DOI: 10.11134/btp.3.2025.4

UDC 579.61 *Original Article*

STUDY OF BACTERIAL LYSATE IN THE FIGHT AGAINST ANTIBIOTIC RESISTANCE

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ABSTRACT

Bacterial lysates are widely used today as immunomodulatory agents that enhance the effectiveness of antibacterial therapy through synergistic interactions with antibiotics. The aim of this study was to evaluate the pharmacological properties of a bacterial lysate obtained from antibiotic-resistant strains of opportunistic microorganisms such as *Acinetobacter baumanii, Klebsiella pneumoniae SCAID PND1-2022 (246), and Pseudomonas aeruginosa SCAID PHRX1-2019* to assess its potential in combating the growing problem of antimicrobial resistance. The study analyzed the properties of the lysate and its effects on the body using in vivo studies at doses of 300.0 and 500.0 mg/kg. No signs of acute toxicity were observed, suggesting that this substance is safe for use. Furthermore, destroyed bacterial cells retain the ability to induce a specific immune response, stimulating immune system activation. Given the inherent resistance of bacterial strains to antibiotics, it can be concluded that the use of bacterial lysate promotes the development of an adaptive immune response directed against highly resistant pathogens, including superbugs.

Key words: antibiotic resistance, lysate, preclinical trials, immunomodulator, opportunistic, acute toxicity.

INTRODUCTION

One of the urgent problems of public health is antibiotic resistance in strains of opportunistic microorganisms. For this reason, it is becoming increasingly difficult to select a treatment protocol and successful recovery of patients every day. This problem is partly anthropogenic, namely due to non-compliance with the recommendations of the attending physician, premature interruption of antibiotics, and the second main reason is the natural process of survival of microorganisms - parallel transfer of plasmids. To minimize the spread of resistance genes through parallel transfer and the formation of immunity to superbugs, it is necessary to strengthen local control over the monitoring of infectious pathogens and introduce the use of preventive treatment measures into practice. To find the optimal solution to the problem, it is worth considering the method of using a lysate of bacteria with multiple resistance to antibiotics, due to which the immunity of the patient receiving this type of prophylactic treatment will be able to quickly respond to infections with polytolerant microorganisms. Bacterial lysate clinically proves its effectiveness by improving specific immunity in studies by Kamil Janeczek, Andrzej Emeryk [1]. Sublingual administration of bacterial lysate preparations provides significant effectiveness in alleviating SAR symptoms in children sensitized to grass pollen allergens. Primary mediastinal B-cell lymphoma (PMBL) probably affects mucosal immunity by attenuating the TH2 cell response and thus restoring the TH1/TH2 balance.

Bacterial lysates were first isolated in the 1960s and introduced for the treatment and prevention of human respiratory tract infections (RTIs). According to Bommu. Rajasekhar [2], bacterial lysate enhances both innate and adaptive immune responses, demonstrating efficacy in restoring epithelial barrier integrity, activating ILC3 and dendritic cells, and stimulating the Th1 response. In addition, they stimulate the production of specific serum IgG and salivary IgA, providing cross-protection against other pathogens through trained immunity. Also, according to Fulvio Braido, bacterial lysates, through DC activation, induce a predominant T helper 1 (TH1) response, with an increase in IL-12 and interferon-γ (IFNγ),14,15 attenuating the TH2 pattern of the immune system [3]. In parallel with the enhancement of the TH1 response, oral administration of bacterial lysates promotes the production of IL-10, promoting the conversion of FoxP3– T cells into FoxP3+ regulatory T cells. However, the combined effects of TH1/TH2 balance and stimulated differentiation of DCs and T regulatory cells lay the foundation for the potential use of bacterial lysates in the prevention of allergic diseases. According to recent studies by Mikhail Kostinov, the addition of a bacterial lysate-based immunomodulatory agent to the treatment regimen for moderate to severe COVID-19 induces the production of pharyngeal and salivary sIgA, which means an enhancement of the mucosal response and protection against various pathogens, including viruses, which is mediated by its neutralizing properties, as well as its ability to prevent pathogen adhesion to mucosal surfaces and opsonize pathogenic microorganisms, thereby preventing their penetration into epithelial cells [4].

Despite the recent introduction of many drugs for the treatment of RTIs, prophylaxis with bacterial lysates still attracts the interest of physicians as a promising tool to reduce acute infection and limit the use of antibiotics, topical corticosteroids and anti-inflammatory drugs. In this regard, the European Medicines Agency [5, 6] recently reviewed the evidence on the safety and efficacy of bacterial lysates and recommended their use for the prevention of recurrent respiratory infections. The use of bacterial lysate is recommended as a prophylaxis, as well as in synergy with a targeted antibiotic to increase the effect of the main therapeutic drug.

More recently, it was reported that in vitro treatment of immature DCs with fragments of bacterial strains obtained by mechanical lysis can induce DC maturation and secretion of a wide range of cytokines and chemokines. Lanzilli et al. [7], in an ex vivo study, found that treatment with a polyvalent me-

DOI: 10.11134/btp.3.2025.4

chanical bacterial lysate (PMBL) altered circulating lymphocyte subsets in elderly patients with chronic obstructive pulmonary disease (COPD).

In connection with the worldwide conducted research of the preparation from the bacterial lysate - OM-85, the world is opening up a detailed study of the effect of the bacterial lysate on the body. According to currently known data, bacterial lysate imitates the natural effect of microbes, promoting an innate, adaptive, antigen-specific immune response against the introduced antigens. In the case of the emerging problem of antibiotic resistance, the scientific community should consider the option of studying both the effect of the lysate itself and the lysate from objects with resistance. Studies from these types of objects have not been previously conducted, before the start of scientific research, a literature analysis was conducted on the mechanism of the effect of bacterial lysate on the body. Presumably, by affecting TLR-2 and TLR-4 through the resistance antigen, the mechanism of a specific response of the body to antibiotic-resistant microorganisms is triggered. This work has its own uniqueness - a high chance of a breakthrough in the treatment and prevention of diseases caused by antibiotic-resistant microorganisms.

MATERIALS AND METHODS

The objects of the study are the lysate of opportunistic microorganisms *Acinetobacter baumanii*, *Klebsiella pneumoniae* SCAID PND1-2022 (246), *Pseudomonas aeruginosa* SCAID PHRX1-2019.

In the preclinical studies conducted during physical disintegration using the French press method, the OECD Guidelines for the Testing of Chemicals No. «423» Acute toxicity - classical method were used.

The studied pharmacological substance Lysate was administered orally once, since this is the intended method of use in the clinic.

The study used white outbred sexually mature laboratory mice of both sexes in the amount of 20 individuals, weighing 22 g ± 10%. Working solutions of the studied pharmacological substance were prepared by dissolving the sample in the calculated volume of solvent (distilled water) to the specified dosages of 300.0 mg/kg, 500.0 mg/kg, 1000.0 mg/kg and 2000.0 mg/kg of weight. The amount of the sample depended on the weight of the animals included in the experimental groups.

Solutions of the studied pharmacological substance Lysate in the studied dosages were administered to the animals once orally using a gastric tube in a volume of 0.5 ml.

Table 1 shows the orally administered doses of the studied pharmacological substance Lysate. Animals were monitored for lethality or toxic signs on the first day - during the working day every 2 hours, in the following days - every 24 hours.

All animals were observed for 14 days after the administration of solutions of the studied pharmacological substance Lysate. During the observation period, the presence of signs of acute toxic effects associated with the effect of the studied pharmacological substance was assessed. The body weight of the animals was measured immediately before dosing and then weekly throughout the observation period.

RESULTS

Data on animal observation after a single oral administration of the studied pharmacological substance Lysate in dif-

Table 1 – Distribution of mice into groups for the study of acute toxicity of the studied pharmacological substance Lysate upon oral administration

The substance under study	Groups	Volume of injection, ml	Dose, mg/kg	Number of animals in a group
Pharmacological substance Lysate	Experimental 1	0,5	300,0	5
	Experimental 2	0,5	500,0	5
	Experimental 3	0,5	1000,0	5
	Experimental 4	0,5	2000,0	5

Table 2 – Clinical symptoms and mortality of mice after a single oral administration of solutions of the studied pharmacological substance Lysate in different dosages

Groups, doses	Animal code	Hours				Days												
		1	2	4	6	2	3	4	5	6	7	8	9	10	11	12	13	14
1 Group – 300,0 мг/кг	PHT-159-1-1	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	PHT-159-1-2	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	PHT-159-1-3	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	PHT-159-1-4	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	PHT-159-1-5	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

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Groups, doses	Animal code	Hours				Days												
		1	2	4	6	2	3	4	5	6	7	8	9	10	11	12	13	14
2 Group – 500,0 мг/кг	PHT-159-2-1	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	PHT-159-2-2	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	PHT-159-2-3	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	PHT-159-2-4	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	PHT-159-2-5	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
3 Group – 1000,0 мг/кг	PHT-159-3-1	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	PHT-159-3-2	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	PHT-159-3-3	√	√	√	√	√	√	√	√	F	F	F	F	F	F	F	F	F
	PHT-159-3-4	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	PHT-159-3-5	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
4 Group – 2000,0 мг/кг	PHT-159-4-1	√	√	√	√	√	√	√	√	F	F	F	F	F	F	F	F	F
	PHT-159-4-2	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	PHT-159-4-3	√	√	√	√	√	√	√	√	√	F	F	F	F	F	F	F	F
	PHT-159-4-4	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	PHT-159-4-5	√	√	√	√	√	√	√	√	√	√	√	F	F	F	F	F	F

X – death;	I – in a state of prostration;
√ – norm;	J – tremor;
A – uncoordinated movements;	K – difficulty breathing;
B – lacrimation;	L – hunched posture;
C – salivation/foam;	M – inhibited response to external stimuli;
D – loose stools;	O – refusal to eat;
E – vomit;	P – paleness of the mucous membranes;
F – alopecia;	Q – convulsions;
G – huddling together;	W – decreased skeletal muscle tone.

ferent dosages are presented in Table 2.

During the observation period (14 days) after the administration of the studied doses of solutions of the studied pharmacological substance, no death of mice was recorded. During the observation, a satisfactory condition of animals was noted that received solutions of the studied pharmacological substance Lysate in doses of 300.0 mg / kg; 500.0 mg / kg; 1000.0 mg / kg and 2000.0 mg / kg, signs of severe toxic poisoning and changes in the behavior of animals were not observed. At doses of 1000.0 mg / kg and 2000.0 mg / kg of weight, signs of alopecia were observed in individual animals.

The dynamics of changes in the body weight of animals during the experiment are presented in Table 3.

When studying the dynamics of body weight of mice with oral administration of the studied doses of solutions of the studied pharmacological substance, a positive physiological increase in body weight was observed over 14 days, no reliably significant changes were detected.

In mice receiving the maximum dose of 2000.0 mg / kg

DOI: 10.11134/btp.3.2025.4

Table 3 – Changes in the body weight of mice after a single oral administration of solutions of the studied pharmacological substance Lysate in different doses, (M ± m).

№ groups	Dose of the substance Lysate, mg/kg of weight	Average weight of animals, g		
		before the experiment began	in 7 days	in 14 days
1	300,0	22,6 ± 0,73	23,3 ± 0,73	24,1 ± 0,42
2	500,0	22,9 ± 0,18	23,4 ± 0,41	23,9 ± 0,20
3	1000,0	22,7 ± 0,30	23,3 ± 0.30	24,0 ± 0,23
4	2000,0	22,8 ± 0,52	23,6 ± 0,54	24,6 ± 0,59
Note: * – the difference is reliable in relation to the values of body weight before the start of the experiment ($p < 0.05$)				

of the drug, the urinary bladder was filled with light transparent contents with a typical urine odor, the ureters were passable. Histological studies of those receiving this dose are shown in Figure 1. In this figure 1, in sample A, dystrophic changes in the epithelial cells of the proximal tubules (blue pointer) are noted in the cortex. Homogeneous detritus is diffusely present in the lumen of the distal tubules, and in the preserved tubules, the epithelial cells are pushed out into the lumen (orange pointer). In the glomeruli, focal activation of mesangiocytes (green pointer) is observed. In sample B, homogeneous detritus is diffusely present in the lumen of the distal tubules; in the preserved tubules, epithelial cells are pushed into the lumen (orange pointer). In the medulla, there are diffuse foci of neutrophilic inflammation, less frequently macrophages, including large ones, predominantly along the periphery of inflammatory cell infiltrates (yellow pointer).

All animals in this group showed similar changes in the cortex and medulla of the kidney. Dystrophic changes in the epithelial cells of the proximal tubules are observed in the cortex. Homogeneous detritus is diffuse in the lumen of the distal tubules, and epithelial cells are pushed into the lumen in the preserved tubules. Focal activation of mesangiocytes is observed in the glomeruli. In the medulla, there are diffuse foci of neutrophilic inflammation, less frequently macrophages, including large ones, mainly along the periphery of inflammatory cell infiltrates.

DISCUSSIONS

The pharmacological substance of bacterial lysate obtained mechanically through a French press disintegrator, as an immunomodulator, shows its safety in doses of 300.0 mg/kg, 500.0 mg/kg. However, when administering a dose of 2000.0 mg/kg and 1000.0 mg/kg, signs of alopecia are observed and macro- and microscopic changes in the urinary system (formation of inflammatory cell, mainly neutrophilic infiltration) and microscopic changes in the spleen (depletion of the lymphoid tissue pool) are noted. Taking into account the preservation of all indicators within the norm when administering a dose of 500.0 mg/kg, this dosage is safe for administration. The absence of mortality in the study groups proves the safety of the pharmacological substance of bacterial lysate.

CONCLUSION

The proposed mechanism of action of bacterial lysate on the body is immunomodulation. Through the activation of macrophages and dendritic cells, followed by the activation of Th1, regulatory T cells and cytotoxic CD8+ cells, bacterial lysate has an immunomodulatory effect in synergy with the targeted therapeutic drug.

When obtaining a bacterial lysate, the minimum toxicity is shown by the volume of drug administration - 500 mg/g. This dosage of bacterial lysate activates specific immunity, increas-

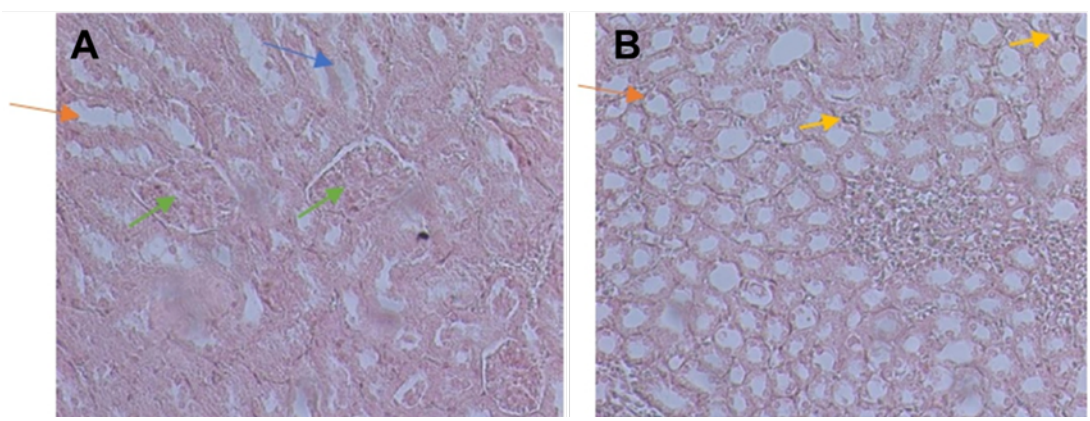

Figure 1 - Histostructure of the kidney tissue of mice 14 days after a single intragastric administration of bacterial lysate 2000.0 mg / kg (Staining with hematoxylin and eosin, eyepiece ×10, objective ×20)

DOI: 10.11134/btp.3.2025.4

ing the body's resistance to antibiotic-resistant microorganisms. The immunomodulatory activity of this bacterial lysate is hypothetical and based mainly on literature data. More detailed and expanded studies of changes in functional immune parameters are needed for a full assessment of the biochemical reactions to the body.

ACKNOWLEDGEMENT

Funded within the framework of the scientific and technical program of the Ministry of Science and Higher Education of the Republic of Kazakhstan "Improving measures to ensure biological safety in Kazakhstan: counteracting dangerous and especially dangerous infections" (IRN BR218004/0223).

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DOI: 10.11134/btp.3.2025.4

УДК: 579.61

ИЗУЧЕНИЕ БАКТЕРИАЛЬНОГО ЛИЗАТА В БОРЬБЕ С АНТИБИОТИКОРЕЗИСТЕНТНОСТЬЮ

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АННОТАЦИЯ

В современных условиях бактериальные лизаты находят широкое применение в качестве иммуномодулирующих средств, усиливающих эффективность антибактериальной терапии за счёт синергетического взаимодействия с антибиотиками. Целью исследования является изучение фармакологической субстанции бактериального лизата, полученного из антибиотикорезистентных штаммов условно-патогенных микроорганизмов *Acinetobacter baumanii*, *Klebsiella pneumoniae* SCAID PND1-2022 (246), *Pseudomonas aeruginosa* SCAID PHRX1-2019 для оценки его потенциала в борьбе с растущей проблемой антимикробной резистентности. В работе проанализированы свойства лизата и его воздействие на организм методом изучения *in vivo* при введении в дозировках 300,0 и 500,0 мг/кг. В результате наблюдается отсутствие признаков острой токсичности, что позволяет считать данную субстанцию безопасной для применения. Кроме того, разрушенные клетки бактерий сохраняют способность индуцировать специфический иммунный ответ, стимулируя активацию иммунной системы. Учитывая исходную устойчивость бактериальных штаммов к антибиотикам, можно сделать вывод, что применение бактериального лизата способствует формированию адаптивного иммунного ответа, направленного против высокорезистентных возбудителей, включая супербактерии.

Ключевые слова: антибиотикорезистентность, лизат, доклинические испытания, иммуномодулятор, условно-патогенные, острая токсичность.

ӘОК: 579.61

АНТИБИОТИКТЕРГЕ ТӨЗІМДІЛІКПЕН КҮРЕСУДЕ БАКТЕРИЯЛЫҚ ЛИЗАТТЫ ЗЕРТТЕУ

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ТҮЙІН

Қазіргі жағдайда бактериялық лизаттар антибиотиктермен синергетикалық өзара әрекеттесу арқылы антибиотикалық терапияның тиімділігін арттыратын иммуномодуляциялық агенттер ретінде кеңінен қолданылады. Зерттеудің мақсаты- *Acinetobacter baumanii, Klebsiella rpeimopiae SCAID PND1-2022 (246), Pseudomonas aeruginosa SCAID PHRX1-2019* шартты патогендік микроорганизмдердің антибиотикке төзімді штамдарынан алынған бактериялық лизаттың фармакологиялық субстанциясын зерттеу, оның өсіп келе жатқан проблемамен күресу әлеуетін бағалау микробқа қарсы төзімділік. Жұмыста 300,0 және 500,0 мг/кг дозада енгізген кезде лизаттың қасиеттері және оның ағзаға in vivo зерттеу әдісімен әсері талданады. нәтижесінде жедел уыттылық белгілерінің болмауы байқалады, бұл осы субстанцияны қолдануға қауіпсіз деп санауға мүмкіндік береді. Сонымен қатар, бактериялардың жойылған жасушалары иммундық жүйенің белсендірілуін ынталандыру арқылы белгілі бір иммундық жауапты индукциялау қабілетін сақтайды. Бактериялық штамдардың антибиотиктерге бастапқы төзімділігін ескере отырып, бактериялық лизатты қолдану жоғары төзімді патогендерге, соның ішінде супербактерияларға қарсы бағытталған адаптивті иммундық жауаптың пайда болуына ықпал етеді деген қорытынды жасауға болады.

Кілтті сөздер: антибиотикке төзімділік, лизат, клиникаға дейінгі зерттеулер, иммуномодулятор, шартты-патогенді, жедел уыттылық.