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Dynamics of lipid peroxidation in the exhaled breath condensate in healthy people during hypo- and hyperosmolar bronchial provocation tests

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ABSTRACT

The dynamics of lipid content in the exhaled breath condensate (EBC) as well as the degree of lipid oxidation in healthy persons before and after executing of bronchial provocation tests with osmotic stimulus had been studied. Within three-days study the content of non-oxidized lipids and dien conjugates in EBC samples significantly increased. The bronchial provocation tests with hyperosmolar stimulus (inhalation of 4,5% solution of NaCL and incremental exercise) result in the decrease of lipid content in EBC while the hyposmolar one (inhalation of distilled water) leads to opposite effect. The content of diene conjugates in EBC samples after hyposmolar and hyperosmolar aerosol inhalations had been increased but was not influenced by the incremental exercise. Bronchial provocation tests did not influence the content of conjugated trienes and ketodienes in EBC. The results obtained show the influence of the osmotic stimuli on the peroxidation of airway lipids in healthy persons.

Keywords: exhaled breath condensate, UV absorption spectra, lipid peroxidation, bronchial provocation tests, osmolarity

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INTRODUCTION

Oxidative stress represents a general molecular mechanism of the development of various inflammatory lung diseases [1, 2]. The environmental changes result in the development of the nonspecific stress of the respiratory system, including the activation of processes of free radical oxidation of lipids, whose products act as mediators of inflammation. One of the physical factor of the environment affecting the functioning of the respiratory tract is the humidity. The influence of low or high humidity of air, as well as of excessive physical exercise result in hyperventilation and violations of the osmolarity of fluids of respiratory tract, capable to provoke asthma-like symptoms in highly sensitive persons [3, 4]. Earlier, we have shown the activation of lipid peroxidation in patients with asthma at the influence of hypo- and hyperosmolar stimuli and developed the modification of the non-invasive method of study of oxidation of airwave lipids, consisting of the registration of the UV absorption spectra of lipid extracts from exhaled breath condensate (EBC) samples [5]. The present study was aimed to investigate the dynamics of the content of products of lipid peroxidation in EBC in healthy persons during a three-day execution of bronchial provocation tests with osmotic stimuli.

MATERIALS AND METHODS

Subjects

The study involved 18 healthy non-smoking young men (mean-by age 19.2±0.2 years; height 176.6±1.4 cm; weight 70.7±1.5 kg). On average, they had normal values of lung function: forced vital capacity (FVC) was 100.2±2.11% of predicted, forced expiratory volume for 1 second (FEV_1) - 103.4±2.58%. Inclusion criteria were: FEV_1 more than

75% of predicted values, the absence of an acute respiratory infection in the last 4 weeks and concomitant pathology of other organs and systems, which could affect the results of study. None of them did not accept any drugs in 2 weeks prior to the coming testing. The study was conducted with the approval of the local committee on biomedical ethics of the Far Eastern Scientific Center of Physiology and Pathology of Respiration. All the participants were read and signed the informed consent protocol.

Study Design

During the 3 days in the morning from 9:00 to 12:00 daily the samples of EBC were collected, lung function was studied; bronchial provocation tests were performed, and the samples of EBC were recollected immediately after tests. On the day of the testing the people under the procedures for 1.5 hours before running the bronchial provocation test were limited to hot meal and beverages, as well as foods that could affect the collected biomaterial. Any physical activity, exposure to cold and air pollutants and change of location was also prohibited.

Collection of EBC samples

EBC collection was performed using ECoScreen Turbo (VIASYS Healthcare GmbH, Germany) through a disposable breathing circuit, which allows the inhale-breath from the surrounding atmosphere, and exhale in device, condensing exhaled air vapors at -20°C. The temperature and relative air humidity were recorded daily before the study using an electronic thermometer (weather station ea2 bl508 slim) (measurement accuracy of the temperature sensor 0,1°C) and hygrometer (VIT2, Russia) located near the apparatus. The variations of these indices were within 24-25°C and 55-65%, respectively. The study began in the morning (9:00-11:00), in 1.5-2 hours after a light breakfast. The person twice rinsing the mouth with distilled water. Afterwards joined to the mouthpiece, tightly clasping his lips, and for 20 minutes ventilated air during quiet breathing arbitrary. Nasal breathing was eliminated by imposing nose clip. To standardize the sampling of biological material the timer was used. The patient was warned about disconnecting from the device in the case of urge to cough or excessive salivation. The testing was resumed after the cause has disappeared. In case of any symptoms of breathing difficulty the procedure was stopped. At the end of the set time for the collection of EBC the flask with biological material immediately pulled from the unit and close lid. After thawing, the liquid condensate was seized with a sterile disposable syringe immediately placed in a freezer at -70°C and stored until analysis. Before assay microtubes with EBC samples were thawed and vigorously shaken at the centrifuge-vortex ELMI CM -70V.07 [6-8].

The methods for lipid extraction and determination of the content of lipid peroxidation products in the EBC samples were previously described [5].

Study of lung function

The lung function was investigated by means of spirometer Easy on-PC (nddMedizintechnik AG, Switzerland) in accordance with the international standards of spirometry conducting [9].

Conducting of bronchial provocation tests

Bronchial provocation test with inhalation of distilled water consisted of two consecutive inhalation of 30 mL of sterile 0,9% NaCl solution, and the same amount of distilled water, respectively, during 3 minutes each, with an arbitrary quiet breathing in a seated position. Nasal breathing was ruled out by the nose clip. The volume and temperature of inhaled solutions were the same for all persons. The total dose of aerosol delivered to the person was measured by weighing the cup and the tube, excluding the valve, before and after inhalation procedure and consisted of 4.72±0.40 g averaged. Ultrasonic nebulizer "Thomex L-2" (Poland) was used to generate the aerosol. The average particle diameter of the aerosol was 3 micrometers (particle diameter range 0,5÷10 mm), the performance 0÷4,5 cm^3/min, the performance boost 20 dm^3/min at the stable temperature 37,3°C (310±4K), the operating capacity of the vessel for the solution - 30 cm^3.

The procedure used for executing of bronchial provocation test with inhalation of hyperosmolar (4,5%) NaCl solution was similar to the above procedure and included inhalations of 30 ml of sterile 0,9% NaCl solution and the same amount of 4,5% NaCl solution. The total dose of aerosol delivered to the patient was 5,33±0,37g averaged.
8 minutes graduated exercise performed with a help of treadmill LE 200 CE, included in the research complex for ergospirometry with incremental exercise Oxycon Pro (VIASYS Healthcare GmbH, Germany) in accordance with ATS/ACCP standards was used to create the conditions of physiological hyperosmolar airway state [10]. The submaximal heart rate corresponding to the age of the persons (170-190 in 1 min) was reached in all subjects during test with incremental exercise. Oxygen saturation at baseline pulse oximetry was 99,0±0,3%, after the exercise 98,8±0,3%, the distance traveled - 898,1±16,6 m. The average work capacity was 110,8±2,8% of the predicted value.

Statistical analysis

Data analysis was executed using standard methods of variation statistics. The values obtained were expressed as mean values of the index (M) and standard error of the mean (m). To determine the significance of the observed differences we used paired Student's t test and to assess the daily spread of values we calculated the coefficient of variation (CV), as the ratio of standard deviation to the mean. In order to determine the degree of relationship between two random variables we performed the classical correlation analysis and calculated Pearson correlation coefficient (r). The significance level (p) <0,05 was accepted for assessment of the significance of differences between the values of all the indices.

RESULTS AND DISCUSSION

Day-to-day dynamics of EBC volume

The volume of collected EBC varies significantly throughout three days of study and during the execution of bronchial provocation tests (table 1). At the same time within one period of study the coefficient of variation for the mean values of the collected volume of EBC does not exceed 10-15%. The volume of collected EBC after aerosol inhalation was on average significantly smaller than after incremental exercise. After the inhalation of distilled water obtained EBC volume was reduced on 0.5-2.0 mL in 42% and after the inhalation of 4,5% solution of NaCl in 58% cases. According to [11] there is direct correlation between the volume of collected EBC and the volume of ventilated air. This may explain the day-to-day variations in the volume of collected EBC, but not the negative dynamics after the bronchial provocation tests. It seems possible that the inhalation of 4,5% solution of NaCl results in transient hyperosmolarity of mucosa of respiratory tract with a decrease of the liquid production. This does not happen in the case of physiological state of hyperosmolar during incremental exercise. It is difficult to explain a significant reduction in the volume of produced fluid after inhalation of distilled water. We made an attempt to evaluate the influence of the volume of inhaled solution delivered to the patient during the aerosol tests and the amount of the collected EBC, but did not find any association between these values, as well as with airway patency and reactivity (FEV_1, ΔFEV_1).

Table 1 The volume (V) of the collected EBC before and after the bronchial provocation tests within three-day survey (M±m)

Indices	Distilled water	Variation coefficient	4,5% NaCl	Variation coefficient	Incremental exercise	Variation coefficient
V before probe, mL	4,12±0,09	0,10	3,93±0,09	0,10	3,87±0,07, p=0,044	0,07
V after probe, mL	3,82±0,09, p_2=0,032	0,11	3,55±0,12, p_2=0,042	0,15	3,79±0,11	0,13
ΔV	-0,30±0,16		-0,37±0,13		-0,12±0,12	

Herein after p - significance of the differences of the values of the index in the test with those in the test with distilled water; p_1 - significance of differences between the values of the indices in the tests 4,5% NaCl and incremental exercise; p_2 - significance of differences of the values of the index after the test compared to baseline values.

Day-to-day dynamics of lipid peroxidation products

During three days of study lipid content in the analyzed samples of EBC and degree of their oxidation varied (table 2). On the set of the values all the samples were normally distributed (Gaussian). The values of absorbance at 206 nm corresponding to the maximum of absorbance of non-oxidized lipid varied in the range 0.1-0.9, the absorbance at 233 nm (conjugates diene-tions) from 0,01-0,5 and the absorbance at 278 nm (conjugated trienes and ketodienes) from 0,001 to 0,02. We calculated the coefficient of variation for each of the indices presented. The dien conjugates content was the least variable. The deviation of its values from the averages in the group over the study days was 23-27%.

Within three days of study the clear trend towards an increase of the content of non-oxidized lipids and dien conjugates in the EBC samples was revealed. It is quite possible that this trend could be due to the different amount of collected biological material due to the differences in the pattern of breathing (superficial or more deep breathing) as the patients breathed in the random mode, only enter a time limit. As shown by studies conducted previously [12-14], the concentration of volatile compounds in EBC in healthy individuals, in particular H_2O_2 varies with the changes of breathing pattern. In our study, the volume of collected EBC in a three-day interval differed significantly. At the same time, we have not found any connection between the volume of collected EBC and the main parameters of lung function. Despite the fact that the volume depends on the minute ventilation and breathing depth, breathing pattern does not significantly affect pH, protein content, nitrite concentration, leukotrienes and malonic dialdehyde content [15-18]. We have identified significant day-to-day dynamics of the baseline content of oxidized lipids in the EBC which may be caused by the bronchial provocation tests held on the eve (table 2).

Table 2 Day-to-day dynamics of baseline lipid peroxidation in EBC before the bronchial provocation tests (M±m)

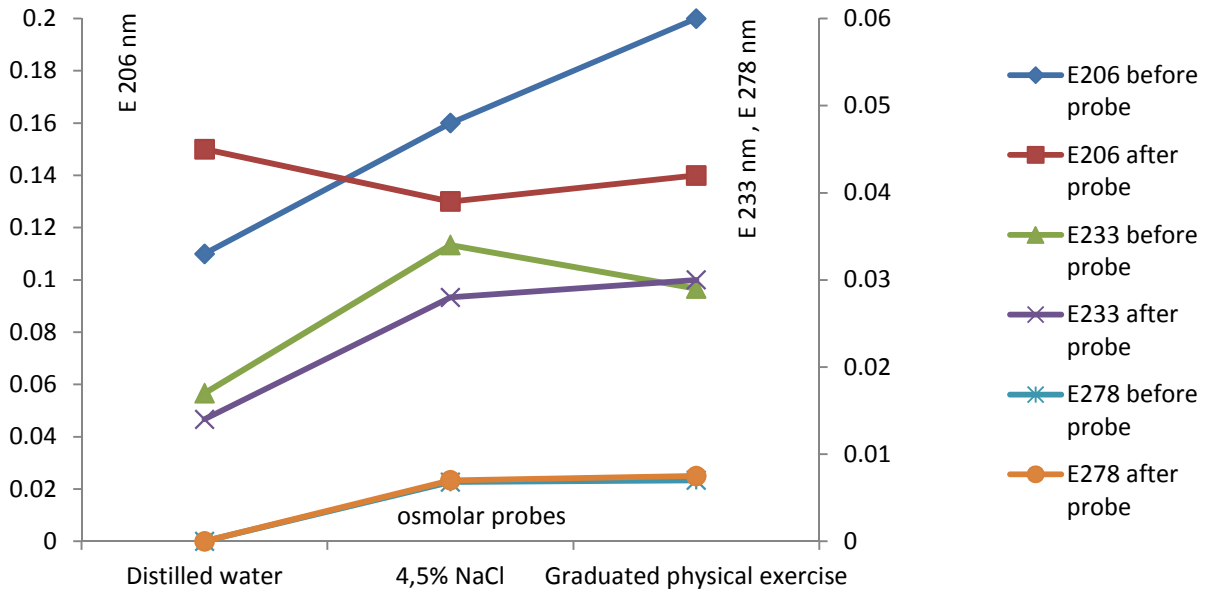
Indices	Distilled water	Variation coefficient	4,5% NaCl	Variation coefficient	Incremental exercise	Variation coefficient
E206 nm	0,11±0,03	0,83	0,16±0,06	0,84	0,20±0,03; p=0,046	0,67
E233 nm	0,017±0,001	0,27	0,034±0,003 p=0,0017	0,23	0,029±0,002 p=0,006	0,23
E278 nm	Lack of absorbance	-	0,007±0,001	0,51	0,007±0,001	0,61
E233/E206	0,18±0,02	0,44	0,26±0,08	0,71	0,22±0,03	0,56
E278/E206	-	-	0,06±0,01	0,54	0,05±0,01	0,62

Oxidative modification of airway lipids after the osmolar bronchial provocation tests

Figure 1 illustrates the changes in the content of oxidized and non-oxidized lipids in the EBC samples after the conducting of osmolar bronchial provocation tests. The hyperosmolar test (4,5% NaCl and incremental exercise) was followed by the decrease of the content of non-oxidized lipids (E206 nm) in EBC samples to 20 and 30%, respectively, while the hypoosmolar test with distilled water resulted in the 25% increase of this index. However, due to the high interindividual variations of the index mean group values did not reach significant difference.

The content of diene conjugates (E233) after the test was reduced to a greater extent in response to an aerosol inhalation (distilled water and 4,5% NaCl) compared with incremental exercise. The mean group values of dien conjugates content in EBC samples after the tests with distilled water, 4,5% NaCl and incremental exercise consisted of 0,014±0,002, 0,028±0,005 and 0,030±0,002, respectively. It is interesting to note the higher values of this index both baseline and after the hyperosmolar test with 4,5% NaCl (p=0.039) and incremental exercise (p=0.009) compared with hyposmolar one. The bronchial provocation tests did not influence the content of conjugated trienes and ketodienes (E278) in the EBC samples.

Fig. 1 Non-oxidized and oxidized lipid content in EBC before and after the bronchial provocation tests



The relationship between the content of oxidized lipids in EBC and the patency of airways

We have investigated the relationship between the degree of oxidation of lipids in EBC samples and the baseline FEV_1 and its change in response to osmotic stimuli. Results are presented in the table 3. For three-day study FEV_1 values varied within 160 mL, slightly greater than the daily reproducibility range adopted for this index (150 mL). The mean group values of FEV_1 had been decreased in response to aerosol tests especially in the case of inhalation of distilled water, while in response to incremental exercise the index had been increased in some extent.

Table 3 Day-to-day baseline FEV_1 and its dynamics in response to bronchial provocation tests (M±m)

Indices	Distilled water	4,5% NaCl	Incremental exercise
FEV_1, л	4,54±0,13	4,43±0,15	4,59±0,13; p_1=0,009
ΔFEV_1, %	-1,79±0,82	-1,08±0,74	0,81±0,85; p=0,039

There was no close relationship between the baseline content of oxidized lipids in the EBC samples and FEV_1. At the same time, the baseline FEV_1 closely correlated with non-oxidized lipid content (E 206 nm) in EBC after the test with distilled water (r=-0.81; p=0.003) and with the ratio E233/E206 after the test with 4,5% NaCl (r=-0.73; p=0.042). The values of E_{233} and E_{278} nm in the case of incremental exercise test were largely dependent on the ΔFEV_1 (r=0.65; p=0.006; r=0.62; p=0.01, respectively) than the initial FEV_1.

The results obtained show the influence of the osmotic stimuli on the peroxidation of lipids of EBC in healthy persons and proves their importance for the adaptation of the respiratory tract to the environmental changes.

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