total analysis time averaged 450 minutes, the longest apnea obstruction 60. Apnea index / gipoapnoe (AHI) was 56.6 / h. It should be noted that the PSC has revealed OSA in 35 of 36 patients complaining of respiratory discomfort during the night, which indicates the efficiency of this method and gives us the quality and quantity of sleep efficiency and the level of hypoxia.

Morphologic study of peripheral blood samples by SEM revealed that the greatest changes are observed in 3 patients, a group with a clear increase in the percentage of pathological forms of erythrocytes, which is proportional to depend on the severity of the underlying disease. There was a constant aggregation of red blood cells with a predominance of pathological forms: macrocytes, echinocytes, stomatocytes et al. There was swelling of red blood cells, plasma membrane detachment frequent, with severe gravity - in some cases there is a «shadow» of the complete separation of red blood cells and their membranes. Revealed erythrocytes in comorbidity indicate excessive hemolysis, which is exacerbating the already existing

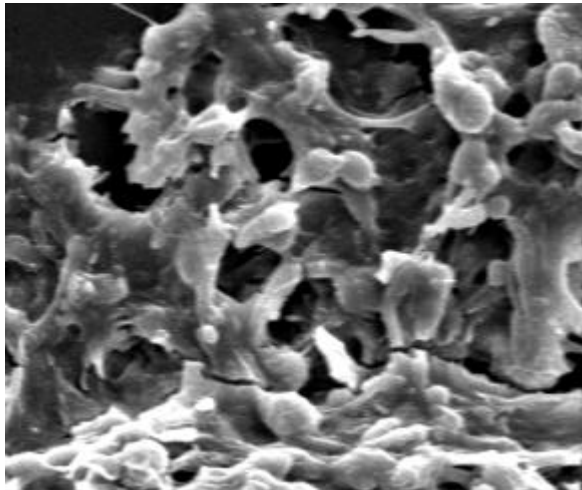


Fig. 1a. SEM. B., 37 years old. BAL. The change in the surface epithelium.

Destruction of ciliated cells (CC). Buildup on their red blood cells, platelets, leukocytes and macrophages. Sharp extrusion of goblet cells (GC) - the desolate cells. Angiogenesis. x 400

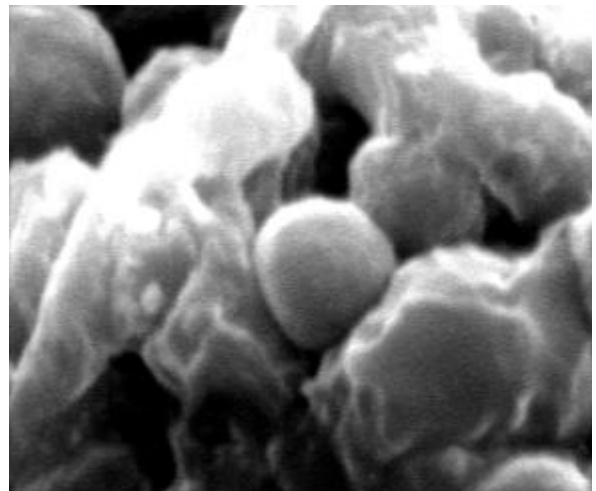


Fig. 1b. SEM. BAL. BC and filled with secret sticking to them macrophages. x 3000.

tissue hypoxia, which generally leads to the development of systemic respiratory failure and not responsive to anti-asthma therapy. Observed in the study of platelet aggregation and accumulation of aggregated groups of platelets, the most pronounced changes were observed in patients 3 groups. Modified forms of platelets differed pretentiousness their shape and pseudopodia, local membrane protrusions and membrane separation from the main cytoplasm. In some parts of the modified form of platelets with numerous spines, had a significant extension and aggregation with others via pseudopodia and directly own platelets.

Therefore, patients with bronchial asthma who have been combined pathology other nosologies deserve special attention of doctors in various specialists.. For these groups of patients need to use a special individual approach aimed at treatment and prevention of BA is not only complex, but also common therapy in general.

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КЛІНІЧНІ, ЕПІДЕМІОЛОГІЧНІ ТА МОРФО- ФУНКЦІОНАЛЬНІ ОСОБЛИВОСТІ БРОНХІАЛЬНУ АСТМУ З ПОЄДНАНОЮ ПАТОЛОГІЄЮ.

Для проведення ретроспективного аналізу медичної документації нами був розроблений алгоритм, в якому фіксувався діагноз, що відображає форму, ступінь тяжкості, стадію БА, сенсibiliзацію до різних груп алергенів, можливі супутні захворювання, стать, вік, основні клініко-лабораторні показники, одержувана терапія, терміни знаходження хворого в стаціонарі. В результаті ретроспективного аналізу 1241 історії хвороби пацієнтів, що знаходилися на стаціонарному лікуванні у відділенні «Бронхіальна астма» Інституту імунології Росії, за 2007-2011 рр. було виявлено, що близько 7% (86 осіб) пацієнтів з БА надійшло у стаціонар з позалікарняною пневмонією (ВП). Проведено дослідження ультраструктури поверхні слизових оболонок, і особливо, різних популяцій клітин носа і бронхів при середньому і важкому перебігу бронхіальної астми при приєднанні пневмонії (ВП). Для вивчення скануючої електронної мікроскопії (СЕМ) цих відділів нами застосовувалися щадні методи збору матеріалу слизових оболонок дихальних шляхів.

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КЛИНИЧЕСКИЕ, ЭПИДЕМИОЛОГИЧЕСКИЕ И МОРФО-ФУНКЦИОНАЛЬНЫЕ ОСОБЕННОСТИ БРОНХИАЛЬНОЙ АСТ- МОЙ С СОЧЕТАННОЙ ПАТОЛОГИЕЙ.

Для проведения ретроспективного анализа медицинской документации нами был разработан алгоритм, в котором фиксировался диагноз, отражающий форму, степень тяжести, стадию БА, сенсibiliзацию к различным группам аллергенов, возможные сопутствующие заболевания, пол, возраст, основные клиничко-лабораторные показатели, получаемая терапия, сроки нахождения больного в стационаре. В результате ретроспективного анализа 1241 истории болезни пациентов, находившихся на стационарном лечении в отделении «Бронхиальная астма» ГНЦ Института иммунологии ФМБА России, за 2007-2011 гг. было выявлено, что около 7% (86 человек) пациентов с БА поступило в стационар с внебольничной пневмонией (ВП). Проведено исследование ультраструктуры поверхности слизистых оболочек, и в особенности, различных популяций клеток носа и бронхов при среднем и тяжелом течении бронхиальной астмы при присоединении внебольничной пневмонии (ВП). Для изучения сканирующей электронной микроскопии (СЭМ) этих отделов нами применялись щадящие методы сбора материала слизистых оболочек дыхательных путей.

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Bronchial asthma and allergic rhinitis in different age groups

Aim: to study the epidemiological, clinical, and morphological features of allergic rhinitis (AR) and bronchial asthma (BA). Materials and methods: common clinical, allergological, morphological (cytological and electron-microscopic methods) examination of patients with AR and BA. Results: the study was conducted among 685 adolescents with AR and BA between the ages of 14 to 18 years and 870 adults aged 18 to 65 years who were divided into groups. Divided by severity of adults: light level - 23%, medium - at 72%, and severe degree - at 5%. Divided by severity of teenagers: light level - 33,1%, medium - at 42,1%, and severe degree - at 24,8%. In the group of adolescents onset of the diseases occurred mostly from 5 to 15 years, and also a hormonal imbalance was detected that increased the severity of illness at this age. The results of allergological examination in the spectrum of allergens in patients with more severe BA revealed sensibilization to multiple allergen groups. In adolescents with AR and BA, there was found unique clinical and morphological changes in the mucous membranes of the respiratory tract. It was installed expressed morphological changes that have a direct relationship to the severity of the diseases. Conclusion: revealed similarities and differences in the clinical course, allergy data and morphological changes in patients of all ages with AR and BA.

In the structure of respiratory pathology allergic rhinitis (AR) and bronchial asthma (BA) are playing the leading roles. Also these diseases involve both the upper and lower airways, and have similar pathogenesis [1,5]. Despite the fact that the initial contact with the allergen occurs in the mucous membrane of the nose, the further process of AR and BA affects the entire respiratory tract.

Nowadays the number of patients with these diseases is increasing worldwide; this process affects negatively quality of life, efficiency in work and study. Also other organs and systems are involved in this pathological process [5]. All these aspects make scientific investigations of great social sharpness in the 21st century.

AR and BA are similar allergic diseases, which are based on an inadequate response of airways on foreign agent (allergen). Hyperresponse leads to increased release of inflammatory mediators that lead to the clinical manifestations (reduced air speed and difficulty of passing it through

airways, secretion hyperproduction, spasm of the respiratory muscles) [3]. The degree of foreignness of allergen-specific characteristics is determined individually; the nature of this determination remains unknown.

Recurrent and long-lasting acute respiratory viral diseases (ARVD), insufficient diagnostics of allergic diseases, environment and appearance of new allergens leads in childhood to development of the AR, and in juvenile period to possibility to transformation in asthma [4,6]. All of these conditions have motivated us to investigate the morphology of the nose cavity in patients with BA and patients with AR. It should also be noted that in juvenile period AR and BA are very closely related. However, the mechanisms of these phenomena are not well investigated.

MATERIALS AND METHODS.

Epidemiology of the diseases was studied, clinical data of outpatients of different age

groups was analyzed. The triggers and clinical characteristics of the diseases were assessed, as well as laboratory tests and instrumental methods results. Also we analyzed claiming rate for medical service, when the AR and BA were diagnosed, the basic therapy competence and adequacy. Studied the causes of onset and clinical features, the results of clinical, laboratory and functional studies, in seeking medical care, the timing of diagnosis of AR and BA, the amount and adequacy of standard therapy. Depending on age and clinical criteria patient were divided in to clinical groups. In our work we used standard laboratory tests and instrumental methods. Special laboratory tests included immunogram, assessment of hypothalamic-pituitary-thyroid system hormone level in the serum (T3, T4, TG, insulin, cortisol, testosterone, somatotropin, TPO, TTH, ACTH) using radioimmunoassay and enzyme immunodetection methods. Provocative nasal and inhalation tests with carbaholinum were carried using existing protocols. Morphological examination of scrapings of the nasal cavity (SNC), bronchoalveolar lavage (BAL) was performed by scanning and transmission electron microscopy (SEM, TEM) using conventional methods, the same methods were used for semithin sections (STS) assessment. STS were made by using ultratome LKB (Sweden) and stained with methylene blue combined method - azure II - basic fuchsin.

Specific allergy assessment of patients included getting the natural history, performing prick - tests with atopic allergens, determination of allergen-specific IgE - antibodies to the main groups of non-bacterial allergens in blood serum. Allergen-specific immunotherapy (ASIT) was held in the hospital using allergens *Dermatophagoides pteronissinus*. The average total dose was 6000 PNU for one ASIT course. Evaluation of clinical efficacy was carried out after the end of each course using generally accepted standards; the morphological evaluation (re-study SNC) was carried out only after the end of the third year of ASIT. All data were

analyzed with Sigma Stat 3.5 (Systat Software, Inc.). A value of $P \leq 0.05$ was considered significant.

RESULTS AND DISCUSSION.

According to the results of retrospective analysis in juvenile patients atopic asthma (88.2%) is the most common form. Debut of asthma in juvenile period occurs in 73.8% patients under the age of 15 years. We clarified that that juvenile have moderate severity of asthma (40.3%), mild severity was observed in 32.8% of patients and the severity stage of the disease was observed in 26.9% of patients. In adults (from 20 to 55 years) the severity was observed in 20%, 50% and 30% of patients respectively. Gastrointestinal tract diseases, endocrine system diseases and vegetative-vascular dystonia were the comorbidities which were found in the half of patients (tabl. 1).

Furthermore, 78% of patients with BA possessed strong family allergic history, a history of frequent ARVD, the presence of bad habits (smoking - 42.2%, alcohol abuse - 10.6%, all the data according the juvenile group).

Positive skin prick-tests with different groups of allergens were detected in 87% of the patients (Table 2).

The widest range of domestic and epidermal allergens was found in patients with more severe BA and with combination with the AR. Nevertheless in patients with AR or mild asthma were found significantly low number of allergens. These data suggest maintaining persistent allergic inflammation in patients with a wide variety of year-round allergies. In patients with more severe asthma polisensibilization was more common. In this group of patients the leading role played dust mites, pollen of trees, compositae and gramineae. Despite the fact that the pollen sensibilization was prevalent, a significant percentage of patients had domestic, epidermal and fungal sensibilization.

In 84% of patients with BA was diagnosed 3rd or 4th class of allergen-specific IgE

Table 1. Comorbidities in the examined groups of patients

Diseases	1 Group n=195	2 Group n=250	3 Group n=425	4 Group n=57
	%	%	%	%
Gastrointestinal tract diseases	65	53	46	45
Chr. tonsillitis	8	9	8	40
Sinusitis	10	8	12	33
Polypoid rhinosinusitis	10	6	11	5
Vegetative-vascular dystonia	53	47	36	46
Endocrinopathy	7	9	10	16
Atopic Dermatitis	25	32	27	37
Alimentary allergy	15	23	22	25

antibodies in the serum of different groups of allergens, corresponding with a history of clinical symptoms and results of skin testing.

Important diagnostic value have assessment of respiratory function and provocative tests with carbaholinum, cold air, exercise.

The most significant changes in respiratory function parameters (obstructive type changes) reported in juvenile and adults without remission. Obstructive changes were observed mainly at the level of small and medium-sized bronchi and respiratory function parameters match the clinical data: history and auscultation of the lungs.

While examining the levels of hormones most significant deviations were observed in juveniles with BA compared to adults. There was a statistically significant ($p < 0.05$) increase in testosterone levels and decrease of cortisol levels in half the patients (52.4%) with acute and severe stage (fig. 1).

Morphologic study of the nasal mucosa in patients of all ages groups with BA showed changes, which are characterized as a destructive process, and abnormal secretion process. These changes were followed by the activation of immunocompetent cells, the severity of which

Table 2. The spectrum of positive reaction on allergens.

Positive prick-tests with allergens	1 Group n=195	2 Group n=250	3 Group n=425	4 Group n=57
	%	%	%	%
Dust mites	76	80	70	70
Feather pillows	53	41	47	15
Library dust	43	39	38	30
Cats hair	53	41	40	36
Dog hair	15	14	13	23
Sheep wool	9	8	6	6
Horse dander	1	3	1	7
Tree group	11	13	14	20
Gramineae group	13	15	17	16
Weeds group	31	28	29	25

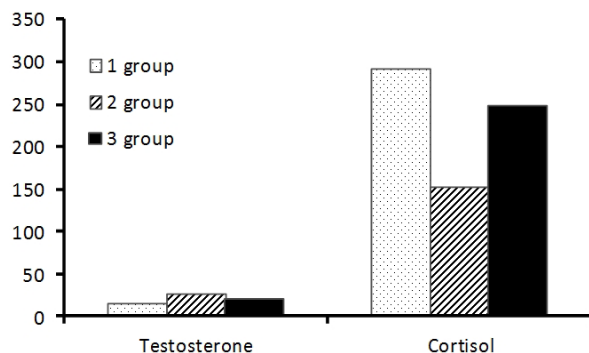


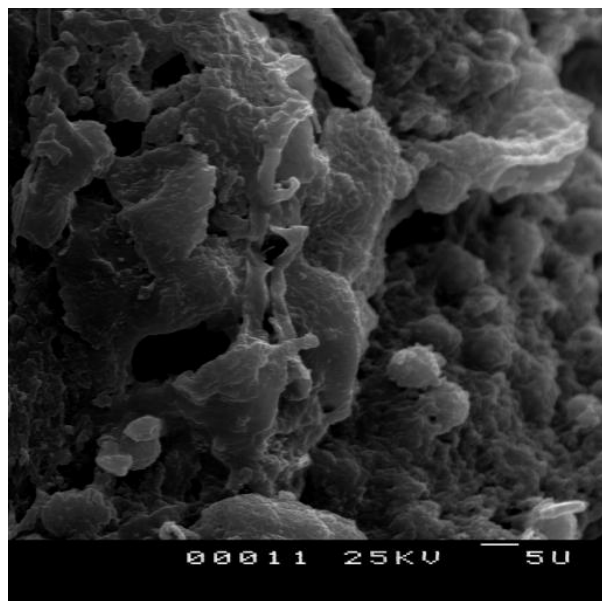
FIGURE 1. Hormone levels in patients examined, nmol / L (n = 33) $p < 0.05$

depends on the severity and duration of asthma.

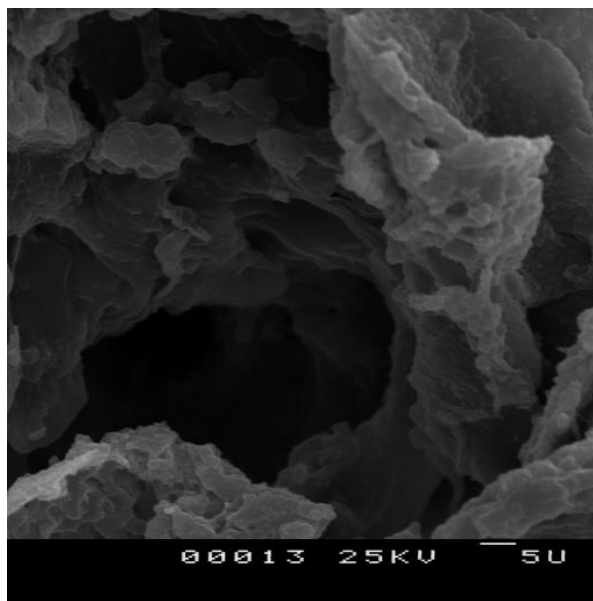
The STS showed alteration of the epithelial layer and cellular elements of the stroma. In ciliate cells degradation processes in all parts of the cell was observed: the disruption of cilia and their smoothing, increased intercellular spaces and changing the configuration of the cells. A large number of goblet cells were located near the basement membrane, and in the apical part of the epithelium goblet cells were found in the extrusion stage. Many patients, especially in the

severe form of the disease, had sharp extrusion of secretion, which is most manifested in the mucosa of the nasal cavity than in the lower respiratory tract.

SEM showed that the surface of the epithelial cells of the upper and lower respiratory tract in AR and BA tend to have the same type of changes, what along with clinical indicators confirms the general pattern of hyperreactivity of these parts, which was determined by provocation tests, proving the similarity of epithelium damage mechanisms [6,8,9]. Using SEM as well as STS we found out that the most damage have the ciliated cells throughout the respiratory tract, but mostly are damaged cells in the nasal mucosa. These changes are characterized by disorientation, deformation of the cilia, up to their smoothing. Using TEM we found intracellular destructive processes in the cell population, which often lead to atrophy. Significant changes are observed in the goblet cells, which playing their protective function, respond to injury by promoted secretion, which we have shown by both light and electron microscopy. This reaction is more expressed in



a



b

FIGURE 2. SEM. SNC. Fixation – 2,5% glutaraldehyde. Application of Aurum. Patient B. 14. Goblet cells in the process of accumulation. x 1500 (a) and extrusion of secretion. x 1500 (b)

the nasal mucosa, which primarily response to pathological impacts [4,7,9].

In some parts of the epithelium there is such great secretion extrusion of goblet cells, that mucin granules cover with a thick layer these damaged areas (fig. 2 a,b).

The intensity of these changes is directly related with the severity of the disease, the period of its debut and duration of the illness. Along with this, the SEM and TEM make it possible to characterize microvascular changes in different parts of the respiratory tract. Observing increased vascular pattern, its roughness, contraction and expansion, unevenly located in different areas we can detect the severity of symptoms and the severity of the pathological process what help us to choose the most efficient method of treatment.

Practical work shows that treatment, which is focused on on-time basic therapy and ASIT realization is the most effective method for the treatment of allergic respiratory diseases. After the ASIT we observe the intensive decrease of reactivity of the mucous membranes of the nose and bronchi, increased regeneration processes of the respiratory epithelial barrier, which are more expressed in young age group compared with adults. These data are confirmed by us using the clinical and morphological methods. Managing ASIT on-time not only reduces the development of clinical symptoms of rhinitis and asthma, but also reduces the amount of consumed drugs and the risk of developing severe forms of the disease and improving the quality of life of the patient.

With the special complex clinical and immunological, allergy and morpho-functional studies in patients with AR and BA in different types of severity, we confirmed the unity of the mechanisms of allergic inflammation of the respiratory tract in AR and BA. We should also note that in the development of AR and BA in juveniles a leading role play: compromised allergic history, inadequacy diagnostics and inadequate treatment of associated allergic diseases, endocrine imbalance, frequent ARVD, bad habits gaining.

It is shown that on-time administration of basic treatment and early ASIT promotes rapid morphological regeneration of the epithelium of the mucous membranes of the respiratory tract. It is important that airways reactivity reducing while using the therapy and regeneration is more intensive in juveniles compared to adult patients. All these promote the most favorable prospects of treatment of AR and BA in patients with on-time and adequate therapy.

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БРОНХИАЛЬНА АСТМА ТА АЛЕРГІЧНІ РИНИТИ В РІЗНИХ ВІКОВИХ ГРУПАХ

На базі відділення «Бронхіальна астма» ФГБУ «ГНЦ» Інститут імунології» ФМБА Росії і в ФГБУ НДІ морфології людини РАМН проведено поглиблене комплексне клініко-лабораторне, алергологічне, імунологічне та морфо-функціональне обстеження хворих у віці від 14 до 65 років. Залежно від віку та особливостей клінічного перебігу алергічного риніту і бронхіальної астми, пацієнти були розподілені на групи. Виявлено комплекс даних, що свідчать про єдність механізмів розвитку алергічного запалення і гіперреактивності дихального тракту при АР і БА. Представлено аналіз підліткової БА. Показано, що своєчасне призначення базисної терапії та раннє проведення АСИТ сприяє якнайшвидшій морфологічній регенерації епітелію слизових оболонок дихальних шляхів.

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Гуменюк С.А.,**

БРОНХИАЛЬНАЯ АСТМА И АЛЛЕРГИЧЕСКИЕ РИНИТЫ В РАЗЛИЧНЫХ ВОЗРАСТНЫХ ГРУППАХ

На базе отделения «Бронхиальная астма» ФГБУ «ГНЦ «Институт иммунологии» ФМБА России и в ФГБУ НИИ морфологии человека РАМН проведено углубленное комплексное клиничко-лабораторное, алергологическое, иммунологическое и морфо-функциональное обследование больных в возрасте от 14 до 65 лет. В зависимости от возраста и особенностей клинического течения аллергического ринита и бронхиальной астмы пациенты были распределены в группы. Вывявлен комплекс данных, свидетельствующих о единстве механизмов развития аллергического воспаления и гиперреактивности дыхательного тракта при АР и БА. Представлен анализ подростковой БА. Показано, что своевременное назначение базисной терапии и раннее проведение АСИТ способствует скорейшей морфологической регенерации эпителия слизистых оболочек дыхательных путей.

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Multisensory educational system “electronic tongue” for the diagnosis of the functional state of the human body on the characteristics of exhaled breath condensate

The structure of a learning diagnostic system, produced on basis of a new concept of multi-parameter electrochemical analysis, simulating the organization and functioning of biological sensory systems, is developed. System features: 1) the use of an array of multi-selective sensors with cross-sensitivity to different chemical components in the investigated medium, 2) evaluation of the measurement results using the methods of processing large amounts of multi-dimensional information and pattern recognition. Such analysis systems are called “electronic tongue”. The purpose of this paper is to investigate the possibility of application of analytical trained multisensor system for non-invasive monitoring of individual functional state of the human body using exhaled breath condensate indicators. The diagnostic system consists of two units - the measuring and informational. They realize the basic functions of the system: learning and recognition of multi-dimensional “images” formed by the sensor module. Studies performed on the test subjects have shown that each of them has its own individual reproduced “image” of exhaled breath condensate and can be identified on this “image”. The results obtained indicate the possibility of developing on this basis a new non-invasive method of rapid diagnosis of the functional state of the person on indicators of exhaled breath condensate.

INTRODUCTION

Human activity is largely determined by the metabolic processes in the organs and tissues. Their products are distributed throughout the internal environment (cells, extracellular space, blood plasma, exhaled air, urine, etc.). They are indicators of the functional state of physiological systems and characterize the “image” of the functional state of the organism. Therefore the analysis of the chemical composition liquid and gaseous media of the body is the leading technology of diagnosis, prognosis and monitoring of the treatment process [6, 7]. This procedure requires the precise measurement of large quantities of chemical components. For example, in human exhaled air detected over 600 chemicals indicators its functional state.

A significant number of laboratory diagnostic tests, that determine the content of chemical substances (indicators of disease) in the body,

are made using electrochemical methods. These methods are used in most modern analytical devices to measure the content of organic and inorganic materials in different media of the body. Their sensors (in most cases) are sensitive not only to main test substance, but also a number of related (“interfering”) substances [1, 2, 5]. This increases the error of measurements. Meanwhile, biological sensory systems with high accuracy recognize complex “images” of the object being analyzed, precisely because their sensors have cross-sensitivity to a range of substances contained in it. These properties are determined their two features: a - their receptors are cross sensitivity, b - in the process of identification of the object involved “information system” - the brain, which carries out the processes of learning and pattern recognition. These principles of sensory analysis systems of humans and animals were

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the basis of a new, intensely developed areas multiparametric electrochemical analysis of complex multicomponent biological media. Analytical systems created on this basis were called “electronic nose”, “electronic tongue”. They have been successfully used to control of the liquid and gaseous body media, environment and the food [3, 8, 4]. Their features are: 1) the measurement is made with the use of sensors with cross-sensitivity to different groups of important chemical components of the medium, 2) evaluation of the results of measurements carried out with the help of pattern recognition methods, processing of large arrays of multidimensional data and mathematical models of neural networks.

The purpose of this study is development multi-sensor analysis system using an array of sensors with cross-sensitivity to different groups of important chemical components of exhaled breath condensate, able to create an “image” of the functional state of the human body for later use it for diagnosis of various diseases.

DESCRIPTION OF THE DIAGNOSTIC SYSTEM

The diagnostic system consists of two units - the measuring and informational. The first unit includes of sample preparation module, sensors module, measurement module. The second unit includes modules: learning, pattern recognition and visualization of images.

Sample preparation module is designed to obtain condensate from exhaled air of test subject.

Sensor module consists of six electrochemical electrodes (sensors), each of which has a high sensitivity to the main determining ion (Na^+ , K^+ , Ca^{+2} , NO^{-3} , NH_4^+ , H^+) and the cross-sensitivity to biologically important components (other inorganic ions, proteins, fats, organic acids, products of lipid peroxidation, the white blood cells, foreign substances). Each of the sensors, placed in exhaled air condensate, generates its electrical potential. Their composition forms the “image” of the condensate of the diagnosed

person. The composition and number of sensors are selected based on their cross-sensitivity to diagnostically significant components of the object.

Measurement module (based on microprocessor) performs high-precision measurement of the electrical potentials of the electrodes (measuring range $+4.0$ V, permissible limit of error ± 0.005 mV) and the transfer of this information to the computer. This information is statistically processed and displayed graphically on the computer screen.

Learning and recognition module is presented in the form of a mathematical model. During training, the system creates a database (“images”) in the form of a particular composition of electrical potentials of the electrodes that characterize the functional state of certain test subjects. In the recognition process, the composition of electrical potentials (registered by from the test subject) compared with the composition of known potential “images” in the database. Then, in accordance with a predetermined criterion (permissible deviations potentials recorded by the electrodes from their average values in the database), the decision is made about which the “image” of the database corresponds to the functional state of the test subject.

EXPERIMENTAL RESEARCH

Studies were carried out on three test subjects during five days. Following conditions are met: 1) the absence of signs of acute illness in the subjects, 2) lack of physical activity for 3 hours prior to the collection of condensate, 3) the experiment began not earlier than 3 hours after a meal, at one and the same time (12 hours). A sample of the condensate was collected for 10 minutes. The value of electrode potential in the obtained sample was measured for each of the sensors (Na^+ , K^+ , Ca^{+2} , NO^{-3} , NH_4^+ , H^+). The results of measurements were processed statistically. They are presented in Figure 1 as a visual “images” of the condensate - hexagons

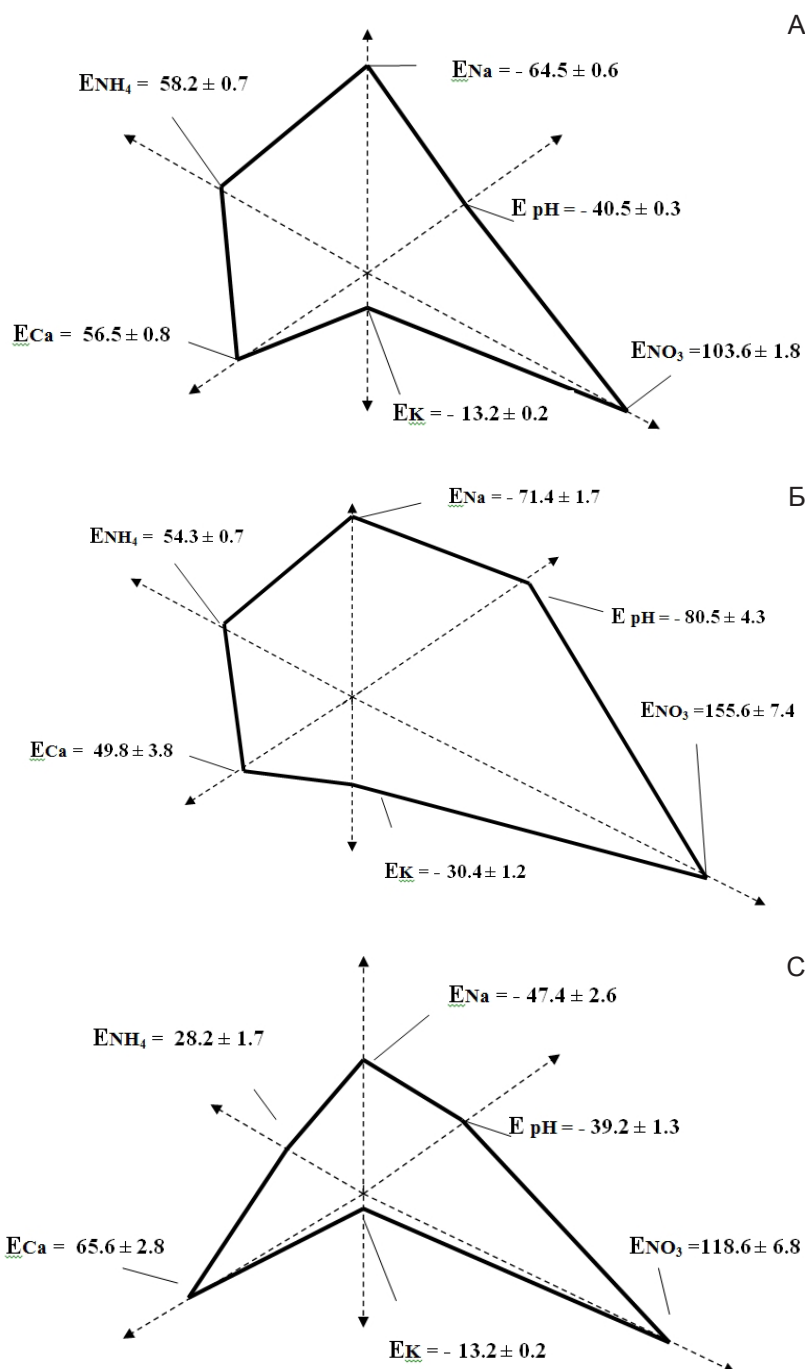


Figure 1. A visual representation of the “image” of condensate test subjects (A, B, C) in the form of diagrams - the hexagon. On the axes (dotted lines) are indicated average values ($n = 5$) electrical potentials of six electrodes. E_x - symbol represents recorded electrode potential (mV) with the basis of the ion (e.g., E_{Na} - is the total electric potential of the sodium electrode that includes potential generated ions Na, and additional potentials caused sensitivity of the electrode a number of chemical components in the sample). To the right of this symbol is shown the arithmetic mean value of the total electric potential of each electrode and the value of its mean square deviation

with rays (the coordinate axes), directed from the center to the point of intersection of the facets of the hexagon. The length of the rays at the points of intersection facets hexagon corresponds to the arithmetic mean of the measured electric potential (average of 5 samples) generated by each electrode.

The presented data shows that by using a selected set of potentiometric sensors with cross-sensitivity, may be obtain reproducible individual "images" of the condensate tested subjects (standard deviation of less than 5% of the arithmetic average of the electrode potential). These «images» in the process of «training» are stored in the computer's memory. The character of their changes, depending on the functional state of the tested subjects, will be the subject of follow-up studies.

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Кислякова Л.П., Кисляков Ю.Я., Зайцева А.Ю., Гуляев В.И.

МУЛЬТИСЕНСОРНАЯ ОБУЧАЕМАЯ СИСТЕМА «ЭЛЕКТРОННЫЙ ЯЗЫК» ДЛЯ ДИАГНОСТИКИ ФУНКЦИОНАЛЬНОГО СОСТОЯНИЯ ЧЕЛОВЕКА ПО ХАРАКТЕРИСТИКАМ КОНДЕНСАТА ВЫДЫХАЕМОГО ВОЗДУХА

Разработана структура обучаемой диагностической системы, создаваемой на основе новой концепции многопараметрического электрохимического анализа, основанного на имитации способов организации и функционирования биологических сенсорных систем. Особенности системы: 1) использование массива полиселективных сенсоров с перекрестной чувствительностью к различным химическим компонентам исследуемой среды, 2) оценка результатов измерений с применением методов обработки больших объемов многомерной информации и распознавания образов. Подобные аналитические системы получили название «электронный язык». Целью настоящей работы является исследование возможности применения обучаемой мультисенсорной аналитической системы для не-

инвазивного индивидуального контроля функционального состояния организма человека по индикаторам конденсата выдыхаемого воздуха. Диагностическая система состоит из двух модулей: измерительного и информационного. Они реализуют основные функции системы: обучения и распознавания многомерных «образов», формируемых сенсорным модулем. Исследования, проведенные на испытуемых, показали, что каждый из них имеет свой индивидуальный, воспроизводимый «образ» конденсата выдыхаемого воздуха и может быть идентифицирован по этому «образу». Полученные результаты свидетельствуют о возможности разработки на их основе нового неинвазивного метода экспресс-диагностики функционального состояния человека по показателям конденсата выдыхаемого воздуха.

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Universal mobile analytical complex for research of respiration and gas exchange in man and animals

Experimental model of universal mobile and compact analytical complex for continuous monitoring of O_2 consumption and CO_2 emissions in breathing humans and laboratory animals has been developed. These gas exchange parameters are calculated based on the simultaneous recording of expiratory flow rate and partial pressures of oxygen (pO_2) and carbon dioxide (pCO_2) in the exhaled air during each respiratory cycle (for a man), and the dynamics of the partial pressures of O_2 and CO_2 in a metabolic chamber (for animals and other biological objects). An analytical complex has a small size, light weight, and measure human respiration and gas exchange immediately during each breathing cycle. The analytical complex consists of two units - the measuring and informational. The first unit consists of a sample preparation module, the sensor module, data logging module and the module data transmission through the channels wired and wireless communications in the information unit. The second unit consists of a module data reception from the measuring unit and the processing module, for the analysis and storage of data. The experimental results showed that the generated mobile analytical complex allows to measure with high precision volumetric expiratory flow rate and the partial pressure of O_2 and CO_2 in the breath of man, as well as the partial pressures of O_2 and CO_2 in a metabolic chamber with the animal. The complex has been tested in human studies and in studies on laboratory animals.

INTRODUCTION

The state of energy and plastic metabolism, determined by the intensity of O_2 consumption and CO_2 emissions, is the leading method for diagnosing the functional state of humans and animals [1, 3, 4]. For the determination of these parameters is necessary for a certain period of time continuously, synchronously and accurately carry out measurements space velocity of breathing and the content of O_2 and CO_2 in air exhaled during each breathing cycle.

Currently on the market there are a considerable number of instruments for recording these parameters (EOS-Sprint, ER 800, ER 900, Ergo-line, Oxycon 5, Meta Max 3B, Rapidlab 840, Synthesis 15, Omni 3). The main disadvantages of most of these devices - a complicated procedure of removing moisture from exhaled air, large size, stationary conditions of exploitation, the high cost of the devices themselves and expendable materials.

The purpose of this work - the development of universal, high-speed, compact, mobile and economic access analytical complex for the diagnosis of energy metabolism in humans and animals with the use of new technology, constructive and informational solutions.

DESCRIPTION OF THE DIAGNOSTIC SYSTEM

The analytical complex consists of two units - the measuring and informational. The measurement unit is intended for registration the parameters of external respiration and gas exchange of subjects and transfer this data into information block. The information unit is intended to receive data from the measuring unit and determination on the basis of their state of energy and plastic metabolism, physical efficiency and physical health subjects on the whole complex of recorded and calculated parameters.

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The first unit consists of a sample preparation module, the sensor module, electronic data logging module and the data transmission module through the channels a wired or wireless communications to the information unit. The second unit consists of a module data reception from the measuring unit and the processing module for the analysis and storage of data.

Sample preparation module provides a continuous flow of exhaled air in the sensor module. It is thermostatic breathing tube that contains sensors for monitoring the parameters of respiration and gas analysis.

Sensor module consists of three electrodes (sensors), each of which has a high sensitivity to the main determining respiration and gas exchange parameters: volume expiratory flow rate q (l / sec), the partial pressure of O_2 and CO_2 in expired air (mm Hg). Control of the partial pressures of O_2 (amperometry method) and CO_2 (infrared spectroscopy method) is carried out continuously during each exhalation. Each of the sensors generates its electrical potential.

Electronic data logger module (based on microprocessor) performs high-precision measurement of the electrical potentials of the electrodes (measuring range +12.0 V, permissible limit of error ± 0.005 mV) and transmission of these data in the information

unit. This information is statistically processed and displayed graphically on the computer screen.

Transmitting and receiving modules send the data from the measuring unit in the information unit through the channels wired or wireless connection.

Module handling, analysis and storage of recorded data provides their mathematical treatment, visual display of the calculated indicators and storing them in an array of data, tables and graphs.

The basic continuously measured parameters: the volume rate of exhaled air during the respiratory cycle, the content of O_2 and CO_2 in exhaled air and the environment, temperature of the environment, atmospheric pressure.

Definable parameters: indicators of pulmonary ventilation, the intensity of O_2 consumption, CO_2 evolution intensity, the respiratory coefficient.

EXPERIMENTAL RESEARCH

Technical characteristics analytical complex (error measurements and dynamic parameters) determined using the calibration gas mixtures and the stand, simulated gas flow rate of during exhalation, are presented in Table 1.

Table 1. Technical characteristics of sensors.

Registered parameters	Range	Error limit of measurement
pO_2 , mmHg	50–200	+0.2
pCO_2 , mmHg	0–60	+0.3
Expiratory flow rate, l / s	0 – 12	+ 2%

Investigate the dynamic characteristics of sensors with step changes in the composition and flow rate of gas mixtures showed that the time to reach steady state of their evidence less than 0.1 seconds.

The developed analytical complex allows to carry out continuous measurements recorded parameters of human breathing (volumetric expiratory flow, the partial pressures of O_2

and CO_2 in exhaled air) during each exhalation individually for each measured parameter (Fig. 1), in any combination of the two analyzed parameters (Fig. 2), as well as to determine their average values for the three parameters being measured in a given period of time of continuous respiration (Fig. 3), which allows to determine the intensity of O_2 consumption and CO_2 emissions.

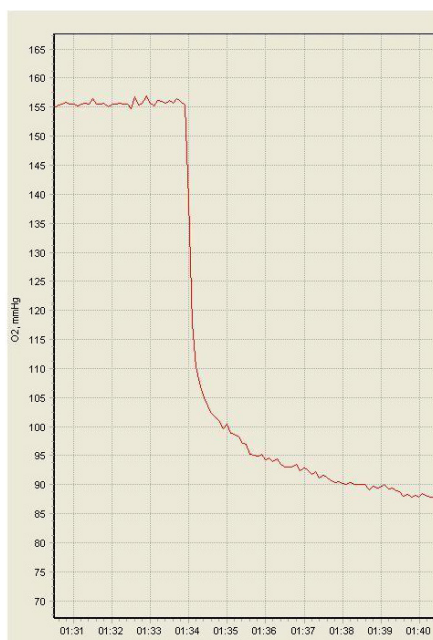


Figure 1. Measurements of the partial pressure of O_2 in the flow of exhaled air during prolonged exhalation. On the horizontal axis - time in minutes and seconds

For laboratory animals these indicators can be determined for a certain period of time in a closed space metabolic chamber (Fig. 4).

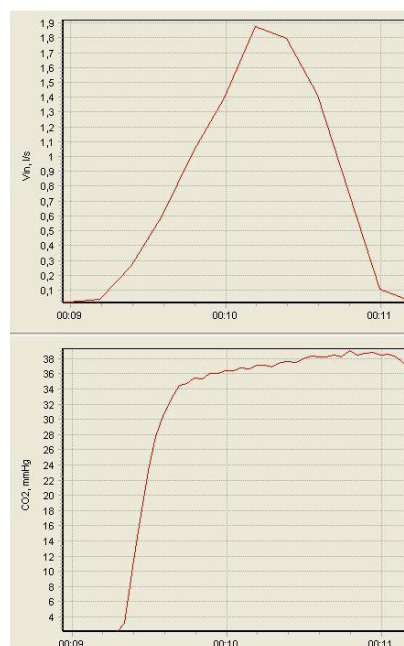


Figure 2. Simultaneous recording of expiratory flow rate (V_n) and the partial pressure of CO_2 in exhaled air. On the horizontal axis - time in minutes and seconds

Demonstrated research results indicate the possibility of using the analytical complex in physiological studies of the functional state

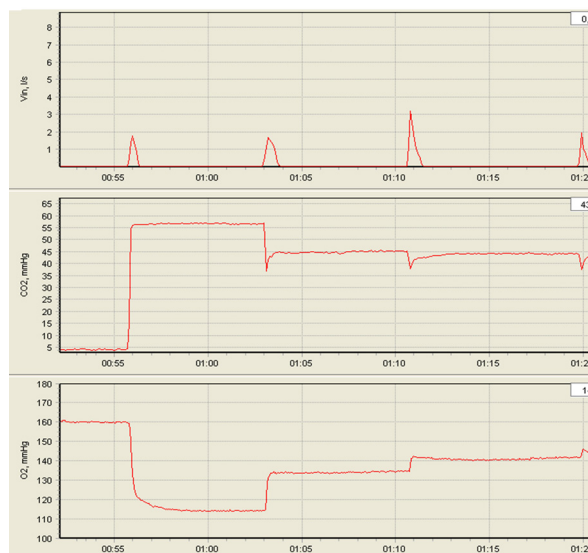


Figure 3. Simultaneous recording of three parameters (volume expiratory flow rate (V_n), the partial pressure of O_2 and CO_2 in exhaled air) after a delay of breathing (55 seconds). On the horizontal axis - time in minutes and seconds

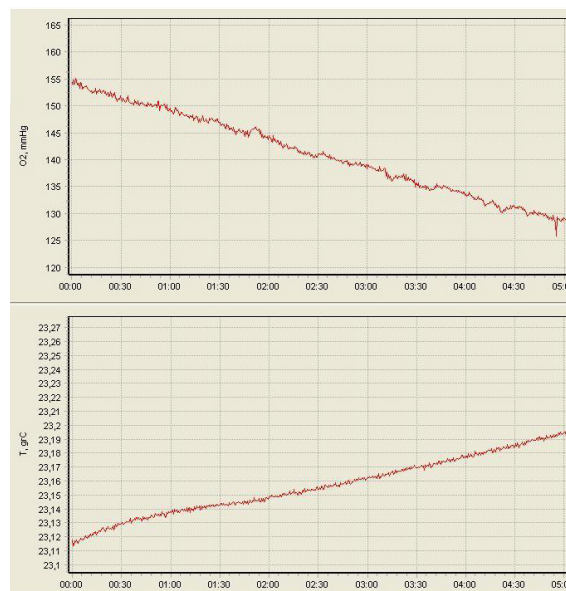


Figure 4. Changes O_2 partial pressure and temperature in the metabolic chamber for 5 minutes stay a rat in it. On the horizontal axis - time in minutes and seconds

of gas exchange and respiratory systems of humans and animals, as well as to measure the basic parameters of the gas exchange of the body (the intensity of O_2 consumption and CO_2 emissions) characterizing its metabolism in normal, pathological and extreme situations [2]. *The project was supported by grants of the Program of the Presidium Russian Academy of Sciences «Establishment and improvement of methods of chemical analysis and investigation of the structure of substances and materials».*

Kislyakov Yu.Ya., Kislyakova L.P., Zaiceva A.Yu., Gulyaev V.I.

UNIVERSAL MOBILE ANALYTICAL COMPLEX FOR RESEARCH OF RESPIRATION AND GAS EXCHANGE IN MAN AND ANIMALS

Разработан экспериментальный образец универсального мобильного малогабаритного аналитического комплекса для непрерывного контроля параметров дыхания, потребления O_2 и выделения CO_2 человеком и лабораторными животными. Показатели газообмена вычисляются на основе данных одновременной регистрации объемной скорости выдоха и парциальных давлений кислорода (pO_2), углекислого газа (pCO_2) в выдыхаемом воздухе в процессе каждого дыхательного цикла (для человека) и по динамике парциальных давлений O_2 и CO_2 в метаболической камере (для животных и других биологических объектов). Аналитический комплекс имеет малые габариты, небольшой

вес и измеряет параметры дыхания и газообмена человека непосредственно в процессе каждого дыхательного цикла. Он состоит из двух блоков –измерительного и информационного. Измерительный блок включает модуль пробоподготовки, сенсорный модуль, модуль регистрации данных и модуль передачи данных в информационный блок по каналам проводной или беспроводной связи. Информационный блок включает модуль приема данных от измерительного блока и модуль обработки, анализа и хранения зарегистрированных данных. Проведенные экспериментальные исследования показали, что созданный мобильный аналитический комплекс позволяет измерять с высокой точностью объемную скорость выдоха и парциальные давления O_2 и CO_2 в выдыхаемом воздухе человека, а так же парциальные давления O_2 и CO_2 в метаболической камере с животным. Комплекс был испытан в исследованиях на людях и лабораторных животных.

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Зміни клітинного інфільтрату в слизовій оболонці бронхів у хворих на хронічне обструктивне захворювання легень залежно від рівня антипротеолітичного захисту

Вперше вивчалися генотип та сироватковий рівень альфа-1-антитрипсину (ААТ), експресія муцинів MUC-2, MUC-3, MUC-4 в слизовій оболонці бронхів хворих на хронічне обструктивне захворювання легень (ХОЗЛ) під час інфекційного та неінфекційного загострення. Встановлено, що при загостренні ХОЗЛ на фоні зниження антипротеолітичного захисту в різних типах епітеліальних клітин слизової оболонці бронхів відбувається різного ступеню виразності зниження експресії антигенів MUC2 та MUC3. Виявлена експресія антигенів MUC2 та MUC3 у веретеноподібних клітинах стромы (фібробластах) при загостренні ХОЗЛ дозволяє засвідчити факт епітеліально-мезенхімальної трансформації, а саме, зміну якості трансформованих епітеліальних клітин, в зв'язку із чим вони по-іншому, ніж звичайні епітеліальні клітини, реагують на молекулярні фактори, які відіграють роль у розвитку запального процесу та прогресуванні фіброзу при ХОЗЛ.

ВСТУП

В'язкий слизовий гель, головною складовою частиною якого є муцини, покриває більшість епітеліальних поверхонь і служить селективним захисним бар'єром між плазматичною мембраною і оточенням клітини [7]. Дослідження функції муцинів можуть пояснити деякі клітинні механізми прогресування запалення дихальних шляхів (ДШ) [9]. Зміни продукції муцинів та дискринія є загальною ознакою запального процесу при хронічних обструктивних захворюваннях легень (ХОЗЛ), що логічно обґрунтовує важливість дослідження впливу муцинів на процеси прогресування запального процесу в ДШ [1, 6]. Відомо, що під дією прозапальних цитокінів посилюється експресія гена, що кодує муцин, а нейтрофільна еластаза і катепсин G стимулюють проліферацію секреторних клітин слизових залоз, що призводить до ремоделювання ДШ, в тому числі до клітинної гіперплазії

[12]. Функцію основного інгібітору нейтрофільної еластази та інших пошкоджуючих протеаз (трипсіна, хімотрипсіна, катепсіна G, протеїнази-3, тромбіну, калікреїну та інш.) виконує альфа-1-антитрипсин (ААТ), який грає провідну роль в гальмуванні тканинного протеолізу [11]. Дефіцит ААТ залишається мало діагностованою проблемою, знання про його наявність є дуже важливим для хворого. Вивчення функціонального стану муцинів на молекулярному рівні та факторів, які на нього впливають, має важливе значення для подальших досліджень, спрямованих на попередження прогресування та лікування ХОЗЛ.

Метою нашого дослідження було комплексне вивчення імуногістохімічних змін клітинного інфільтрату за вмістом муцинів MUC 2, 3, 4 у слизовій оболонці (СО) бронхів при ХОЗЛ в осіб з різними сироватковим рівнем ААТ і його генотипом.

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МЕТОДИКА.

Обстежено 30 пацієнтів, які знаходилися на стаціонарному лікуванні у пульмонологічному відділенні обласної клінічної лікарні м. Чернівці. Вік обстежених коливався від 26 до 77 років (в середньому $53,7 \pm 2,1$ року). У дослідження включалися хворі, які звернулися в стаціонар в період загострення, яким був виставлений діагноз ХОЗЛ (виставлявся на підставі критеріїв діагностики “Key indicators for considering a diagnosis of COPD” [2, 5]). З метою визначення ступеня та характеру запалення бронхів всім хворим проводили фібробронхоскопію (ФБС) за допомогою фібробронхоскопа Olympus. У зв'язку із необхідністю збереження для імуногістохімічних досліджень цілісності антигенів у структурах бронхів виконували прижиттєву біопсію СО бронхів за загальноприйнятою методикою. Рівень біопсії – шпори сегментарних бронхів. Під час фібробронхоскопії спеціальними щипцями забирали шматочок стінки бронху із макроскопічно-змінених ділянок. Висікався шматочок тканини, що містив лише слизову та підслизову оболонки, не травмуючи хрящові кільця. Свіжий матеріал фіксували протягом 22 годин в нейтральному забуференому 10% водному розчині формаліну, після чого здійснювали зневоднювання у висхідній батареї етанолу і заливку в парафін. З парафінових блоків на санному мікромомі виготовляли зрізи товщиною 5 мкм. Парафінові зрізи монтували на неімуногенні предметні скельця SuperFrost®Plus (Germany). Вивчали оптичну густину (в умовних одиницях) специфічного забарвлення структур бронхів у хворих на ХОЗЛ при застосуванні імуногістохімічних методик на антигени MUC-2, MUC-3, MUC-4 за допомогою первинних моноклональних АТ до цих протеїнів та системи візуалізації Dako EnVision+System, Peroxidase (AEC). Інтенсивність забарвлення (оптична густина) оцінена об'єктивно на цифрових копіях оптичних зображень мікропрепаратів за допомогою методу комп'ютерної мікроденсито-

метрії у середовищі графічної комп'ютерної програми GIMP, версія 2,82 (ліцензія GPL) зондовим способом у градаціях інтенсивності забарвлення (від 0 до 255) з логарифмічним перерахунком в умовні одиниці оптичної густини (ум.од.опт.густина) з градацією від «0» (абсолютна прозорість) до «1» (абсолютна непрозорість).

Сироватковий рівень ААТ визначали за допомогою імуноферментного аналізу за допомогою панелі Immunokit на базі імунологічної лабораторії Університетської клініки при Донецькому національному медичному університеті ім. М. Горького. У всіх хворих проводилося генотипування S- і Z-дефіцитних алелей - методом полімеразної ланцюгової реакції з висушених зразків крові в Національному інституті туберкульозу та хвороб легень (Варшава, Польща).

Статистична обробка результатів проводилася з використанням методів інтервованої системи статистичної обробки стандартного пакету Microsoft Excel для вірогідності 95% і 99%. Для протяжних змінних обчислювали середні величини, їх стандартні відхилення й помилки ($M \pm m$).

Обстежені хворі були розподілені на 2 групи: I група - хворі на ХОЗЛ III стадії без ознак інфекційного загострення – 14 пацієнтів та II група - 16 осіб, хворі на ХОЗЛ та ознаками інфекційного загострення I типу за Антонісеном та стадії III [5]. Серед хворих I групи жінок було 6 (42,8%), II групи – 7 (43,75%) осіб жіночої статі. За ставленням до тютюнокуріння хворі I групи розподілялися так: 11 - активні курці в минулому, 3 - залишилися ними і дотепер, стаж куріння – $41,2 \pm 5,1$ роки. Індекс „пачка/рік” становив $29,5 \pm 2,4$ роки. Середній показник ОФВ₁ у пацієнтів I групи становив $38,9 \pm 4,3\%$ до належної величини. До II групи увійшли 16 хворих на ХОЗЛ, середній вік $65,4 \pm 4,3$ роки. Середня тривалість захворювання – $18,3 \pm 1,7$ роки. Активних курців – 5 осіб, в минулому - 15 осіб, індекс „пачка/рік” становив $48,3 \pm 3,8$ роки. Середній показник ОФВ₁ у пацієнтів I

групи складав $37,6 \pm 2,7\%$ від належних.

Результати та їх обговорення. Генотипування ААТ показало, що більшість обстежених пацієнтів виявилися гомозиготними по М-аллелю (93,33%), у двох пацієнтів II групи був виявлений генотип RiMZ (6,67%).

Серед пацієнтів I групи нормальний рівень ААТ (120 - 200 мг/дл) спостерігався у 11 осіб (78,57%), суміжні значення ААТ (100 - 120 мг/дл) – у 2 осіб (14,29%), у одного хворого (7,14%) рівень ААТ був низьким (менше 100 мг/дл). Відповідно пацієнтів II групи: 10 осіб (62,5%) мали нормальні показники ААТ у сироватці крові, 4 осіб (25%) – суміжні, 2 (12,5%) – низькі.

Серед пацієнтів з нормальним рівнем ААТ кількість курців та некурців розподілилася в процентному співвідношенні 59,09% до 40,91%. Серед хворих з суміжними значеннями ААТ теж превалювали курці - 80,0% до 20,0%. Усі пацієнти з низьким рівнем ААТ виявилися курцями (100%). Таким чином, куріння може розглядатися як фактор ризику, який гальмує генетично нормальну продукцію ААТ. Наші дані зіставленні з міжнародними [3, 5, 11]. У курящих осіб з дефіцитом ААТ активність протеолітичних ферментів може бути значно вище норми, а знижена

антіпротеазна активність не в змозі захистити легеневу тканину від ранньої деструкції.

При використанні імуногістохімічних методів на антигени MUC-2, MUC-3, MUC-4 у хворих на ХОЗЛ I групи отримані наступні результати. Позитивне імуногістохімічне забарвлення (експресія) на антиген MUC-2 спостерігається в поодиноких клітинах, які злучені у просвіт бронхів (рис.1), оптична густина такого забарвлення у середньому становить $0,128 \pm 0,010$ ум.од.опт.густини. Точно такі ж цифри отримані для покривного епітелію бронхів (табл.).

Експресія антигену MUC-3 відмічається в покривному епітелії бронхів з виразністю від середньої до слабкої із середніми цифрами оптичної густини забарвлення $0,201 \pm 0,021$ ум.од.опт.густини. Максимальна експресія антигену MUC-3 в покривному епітелії бронхів показана на рисунку 2.

У келихоподібних клітинах експресія антигену MUC-3 не виявлена. У слизових залозах експресія MUC-3 відсутня у більшості клітин, а в тих клітинах, в яких вона присутня, її можна оцінити як помірну.

У стромі бронхів експресія MUC-3 визначається в веретеноподібних клітинах під субепітеліальною базальною мембраною

Таблиця. Оптична густина (в умовних одиницях) специфічного забарвлення структур бронхів у хворих на ХОЗЛ при застосуванні імуногістохімічних методик на антигени MUC-2, MUC-3, MUC-4 ($X \pm s_x$)

Імуногістохімічна методика/локалізація	I група хворих (n=14)	II група хворих (n=16)	Вірогідність розбіжностей (P) у середніх тенденціях за методом Mann-Whitney
MUC-2 / покривний епітелій	$0,128 \pm 0,010$	Не визначається	P не обраховано
MUC-2 / келихоподібні клітини	$0,247 \pm 0,016$	$0,196 \pm 0,015$	$P < 0,05$
MUC-2 / епітелій слизових залоз	$0,212 \pm 0,018$	$0,103 \pm 0,008$	$P < 0,01$
MUC-2 / MUC-2-позитивні веретеноподібні клітини стромы	$0,208 \pm 0,025$	$0,206 \pm 0,028$	$P > 0,05$
MUC-3 / покривний епітелій	$0,201 \pm 0,021$	$0,049 \pm 0,006$	$P < 0,001$
MUC-3 / келихоподібні клітини	Не визначається	Не визначається	P не обраховано
MUC-3 / епітелій слизових залоз	$0,192 \pm 0,009$	$0,065 \pm 0,007$	$P < 0,001$
MUC-3 / MUC-3-позитивні веретеноподібні клітини стромы	$0,286 \pm 0,024$	$0,289 \pm 0,028$	$P > 0,05$
MUC-4 / будь-які структури	Не визначається	Не визначається	P не обраховано

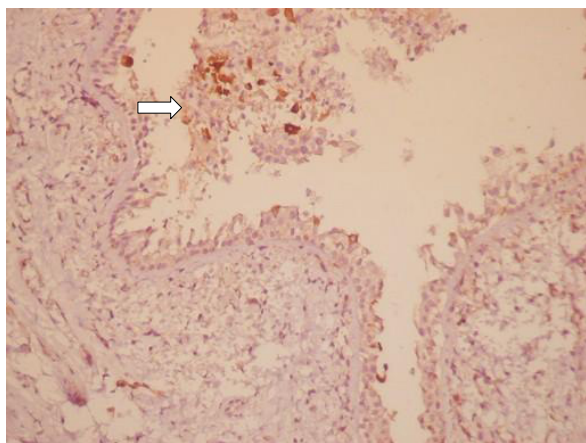


Рис. 1. Експресія антигену MUC-2 в десквамованому епітелії бронха у хворих на ХОЗЛ І групи. Імуногістохімічний метод, візуалізація з діамінобензидином. Об.20^х.Ок.10^х

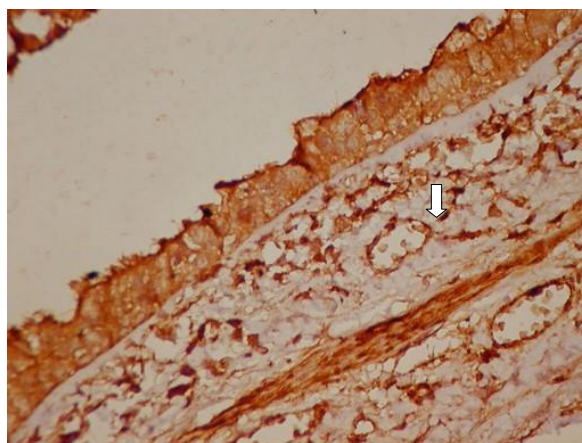


Рис. 2. Експресія антигену MUC-3 в покривному епітелії бронха та у веретеноподібних (фібробластоподібних) клітинах строми бронха у хворих на ХОЗЛ І групи. Імуногістохімічний метод, візуалізація з діамінобензидином. Об.20^х.Ок.10^х

(рис.2) з виразністю від помірної до сильно вираженої із середніми цифрами оптичної густини забарвлення $0,286 \pm 0,024$ ум.од.опт. густини.

Щодо MUC-4, то слід відмітити, що в наших дослідженнях експресія цього антигену, як правило, не визначалася в жодній структурі. Лише у двох пацієнтів експресія антигену MUC-4 була виявлена у покривному епітелії бронхів, причому вона була ледве помітною.

Імуногістохімічне дослідження експресії антигенів MUC-2, MUC-3, MUC-4 у хворих на ХОЗЛ II групи дало наступні результати.

Експресія антигену MUC-2 в покривному епітелії практично не визначається. Отже, продукція цього білка в покривному епітелії при інфекційному загостренні ХОЗЛ порушена. Окрім того, за інтенсивністю забарвлення слід констатувати зниження експресії антигену MUC-2 в келихоподібних клітинах та епітелії слизистих залоз), у цифрах це показано в таблиці.

У покривному епітелії та епітелії слизистих залоз також знижена експресія антигену MUC-3 (табл.). У келихоподібних клітинах при інфекційному загостренні ХОЗЛ, так само, як і при неінфекційному, експресія антигену MUC-3 не визначалася.

На відміну від вищевказаних епітеліальних клітин в веретеноподібних клітинах строми бронха зниження експресії антигенів MUC-2 та MUC-3 при інфекційному загостренні ХОЗЛ не знайдено. Отже, продукція вказаних молекул у клітинах, які, вірогідно, піддалися так званій епітеліально-мезенхімальній трансформації (EMT), не змінена при інфекційному загостренні ХОЗЛ у порівнянні з ХОЗЛ без інфекційного загострення. Ймовірно, це додатково підтверджує зміну якості даних трансформованих епітеліальних клітин, в зв'язку із чим вони по-іншому, ніж звичайні епітеліальні клітини, реагують на молекулярні фактори, які відіграють роль у розвитку запального процесу та прогресуванні фіброзу при ХОЗЛ.

Таким чином, при загостренні ХОЗЛ спостерігається експресія секреторних муцинів - MUC2 та MUC3, що сприяють утворенню гелевого компоненту слизу. Синтез MUC2 і MUC3 зменшується в ДШ при інфекційному загостренні ХОЗЛ III в порівнянні з їх експресією при неінфекційному загостренні ХОЗЛ III в усіх структурах, окрім веретеноподібних клітин строми – фіброблестах. Хоча остаточна роль муцинів в ДШ не відома, вони можуть

функціонувати в якості рецепторів або рецепторних лігандів та активувати внутрішньоклітинні сигнальні каскади, що впливають на функції епітеліальних клітин, сприяючи, в тому числі розвитку ЕМТ [4,10, 13].

ВИСНОВКИ

1. Генотипування ААТ показало, що більшість обстежених пацієнтів виявилися гомозиготними по М-аллелю (93,33%), у двох був виявлений генотип RmZ (6,67%).

2. Усі пацієнти з низьким рівнем ААТ виявилися курцями, що дозволяє розглядати куріння як фактор ризику, який гальмує продукцію ААТ.

3. При інфекційному загостренні ХОЗЛ адекватний антипротейолітичний захист спостерігався у 62,5% обстежених, при неінфекційному загостренні - у 78,57%. На фоні зниженого антипротейолітичного захисту відмічається більш виражена проліферація секреторних клітин слизових залоз, що призводить до ремоделювання ДШ.

4. При інфекційному загостренні ХОЗЛ в різних типах епітеліальних клітин слизової оболонки бронхів знижується експресія антигенів MUC2 та MUC3.

5. Імуногістохімічне дослідження антигенів MUC2 та MUC3 у веретеноподібних клітинах строми дозволяє засвідчити факт так званої епітеліально-мезенхімальної трансформації в слизовій оболонці бронхів при загостренні ХОЗЛ.

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ИЗМЕНЕНИЯ КЛЕТОЧНОГО ИНФИЛЬТРАТА В СЛИЗИСТОЙ ОБОЛОЧКЕ БРОНХОВ БОЛЬНЫХ ХРОНИЧЕСКИМ ОБСТРУКТИВНЫМ ЗАБОЛЕВАНИЕМ ЛЕГКИХ В ЗАВИСИМОСТИ ОТ УРОВНЯ АНТИПРОТЕОЛИТИЧЕСКОЙ ЗАЩИТЫ

Впервые изучался генотип и сывороточный уровень альфа-1-антитрипсина (ААТ), экспрессия муцинов MUC-2,

MUC-3, MUC-4 в слизистой оболочке бронхов больных хроническим обструктивным заболеванием легких (ХОЗЛ) во время инфекционного и неинфекционного обострения. Установлено, что при обострении ХОЗЛ на фоне снижения антипротейолитической защиты в различных типах эпителиальных клеток слизистой оболочки бронхов происходит различной степени выраженности снижение экспрессии антигенов MUC2 и MUC3. Выявлена экспрессия антигенов MUC2 и MUC3 в веретеноподобных клетках строны (фибробластах) при обострении ХОЗЛ позволяет засвидетельствовать факт эпителиально-мезенхимальной трансформации, в связи с чем они по-другому, чем обычные эпителиальные клетки, реагируют на молекулярные факторы, играющие роль в развитии воспалительного процесса и прогрессировании фиброза при ХОЗЛ. Ключевые слова: альфа-1-антитрипсин, муцины, слизистая оболочка бронхов, воспаление.

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CHANGES OF THE CELLULAR INFILTRATE IN THE MUCOUS TUNIC OF THE BRONCHI IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE IN DEPENDENCE ON ANTIPROTOLITHIC PROTECTION

For the first time the genotype, serum levels of alpha-1-antitrypsin (AAT), the expression of mucin has been carried out of the mucous tunic of the bronchi in patients with chronic obstructive pulmonary disease (COPD) during an infectious and noninfectious exacerbation. It has been established that during an exacerbation of COPD in patients with reduced antiprotolitic protection in different types of epithelial cells of the mucous tunic of the bronchi there occurs a decline of the expression of antigens of MUC2 and MUC3 of a various degree of a marked character. The expression of antigens MUC2 and MUC3 detected in the fusiform cells of the stroma (fibroblasts) during a COPD exacerbation enables to certify a fact of an epithelial-mesenchymal transformation, as a result of which they react differently than ordinary epithelial cells to molecular factors which play a certain role in the development of an inflammatory process and progression of fibrosis in COPD.

Key words: alpha-1-antitrypsin, mucins, mucous tunic of the bronchi, inflammation.

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Влияние антиортостатического положения на регуляцию дыхания и компенсаторные реакции дыхательной системы

Показано, что у наркотизированных животных в антиортостатическом положении происходит увеличение резистивного и эластического компонента сопротивления дыханию, что свидетельствует об увеличении механической нагрузки на дыхательную систему. В компенсации респираторных эффектов антиортостатиза участвуют механорефлекторные механизмы, реализующиеся через афферентную систему легких и обеспечивающие обратную связь между изменением объема легких и центральной инспираторной активностью. Основным рефлекторным механизмом, вызывающим компенсаторное увеличение общего инспираторного усилия при переходе в антиортостатическое положение, является ослабление тормозной афферентной импульсации, поступающей в дыхательный центр от рецепторов растяжения легких, вследствие снижения легочных объемов.
Ключевые слова: регуляция дыхания, антиортостатическое положение, сопротивление дыханию, афферентная импульсация

ВВЕДЕНИЕ

Согласно современным представлениям, афферентная импульсация от механорецепторов легких играет исключительно важную роль в регуляции дыхания и формировании компенсаторных реакций дыхательной системы [2]. Нарушения биомеханики дыхания, связанные с разнообразными видами патологии или действия экстремальных факторов сказываются на физиологических механизмах, способствующих оптимизации дыхания.

Многочисленные данные свидетельствуют, что при изменении положения тела в пространстве по отношению к вектору гравитации (при постральных воздействиях) функциональные сдвиги затрагивают в первую очередь, систему кровообращения и дыхания, которые являются звеньями единой газотранспортной системы организма [2, 5, 9, 10]. Тем не менее, до сих пор нет общей картины развития взаимозависимых реакций дыхания и кровообращения на постральные воздействия, которая позволила бы наиболее полно представить механизмы регуляции респираторной системы в условиях измененной гемодинамики. Поскольку регионарное

перераспределение крови, возникающее при постральных воздействиях, изменяет кровенаполнение легочных сосудов и нарушает легочную гемодинамику [5], то совершенно очевидно, что происходящие сдвиги кровообращения в легких будут непосредственным образом отражаться на функциональном состоянии и регуляторных механизмах дыхательной системы. Однако в большинстве работ функция сердечно-сосудистой и дыхательной системы исследуется отдельно, а полученные результаты впоследствии обобщаются и рассматриваются как целостные реакции организма на влияние гравитационного фактора.

Исследованию регуляторных механизмов функционирования кардиореспираторной системы при естественных для человека физиологических позах (стоя, сидя, лежа) посвящено значительное количество работ. Между тем, механизмы регуляции дыхания в антиортостатическом положении (АОП) (наклон туловища головой вниз по отношению к горизонту) рассматриваются лишь в единичных работах. Следует отметить, что влияние антиортостатического положения на функ-

цию кардиореспираторной системы является одной из актуальных проблем последних десятилетий. Так, центральная гиперволемиа, возникающая при АОП также наблюдается при некоторых видах сердечно-легочной патологии, при астматических приступах, у больных с хроническими обструктивными заболеваниями легких, в положении Тренделенбурга. Кроме того, в практике космической медицины АОП применяется в качестве функциональной пробы, воспроизводящей эффекты перераспределения крови и жидкостных сред организма в невесомости [4, 15]. Исследования функции внешнего дыхания в условиях антиортостатической гипокинезии обнаружили изменения структуры легочных объемов и емкостей, вязкостно-эластического сопротивления легких, паттерна дыхания и газообмена [1, 11, 12, 18]. Это свидетельствует об ухудшении функционального состояния респираторной системы. Однако роль нейрогенных компонентов лежащих в основе формирования компенсаторных реакций системы дыхания на гравитационные воздействия окончательно не выяснена и требует дальнейшего углубленного изучения.

Цель настоящего исследования состояла в изучении роли афферентной системы легких в формировании компенсаторных реакций дыхательной системы на антиортостатическую нагрузку.

МЕТОДИКА

Эксперименты с соблюдением биоэтических правил были выполнены на 15 наркотизированных уретаном (1000 мг/кг) крысах линии Wistar, массой 280 ± 20 г. Антиортостатическое положение создавали наклоном поворотного стола на -30° к горизонтальной плоскости. Длительность экспозиции в АОП -30° составляла 30 минут. Постуральные реакции дыхательной системы исследовали до и после деафферентации легких (билатеральная цервикальная ваготомия).

В ходе эксперимента с использованием

метода пневмотахографии регистрировали объемно-временные параметры дыхания: частоту дыхания (ЧД), дыхательный объем (ДО), внутригрудное давление (ВГД), рассчитывали минутный объем дыхания (МОД). Для оценки резервных возможностей дыхательной системы использовали окклюзионное давление ($P_{0.1}$). Реакцию на инспираторную окклюзию оценивали по пиковым значениям внутригрудного давления при кратковременном перекрытии (0,1 с) дыхательных путей в начале вдоха.

Графическую регистрацию и первичный анализ экспериментальных данных производили с использованием аппаратно-программного комплекса "Biograph" (ГУАП, Санкт-Петербург), совмещенного с персональным компьютером IBM PC. Статистическая обработка данных производилась компьютерными средствами с использованием программы Microsoft Excel, вычисляли среднюю величину и ошибку средней регистрируемых показателей. Достоверность различий оценивали с помощью t-критерия по Стьюденту. Различия считали достоверными при $p < 0,05$.

РЕЗУЛЬТАТЫ И ИХ ОБСУЖДЕНИЕ.

Эксперименты, проведенные на вагально-интактных животных, показали, что изменение паттерна дыхания начинается сразу после перевода животного в АОП- 30° . Через 30 минут после перевода животного из горизонтального в антиортостатическое положение наблюдалось снижение максимальной скорости инспираторного потока на 28% ($p < 0,05$), ДО на 18 % и МОД на 14 % ($p < 0,05$), соответственно. Отмечалась тенденция к снижению ЧД на 6% за счет удлинения фазы вдоха, однако эти изменения не являлись статистически значимыми ($p > 0,05$). Одновременно с уменьшением объемно-временных параметров дыхания наблюдался резкий рост инспираторного ВГД в среднем до 216 ± 17 % (рис. 1). В некоторых экспериментах наблюдалось почти пятикратное увеличение этого показателя.

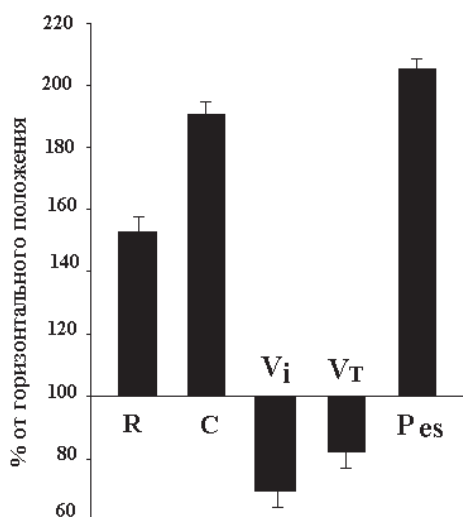


Рис. 1. Изменения основных параметров дыхания через 30 минут действия антиортостатиза.

По оси абсцисс: R – резистивное сопротивление дыханию; C – эластическое сопротивление дыханию; Vi – скорость вдоха; VT – дыхательный объем; Pes – внутригрудное давление. По оси ординат – изменения в процентах по отношению к горизонтальному положению.

В АОП-30° наблюдался значительный рост резистивного и эластического компонента сопротивления дыханию: на 50% и 90% ($p < 0,05$), соответственно, по сравнению с горизонтальным положением.

Рост инспираторного внутригрудного давления в АОП, свидетельствует о компенсаторном усилении сократительной активности дыхательной мускулатуры, направленном, по всей вероятности, на преодоление добавочного сопротивления дыханию и обеспечения адекватного уровня вентиляции легких в этих условиях. Анализируя механизмы компенсаторного роста ВГД в АОП необходимо

обратить внимание на изменение величины легочных объемов, вызванное как увеличением сопротивления дыханию, так и изменением биомеханических условий осуществления вдоха в АОП. Смещение диафрагмы в краниальном направлении способствует уменьшению конечно-экспираторного объема легких, а увеличение сопротивления дыханию вызывает снижение дыхательного объема и замедление скорости инспираторных потоков. Следствием таких изменений является ослабление активации рецепторов растяжения легких. Это снижает интенсивность афферентной импульсации, поступающей от рецепторов растяжения легких в дыхательный центр и вызывающей торможение центральной инспираторной активности. В результате увеличивается продолжительность инспираторных разрядов, возрастает электрическая активность и сила сокращений дыхательных мышц, что позволяет сохранить вентиляцию легких на адекватном уровне, не допуская резкого снижения дыхательного объема.

Наличие подобного рефлекторного механизма компенсации реакций дыхательной системы на антиортостатическое воздействие подтверждается данными, полученными в экспериментах на ваготомированных животных.

После билатеральной цервикальной ваготомии, произведенной в горизонтальном положении (табл. 1) происходило снижение МОД, в основном за счет уменьшения ЧД, при этом наблюдалось увеличение инспираторного ВГД.

Перевод ваготомированных животных в антиортостатическое положение вызывал

Таблица 1. Влияние билатеральной ваготомии на паттерн дыхания анестезированных крыс в горизонтальном положении.

	ДО, мл	ЧД, мин ⁻¹	МОД, мл мин ⁻¹	ВГД мм вод.ст.
Интактные	2,5 ± 0,17	72 ± 2,3	180 ± 7,6	-4,1 ± 0,19
Ваготомированные	4,3 ± 0,21*	30 ± 2,2*	129 ± 6,9*	-5,2 ± 0,21*

ДО – дыхательный объем, ЧД – частота дыхания, МОД – минутный объем дыхания, ВГД – внутригрудное давление; * – достоверное различие ($p < 0,05$).

сходную реакцию, как и у вагально интактных животных: наблюдалось увеличение ВГД, снижение ДО и МОД. Однако после деафферентации легких прирост ВГД в АОП происходил только на 65%, тогда как у интактных животных - на 116% (рис. 2).

Одновременно наблюдалось более значительное снижение минутной вентиляции и дыхательного объема. Так, после билатеральной ваготомии в АОП, ДО и МОД снижался в среднем на 30% ($p < 0,05$), тогда как у животных с интактными блуждающими нервами, снижение происходило на 14 и 18 % ($p > 0,05$), соответственно. Эти данные свидетельствуют об ослаблении компенсаторной реакции дыхательной системы на антиортостатическое воздействие после ваготомии: недостаточный компенсаторный прирост сократительной активности дыхательной мускулатуры явился причиной более значительного снижения вентиляции легких в антиортостатическом положении.

Инспираторная окклюзия у интактных животных в горизонтальном положении вызывала рост инспираторного ВГД в пять

раз ($536 \pm 50\%$) по сравнению со свободным дыханием, тогда как в АОП - только в три раза ($320 \pm 35\%$). Разница в приростах составляла 200%, что указывает на резкое ослабление компенсаторной реакции дыхательной системы в антиортостатическом положении (рис.3). Причиной угнетения компенсаторного ответа в этих условиях является, по всей вероятности, ослабление вагальной объемно-зависимой связи, которая обеспечивается рефлексом с механорецепторов легких. Это подтверждается результатами, полученными на ваготомированных животных, у которых полностью отсутствовали различия в реакциях на конечно-экспираторную окклюзию, как в горизонтальном, так и в антиортостатическом положении (рис. 3). Показатель $P_{0.1}$ у данной группы животных в горизонтальном, и антиортостатическом положении соответствовал $300 \pm 20\%$. У вагально-интактных животных такой же прирост $P_{0.1}$ ($320 \pm 35\%$) в ответ на окклюзию наблюдался только в условиях АОП. По-видимому, в антиортостатическом положении вагально опосредованная объемно-зависимая связь в системе дыхания

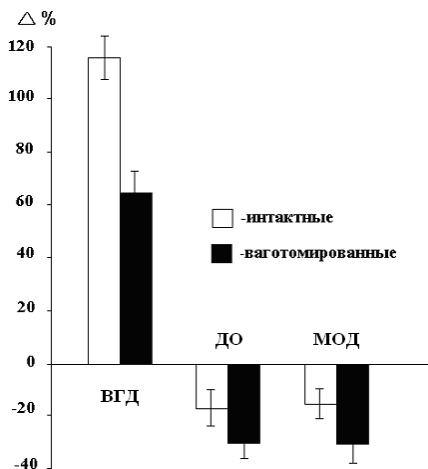


Рис. 2. Изменения параметров дыхания в антиортостатическом положении у интактных и ваготомированных животных.

По оси абсцисс: ВГД – внутригрудное давление; ДО – дыхательный объем; МОД – минутный объем дыхания. По оси ординат: изменение параметров в антиортостатическом положении -30° по отношению к горизонту.

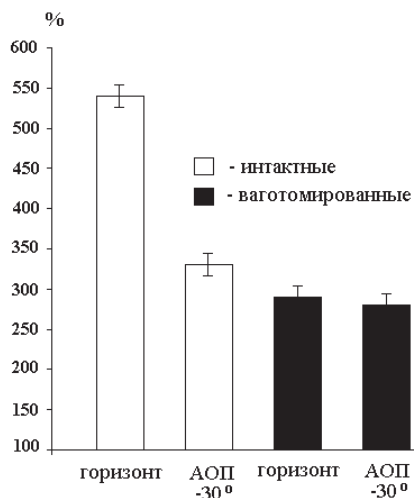


Рис. 3. Максимальное внутригрудное инспираторное давление в первом окклюзионном вдохе у интактных и ваготомированных животных в горизонтальном и антиортостатическом положении (АОП- 30°).

По оси ординат – инспираторное внутригрудное давление в % по сравнению с интактным дыханием.

настолько ослабевает, что развивается состояние, близкое к функциональной ваготомии.

Таким образом, результаты экспериментов доказывают, что афферентная система легких, обеспечивающая обратную связь между изменением объема легких и центральной инспираторной активностью, принимает непосредственное участие в компенсации респираторных эффектов антиортостаза. Увеличение механической нагрузки на дыхательную систему происходит вследствие роста эластического и резистивного сопротивления дыханию [11, 12] основной причиной которого является повышение кровенаполнения легких [6, 8, 13, 16]. Известно, что переполнение легочных сосудов кровью вызывает снижение растяжимости стенок дыхательных путей и сужение их просвета, что способствует росту как эластического, так и резистивного компонента сопротивления [3, 7, 17, 18]. Увеличение сопротивления дыханию вызывает компенсаторный рост внутригрудного давления, которое отражает общее инспираторное усилие. Благодаря усилению сократительной активности инспираторных мышц снижение дыхательного объема и вентиляции легких в антиортостатическом положении является незначительным.

Кроме того, полученные данные указывают на то, что вследствие ослабления обратной вагальной связи между центральной инспираторной активностью и изменением объема легких (рефлексы Геринга-Брейера) в антиортостатическом положении резко ослабевают компенсаторные возможности системы внешнего дыхания. В результате одинаковая по величине механическая нагрузка вызывает меньший прирост инспираторного давления в антиортостатическом положении по сравнению с горизонтальным.

Необходимо отметить, что увеличение сопротивления дыханию, которое наблюдается в АОП, не только уменьшает скорость и объем вдоха, но и активирует рецепторы растяжения межреберных мышц – мышечные веретена, которые через фузимоторную систему ин-

нервации усиливают сокращение волокон межреберных мышц. Для реализации этого компенсаторного механизма не требуется усиления ЦИА, она осуществляется на более низком сегментарном и межсегментарном уровне (спинной мозг).

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