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EFFECT OF METHIONINE ON THE LUNGS RESPIRATORY PART IN RATS OF DIFFERENT AGES*

***Roman Yanko*[1], *Elena Chaka*[2], *Mikhail Levashov*[3]**

[1] ORCID: 0000-0002-0397-7517
[2] ORCID: 0000-0001-7425-2751
[3] ORCID: 0000-0003-1354-2047
Department of Clinical Physiology of Connective Tissue
Bogomoletz Institute of Physiology, National Academy of Sciences of Ukraine, Kiev, Ukraine

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Abstract

The aim of this work was to study the lungs respiratory part in rats of different ages after methionine administration. The experiments were conducted on 48 male 3 and 15 months old Wistar rats. Animals received 250 mg/kg of methionine daily for 21 days. Lungs tissue samples were taken at the end of the experiment. We used histological, morphometric, biochemical and statistical research methods. It was found that in the lungs of 3-month experimental rats, the area of the alveolus (in 19%) and the relative area of air spaces (in 11%) decreased, and the relative area of the parenchyma and stroma – increased (in 16%) at the end of the experiment. Elevated levels of hydroxyproline in the lung tissue of these rats may indicate an increase in the number of connective tissue elements. In 15-month-old animals treated with methionine the morpho- and biochemical signs of improvement in the functional capabilities of the lungs were observed. Thus, the administration of methionine with young rats reduces the activity of the lungs, and the adult animals, on the contrary, increases.

Introduction

Amino acids are structural units of proteins, but also have their own biological roles as active substances, that are not fully discovered yet. The irreplaceable sulfur-containing amino acid methionine, which occupies a central place in amino acid metabolism, is of particular interest in this regard (BROSNAN et al. 2007).

Address: Roman Yanko, Bogomoletz Institute of Physiology, Bogomoletz street 4, Kiev, Ukraine, 01024, e-mail address: biolag@ukr.net

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Methionine has a wide range of effects on metabolic processes in the body. It is essential for cell proliferation, in particular for protein and RNA synthesis (GELTINK and PEARCE 2019). The effect of methionine on the cardiovascular, digestive, endocrine and other systems is well studied (AISSA et al. 2017, LATIMER et al. 2018). But, at the same time, the effect of methionine on the morphofunctional state of the lungs remains poorly understood.

Most of the existing literature data are devoted to clinical and experimental studies of the methionine effect on the lung condition in a particular pathology and the effectiveness of its use for the correction of existing disorders (GNANADHAS et al. 2015, YOON et al. 2016). It was shown that the levels of collagen and elastin transcription, as well as the content of phosphatidylcholine, which is the main component of pulmonary surfactant, decreased in mice on a methionine restriction diet. A lack of methionine has been shown to alter significantly the lung response to cigarette smoke, increasing the risk of inflammation (JUBINVILLE et al. 2020). Model studies of chronic asthma have shown that S-adenosylmethionine (a methionine derivative) protects mouse lung tissue from damage and fibrosis by reducing oxidative stress (YOON et al. 2016). It was found that methionine reduces the risk of developing lung cancer (TAKATA et al. 2012), increases the effectiveness of antibiotic therapy for chronic infections of the respiratory system (GNANADHAS et al. 2015). At the same time, a number of studies have shown that prolonged exposure to high doses of methionine can lead to serious damage to elastic fibers and lung structure in general (STARCHER and HILL 2005).

Of particular interest is the question of effectiveness of the use of methionine to prevent the development of pathology or in healthy individuals, as a means of preadaptation and increasing the body's resistance to effects of various harmful environmental factors (YANKO et al. 2020). Until now, the question how the pronounced effect of using methionine to increase the functional activity of healthy lungs, remains open.

The results of the use of methionine in experimental practice are significantly influenced, on the one hand, by the dosage and duration of methionine administration, and, on the other, by the age and sex of the experimental animals. It is known that the lungs, like other organs, react differently to the same influences in the process of ontogenesis. This fully applies to age-related differences in the response of the lungs to the administration of methionine.

The aim of this work was to study the methionine effect on the lungs respiratory part of healthy rats of different ages.

Materials and Methods

Research object and experiment design

The study was conducted on 48 male Wistar rats at the age of 3 and 15 months. The rats were divided into 4 groups (12 animals each): I and III – control 3 and 15 month old animals, II and IV – experimental 3 and 15 month old rats, respectively. Control rats received 240–250 mg/kg methionine, which was included to the standard diet. The experimental rats received an additional oral 250 mg/kg of body weight dose of methionine (Sigma-Aldrich, Germany). Thus, the total amount of methionine that the experimental animals received was ≈500 mg/kg of body weight. Such a dose of methionine can be considered as a prophylactic one, since it does not lead to a significant increase in its content in the body and the occurrence of homocystenemia, but is sufficient to correct a possible deficiency of an amino acid in the body to the values of the physiological norm (Zhang et al. 2004). The animals received methionine together with the cheese mass, with visual control of the complete consumption of the portion. The control rats received a similar portion of the cheese mass without methionine. Animals of all groups were kept in standardized conditions, on a standard diet. The total duration of the experiment was 21 days.

The rats were removed from the experiment by decapitation under ether narcosis. At the end of the experiment, the body and lung weights of the rats were measured and the lung index was determined. All research protocols corresponded to the provisions of the Council of Europe Convention on Bioethics (1997), the Helsinki Declaration of the World Medical Association (1996), the European Convention for the Protection of Vertebrates, which are used for experimental and other scientific purposes (Strasbourg, 1985), the general ethical principles of animal experiments, adopted by the First National Congress of Ukraine on Bioethics (2001), as well as a committee with biomedical ethics of the A. A. Bogomoletz Institute of Physiology, National Academy of Sciences of Ukraine.

Histological studies

Histological preparations of lungs tissue were prepared according to a standard procedure: fixed in Buen's liquid, dehydrated in spirits of increasing concentration and dioxane and poured into paraffin. The obtained preparations were used for morphological and morphometric studies. The sections were stained with Bemer's hematoxylin and eosin, and for the detection of connective tissue elements – by the Van Gyzon and Mason method (Kiernan 2015). Microscopic preparations were photographed on a microscope “Nikon Eclipse E100” (Japan) using a digital camera. The morphometry of the preparations digital images was performed using the computer program “Image J”.

The mean diameter of the alveolar lumen, the depth and area of the alveolus, the alveolus entrance width, the thickness of the interalveolar septum, the diameter of the respiratory bronchioles, the alveolar courses and sacs were measured on the lungs tissue histological sections. The relative area of parenchymal tissue, stroma, air spaces were measured and their ratio was determined. Morphometric measurements were performed on sections where the alveolar passages and alveolus are clearly visible (WEIBEL 1964, YANKO et al. 2021).

Biochemical studies

For biochemical studies, a sample of lung tissue was washed with saline from blood residues and dried to constant weight. The concentration of total hydroxyproline in the lung tissue was determined photometrically by oxidation of hydroxyproline with chloramine T (KLIMENT et al. 2011).

Data Analysis

Statistical processing was carried out using variation statistics methods using the computer program Statistica 6.0. The normal distribution of digital arrays was verified using the Pearson criterion. When the distribution was normal, the Student’s *t*-test was used to estimate the difference in the reliability of the difference between the control and experimental groups. Differences were considered significant at $p < 0.05$.

Results and Discussion

Lung mass and lung index in 3-month-old rats, after methionine administration, were at the level of control values. Whereas in 15-month-old animals these indicators, compared with the control, were lower by 15% and 13% respectively. From this it follows that the lungs of adult rats are more sensitive to the effects of methionine.

The lungs respiratory part (LRP) was represented by respiratory bronchioles (RB), alveolar courses (AC), alveolar sacs (AS) and alveolus. It is difficult to identify structural differences between AC and AS in histological sections, as well as the differences between peripheral RB and AC, they is why they are generally considered to be the one group (Figure 1).

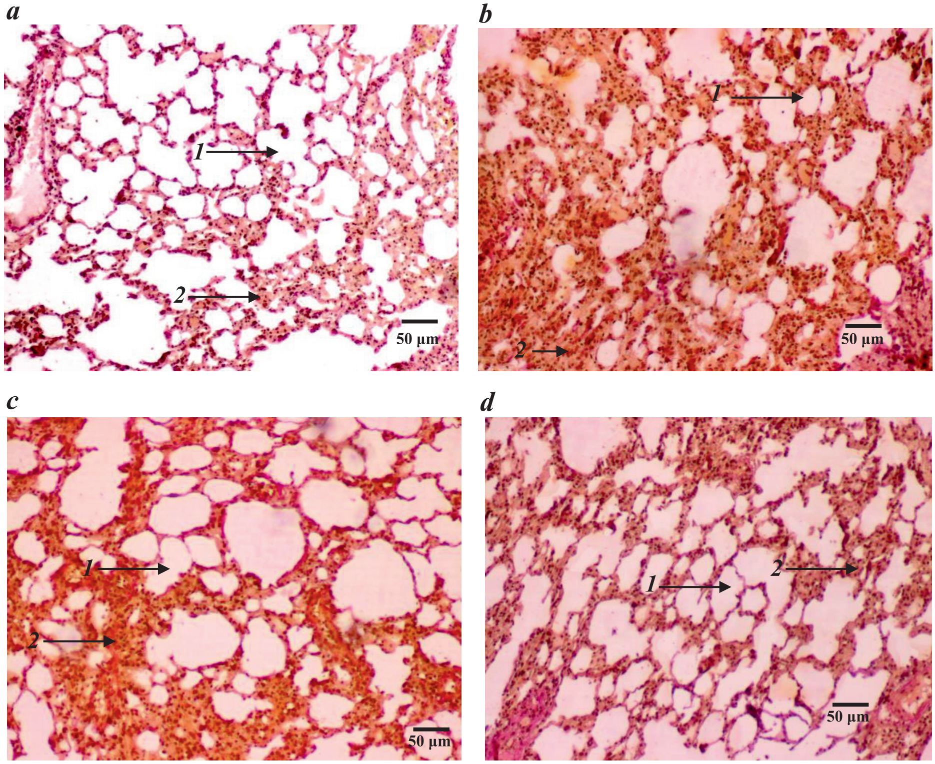

Fig. 1. Micrograph of the lungs respiratory part in control (*a* – 3 months old; *c* – 15 months old) and experimental rats (*b* – 3 months old; *d* – 15 months old). Van Gieson staining, > 200

Note. Micrographs show a decrease in the size of the alveolus and the relative area of air spaces (*1*), as well as in increase in the relative area of the parenchyma and stroma (*2*) in 3-month-old rats, after the administration of methionine (*b*). In 15-month-old experimental animals, on the contrary, the size of the alveolus, the relative area of air spaces (*1*) increased, and the relative area of connective tissue and parenchyma in the lungs (*2*) decreased (*d*).

Morphological differences in LRP structure of control rats of different ages were revealed. Thus, in the lungs of 15-month-old rats, compared to 3-month-old rats, the area of alveolus was smaller by 20%, their depth – by 13%. The thickness of the interalveolar septum was larger by 26%. The relative area of the parenchyma and stroma was 17% larger and airspace was 13% smaller. The concentration of hydroxyproline in the lungs tissue of adult rats was 88% higher than in young animals (Table 1). This nature of differences in the main morphometric parameters of LRP of young and adult rats corresponds to the general pattern of decrease in the functional activity of the lungs with age.

Morphometric parameters of LRP, after methionine administration, depended on the age of the animals and changed in different ways. Thus, in 3-month-old experimental rats, the average area of the alveolus was smaller than in control animals of the same age by 19%, their depth – by 13% and the width of the entrance to the alveolus – by 14%. In 3-month-old rats, a significant increase in the relative area of the parenchyma and stroma (by 16%), a decrease in the relative area of air spaces (by 11%) and the ratio of the area of air spaces to the area of the parenchyma and stroma (by 23%) were observed comparing to the control. This indicated a decrease in the air content of the alveolus and deterioration in the conditions for the processes of intrapulmonary gas exchange (Table 1).

Table 1

Morphometric indicators of the lungs respiratory part (n = 12, M ± m)

Indicators	3 month old rats		15 month old rats	
	control	experience	control	experience
Mean diameter of alveolus lumen, μm	24.0±0.5	21.9±0.3	22.4±0.5	23.3±0.8
Cross-sectional area of alveolus [μm^2]	746±22	600±23*	600±15**	672±21*
Depth of alveolus [μm]	22.6±0.6	19.6±0.7*	19.7±0.9**	22.7±1.0*
Width of the entrance to the alveolus [μm]	13.5±0.4	11.6±0.4*	12.3±0.4	13.9±0.6*
Diameter of lumen of respiratory bronchioles, alveolar courses and alveolar sacs [μm]	63.1±2.9	58.1±1.5	67.8±2.1	68.8±2.7
Thickness of interalveolar septum [μm]	3.4±0.1	3.2±0.2	4.3±0.1**	3.2±0.1*
Relative area of parenchyma and stroma [%]	41.8±1.7	48.4±1.1*	49.1±1.7**	45.5±1.7
Relative area of air spaces [%]	58.2±1.3	51.6±1.1*	50.9±1.9**	54.5±3.6
The ratio of the area of air spaces / the area of parenchyma and stroma	1.39±0.12	1.07±0.08*	1.04±0.08**	1.20±0.07*

*- $p < 0.05$ – significance of differences in comparison with control
** - $p < 0.05$ – significance of differences in comparison with the control of 3-month-old rats

The changes in the LRP of 15-month-old rats treated with methionine were opposite. They were expressed as a significant increase in the area of the alveolus (by 12%), their depth (by 15%), the width of the entrance to the alveolus (by 13%); tendencies towards decrease in the relative area of the parenchyma and stroma, as well as increase in the relative area of air spaces in comparison to the control. This led to significant increase in the ratio of the area of air spaces to the area of the parenchyma and stroma of the lungs (by 15%). It should be noted that although the total area of the alveolar surface increased after the administration of methionine, it never went beyond the normal range and did not reach the size characteristic of emphysematous state. The thickness of the interalveolar septum in 15-month-old rats, after the administration of methionine, significantly decreased by 26% compared to the control animals (Table 1).

As it is known, the interalveolar septum consists of the epithelial layers of the alveolus, subepithelial basement membranes, a network of blood capillaries, as well as elastic, reticular and collagen fibers – its most pronounced structural component (KNUDSEN and OCHS 2018). It is obvious that a decrease in the thickness of the interalveolar septum, first of all, can be associated with a decrease in the content of connective tissue elements in it – elastic, reticular and collagen fibers. Such changes in the air-blood barrier lead to improved alveolar-capillary gas exchange. A similar nature of changes in the morphometric parameters of LRP in adult experimental rats, which were noted in our studies, may indicate improvement in the conditions of intrapulmonary gas exchange and functional activity of the lungs in general.

Staining by the Van Gieson method (KIERNAN 2015) revealed an increase in the number of collagen fiber bundles in the LRP of 3-month-old rats and decrease in adult animals. The majority of connective tissue elements was located around the RB, blood vessels, to a lesser extent, in the interalveolar septum (Figure 1).

Determination of concentration of hydroxyproline, an amino acid marker of collagen fibrillar protein, is often used to assess the condition of the connective tissue of various organs (LI and WU 2018). We revealed a significant increase in the concentration of total hydroxyproline (by 39%) in the lung tissue of young rats treated with methionine. Concentration of hydroxyproline in the lungs of 15-month-old rats, on the contrary, was by 35% lower ($p < 0.05$) than in control animals, that may indicate violation of the dynamic balance between the processes of collagen catabolism and biosynthesis (Figure 2). The multidirectional nature of changes in the

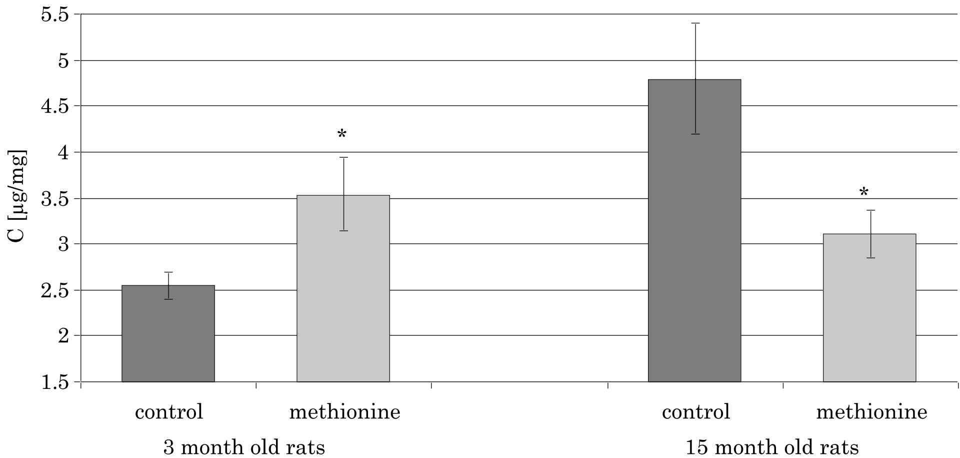

Fig. 2. Concentration of hydroxyproline in the lungs tissue in control and experimental rats
* $p < 0.05$ – reliability compared to control

concentration of total hydroxyproline in the lung tissue indicates the predominance of collagen catabolism processes and decrease in relative mass of connective tissue in the lungs of adult rats and, on the contrary, increase in its mass in young animals. These data may be important to explain the pathogenesis of disorders of the LRP.

The reasons of the identified age differences in the effect of methionine on the LRP state require further study and clarification. As you know, with age, a body's response to drugs can change. In old age, the effect of use of drug can both increase and decrease in comparison with young age. It is also known that age-related decrease in metabolic rate leads to slow-down in drug inactivation. The drugs can be contained in plasma in high concentrations and for a longer time. It is also necessary to take into account the fact that the ability to activate methionine-dependent enzymes in an aging organism may also decrease, and the number of receptors may decrease as well. Therefore, the amount of methionine supplied with a standard diet may not be sufficient. At a young age, with a balanced diet, methionine is quite enough, and its excessive administration leads to its accumulation in the body, and as a consequence to adverse consequences in the functioning of the lungs. Obviously, all this requires an age-dependent correction of the standard doses of the drug.

To date, there is no information about the features of the effect of methionine on the histomorphological and biochemical parameters of the lung state in animals of different ages. Therefore, further detailed study of the functions and mechanisms of the methionine effect on morphological changes in the lungs is urgent.

Conclusions

Thus, the administration of an additional amount of methionine (at a dose of 250 mg/kg) to standard diet of rats had different effects on the LRP of rats of different ages. The nature and severity of changes in the main histo- and biochemical parameters of LRP in 3-month-old rats indicated decrease in its activity. This was evidenced by decrease in the size of the alveolus and the relative area of air spaces, as well as in increase in the relative area of the parenchyma and stroma. Increase in the concentration of hydroxyproline in lung tissue of these rats may indicate increase in the number of connective tissue elements, which may reduce the efficiency of alveolar-capillary gas exchange. In 15-month-old animals treated with methionine, on the contrary, morpho- and biochemical signs of improvement in functional capabilities of the lungs were observed: the size of the alveolus increased, their depth, the width of the entrance to the alveolus, the relative area of air spaces increased, the thickness of the interalveolar septum and the concentration of hydroxyproline in the lungs decreased. Such differences in the LRP response of young and old rats to the administration of methionine should be taken into account when prescribing methionine-containing drugs to people of different ages with impaired lung function.

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