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OSTEOPROTEGERIN-MEDIATED LYMPHOID (LEUKOCYTIC) REGULATION OF CYTOKINES SYNTHESIS BY BRONCHI EPITHELIUM CELLS IN PATIENTS WITH COPD, WHO HAVE TRANSFERRED PULMONARY TUBERCULOSIS

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ОСТЕОПРОТЕГЕРИН–ОПОСРЕДОВАННАЯ ЛИМФОИДНАЯ (ЛЕЙКОЦИТАРНАЯ) РЕГУЛЯЦИЯ СИНТЕЗА ЦИТОКИНОВ КЛЕТКАМИ ЭПИТЕЛИЯ БРОНХОВ У БОЛЬНЫХ ХОЗЛ, ПЕРЕНЕСШИХ ТУБЕРКУЛЕЗ ЛЕГКИХ

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РЕЗЮМЕ

У больных хроническим обструктивным заболеванием легких (ХОЗЛ), перенесших туберкулез (ТВС) легких, изучены регионарный (в индуцированной мокроте) уровень остеопротегерина, а также остеопротегерин–опосредованная лимфоидная (лейкоцитарная) регуляция синтеза цитокинов клетками эпителия бронхов. Установлено, что LPS-индуцированные мононуклеарные лейкоциты имеют способность оказывать остеопротегерин-дозозависимое дифференцированное влияние на функциональную (синтез цитокинов IL-1β, IL-4 и TNF-α) активность клеток бронхиального эпителия: стимулировать синтез цитокинов IL-1β и TNF-α, а также ингибировать синтез цитокина IL-4. Перенесенный ТВС легких в сочетании с существенно повышенным эндобронхиальным уровнем остеопротегерина формируют условия повышенного риска цитокино–зависимого прогрессирования ХЛЗЛ. Дано патофизиологическое обоснование целесообразности лечебной коррекции повышенного эндобронхиального уровня остеопротегерина для уменьшения местного (эпителий бронхов) цитокинового дисбаланса у больных ХОЗЛ.

ОСТЕОПРОТЕГЕРИН–ОПОСЕРЕДКОВАНА ЛІМФОЇДНА (ЛЕЙКОЦИТАРНА) РЕГУЛЯЦІЯ СИНТЕЗА ЦИТОКІНІВ КЛІТИНАМИ ЕПІТЕЛІЮ БРОНХІВ У ХВОРИХ З ХОЗЛ, ЯКІ ПЕРЕНЕСЛИ ТУБЕРКУЛЬОЗ ЛЕГЕНІВ

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РЕЗЮМЕ

У хворих з хронічним обструктивним захворюванням легень (ХОЗЛ), які перенесли туберкульоз (ТВС) легень, вивчені регіонарний (в індукованому мокротинні) рівень остеопротегерину, а також остеопротегерин–опосередкована лімфоїдна (лейкоцитарна) регуляція синтезу цитокінів клітинами епітелію бронхів. Установлено, що LPS-індуковані мононуклеарні лейкоцити мають здатність чинити остеопротегерин-дозозалежний диференційований вплив на функціональну (синтез цитокінів IL-1β, IL -4 і TNF-α) активність клітин бронхіального епітелію: стимулювати синтез цитокінів IL-1β і TNF-α, а також інгібувати синтез цитокіну IL-4. Перенесений ТВС легень у поєднанні із суттєво підвищеним ендобронхіальним рівнем остеопротегерину формує умови підвищеного ризику цитокіно–залежного прогресування ХЛЗЛ. Дано патофізіологічне обгрунтування доцільності лікувальної корекції підвищеного ендобронхіального рівня остеопротегерину для зменшення місцевого (епітелій бронхів) цитокінового дисбалансу у хворих з ХОЗЛ.

Key words: osteoprotegerin, cytokines, bronchial epithelium, chronic obstructive pulmonary disease, pulmonary tuberculosis.

Last fifteen years the doctrine about cytokine-dependent of development mechanisms of mineral density of bone tissue disturbances, as a whole, and formations of systemic manifestations of chronic obstructive pulmonary diseases (COPD), in particular, was essentially added at the expense of discovery of the new representative of TNF-б family playing an important part both in formation of osteoclasts, and in processes of remodeling in a bone, and received the name «osteoprotegerin» [6]. It is determined that osteoprotegerin, also known as osteoclast-inhibiting factor or osteoclast-bonding factor, is a key part of differentiation and activation inhibition of osteoclasts and consequently has great importance in process of bone tissue resorption [12]. Osteoprotegerin mRNA is intensively expressed in various tissues, for example, in lungs, heart, kidneys, bones, liver, placenta, brain in adult people [5].

At research of pathogenesis of osteopenic syndrome formation in patients with COPD by Kochetkova E. A. and et al. (2010) is revealed the interrelation of reduction of osteoprotegerin level in blood serum, increase of

TNF-α levels and a marker of bone resorption βCL (β CrossLaps), and also decrease of mineral density of bone tissue [3]. In research of Eagan T. and et al. (2010) in patients with COPD negative correlational bond between an index of body mass and osteoprotegerin level [8] is revealed also. Decrease of osteoprotegerin in patients with middle-severe and severe stage of COPD, more expressed at severe degree of COPD and at smoking patients is revealed in Burya K. A. research and et al. (2011) [4].

The specified facts have allowed formulating the scientific concept of osteoprotegerin-dependent mechanisms of osteopenic syndrome formation in patients with COPD. On the other hand, in patients with COPD is revealed systemic-regional dyscrinia of osteoprotegerin content: decrease in the system blood flow, «regulated» by severity level of disease, and also essential increase of regional (endobronchial) content of cytokine [13]. Mechanisms of different-directed dynamics of osteoprotegerin content in general blood flow and in loko morbi (at level of bronchial tissues) are remained by a subject of scientific discussion.

So, To M. and et al. (2011) is determined that by data of immunocytochemical researches osteoprotegerin is expressed in peripheral tissues of lungs, and also by macrophages and neutrophils, which were received from sputum. It is revealed also that concentration of osteoprotegerin in the induced sputum of patients with COPD was considerably higher, than in patients with bronchial asthma, healthy smokers and healthy non-smoking. Thus concentration of osteoprotegerin in sputum of patients with COPD negatively correlates with V_1X and positively correlates with tidal lung volume [13]. It is revealed also that components of OPG/RANK/RANKL-system are released and from T-lymphocytes. Ability of the activated T-cells to release this protein explains systemic or local loss of bone tissue at many diseases with immunopathological pathogenesis [10]. Active participation of OPG/RANK/RANKL-system in regulation of the immune answer, including influence on functional activity both T- and B-systems of cellular immunity is revealed [11, 14]. Genetic relation between OPG/RANK/RANKL-system and system of cellular immunity is found [14].

In the light of the above-stated it is possible to assume that the further decoding of the biological importance of increase of osteoprotegerin content at the level of bronchial tissues will allow essentially dilating understanding of cytokine-dependent pathogenetic mechanisms of progressing COPD that can be basis for working out of new ways of the differentiated pathogenetic therapy of chronic obstructive pulmonary diseases.

Research main objective was the scientific substantiation of expediency of use and an assessment of clinical efficacy of the optimized basic therapy for correction of regional (endobronchial) osteoprotegerin-dependent imbalance of cytokine homeostasis at chronic obstructive pulmonary disease in persons who have tolerated pulmonary tuberculosis. In the present work we introduce results of studying at similar patients of osteoprotegerin regional (in the induced sputum) level, and also the osteoprotegerin-mediated lymphoid (leukocytic) regulation of cytokines synthesis by cells of bronchial epithelium.

MATERIAL AND METHODS

There were 83 patients with COPD (the I-II degree of severity, stable current) under observation, whom were subdivided as follows: the 1st group included 41 patients with COPD, the 2nd group was compounded by 42 patients with COPD, who has tolerated various forms of pulmonary tuberculosis. 28 healthy persons served in the conforming age range as control group. The 2nd control group was compounded by 14 healthy persons in whom bronchoalveolar outwashes received at the diagnostic bronchoscopy made concerning disputable clinical situations (consripts) and whom pathological changes in broinchopulmonary system after complex examination have not been found.

Concentration of osteoprotegerin in the induced sputum (which is collected after repeated inhalation of hypertonic solution of Sodium chloridum through nebuliser is defined by an immuno-enzyme method with use of commercial sets Human Osteoprotegerin (OPG) ELISA Kit Company Biomedica Medizinprodukte GmbH and Co KG (Austria). The assessment of results was carried out by photometric.

For cultivation of cells of bronchial epithelium the method of short-term organic cultures, providing cultivation of cells in vitro is used. Cultivation was made in the presence of antibiotics (benzylpenicillin sodium salt 1000 units and streptomycin sulphate of 0,01 g on 1 ml of culture medium). Cells of bronchial epithelium from sputum received. Some experiments were collaterally carried out including preicubation of suspension of LPS-induced mononuclears with osteoprotegerin (it was used Osteoprotegerin (OPG) human, recombinant, Sigma-Aldrich, USA) with the subsequent injection in the culture medium at the cultivation beginning.

Mononuclear leukocytes were discharged from heparinized blood with centrifugation on a gradient of density of ficol-verografinum. LPS is received from strains of E.coli K 30 and C 600 (lux) (R-mutants) on Westphal's O. method (1984 [17]. Mild hydrolysis of native LPS was made by acetic acid [2]. Concentration of cytokines is defined by a method of hard-phase immuno-enzyme analysis. For definition of TNF-α and IL-4 levels test-systems and reagents of test-system ProCon of Open Company «Protein contour» (Russia), level IL-1β– test-system of Open Company «Cytokine» (Russia) have been used.

RESULTS AND DISCUSSION

Results of research of osteoprotegerin level in the

induced sputum in patients of the 1st and 2nd groups are introduced in a tab. 1.

Table 1

Osteoprotegerin level in the induced sputum in patients of the 1st and 2nd groups, pg/ml on a protein unit

Group	Statistical index	Osteoprotegerin
1st group (COPD)	M ± m n p	14660,49 ± 563,66 41 < 0,001
2nd group (COPD and pulmonary tuberculosis in the anamnesis)	M ± m n p p1	19772,00 ± 701,49 42 < 0,001 < 0,001
Healthy persons	M ± m n	2565,45 ± 109,28 28

Note: p – reliability of differences calculated in comparison with group of healthy persons, p1 – reliability of differences calculated in comparison with the 1st group of patients.

It is known, that osteoprotegerin is «receptor-trap», which binds RANKL and thus warns activating influence of the last on RANK, thus being an inhibitor of osteoclastogenesis [9]. The analysis of the data introduced in a tab. 1 testifies that in patients of the 1st and 2nd groups the level of osteoprotegerin in the induced sputum, unlike a system blood flow, is increased accordingly in 5,6 times and 7,4 times (p<0,001). Thus osteoprotegerin level in the induced sputum in patients of the 2nd group on 32,3 % (p1<0,001) higher than in patients of the 1st group. Thus, tolerated pulmonary tuberculosis is «the aggravation factor» the risk of formation an osteopenic syndrome in patients with COPD. Earlier the increase of osteoprotegerin level in the induced sputum in patients with COPD is revealed by To M. and et al. (2011) [13].

Having assumed as a basis that OPG/RANK/RANKL–system plays an important role not only in development of systemic and local osteopenia, but also in regulation of immune system, we carried out a series of vitral experiments characterizing osteoprotegerin-mediated lymphoid (leukocytic) regulation of synthesis of cytokines by cells of bronchial epithelium in almost healthy persons (the 2nd control group) and in patients with COPD. The main tasks at carrying out of vitral experiments with cells of persons of the 2nd control group were studying of the biological phenomenon – influence possibilities of osteoprotegerin on lymphoid (leukocytic) regulation of synthesis of cytokines by cells of bronchial epithelium, and also selection of an optimum dose of osteoprotegerin for including in an experimental model.

It is determined by us (tab. 2) that in persons of the 2nd control group under the influence of injection in culture medium of meal LPS-induced autologic mononuclear leukocytes (experiment 2), and also under influence of preincubation of mononuclears with 1000 pg/ml of medium of human osteoprotegerin level of a proinflammatory cytokine IL-1в in supernatant of culture of bronchial epithelium cells is not essentially changed. It is determined also that under influence of preincubation of mononuclears with human osteoprotegerin in doses of 5000 pg/ml, 10000 pg/ml of medium, 20000 pg/ml of medium and 30000 pg/ml of medium the researched index statistically significantly increases (in comparison with experiment 1) accordingly on 32,2 %, 97,5 %, 301,8 % and 336,4 % (p<0,001).

Thus, it is determined by us that in physiological conditions (vitral research was carried out with cells practically healthy persons), mononuclear leukocytes have ability to render dose-dependent osteoprotegerin-induced influence on functional (cytokine synthesis IL-1в) activity of bronchial epithelium cells. Attracts attention that level of proinflammatory cytokine IL-1β in experiment 6 and 7 does not essentially differ that can testify about existence of "upper range" of biologically active dosage (20000 pg/ml of medium) osteoprotegerin concerning modulating influence on lymphoid (leukocytic) regulation of synthesis of proinflammatory cytokine IL-1β by cells of bronchial epithelium.

Cytokine level IL-4 in supernatant of cells culture of bronchial epithelium in persons of the 2nd control group in experiment 2 is essentially increased (on 138,3 %, p<0,001). Under influence of preincubation LPS-induced autologic mononuclear leukocytes with 1000 pg/ml of medium, 5000 pg/ml of medium and 10000 pg/ml of medium of human osteoprotegerin the investigated index in comparison with experiment 2 is statistically significantly reduced (accordingly on 17,0 %, 45,8 % and 49,9 %, p1<0,001) that testifies about osteoprotegerin-dependent cancellation of stimulating synthesis IL-4 by cells of bronchial epithelium of influence LPS-induced of autologic mononuclear leukocytes. It is determined also that under influence of preincubation LPS-induced mononuclears with human osteoprotegerin in doses 20000 pg/ml of medium

Table 2

Influence of various doses of osteoprotegerin of human recombinant (Sigma-Aldrich, USA) on the LPS-induced lymphoid (leukocytic) regulation of cytokines synthesis by cells of bronchial epithelium in persons of the 2nd control group, pg/ml

Stages of vitral experiment	Statistical indexes	Level of cytokines in supernatant of culture medium of cells culture of bronchial epithelium		
		IL-1β	IL-4	TNF-α
Experiment 1 (cytokine level in culture medium)	M ± m n	9,26 ± 0,27 14	6,11 ± 0,28 14	14,43 ± 0,59 14
Experiment 2 (meal of autologic mononuclears is introduced into culture medium)	M ± m n p	9,75 ± 0,38 14 < 0,5	14,56 ± 0,40 14 < 0,001	18,37 ± 0,79 14 < 0,001
Experiment 3 (preincubation of mononuclears with OPG (1000 pg/ml of medium) - washing up of cells - in culture medium)	M ± m n p p_1	10,06 ± 0,41 14 < 0,2 < 0,5	12,08 ± 0,40 14 < 0,001 < 0,001	19,53 ± 0,74 14 < 0,001 < 0,5
Experiment 4 (preincubation of mononuclears with OPG (5000 pg/ml of medium)	M ± m n p p_1 p_2	12,89 ± 0,47 14 < 0,001 < 0,001 < 0,001	7,89 ± 0,44 14 < 0,01 < 0,001 < 0,001	23,62 ± 0,83 14 < 0,001 < 0,001 < 0,01
Experiment 5 (preincubation of mononuclears with OPG (10000 pg/ml of medium)	M ± m n p p_1 p_2 p_3	19,26 ± 0,75 14 < 0,001 < 0,001 < 0,001 < 0,001	7,29 ± 0,32 14 < 0,01 < 0,001 < 0,001 < 0,5	34,19 ± 1,44 14 < 0,001 < 0,001 < 0,001 < 0,001
Experiment 6 (preincubation of mononuclears with OPG (20000 pg/ml of medium)	M ± m n p p_1 p_2 p_3 p_4	39,18 ± 1,48 14 < 0,001 < 0,001 < 0,001 < 0,001 < 0,001	4,18 ± 0,15 14 < 0,001 < 0,001 < 0,001 < 0,001 < 0,001	51,63 ± 1,64 14 < 0,001 < 0,001 < 0,001 < 0,001 < 0,001
Experiment 7 (preincubation of mononuclears with OPG (30000 pg/ml of medium)	M ± m n p p_1 p_2 p_3 p_4 p_5	42,55 ± 1,30 14 < 0,001 < 0,001 < 0,001 < 0,001 < 0,001 < 0,1	4,57 ± 0,22 14 < 0,001 < 0,001 < 0,001 < 0,001 < 0,001 < 0,2	54,48 ± 1,42 14 < 0,001 < 0,001 < 0,001 < 0,001 < 0,001 < 0,5

Note: p – reliability of differences calculated in comparison with experiment 1, p1 – reliability of differences calculated in comparison with experiment 2, p2 – reliability of differences calculated in comparison with experiment 3, p3 – reliability of differences calculated in comparison with experiment 4, p4 – reliability of differences calculated in comparison with experiment 5, p5 – reliability of differences calculated in comparison with experiment 6.

(experiment 6) and 30000 pg/ml of medium (experiment 7) the researched index is statistically significantly reduced below of the initial (in experiment 1) level accordingly on 31,6 % and 25,2 % ($p<0,001$).

Thus, it is determined by us that in physiological conditions mononuclear leukocytes have ability to render dose-dependent osteoprotegerin-induced influence by cells of bronchial epithelium and on synthesis of cytokine IL-4.

Considering that IL-4 is concerned first of all to anti-inflammatory cytokines [1], dose-dependent inhibition of lymphoid (leukocytic) regulation synthesis of cytokine IL-4 by cells of bronchial epithelium under influence of osteoprotegerin is regarded by us as one more (along with dynamics of proinflammatory cytokine IL-1β level) acknowledgement of possible participation of the osteoclastinhibiting factor (osteoprotegerin) in formation of cytokine imbalance in loko morbi (at level of bronchial tissues) towards prevalence of cytokines level with proinflammatory activity.

Last decade proinflammatory cytokine TNF-α began to relate to high-informative laboratory markers of bone resorption, and also to active «participants» of pathogenesis of an osteopenic syndrome at COPD [3]. It is determined by us that in persons of the 2nd control group under the influence of injection in culture medium of meal of LPS-induced autologic mononuclear leukocytes (experiment 2) cytokine level TNF-α in supernatant culture of cells of bronchial epithelium is authentically increased (on 27,3 %, $p<0,001$).

Under influence of preincubation LPS-induced autologic mononuclear leukocytes with 1000 pg/ml of medium of human osteoprotegerin the researched index is not essentially changed (in comparison with experiment 2), and with concentrations of 5000 pg/ml of medium, 10000 pg/ml of medium, 20000 pg/ml of medium and 30000 pg/ml of medium – increases accordingly on 28,6 %, 86,1 %, 181,1 % and 196,6 %, ($p1<0,001$) that testifies about dose-dependent osteoprotegerin-dependent stimulating influence of LPS-induced autologic mononuclear leukocytes on synthesis of proinflammatory cytokine TNF-α by cells of bronchial epithelium. Absence of authentic difference of osteoprotegerin-dependent stimulating influence on the researched index at comparison of doses of osteoclastinhibiting factor (osteoprotegerin) 20000 pg/ml of medium and 30000 pg/ml of medium attracts attention. It can testify about existence of «the upper slat» of biologically active dosage (20000 pg/ml of medium) osteoprotegerin concerning modulating influence on lymphoid (leukocytic) regulation of synthesis of proinflammatory cytokine TNF-α by cells of bronchial epithelium.

Thus, it is determined by us that in physiological conditions (research was carried out with cells of practically healthy persons) mononuclear leukocytes have ability to render dose-dependent osteoprotegerin-induced influence and on synthesis of proinflammatory cytokine TNF-б by cells of bronchial epithelium.

Research results of osteoprotegerin influence on the LPS-induced lymphoid (leukocytic) regulation of cytokines synthesis by cells of bronchial epithelium in patients of the 1st and 2nd groups are introduced in a tab. 3.

It is determined by us (tab. 3) that in patients of the 2nd group at all stages of experiment IL-1в level in supernatant culture medium of cells culture of bronchial epithelium is statistically significantly higher than in patients of the 1st group. These facts testify that COPD current in persons who have tolerated pulmonary tuberculosis and with authentic higher level of osteoprotegerin in the induced sputum is characterized by formation of higher regional (tissues of bronchial epithelium) proinflammatory cytokine (on IL-1в level) potential. It is determined also that under the influence of injection in culture medium of meal of LPS-induced autologic mononuclear leukocytes (experiment 2) IL-1в level in supernatant of cells culture of bronchial epithelium in patients both the 1st, and 2nd groups does not essentially change. Under influence preincubation of LPS-induced autologic mononuclear leukocytes with 20000 pg/ml of medium of human osteoprotegerin the researched index increases (in comparison with experiment 2) in patients of the 1st and 2nd groups accordingly on 37,6 % and 24,2 %, ($p1<0,001$) that confirms osteoprotegerin-dependent increase of proinflammatory (on IL-1в level) cytokine potential in patients with COPD.

In comparison with persons of the 2nd control group (almost healthy people) in experiment 1 investigated index in patients both the 1st, and 2nd groups is essentially higher, and in experiment 6 – is lower, it attracts attention. It is possible to assume that as in patients with COPD immune (leukocytic) regulation of functional activity of cells of bronchial epithelium is carried out in the conditions of constantly increased osteoprotegerin level, it can be accompanied by depletion of functional reserves (osteoprotegerin-dependent synthesis of cytokines) cells of bronchial epithelium.

In patients of the 2nd group at all stages of experiment IL-4 level in supernatant culture medium of cells culture of bronchial epithelium is statistically significantly lower than in patients of the 1st group. Thus, COPD current in persons who have tolerated pulmonary tuberculosis and with authentic higher level of osteoprotegerin in the induced sputum is characterized by imbalance formation of cytokine homeostasis on the level of bronchial tissues not only at the expense of increase of the level of proinflammatory cytokines, but also at the reduction of the level of cytokines with anti-inflammatory activity. Under the influence of injection in culture medium of meal of LPS-induced autologic mononuclear leukocytes (experiment 2) IL-4 level in supernatant of cells culture of bronchial epithelium in patients of the 1st and 2nd

groups isn't essentially changed. Under influence of preincubation of LPS-induced autologic mononuclear leukocytes with 20000 pg/ml of medium of human osteoprotegerin the investigated index is reduced so that is defined in a range of sensitivity of test-system only in the part of assays.

Table 3

Influence of osteoprotegerin of human recombinant (Sigma-Aldrich, USA) on the LPS-induced lymphoid (leukocytic) regulation of synthesis of cytokines by cells of bronchial epithelium in patients of the 1st and 2nd groups, pg/ml

Index	Group	Statistical index	Stages of vitral experiment		
			Experiment 1 (cytokine level in culture medium)	Experiment 2 (meal of autologic mononuclears)	Experiment 6 (preincubation of mononuclears with osteoprotegerin (20000 pg/ml of medium) washing up of cells in culture medium)
IL-1β	1st group	M ± m	19,57 ± 0,80	20,55 ± 0,77	28,27 ± 1,05
		n	26	26	26
		p	–	–	–
		p_1	–	< 0,5	< 0,001
		p_2	–	–	< 0,001
	2nd group	M ± m	26,26 ± 1,14	27,35 ± 0,88	33,96 ± 1,34
		n	28	28	28
		p	< 0,001	< 0,001	< 0,001
		p_1	–	< 0,5	< 0,001
		p_2	–	–	< 0,001
IL-4	1st group	M ± m	5,21 ± 0,20	5,01 ± 0,20	In a range of sensitivity of test-system it was defined in 12 from 26 assays
		n	26	26	
		p	–	–	
		p_1	–	< 0,5	
		p_2	–	–	
	2nd group	M ± m	4,16 ± 0,21	3,92 ± 0,18	In a range of sensitivity of test-system it was defined in 9 from 28 assays
		n	28	28	
		p	< 0,001	< 0,001	
		p_1	–	< 0,5	
		p_2	–	–	
TNF-α	1st group	M ± m	17,83 ± 0,94	18,57 ± 0,78	31,31 ± 1,22
		n	26	26	26
		p	–	–	–
		p_1	–	> 0,5	< 0,001
		p_2	–	–	< 0,001
	2nd group	M ± m	23,67 ± 1,14	25,68 ± 0,93	34,07 ± 1,73
		n	28	28	28
		p	< 0,001	< 0,001	< 0,2
		p_1	–	< 0,2	< 0,001
		p_2	–	–	< 0,001

Note: p – reliability of differences calculated in comparison with the conforming stage of experiment in patients of the 1st group, p1 – reliability of differences calculated in comparison with experiment 1 in the same group of patients, p2 – reliability of differences calculated in comparison with experiment 2 in the same group of patients.

TNF-α level in supernatant of culture medium of cells culture of bronchial epithelium in patients of the 2nd group on the first and second stages of experiment (experiment 1 and 2) is statistically significant higher than in patients of the 1st group. In experiment 6 the investigated index in patients both the 1st, and 2nd groups

essentially increases (in comparison with experiment 2) accordingly on 75,6 % and 43,9 % ($p1<0,001$) that documents the osteoprotegerin-dependent mononuclear-mediated mechanism of synthesis TNF-α by cells of bronchial epithelium.

CONCLUSIONS

1. For the first time it is determined that in physiological conditions (research was carried out with cells of practically healthy persons) the LPS-induced mononuclear leukocytes have ability to render osteoprotegerin-doze-dependent differentiated influence on functional (synthesis of cytokines IL-1β, IL-4 и TNF-α) activity of cells of bronchial epithelium: to stimulate synthesis of cytokines IL-1β and TNF-α, and also to inhibit synthesis of cytokine IL-4.

2. It was revealed low and upper «bound» of biologically active dosage (accordingly <5000 pg/ml of medium and >20000 pg/ml of medium) osteoprotegerin of human recombinant concerning of modulating influence on lymphoid (leukocytic) regulation of synthesis of cytokines IL-1β, IL-4 и TNF-α by cells of bronchial epithelium.

3. It is proved that increase of osteoprotegerin dosage of human recombinant in the experimental cultural vitral biological model is accompanied by statistically significant magnification of modulating influence of osteoprotegerin on synthesis of cytokines by cells of bronchial epithelium (dose-dependent modulating effect).

4. Tolerated pulmonary tuberculosis in a combination with essentially increased endobronchial level of osteoprotegerin form conditions of the increased risk of cytokine-dependent progressing of COPD.

5. The specified scientific facts are regarded by us as pathophysiological substantiation of expediency of medical correction of the increased endobronchial level of osteoprotegerin for reduction of local (bronchial epithelium) cytokine imbalance in patients with COPD, first of all – in persons who have tolerated pulmonary tuberculosis.

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