EVALUATION OF THE FUNCTIONAL ACTIVITY OF T LYMPHOCYTES, CONCENTRATION OF IL-4, IL-10 and IFN-γ AT THE EXPOSURE TO BIOACTIVE COMPOUNDS EXTRACTED FROM *SPIRULINA PLATENSIS* IN PULMONARY TUBERCULOSIS

Lesnic E. [1], Privalova E.[2], Ghinda S. [2], Chiriac T. [3], Rudic V. [3]
[1] – *Nicolae Testemitanu State University of Medicine and Pharmacy, Republic of Moldova*
[2] – *Institute of Phthysiopneumologie „Ch. Draganiuc", Republic of Moldova*
[3] – *Institute of Microbiology and Biotechnology, Republic of Moldova*
e-mail: evelina.lesnic@usmf.md
CZU:615.322:582.232.2+616.24-002.5-08 https://doi.org/10.52757/imb22.63

Summary

This study presents the results of the influence of bioactive compounds extracted from *Spirulina platensis* on the functional activity of T-lymphocytes through BTRL by PHA, concentration of IL-4, IL -10 and IFN-γ in patients with pulmonary tuberculosis. The experiments were realised *in vitro* and were tested sulfated polysiccharides (SPS), BioR and BioR^{Zn}. Results: In patients with allergic reactions to anti-tuberculous treatment BioR^{Zn} decreased the BTRL by PHA, but SPS and BioR increased. In patients with toxic and toxic-allergic reactions SPS and BioR increased and BioR^{Zn} decreased. In patients with toxic and toxic-allergic reactions the concentration of IFN-γ was higher than in those with allergic reactions but IL-4 was higher in patients with allergic and toxic-allergic reactions. The exposure of the T-lymphocytes to SPS statistically decreased the level of IFN–γ, increased IL-4 and IL-10. Conclusions: obtained data confirmed the utility of SPS, BioR and BioR^{Z} as remedies for pathogenic correction of disturbances caused by adverse drag events developed during the anti-tuberculous treatment in patients with pulmonary tuberculosis.

Key words: sulfated polysaccharides from Spirulina, BioR and BioR^{Zn}, pulmonary tuberculosis, T-lymphocytes, IL-4, IL -10 and IFN-γ.

Introduction

Adverse drug events (ADE) during the anti-tuberculous drugs (ATD) can occur up to 20% of patients with pulmonary tuberculosis (PTB) and the most common is the drug ATD toxicity [1]. Drug toxicity (DT) appears the most frequently during the treatment with injectable aminoglycosides, followed by isoniazid, rifampicin and pyrazinamide [2]. Allergic reactions due to ATD are diagnosed in 4-6% and can be mild to severe such life-threatening conditions [1]. The correct management of ADE consists in the identification of the causing ATD, individualisation of the treatment according to the patient's clinical tolerance and metabolic disorders and desenzitization [3]. Studies showed that DT is often conditioned by the immune disorders, which finally worsen the clinical evolution and determine unfavourable outcome [4]. Current medical prescribing practices does not allow the systematic evaluation of the patient's immune state, even if it is an essential method of the personalized medicine for the improvement of the compliance and outcome [3]. Some researches revealead the immune-modulatory and anti-inflammatory effects of bioactive compounds (BAC) extracted from the *cyanobacterium Spirulina (Athrospira) platensis*. Particularly valuable are sulfated polysaccharides (SPS) which are polyanionic complexes located on the external surface of cell membranes and in the extracellular space. SPS is mainly based on fructose, rhamnose, xylose, mannose, glucose and galactose, various isomeric forms and types of glycosidic bonds, and the three-dimensional structure [5]. SPS extracted from *S. platensis* exhibits antiviral, antioxidant, antifibrotic, anticoagulant, antitrombotic, hypolipemiant activity and immune modulating effects through the mechanism of constitution of the tissue barrier. *Sodium spirulan,* a SPS isolated from *S. platensis,* increases the production of endothelial proteoglicans, providing an efficient anti-thrombotic activity, stabilizes the lysosomal membranes and increase the immune defense [6]. Despite the recent advances in ficobiotechnology the effects of BAC isolated from *S. platensis* on the cell-mediated immunity indicators in patients with PTB were not established.

The aim of the study was to investigate the effects of BAC extracted from *S. platensis,* on the indices of the functional activity of T lymphocytes, concentration of IL-4, IL -10 and IFN-γ in patients diagnosed with PTB, which developed ADE with the scope to select the compound with the greatest evidence in the improvement of the immune disorders.

Material and methods

The conducted study was analytical, prospective and case-control. In the study were included 110 patients, who encountered the including criteria: age more than 18 years old, diagnosed with PTB infiltrative form, culture drug-susceptible confirmed TB and new case. All patients provided the signed informed consent. The patients were distributed in 3 groups according to the ADE: 1st group – 37 patients with allergic reaction (type I reaction mediated by IgE), 2nd group – 49 patients with toxic and allergic reactions (type I mediated by IgE and type II mediated by IgG and IgM) and 3rd group -25 patients with toxic reaction (type II, mediated by IgG and IgM). The experiments were conducted *in vitro* using the peripheral lymphocytes selected from the venous blood samples, which were collected, when the adverse drug effects were established, according to the principles of the biological standardization and conduct of experiments, approved by the Research Ethics Committee of "Nicolae Testemițanu" SMPhU, protocol No. 14 from 20/10/2017 and carried out according to the Helsinki declaration with modifications (Somerset West Amendment, 1996). To evaluate the functional activity of T cells, was used the index of blast transformation of lymphocytes reaction (BTRL) to polyclonal mitogens - phytohemagglutinin (PHA), the concentration of interleukins IL-4, IL-10 and IFN-γ before and after the exposure to the BAC: SPS, BioR and $BioR^Z$ obtained at the Institute of Microbiology and Biotechnology from the R. of Moldova.Statistical analysis was performed using the SPSS 23.0 program. To test for significant differences between the parameters of the compared groups, Fisher's exact test and non-parametric Student's t-test were performed. The threshold for statistical significance was $p<0.05$.

Rezultats and discutions

When distributing patients by sex, was established the predominance of men *vs.* women ($p<0,001$ in all groups) with the ratio 1,8 in the 1st group, 1,6 - 2nd group and 2,4 - 3rd group without statistical significance at the comparision of the groups. Analyzing the age, it was found that the majority of patients in all groups were between 18 and 44 years old, and statistically predomminated compared with the patients older 45 years ($p<0,001$ in all groups), without differences between the groups. As well the average age did not differ significantly between the groups. So, according to the distribution acoording to the sex and age, the goups were comparable.

Radiological features of etensive TB affecting more than 3 lung segments statistically predominated in the 3rd *vs.* 1st group ($p<0,001$) and unsignificantly *vs.* 2nd group. Destruction of the lung parenchima statistically predominated in the 3rd *vs.* 1st and 2nd groups ($p<0,001$ in both groups). Disseminative opacitities were identified in every third case from all groups. Microscopic positive for AFB were the majority of patients, due to including criteria in the research of the drug-susuceptible confirmed pulmonary TB. The duration of the hospitalisation for the anti-TB treatment was significantly longer in the 3rd *vs.* 1st and 2nd groups ($p<0,001$ in both groups (Table 1).

Table 1. Distribution of patients by sex, age, radiological and microbiological characteristics

Indicators	1st group	2nd group	3rd group
Men (abs. no., %)	24 (64,9) ◊	30 (61,2) ◊	17 (70,8) ◊
Women (abs. no., %)	13 (35,1)	19 (38,8)	7 (29,2)
Age 18-44 years (abs. no., %)	25 (76,6) ◊	34 (69,4) ◊	15 (60,1) ◊
Age older than 45 year (abs. no., %)	12 (24,4)	15 (31,6)	10 (39,9)
Average age (years)	36,3±2,38	35,4±2,07	36,0±2,78
Extensive TB (abs. no., %)	21 (56,7)	32 (65,4)	18 (72,1) ○
Lung destruction (abs. no., %)	22 (59,5)	32 (65,3) *	21 (87,5) ○
Dissemination (abs. no., %)	11 (29,7)	13 (26,5)	8 (32,1)
AFB positive (abs. no., %)	26 (70,3)	40 (81,6)	20 (83,3)
Total duration of the treatment (days)	74,9±7,76	73,8±5,46 *	89,5±10,91○

Note: Statistical test used Fisher's exact test.

◊ - statistical difference between the rate of men compared with women, and of patients between 18-44 and older 45 years, in each group, respectively;

○ – statistical difference detween the indices from the 1st group and 3 rd group;

** - statistical difference detween the indices from the 2nd group and 3 rd group;*

The rate of patients with previous allergic reactions was low and did not differ between the groups. Comparing the clinical signs attributed to ADE, was estbalished the statistical predominance of pruritus in

the 1st and 2nd groups *vs.* the 3rd group (p<0,01 in both groups), due to including criteria. Nausea and vomiting, increased size of the liver, statisticaly predominated in the 3rd *vs.* 1st and 2nd groups (p<0,001 in both groups), concomitantly the hepatomegaly was more frequently in the 3rd *vs.* 2nd group (p<0,01). Paresthesis and decreased visual acuity predominated in the 3rd *vs.* 1st and 2nd groups. Laboratory disorders such as anemia, elevated alanine transaminase (ALT) and aspartate aminotransferare (AST) statistically predominated in the 3rd *vs.* 1st and 2nd groups (p<0,001 respectively) (Table 2).

Table 2. Clinical and laboratory peculiarities of ADE (abs. no., %)

Indicators	1st group	2nd group	3rd group
Allergic anamnesis (abs. no., %)	2 (5,4)	2 (4,1)	1 (4,2)
Papular pruritus (abs. no., %)	5 (13,5) ●	6 (12,2) ∗	0
Erythematous pruritus (abs. no., %)	9 (24,3) ●	10 (20,4) ∗	0
Paresthesis (abs. no., %)	3 (8,1)	3 (6,1)	3 (12,5)
Nausea, vomiting (abs. no., %)	0	6 (12,2) ●	8 (33,3) ○
Decreased visual acuity (abs. no., %)	0	0	1 (4,2)
Increased liver size (abs. no., %)	2 (5,5) ●	15 (30,6) ∗	15 (62,5) ○
Anemia (abs. no., %)	1 (2,7) ●	3 (6,1)	5 (20,8)
Elevated ALT (abs. no., %)	4 (10,8) ●	10 (20,4)	8 (33,3)
Elevated AST (abs. no., %)	2 (5,4) ●	9 (18,4)	7 (29,2)
Positive Thymol turbidity test (abs. no., %)	1 (2,7)	2 (4,1) 83	1 (4,2)

Note: Statistical test used Fisher's exact test.
● *– statistical difference detween the indices from the 1st group and 2nd group;*
○ *– statistical difference detween the indices from the 1st group and 3 rd group;*
∗ *- statistical difference detween the indices from the 2nd group and 3 rd group;*

The analysis of the complete blood count established a higher number of leucocytes in the 3rd *vs.* 1st group (p<0,01). When analysing the rate of each type of neutrophils, was determined the statistical predomination of segmented, non-segmented and of the cells with toxic granulations in the 3rd *vs.* 1st group (p<0,001 respectively). The rate of eosinophils and lymphocytes was statistically higher in the 1st group *vs.* 3rd group (p<0,001 respectively). Concomitantly the rate of neutrophils with toxic granulations and eosinophils was higher in the 3rd group *vs.* 2nd group and eosinophils in the 2nd *vs.*group 3rd (p<0,05 respectively). The analysis of the BTRL by PHA established a lower proliferative activity in the 3rd *vs.* 1st group (p<0,001) and *vs.* 2nd groups (p<0,01), (Table 3). So, the patients with PTB which developed allergic, toxic and mixed reactions to ATD presented different disorders. Degree of disturbances was higher in patients with toxic ADE compared with those with allergic events.

Table 3. Complete blood count in patients with ADE

Indicators	1st group	2nd group	3rd group
Leucocytes (x10^9/L)	7,5±0,34●	8,4±0,37	9,1±0,75
Segmented neutrophils (%)	53,7±1,50●	62,3±1,39	64,7±1,90○
Nonsegmented neutrophils (%)	3,6±0,48●	3,7±0,65	5,0±0,94○
Neutrophils with toxic granulations, (abs. no. and %)	0●	1 (2,0±2,02)∗	5 (20,8±8,29)
Eosinophils (%)	7,5±0,75●	2,4±0,33∗	1,4±0,30○
Lymphocytes (%)	28,1±1,50●	24,7±1,36	21,7±2,00
BTRL by PHA (%)	61,7±1,42●	54,9±1,12∗	48,1±2,47

Note: Statistical test used nonparametric T Student.
● *– statistical difference detween the indices from the 1st group and 2nd group;*
○ *– statistical difference detween the indices from the 1st group and 3 rd group;*
∗ *- statistical difference detween the indices from the 2nd group and 3 rd group;*

The BTRL by PHA after the exposure to different BAC isolated from *S. platensis* showed different effects as following:

$BioR^{Zn}$ significantly decreased the BTRL of T lymphocytes in the 1st group, SPS and BioR unsignificantly increased.

SPS and BioR significantly increased the functional activity in the 2nd and 3rd groups, and BioR^{Zn} slightly reduced in the same group.

Comparing the groups, the SPS increased more evident the BTRL in 1st *vs.* 2nd and *vs.* 3rd groups and less evident in the 2nd *vs.* the 3rd groups.

BioR significantly increased the BTRL in 1st *vs.* 2nd group, 1st *vs.* 3rd group, as well in the 2nd *vs.* the 3rd group.

BioR^{Zn} significantly decreased the functional activity in the 1st *vs.* the 3rd group, and in the 2nd *vs.* the 3rd group (Table 4).

Table 4. *In vitro* evaluation of the BTRL by PHA at the exposure to the BAC

Indicators	Exposure to SPS		Exposure to BioR		Exposure to BioR^{Zn}	
1st group before after the exposure	68,1±1,21 70,2±0,56		65,6±1,11 67,5±0,64		67,6±0,90 61,9±0,86●	
2nd group before after the exposure	58,3±1,12 61,9±1,11●		59,9±1,04 63,4±0,86●		63,7±0,95 62,1±0,74	
3rd group before after the exposure	48,0±1,11 54,2±3,64●		52,9±2,35 58,4±2,20●		57,1±2,24 56,3±1,68	
Before/after the exposure	before	after	before	after	before	after
1st *vs.* 2nd group	<0,001	<0,001	<0,001	<0,001	<0,01	>0,05
1st *vs.* 3rd group	<0,001	<0,001	<0,001	<0,01	<0,001	<0,01
2nd *vs.* 3rd group	<0,01	<0,05	<0,01	<0,05	<0,01	<0,01

The IFN–γ plays an essential antiproliferative role and interleukins IL-4 and IL-10 determine anti-inflammatory effects. When the ADE were detected the concentration of IFN–γ was statistically higher in the 3rd *vs.* 1st and 2nd grous. The concetration of IL-4 was statistically higher in the 1st *vs.* 2nd group, and achieved the statistical threshold at the comparision with 2nd group. The concetration of IL-10 did not statistically differ among all groups. The exposure SPS statistically decreased the concentration of IFN–γ from the initial one, statistically increased the concentration IL-4 and insignificantly increased IL-10. Comparing the groups, the IFN–γ decreased more evidently in the 1st vs. 2nd and 3rd groups, as well in the 2nd *vs.* 3rd groups. The concentration of IL-4 increased significantly in the 1st *vs.* 2nd and 3rd groups, and the IL-10 did not changed significantly (Table 5).

Table 5. The effect of SPS on the concetration of cytokines

Indicators	IFN –γ ng/L		IL-4 ng/L		IL-10 pg/L	
1st group before after the exposure	71,8±4,13 53,3±4,03●		8,1±0,80 14,0±1,32●		0,81±0,03 0,90±0,02	
2nd group before after the exposure	101,9±5,37 83,1±5,60●		7,0±0,60 9,6±0,90●		0,77±0,01 0,83±0,02	
3rd group before after the exposure	124,9±5,34 110,5±5,12●		5,5±0,53 7,1±0,59●		0,82±0,03 0,93±0,02	
	before	after	before	after	before	after
1st *vs.* 2nd group	<0,001	<0,001	>0,05	<0,01	>0,05	>0,05
1st *vs.* 3rd group	<0,001	<0,001	<0,05	>0,001	>0,05	>0,05
2nd *vs.* 3rd group	<0,01	<0,01	>0,05	<0,05	>0,05	>0,05

Spearman's correlation coefficient established moderate negative correlation between the BTLR by PHA and IFN –γ in the 1st and 2nd groups, and strong negative in the 3rd group. So, the high levels of IFN–γ determined a reduced proliferation of lymphocytes. The concentrations of IL-4 and IL-10 positively correlated with BTLR by PHA, at moderate degree in the 3rd group, meaning high concentrations of anti-inflammatory interleukins determined intense proliferation of the lymphocytes (Table 6).

Table 6. Correlation between the values of BTLR by PHA and cytokines

Indicators	IFN –γ r	IL-4 r	IL-10 r
1st group	**-0,51**[3]	0,37[2]	0,25[1]
2nd group	**-0,58**[3]	0,48[2]	0,37[2]
3rd group	**-0,71**[4]	**0,57**[3]	**0,58**[3]

**Spearman's correlation coefficient was calculated to determine the strength of the correlation between the BTLR by PHA and immunological parameters. Interpretation on the Chaddock scale: 1 at r 0.1-0.29 - very weak correlation, 2 at r 0.3-0.49 weak correlation; 3 at r 0.5-0.69 average correlation; 4 at r 0.7 and higher—strong correlation.*

Conclusions

The effects of bioactive compounds SPS, BioR and BioR^{Zn} were selective and differentiated. In patients with allergic reactions BioR^{Zn} decreased the BTRL by PHA, and SPS and BioR increased. In patients with toxic and toxic-allergic reactions SPS and BioR increased and BioR^{Zn} slightly reduced. In patients with toxic and toxic-allergic reactions the concentration of IFN-γ was higher than in those with allergic reactions, IL-4 was higher in patients with allergic and toxic-allergic reactions. Was established negative correlation between IFN–γ, and positive between IL-4, IL-10 and BTRL by PHA. The exposure of the T-lymphocytes to SPS statistically decreased the level of IFN–γ, increased IL-4 and IL-10, with higher evidence in patients with allergic reactions.

The results confirmed the utility of SPS, BioR and BioR^{Z} as remedies for pathogenic correction of the immune disturbances caused by adverse drag events developed during the anti-tuberculous treatment in pulmonary tuberculosis.

References:

1. *WHO*: Guidelines for treatment of drug-susceptible tuberculosis and patient care, 2017. Disponibil : https://www.who.int/tb/publications/2017/dstb_guidance_2017/en/
2. Li Y, Zhu Y, Zhong Q, Zhang X, Shu M, Wan C. Serious Adverse Reactions from Anti-tuberculosis Drugs Among 599 Children Hospitalized for Tuberculosis. Pediatr Infect Dis J. 2017 Aug;36(8):720-725. doi: 10.1097/INF.0000000000001532. PMID: 28060046.
3. Ministerul Sănătății, Muncii și Protecției Sociale al Republicii Moldova. Protocol Clinic Național. Tuberculoza la Adult. PCN-123. Chișinău, 2020. 152 p. Available on: https://msmps.gov.md/wp-content/uploads/2021/02/PCN-123-Tuberculoza_la_adult.pdf.
4. Martino M., Lodi L., Galli L., Chiappini E. Immune Response to Mycobacterium tuberculosis: A Narrative Review. In: *Frontiers in Pediatrics*, Vol. 7, 2019, https://www.frontiersin.org/article/10.3389/fped.2019.00350
5. Rudi L., Chiriac T., Cepoi L. Factorii tehnologici și calitatea biomasei de spirulină. Chișinău, 2020, et. ,,Artpoligraf", 242 p.
6. Se-Kwon Kim, Yong-Xin Li. Medicinal Benefits of Sulfated Polysaccharides from Sea Vegetables. Ed. Advances in Food and Nutrition Research, Academic Press, Volume 64, 2011, Pages 391-402, ISSN 1043-4526, ISBN 9780123876690, https://doi.org/10.1016/B978-0-12-387669-0.00030-2.