UDC 616.24-007.272-036.12+616.233-002.612.047.1.615.-085

O.M. Rekalova, O.R. Panasyukova, Yu.O. Matviienko, N.I. Grabchenko, M.B. Singhayevsky, S.G. Yassir
SO «National institute of phthisiology and pulmonology named after F.G. Yanovsky NAMS of Ukraine»

Efficiency of bacterial immunomodulator blastolen in patients with chronic obstructive pulmonary disease and chronic bronchitis

Key words: *chronic obstructive pulmonary disease, chronic bronchitis, bacterial immunomodulator, immune system.*

In recent years researchers have devoted considerable attention to the development of immunomodulators based on various strains of lactic acid bacteria that have immunomodulatory, antitumor, anti-infectious and anti-radiation properties, and also reduce the undesirable toxic effects of drugs.

Blastolen (Blastomunil) is an immunomodulator of natural origin, active ingredient of which is peptidoglycan from the cell wall of lactic acid bacteria (*Lactobacillus delbrucci*), which has nonspecific antigenic properties and triggers an innate immune reaction, mainly by activation of macrophages. In the international anatomical-therapeutic-chemical classification, the drug has code L03A X21 **: belongs to the group called «Cytokines and immunomodulators», «Other immunostimulants» (L03 «Immunomodulators», L03A «Immunostimulants»).

Blastolen has immunotropic, interferonogenic (promotes the induction of endogenous interferon), indirect antiviral, indirect antibacterial, microcirculatory and reparative properties [2]. The main feature of Blastolen is the activation of mononuclear phagocytes and dendritic cells through the inclusion of evolutionary mechanisms of hereditary immunity [1].

The action of the drug was studied in diseases that are accompanied by a secondary immunodeficiency with leukopenia, namely, with surgical, chemo- radiotherapy of cancer patients and patients with leukemia, in anti-inflammatory, antiviral, antibacterial complex therapy of various acute and chronic human diseases, including bronchopulmonary system, patients with AIDS, trophic ulcers, and attenuated patients [6], [10]. An additional use of the immunomodulator in the complex treatment of patients with mild COPD leads to a more significant inhibition of the systemic inflammatory process, manifested by a predominant decrease in the levels of proinflammatory cytokines and the restoration of the ratio between pro- and anti-inflammatory cytokines, as well as some decrease in the activity of local inflammation (more pronounced when inhalation administration of the drug) [4].

There is an experience of application bacterial immunomodulator in complex therapy of patients with chemoresistant destructive pulmonary tuberculosis, which leads to stimulation of functional activity of monocytes due to the growth of active forms of oxygen, a decrease in the level of circulating immune complexes (medium sized) and a positive therapeutic effect of treatment with a reduction in periods of decrease in infiltrative changes in the lungs, an increase in the proportion of patients who have lost

ISSN 2307-3373

caverns in the lungs, and a reduction in the length of stay in the hospital [3].

Frequent exacerbations of COPD and CB in patients, often observed after an acute respiratory illness, gave the basis for research the effectiveness of the drug Blastolen for the prevention of exacerbations of respiratory system diseases in patients in the phase of remission, which makes the study relevant.

The aim of this work was to evaluate the efficacy of Blastolen on the amount of exacerbations observed during the year and its effect on the state of the immune system of patients with COPD and CB.

Materials and methods

This work was funded by the state budget of Ukraine.

The study included 17 patients, which were observed in the SO «National institute of phthisiology and pulmonology named after F.G. Yanovsky NAMS of Ukraine». The middle age (61, 1 ± 1,9) years (from 49 to 70 years), of whom men – 10 (59%) and women – 7 (41%). Among them were 10 patients with chronic obstructive pulmonary disease (COPD) in remission phase the middle-aged (57.9 ± 2.8) years, of which 3 patients were in group B, 2 patients in group C, 5 patients in group D, and 7 patients with chronic bronchitis (CB) in the remission phase of middle age (63.5 ± 2.3) years.

The clinical diagnosis of COPD and CB was established in accordance with the accepted classification recommendations based on the data of the general clinical, functional, bronchological examination and was verified according to the characteristics of the patient groups in accordance with the Order of the Ministry of Health of Ukraine No. 555 of June 27, 2013 [7]. Patients signed informed consent to participate in the study. All patients underwent a general blood test with determination of lymphocytes, monocytes, neutrophils in peripheral blood.

Immunological study of peripheral blood of patients was carried out twice before the immunomodulator Blastolen administration and 2–3 weeks after the end of the course (only 7–8 weeks after the start of treatment). Immunological examination of patients assessed:

– the state of the phagocytic immune link by the content of granulocytes and monocytes in the blood, their ability to absorb test objects (Staphilococcus aureus), the phagocytosis and phagocytic numbers monocytes and neutrophils, labeled with fluorochromes (FITC), and the levels of their oxygen-dependent metabolism by spontaneous formation of active forms of oxygen (ROS) by cells and

Table 1- Cellular immunity factors in patients with COPD and CB before and after taking of Blastolen (M ± m)

Indicators of peripheral blood	Healthy persons (n = 17)	Patients (n = 17)	
		before	after
		treatment with the immunomodulator	
Number of leukocytes (10^9/L)	6,4 ± 0,3	7,1 ± 0,8	5,8 ± 0,9
Absolute number of lymphocytes (10^9/L)	2,09 ± 0,13	1,94 ± 0,20	1,83 ± 0,51
Relative number of lymphocytes (%)	32,5 ± 1,3	28,7 ± 3,6	30,5 ± 4,6
Absolute number of monocytes (10^9/L)	0,40 ± 0,03	0,30 ± 0,05	0,20 ± 0,05 *
Relative amount of monocytes (%)	6,2 ± 0,4	4,5 ± 0,3 *	4,4 ± 0,9
Absolute number of neutrophilic granulocytes (109/L)	3,94 ± 0,19	4,89 ± 0,71	3,78 ± 0,73
Relative number of neutrophilic granulocytes (%)	61,4 ± 1,3	66,7 ± 3,4	65,2 ± 4,7
Indicator of granulocyte phagocytosis (%)	49,5 ± 2,2	52,4 ± 5,0	48,8 ± 6,5
Phagocytic number of granulocytes (c u.)	15,5 ± 0,8	13,6 ± 2,5	14,5 ± 2,2
Formation of active forms of oxygen by granulocytes: – spontaneous (c u)	14,9 ± 1,2	18,8 ± 3,8	11,1 ± 3,1
– stimulated (c u.)	115,5 ± 16,8	62,1 ± 12,4 *	67,3 ± 22,2
stimulation coefficient (c u)	7,8 ± 1,0	3,6 ± 0,7 *	7,2 ± 1,7 ↑
Indicator of phagocytosis of monocytes (%)	41,6 ± 1,8	46,4 ± 6,9	42,3 ± 5,3
Phagocytic number of monocytes (c u)	11,3 ± 0,6	10,7 ± 2,2	10,7 ± 1,2
Formation of active forms of oxygen by monocytes: – spontaneous (c u)	88,8 ± 5,2	48,5 ± 18,5 *	53,4 ± 15,1
- stimulated (c u)	152,7 ± 13,0	222,7 ± 93,9	243,5 ± 103,9
stimulation coefficient (c u)	1,8 ± 0,2	4,3 ± 0,8 *	4,5 ± 0,9 *

Notes: * – the difference between this indicator and the indicator of a healthy group is statistically verified ($p < 0,05$).
↑↓ – statistically verified (by U – Wilcoxon criterion) direction of change: indicator in the dynamics of treatment: increased / decreased ($p<0,05$).

ISSN 2307-3373

after stimulation with zymosan, with calculation of the coefficient stimulation equal to the ratio of stimulated ROS to spontaneous ROS by the method of flow cytofluorimetry [9].

– the levels of circulating immune complexes (CICs) of medium and small size were evaluated in the microprecipitate test in polyethylene glycol (characterized by functional activity of B-lymphocytes) using the commercial test systems of the «Ham-Medica» (Russia) and taking into account the results of the μQuant analyzer spectrophotometer (BioTek, USA); [8];

– the cytokines and C-reactive protein (C-RP) content in the serum was determined by ELISA using commercial test systems: TNFα, IL-4, IL-8, IL-17A (« Cytokin «, Russia); C-RP («Ham», Russia).

The control group consisted of 17 volunteers (blood donors) without clinical signs of somatic and infectious pathology at the age of 28 to 67 years, the average age (48,9 ± 2,8) years. There were 5 men (29%) and 12 (71%) women.

Patients received injections of Blastolen at 0.6 mg intramuscularly once a week for 5 weeks (5 injections). Six months later and one year after the end of the Blastolen injection course, a telephone questionnaire was conducted.

The obtained digital data were processed using the parametric (one- and two-sided t-test of Student) and nonparametric (one- and two-sample Wilcoxon test) statistics using licensed software products included in the package Microsoft Office Professional 2007, license Russian Academic OPEN NoLevel No. 43437596. The results were presented in the form of n-number of examined patients in the group, arithmetic mean value (M), arithmetic mean value error (m). Calculations of the criterial values were carried out at a given significance level p • 0.05 [4].

Results and discussion

All patients had disorders of the phagocytic link of the immune system, namely, a decrease in the relative and absolute amount of peripheral blood monocytes ($p < 0.05$) (Table 1). At the same time, the decrease in stimulated production of the ROS by granulocytes ($p < 0.05$) was accompanied by a decrease in the stimulation coefficient of these cells ($p < 0.05$), which indicated a decrease in their functional reserve. Despite the relatively low level of spontaneous production of ROS by peripheral blood monocytes ($p < 0.05$), the functional reserve of these cells was maintained.

In the phase of remission, the activation of the anti-inflammatory potential of the immune system was established in patients, as evidenced by an increase in the serum level of IL-4 ($p < 0.05$) on the background of the prolongation of inflammatory changes, which was accompanied by an elevated C-reactive protein (C-RP) ($p < 0.05$) and an increase in the CIC level of small size ($p < 0.05$) in the blood. These changes in the immune system reflected the presence of a chronic inflammatory process in patients in the phase of remission of the disease and indicated the intensity of anti-inflammatory immunity (Table 2).

Application of the bacterial immunomodulator in the phase of remission in patients with COPD and CB led to an increase in the functional reserve of neutrophils with a probable increase in their stimulation factor from (3.6 ± 0.7) cu to (7.2 ± 1.7) cu at a rate of (7.8 ± 1.0) cu, $p < 0.05$.

There was also a decrease in proinflammatory signs in the patients. Thus, a decrease in the level of small CICs in serum from (750.6 ± 78.4) to (525.3 ± 88.7) cu, (norm (544.3 ± 33.1) cu), $p < 0.05$, could be associated with an improvement in the function of phagocytes. There was a decrease in the concentration of C-RP in the blood serum of patients with (18.8 ± 4.4) mg / l to (7.9 ± 2.2) mg / L, $p < 0.05$, although relative to the control value (3.3 ± 0.6) mg / l, it remained elevated, $p < 0.05$.

The positive character of the revealed changes in immunological parameters was confirmed by clinical indications. There was a reduction in the frequency of exacerbations in the last year – from (2.6 ± 0.3) times to (1.2 ± 0.5) times, $p < 0.05$, as well as the duration of exacerbations with (2.7 ± 0.2) weeks to (1.0 ± 0.3) weeks, $p < 0.05$. In general, a positive effect after taking the course of Blastolen with improvement in the general condition and decrease in the duration and frequency of exacerbations was observed in 11 of 17 patients (64.7)%, in 6 of them (35.3%) there was no acute exacerbation per year.

Table 2 – Humoral Immunity Factors in Patients with COPD and CB before and after Blastolen administration (M ± m)

Indicators of peripheral blood	Healthy persons (n = 17)	Patients (n = 17)	
		before	after
		treatment with the immunomodulator	
The level of small CICs (c u)	544,3 ± 33,1	750,6 ± 78,4 *	525,3 ± 88,7
Content of TNFα (pg/ml)	25,3 ± 5,0	24,0 ± 5,5	28,9 ± 7,2
Content of IL-8 (pg/ml)	31,3 ± 3,2	31,3 ± 4,3	36,8 ± 7,3
Content of IL-4 (pg/ml)	2,4 ± 0,3	5,9 ± 1,8 *	5,5 ± 1,3 *
Content of IL-17A (pg/ml)	120,1 ± 29,4	363,9 ± 141,8	324,8 ± 106,2
C-RP (mg/L)	3,3 ± 0,6	18,8 ± 4,4 *	7,9 ± 2,2 *#

Notes: 1. * – the difference between this indicator and the indicator of a healthy group is statistically verified ($p < 0,05$).
2. # – the change in the indicator in the dynamics of treatment is statistically verified ($p < 0,05$).
3. ↑↓ – statistically verified (by U – Wilcoxon criterion) direction of change: indicator in the dynamics of treatment: increased / decreased ($p<0,05$).

ISSN 2307-3373

In two patients noted an aggravation of the process, which quickly passed and did not require the drug to be withdrawn. One patient noted the appearance of pain in the joints after taking of Blastolen (connection is unlikely).

Conclusions

1) The administration of a course of injections of Blastolen is accompanied by immunomodulatory effect on phagocytes (increase in the functional reserve of neutrophils), as well as a decrease in the markers of the inflammatory process – the level of small CIC and the concentration of C-reactive protein in the blood of patients.

2) The application of the injection of Blastolen during the remission of chronic obstructive pulmonary disease or chronic bronchitis leads to a reduction in the duration and frequency of exacerbations of the disease during the year.

Список літератури

1. Болоховська В.А., Васильєв В.І., Нагорна О.В. Бластомуніл. Природний імуномодулятор, виділений з клітин молочнокислих бактерій. ЗАТ «Біофарма». 2010. 29 с.
2. Бластомунил® (Blastomunyl). Инструкция по применению. URL: http://compendium.com.ua/info/168041
3. Рекалова О.М. та ін. Застосування бактеріального пептидоглікану з метою корекції системи гама-інтерферону у хворих на деструктивний туберкульоз легень. Укр. пульмонол. журн. 2016. № 2. С. 31–32.
4. Лемко О.І. та ін. Комплексне відновлювальне лікування хворих на хронічне обструктивне захворювання легень і стадії з використанням імуномодулятора. Укр. пульмон. журнал. 2012. № 3. С. 38–43.
5. Лапач С.Н., Чубенко П.Н. Статистические методы в медико-биологических исследованиях с использованием Excel. Киев, Морион, 2000. 320 с.
6. Спіженко Ю. та ін. Місце імуномодулюючої терапії в комплексному лікуванні пухлинної хвороби та інших захворювань. Ліки України. 2005. № 3. С. 80–85.
7. Наказ МОЗ України № 555 від 27 червня 2013 р. «Про затвердження та впровадження медико-технологічних документів зі стандартизації медичної допомоги при хронічному обструктивному захворюванні легень». URL: http://document.ua/pro-zatverdzhennja-ta-vprovadzhennja-mediko-tehnologichnih-d-doc166218.html.
8. Васильева Е.В. и др. Применение иммуноферментного анализа для оценки гуморального и клеточного иммунного ответа при туберкулезе. Развитие научных исследований и надзор за инфекционными заболеваниями: Материалы междунар. науч. конф. под. ред. А.Б. Жебруна. СПб.: ФГУН НИИЭМ имени Пастера Роспотребнадзора. 2010. С. 6.
9. Ковальчук Л.В. и др. Система цитокинов, комплемента и современные методы иммунного анализа. М., Изд-во Российского гос. мед. ун-та. 2001. 81 с.
10. Мосієнко В.С. та ін. Ефективність вітчизняного імуномодулятора Бластомунілу в комплексному лікуванні пухлинної хвороби та інших захворювань. Клінічна імунологія. Алергологія. Інфектологія. 2007. № 5. С. 91–94.

References

1. Boloxovs'ka VA, Vasy'l'yev VI, Nagorna OV. Blastomunil. Pry'rodny'j imunomodulyator, vy'dileny'j z klity'n molochnoky'sly'x bakterij (Natural immunomodulator, isolated from lactic acid bacteria cells). ZAT «Biofarma». 2010; 29.
2. Blastomuny'l® (Blastomunyl). Y'nstrukcy'ya po pry'meneny'yu (Blastomunyl® (Blastomunyl). Instructions for use). Available from: http://compendium.com.ua/info/168041
3. Rekalova OM, et al. Zastosuvannya bakterial'nogo pepty'doglikanu z metoyu korekciyi sy'stemy' gama-interferonu u xvory'x na destrukty'vny'j tuberkul'oz legen (Application of bacterial peptidoglycan to correct the system of gamma-interferon in patients with destructive pulmonary tuberculosis). Ukr. pul'monol. Zhurn. 2016;2:31–32.
4. Lemko OI, et al. Kompleksne vidnovlyuval'ne likuvannya xvory'x na xronichne obstrukty'vne zaxvoryuvannya legen' i stadiyi z vy'kory'stannyam imunomodulyatora (Comprehensive regenerative treatment of patients with chronic obstructive pulmonary disease and stage using an immunomodulator). Ukr. pul'mon. zhurnal. 2012;3:38–43.
5. Lapach SN, Chubenko PN. Staty'sty'chesky'e metody v medy'koby'ology'chesky'x y'ssledovany'yax s y'spol'zovany'em Excel (Statistical methods in biomedical research using Excel). Ky'ev: Mory'on, 2000. 320 p.
6. Spizhenko Y, et al. Misce imunomodulyuyuchoyi terapiyi v kompleksnomu likuvanni puxly'nnoyi xvoroby' ta inshy'x zaxvoryuvan ('The place of immunomodulating therapy in the complex treatment of tumor disease and other diseases). Liky' Ukrayiny'. 2005;3:80–85.
7. Nakaz MOZ Ukrayiny' № 555 vid 27 chervnya 2013 r. «Pro zatverdzhennya ta vprovadzhennya medy'ko-texnologichny'x dokumentiv zi standarty'zaciyi medy'chnoyi dopomogy' pry' xronichnomu obstrukty'vnomu zaxvoryuvanni legen'» («On approval and introduction of medical and technological documents on standardization of care in chronic obstructive pulmonary disease») URL: http://document.ua/pro-zatverdzhennja-ta-vprovadzhennja-mediko-tehnologichnih-d-doc166218.html.
8. Vasy'l'eva EV, et al. Pry'meneny'e y'mmunofermentnogo analy'za dlya ocenky' gumoral'nogo y' kletochnogo y'mmunnogo otveta pry' tuberkuleze Razvy'ty'e nauchnыx y'ssledovany'j y' nadzor za y'nfekcy'onnыmy' zabolevany'yamy'(The use of enzyme immunoassay to assess the humoral and cellular immune response in tuberculosis. Development of scientific research and surveillance of infectious diseases): Matery'ali mezhdunar. nauch. konf. pod. red. A.B. Zhebruna. SPb.: FGUN NY'Y'ЭM y'meny' Pastera Rospotrebnadzora. 2010; 6 p.
9. Koval'chuk LV, et al. Sy'stema cy'toky'nov, komplementa y' sovremennыe metodы y'mmunnogo analy'za (System of cytokines, complement and modern methods of immune analysis). M.: Y'zd-vo Rossy'jskogo gos. Med. Un-ta, 2001. 81 p.
10. Mosiyenko VS, et al. Efekty'vnist' vitchy'znyanogo imunomodulyatora Blastomunilu v kompleksnomu likuvanni puxly'nnoyi xvoroby' ta inshy'x zaxvoryuvan (The effectiveness of the national immunomodulator Blastomunil in the complex treatment of tumor disease and other diseases). Klinichna imunologiya. Alergologiya. Infektologiya.2007;5:91–94.

Theoretical and practical J. «Asthma and allergy», 2018, 3

O.M. Rekalova, doctor of medical science, senoir research fellow, Head of the Laboratory of Clinical Immunology,
SO «National institute of phthisiology and pulmonology named after F.G. Yanovsky of NAMS of Ukraine»,
10, N. Amosova str., Kyiv, Ukraine, 03038; tel.: +38 (044) 275-42-22;
e-mail: rekalova@ifp.kiev.ua

ISSN 2307-3373