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RESEARCH ARTICLE
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The Effects of Interleukin-2 Substances (Glutamate, Glycine, Arginine) on Biofilm Formation in Multidrug-resistant Uropathogenic *Escherichia coli* in Women with Previous Reproductive Losses

Oksana Gizinger[1], Viktor Radzinskiy[2], Yuri Sorokin[3], Nadezhda Sachivkina[1,*], Gadzhi Letifov[4], Akim Gaziev[1], Natalia Kornova[5], Olga Burgasova[6], Marina Taranova[7], Alfia Ibragimova[8,9] and Anna Bobunova[9]

[1]Department of Microbiology named after V.S. Kiktenko, Institute of Medicine, Peoples' Friendship University of Russia named after Patrice Lumumba, 6 Miklukho-Maklaya str., 117198 Moscow, Russia
[2]Department of Obstetrics and Gynecology with a Perinatology Course Institute of Medicine, Peoples' Friendship University of Russia named after Patrice Lumumba, 117198 Moscow, Russia
[3]Center of Reproductive Health, "Medsi Group" JSC, 123056, Moscow, Russia
[4]Federal State Budgetary Educational Institution of Higher Education «Rostov State Medical University» of the Ministry of Healthcare of the Russian Federation, 29, Nakhichevansky Lane, Rostov-on-Don, 344022, Russia
[5]Federal State Budgetary Educational Institution of Higher Education «South Ural State Medical University» of the Ministry of Healthcare of the Russian Federation 64, Vorovskogo, Chelyabinsk, 454092, Russia
[6]Department of Infectious Diseases with a course in epidemiology, Institute of Medicine, Peoples' Friendship University of Russia named after Patrice Lumumba, 6 Miklukho-Maklaya str., 117198 Moscow, Russia
[7]The Department of Internal, Occupational Diseases and Rheumatology, Sechenov First Moscow State Medical University (Sechenov University), 8/2 Trubetskaya st., 119991 Moscow, Russia
[8]Department of Disaster Medicine, Institute of Medicine, Peoples' Friendship University of Russia named after Patrice Lumumba, 6 Miklukho-Maklaya str., 117198 Moscow, Russia
[9]Department of Foreign Languages, Institute of Medicine, Peoples' Friendship University of Russia named after Patrice Lumumba, 6 Miklukho-Maklaya str., 117198 Moscow, Russia
*Corresponding author: sachivkina@yandex.ru

ABSTRACT

Infectious diseases are very dangerous for the existence of humans and animals. Multidrug-resistant uropathogenic *Escherichia coli* (UPEC) can cause diseases of the vulva and vagina. The study of uropathogenic *E. coli* in women who have previously had reproductive losses is relevant. *In vitro* studies showed that fosfomycin 20mg/disk demonstrated sensitivity in 100 isolates (100%) of uropathogenic multidrug-resistant *E. coli*. Of the 100 *E. coli* isolates studied, 40, 37, 31, 29, 21, 19, and 11% were resistant to amikacin (10 µg/disk), ciprofloxacin(30mg/disc), ampicillin (25µg/disc), ceftazidime (20µg/disc), cefuroxime (20mg/disc), ceftriaxone (20µg/disc), ceftazidime and clavulanic acid (10µg/disc), respectively. Whereas 34% were intermediately resistant to gentamicin (10µg/disc). None of the 69 UPEC isolates (69%) tested in women with vaginitis were resistant to Fosfomycin (20µg/disc). The effect of IL-2 and its combination with the enzyme lysozyme and some low-molecular compounds: glutamate, glycine and arginine on clinical isolates of multidrug-resistant UPEC isolated from 100 patients aged 28 to 35 years with laboratory and clinically confirmed nonspecific vaginitis and with previous reproductive losses was studied. The use of recombinant IL-2, α-lysozyme, glutamate, L-glycine, L-arginine at an early stage of UPEC cultivation inhibits biofilms' formation. With the introduction of recombinant IL-2 and glutamine T=24 hours, the biofilm inhibition was 20-30%, 10-15% at T=48 hours and 5-7% at T= 72 hours. The study showed that the bacteriolytic activity of IL-2 against UPEC in women with vaginitis and reproductive losses increases when IL-2 is used in combination with arginine and lysozyme.

Keywords: Interleukin-2; Lysozyme; Glutamate; L-glycine; L-arginine; Uropathogenic *E. coli* (UPEC); Biofilms; Antibiotic resistance

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INTRODUCTION

Uropathogenic *Escherichia coli* (UPEC) is a significant factor in the pathogenesis of nonspecific vaginitis in women, worsens clinical manifestations, contributes to a decrease in reproductive potential, increases the duration of the disease, and participates in the pathogenesis of reproductive losses and complications of the gestational period (Radera et al., 2022). Besides diseases of the reproductive system, UPEC causes infections of the digestive tract, urinary and nervous systems, and sepsis (Choong and Whitfield, 2000). Most UPEC strains tend to form microbial communities attached to the surface of biofilm, which is a strategy for their survival and correlates with the multidrug resistance of *E. coli* isolates (Maharjan et al., 2018). UPEC can enhance the pathogenic potential of a previously formed microbial community on the surface of mucous membranes (Wiles et al., 2008; Saxena et al., 2019; Shah et al., 2019). One of the pathogenetic factors influencing biofilm formation in *E. coli* is resistance to antibacterial drugs, the state of factors of local antimicrobial protection, the presence of unique factors of adhesion, invasion, and colonization. Pathogenicity factors include pili. The main type of pili specific for uropathogenic *E. coli* is P-fimbriae (Saxena et al., 2019). P-fimbriae are represented by numerous antigenic variants that bind to the same glycosphingolipid receptor. Its specificity is determined by globobiose (galactose disaccharide: a-D-Gal-(1,4)-a-D-Gal), and its strong attachment to the surface of the cell membrane by ceramide (Shah et al., 2019). Influence on signaling systems, and destruction of lipopolysaccharides, globbiness and ceramides are mechanisms that researchers use as targets to suppress the growth of *E. coli* and the development of biofilm (Wiles et al., 2008). The need to influence the processes of biofilm formation with the participation of UPEC not only with the help of antibacterial drugs but also other chemically active compounds indicates the importance of studying UPEC and justifying new approaches to the treatment of nonspecific vaginitis in women of reproductive age (Behzadi et al., 2020). Recent studies have shown that UPEC biofilms are sensitive to N-acetylcysteine, N-(pyridin-4-yl) benzo[d]thiazol-6-carboxamide, nitrogen metabolites, and several low molecular weight compounds that inhibit biofilm formation. Several already-known compounds of natural or genetically engineered origin causes the death of biofilm containing pathogenic and opportunistic microorganisms (Borysowski and Górski, 2010). Previously, data on discovering new bacteriolytic factors that can influence *E. coli* under experimental conditions are presented. For example, the bacteriolytic activity of the complement system protein C3 and the substrate specificity of human recombinant IL-2 because of its ability to attach to the surface of microorganisms and reduce their pathogenic potential were discovered (Borysowski and Górski, 2010). When combined with certain enzymes and low molecular weight compounds, this activity may be enhanced. It was interesting to consider the current situation with strains of UPEC isolated from patients with nonspecific vaginitis and a history of reproductive losses. Data on the effectiveness of IL-2 in various enzymes and low-molecular compounds against isolated UPEC make it possible to study the bacteriolytic activity of UPEC isolated from patients with chronic vaginitis and with previous reproductive losses and to use combinations with IL-2 that enhance bacteriolytic and bactericidal activity against Escherichia coli (Park and Cheong, 2002; Yuzhalin and Kutikhin, 2015; Shimazaki and Takahashi, 2018).

It was previously shown that several enzymes and low molecular weight compounds, such as glycine, arginine, alanine, and valine, increase the bacteriological activity of IL-2 against planktonic forms of *E. coli*. The pH dependence of the bacteriolytic activity of IL-2 solution on the ionic strength of *E. coli* in vitro had also been described (Shimazaki and Takahashi, 2018). It was interesting to see how UPEC isolated from patients with vulvovaginitis who had a history of reproductive losses would behave. Our attention was drawn to IL-2 because of the presence of studies indicating its bactericidal activity in the experiment and the fact that IL-2 is one of the key cytokines of the human immune system that implements the mechanisms for maintaining colonization resistance of the reproductive tract. IL-2 is secreted by Th lymphocytes; it plays an important immunoregulatory role in Th0 differentiation and has an autocrine and paracrine effect on cells of the immune system, IL-2 is responsible for the development of protective immune responses in the body (Levashov et al., 2012; Levashov et al., 2017; Morshed et al., 2024).

The information obtained allows to use it in the treatment of a few diseases of a bacterial, viral, bacterial-viral nature (Yuzhalin and Kutikhin, 2015). The importance of IL-2 in the mechanisms of anti-infective defense is that IL-2, through the IL-2 receptors (rIL-2), binds to the receptors of B lymphocytes, stimulating their differentiation into plasma cells. Plasma cells initiate the primary and secondary immune response with the production of immunoglobulins IgA, IgG, and IgM. Immunoglobulins in the antigen-antibody complex activate the production of protein C3 of the complement system and increase the bactericidal functions of the C5b-C9 complex of the complement system (Borysowski and Górski, 2010). Moreover, IL-2 stimulates the cytotoxicity of NK cells and monocytes and enhances the production of hydroperoxides and nitrogen metabolites, which also normalizes the state of colonization resistance factors of the mucous membranes (Park and Cheong, 2002) (Fig. 1).

On the eve of this work, data on the discovery of the bacteriolytic activity of IL-2 were published (Levashov et al., 2017). It has been shown that IL-2 is active against individual *E. coli* cells, but its effect on clinical isolates of multidrug-resistant UPEC isolated from patients with chronic vaginitis has not been studied, nor has its effect on the processes of biofilm formation been studied.

In vitro, a significant increase in the rate of lysis of *E. coli* in the presence of IL-2 was recorded at a pH of the buffer mixture in the range of 7.8-8.8 (Levashov et al., 2012), so it was appropriate to study the effect of IL-2 on the processes of lysis and its adsorption on the surface of UPEC.

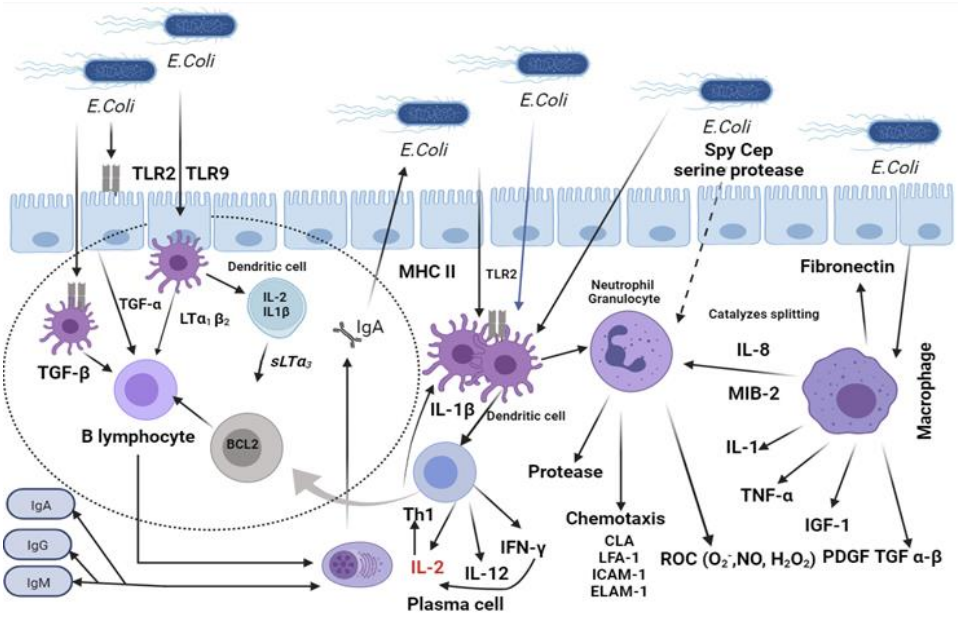


Fig. 1: IL-2 is secreted by Th-lymphocytes, it has an important immunoregulatory role in the differentiation of Th0, has autocrine and paracrine effects on cells of the immune system, accelerates their differentiation into plasma cells, followed by the production of immunoglobulins: IgA, IgG, Ig M, hydroperoxides and nitrogen metabolites.

One promising way to destroy biofilms may disruption of the structural integrity and disorganization, followed by the release of bacteria that will be available for subsequent antibacterial action. For this purpose, enzymes are used that can destroy the polysaccharides of the bacterial matrix and initiate the dispersion of biofilms. Such enzymes can be specific glycoside hydrolases that destroy polysaccharides in bacterial biofilms (Morshed et al., 2024). Glycoside hydrolases realize their action through the hydrolysis of glycosidic bonds: amylases break down α-1,4-; cellulases - β-1,4-; β-galactosidases – β-1,3-glycosidic linkages. Among glycoside hydrolases with antibiofilm activity, α-lysozyme stands out (Van Herreweghe et al., 2010). Lysozyme, an effective bactericidal agent, acts on both gram-positive and gram-negative bacteria, regardless of its muramidase activity. α-lysozyme destroys the 1,4-β-glycosidic bond of N-acetylglucosamine and N-acetylmuramic acid, which occurs via a non-hydrolytic mechanism (Fleming et al., 2017). In several in vitro experiments, lysozyme prevented bacterial adhesion of *E. coli* (Benarafa and Simon, 2017). In several other studies, on the contrary, it was shown that lysozyme (muramidase) has an enhancing effect on the process of biofilm formation. Each study requires additional analysis. Previous studies have shown the high lysis activity of lysozyme against *E. coli* (Levashov et al., 2017). Studying the interaction between the composition of IL-2 and lysozyme is a promising direction, and it is important to consider the adsorption of IL-2 on the surface of UPEC isolated by women with vaginitis.

Low-molecular-weight effectors - glutamate, glycine, arginine, glycine - that can influence the lysis of bacterial cells are under study. For example, glycine has proven antibacterial properties against *Lactobacillus plantarum, Staphylococcus aureus, Corynebacterium callunae, Helicobacter pylori* and several other pathogenic and opportunistic microorganisms (Hammes et al., 1973; Minami et al., 2004; Levashov et al., 2009; Plate and Marletta, 2012; Senyagin et al., 2023). The mechanism of the antibacterial action of glycine is based on blocking the formation of peptidoglycan and the inclusion of glycine in the peptide bridge which links the polysaccharide chains of murein, instead of L-alanine and D-alanine in positions 1, 4, and 5. This sequence of events leads to a decrease in the number of cross-links in peptidoglycans and morphological changes in cells, which, in turn, disrupt bacterial activity (Minami et al., 2004). Of interest is the role of arginine, which is converted into equivalent amounts of NO and L-citrulline by the enzyme NO synthase. NO, binding to the H-NOX (hemenitric oxide/oxygen binding) protein and changing the concentration of the secondary messenger – cyclic diguano-sine monophosphate (GMP), regulates the formation of biofilms in *Legionella pneumophila, Shewanella woodyi, Shewanella oneidensis, Pseudoalteromonas atlantica, E. coli* (Plate and Marletta, 2012).

MATERIALS & METHODS

Microorganisms

UPEC were obtained from one hundred women aged 28 to 35 years with laboratory-confirmed vaginitis of nonspecific etiology and with a history of reproductive losses. In the samples where *E. coli* growth was detected, only one isolate (one colony) was chosen from each woman. The studied biomaterial was inoculated on endo-nutrient media. The cultures were placed in the incubator for 24 hours at 37°C. On Endo medium, lactose-positive *E. coli* grew in the form of flat-convex colonies of a dark red color with a metallic sheen. After incubation, identification was conducted based on morphological, tinctorial (negative Gram stain) properties. At the next stage, bacterial DNA was isolated using standard methods. For PCR amplification, oligonucleotide primers of gene fragments film A, hly A, B, C, pap C were used, which were selected based on the nucleotide sequences of these genes in UPEC, presented in the GenBank database NCBI (USA), EMBL (European Molecular Biological Library). The search for genes was carried out using pro-BLAST grams

(http://blast.ncbi.nlm.nih.gov/Blast.cgi) in the international GenBank database. We used the VectorNTI 10 program (https://catalog.invitrogen.com) to align scanning nucleotide sequences and search for conserved regions. Oligonucleotide sequences were selected manually. To analyze the properties of the primers, we used the OligoAnalyzer 3.1 program (http://eu.idtdna.com/analyzer/Applications/OligoAnalyzer/default.aspx). Checking the complementarity of primers according to genes was performed using the program Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast).

Composition of the reaction mixture was as follows: 0.5mM Tris-Cl buffer, pH 8.6; 0.5M KCl; 15mM MgCl2; 2.5mM deoxynucleotide triphosphates; 0.2mM forward and reverse primers each; Taq DNA polymerase - 50µl. PCR was conducted in a Tertsik device (NPO DNA-Technology, Russia). Registration of PCR results was carried out by electrophoresis of amplification products on an agarose gel stained with ethidium bromide, for which agarose gels containing 2-3% agarose and ethidium bromide were used. Electropherogram analysis was performed using the Gel Imager video system and Gel Analyzer software.

Enzyme, IL-2, Low Molecular Weight Peptides used in the Study: Lysozyme, Arginine, Glycine, Sodium L-Glutamate

Lyophilized enzyme lysozyme was obtained from lyophilized chicken egg lyso-zyme (95% purity, Sigma Aldrich, USA). A lysozyme was prepared immediately before the experiment, dissolving in a buffer mixture for measuring activity. Protein concentration was determined using the micro biuret method using a modified Benedict reagent (Hammes et al., 1973). As a source of IL-2, a solution of recombinant purified interleukin-2 for intravenous and subcutaneous administration was used (Biotech, Russia). Peptides are represented by L-arginine (99% of purity Pharma Grade, Sigma Aldrich, USA); glycine (99% of purity, Fluka, Germany), L-monosodium glutamate (99% of purity in Hong Mei, China).

Antibiotics Resistance Tests

Clinical isolates of multi-resistant UPEC were obtained from 100 women aged 28 to 35 years with laboratory-confirmed vaginitis of nonspecific etiology and with a history of reproductive losses. All clinical isolates have been identified under the legal acts applicable in the Russian Federation. For microbiological analysis, the appropriate bacterial suspensions were first pre-pared, to which a bacteriolytic factor was added to the required concentration (for *E. coli* 15 and 0.1µg/mL IL-2 and lysozyme, respectively).

The antimicrobial activity of the complex containing IL-2, the enzyme lysozyme, and one of the low-molecular effectors: L-arginine, glycine, L-sodium glutamate on UPEC (n=100) was determined by the Kirby-Bauer disk diffusion method, according to resistance to two or more of the following antibiotics (DI-PLS-50-01 Research Center for Pharmacotherapy, Russia)

For the study, *E. coli* isolates were grown in M210 heart-brain broth (HIMEDIA® M210, India) for 24h at 37°C. After cultivation, the broth culture was centrifuged for 10min at 2.4 x 10^3rpm. The bacterial suspension for inoculation was prepared from the microbial sediment according to the 0.5 turbidity standard (McFarland, HIMEDIA), equal to 1.5–3.0 10^8 CFU/mL in 0.9% sodium chloride (NaCl). The resulting bacterial suspension with a volume of 100µL was seeded by the lawn culture method on Muller-Hinton agar (Biomerieux SA, Marcy-l'Étoile, France). After seeding with a sterile punch, wells were made in agar with a diameter of 5mm. 100µL of IL-2 solution was added to each well. A 0.9% NaCl solution was selected as a negative control, and a test disk with fosfomycin (20mg/disk) from the set of Indicator disks DI-PLS-50-01 with antimicrobial drugs for medical purposes produced by the Research Center for Pharmacotherapy, Russian Federation, was selected as a positive control.

Study of the Ability of E. coli to form a Biofilm

The formation of UPEC biofilms and the effect of the test substances (IL-2, lysozyme, IL-2, lysozyme with arginine, glycine, monosodium glutamate) on the suppression of biofilm formation processes were assessed using the microplate culture method. The dosages of the substances were selected based on their recommended therapeutic concentration in tissues: IL-2-0.1µg/ml, lysozyme -0.2µg/mL, L-arginine-0.5µg/mL, glycine-0.3µg/mL, L-monosodium glutamate 0.5µg/mL. According to the recommendations of the National Committee of Clinical Laboratory Standards (USA), loss of viability when treated with similar concentrations is considered a criterion for the "susceptibility" of biofilm microorganisms. The *E. coli* cultures studied were grown for 24h at 37°C in 5ml of Mueller-Hinton broth medium (Biomerieux SA, Marcy-l'Etoile, France). After 24 hours, UPEC test cultures were centrifuged at 2.4×10^3rpm. The resulting suspension was diluted with a solution of 0.9% NaCl at a ratio of 1/20 (starting culture). Liquid Mueller-Hinton medium was added to each well of a sterile polystyrene 96-well plate in a volume of 180µL. Preprepared samples of the test culture were added to a volume of 20µL in a dilution of 1:10. A solution of lysozyme, L-arginine, L-glycine, and monosodium glutamate was added to the appropriate wells with a final dilution of 1:10. Test samples consisting of IL-2, glycine and low molecular weight compounds glutamate, L-glycine and L-arginine were introduced into the wells of the plate at the beginning of the experiment, after 24h, after 48h and after 72h from the start of cultivation. The total culture time for UPEC and test substances was 96h at 37°C. The formation of biofilms was monitored using laser scanning confocal microscopy. After cultivation, the wells of the plate were washed three times with phosphate buffer. After washing and drying, 0.1% crystal violet solution was added to each well. After washing, 200µL of 96% ethanol was added to the wells of the plate to extract paint from the biofilm, and the optical density of the solution was measured at a wavelength of 500nm. Four additional holes were seeded with pure medium as a negative control. The intensity of coloring of the contents of the wells corresponded to the degree of film formation. The quantitative expression of the degree

of biofilm formation was the optical density values measured by the spectrophotometric method. Based on the data obtained, the average value for 5 wells was determined and the ability of the microorganism to form biofilm *in vitro* was assessed (Levashov et al., 2009; Lenchenko et al., 2023; Senyagin et al., 2023). All experiments were carried out in two series, each repeated three times.

Study of IL-2 Adsorption on UPEC Surface

1. UPEC bacterial cells were suspended in a buffer mixture of 10mM TRIS-MES-CH3COOH with various pH values to the initial optical density A650=1 ((8.2±1.1) $\times 10^8$ CFU/mL).
2. The resulting mixture of bacterial cells and buffer solution was incubated at 37°C, followed by mixing and subsequent centrifugation at 3500rpm for 4 min.
3. The concentration of free IL-2 was determined by enzyme immunoassay (ELISA) using the Interleukin-2-IFA-BEST (Russia) kit. The amount of bacterial-bound IL-2 was determined by the difference between the amount added and the amount determined by ELISA (free IL-2 concentration). At the same time, the amount of IL-2 bound to one cell was calculated based on the data on the number of CFU in 1mL of bacterial suspension with an initial optical density of A 650=1 (Kot, 2019). The use of this technique was recommended by Levashov et al. (2010), it was shown that the parameters of adsorption isotherms did not change at various times of incubation of *E. coli* with IL-2, so the authors of the method chose the incubation time of 5min (Levashov et al., 2010).

Statistical analysis

Statistical data processing was performed using Microsoft Office XP Excel and STATISTICA 10 computer programs. χ^2 or F-test was used to identify statistically significant differences. Differences between data groups were considered reliable at $P<0.05$.

RESULTS

Resistance distribution data for the *E. coli* cultures studied (n=100) are presented in Table 1. Of the 100 isolates studied, 40 (40%) were resistant to amikacin (10μg/disk); 31 *E. coli* isolates (31%) were resistant to ampicillin (25 μg/disc); 29 (29%) were resistant (20μg/disc) to ceftazidime 20 μg/disk, 11 (11%) were resistant to ceftazidime and clavulanic acid (10μg/disc), 34 (34%) were intermediate to gentamicin (10μg/disc), 21 (21%) isolate were resistant to cefuroxime (20mg/disk), 37 were (37%) resistant to ciprofloxacin (30mg/disk) and 19(19%) were resistant to ceftriaxone (20 μg/disk). None of the 69 UPEC isolates (69%) tested in women with vaginitis and history of reproductive loss were sensitive to Fosfomycin (20μg/disc). Thus, from the presented, tested antibacterial substances, an extremely low number of drugs sensitive to UPEC was identified.

Next, we analyzed the effect of various combinations of IL-2 with low-molecular compounds: lysozyme, arginine, sodium glutamate, and glycine on UPEC isolates to determine whether these combinations have their antibacterial activity. So, we analyzed the effect of various combinations of IL-2 on an isolate sensitive to fosfomycin. An analysis of the results of determining antibacterial activity against 69 UPEC isolates (Kirby-Bauer method) established that in the experiment when using a combination of IL-2 with the enzyme lysozyme and a low-modulus compound arginine, in the group "IL-2 + lysozyme + arginine" there was a zone of UPEC growth inhibition amounted to 12mm for 18 strains; 11mm in 14 strains, 10mm in 11 strains, 9mm in 7 strains, 8mm in 9 strains, 3 UPEC strains showed a growth retardation value of 16mm. The combination "IL-2 + lysozyme + monosodium glutamate" demonstrated a UPEC growth inhibition zone of 12mm in 12 strains, a growth inhibition zone of 11mm was formed in 13 strains, a growth inhibition zone of 10mm was formed in 7 strains, and a zone with a diameter of 8mm was formed in 9 strains. 8mm in 15 strains and two UPEC strains showed a growth retardation value of 16mm. Thus, the combination "IL-2 + lysozyme + monosodium glutamate" showed significantly less activity than "IL-2 + lysozyme + L-arginine" ($P<0.05$). The action of "IL-2+lysozyme+glycine" formed a zone of UPEC growth retardation, which in isolates from women with vaginitis and reproductive losses was significantly less than "IL-2+lysozyme+monosodium glutamate" and "IL-2+lysozyme+arginine": growth delay of 12mm for 4 strains, growth delay of 11mm for 5 strains, 10mm for 7 strains, 9mm for 11 strains and 8mm for 13 strains. The combination "IL-2 + lysozyme" in the experiment formed a zone of UPEC growth inhibition: 12mm for only 1 strain, 11mm for 4 strains, 10mm for 6 strains, 9mm for 12 strains and 8mm for 15 strains. Human recombinant IL-2, used in the experiment as a mono-component, formed a zone of

Table 1: Distribution of resistance in clinical isolates of UPEC to antibacterial drugs in women with non-specific vaginitis and previous reproductive losses.

Antibiotic	Abbreviation	Family	Disc load μg andmg/disk	Limit values of the diameter of growth inhibition zones (mm)S≥	Result	% resistant strains in the experiment
Ceftazidime	CAZ	Tazidime	20μg	19	R	29
Ceftriaxone	CRO	2 generation β-Lactam	20μg	17	R	19
Ceftazidime with clavulanic acid	CAZ with clavulanic acid	3-generation cephalosporin family	30/10μg/	20	R	11
Amikacin	AK	Aminoglycoside	10mg]	22	R	40
Fosfomycin	FOS	Macrolides	20mg	22	S	69
Ampicillin	AMP	Penicillins	25mg	29	R	31
Gentamicin	GN	Aminoglycosides	10mg	24	I	34
Ciprofloxacin	CIP	Fluoroquinolones	30mg	26	R	37
Cefuroxime	CXM	Cephalosporin	20mg	15	R	21

Note: R= resistant S= sensitive I= intermediate.

UPEC growth inhibition measuring 12mm in 3 strains, 8mm in 9 strains, 10mm in 8 strains, 9mm in 10 strains, and 8mm in 14 strains. The enzyme lysozyme formed a growth inhibition zone for UPEC: 12mm for 7 strains, 11mm for 3 strains, 10mm for 6 strains, 9mm for 12 strains, and 8mm for 13 strains. Our study shows that UPEC isolated from women with vaginitis and a history of reproductive problems demonstrated increased susceptibility to complexes of IL 2 and low molecular weight compounds lysozyme, arginine, sodium glutamate, and glycine ($P<0.05$).

Antibacterial activity against UPEC of the 6 objects of study is shown in Fig. 2-7. The ability of UPEC to form biofilm in the presence of IL-2, lysozyme, IL-2 and lysozyme, lysozyme with arginine, glycine, MSG) was assessed using a micro-plate culture method. Dosages of substances were selected based on their recommended therapeutic concentrations in tissues. IL-2 0.1, lysozyme - 0.2, L-arginine-0.5, glycine-0.3 and L-monosodium glutamate 0.5 µg/mL. After preparatory measures, the optical density of the solutions was measured at a wavelength of 500nm. Based on the data obtained, the average value for 5 wells was determined and the ability of the microorganism to form biofilm in vitro was assessed. The maximum inhibitory effect on biofilm was demonstrated when using the mixture "IL-2 + lysozyme + arginine" (Fig. 8).

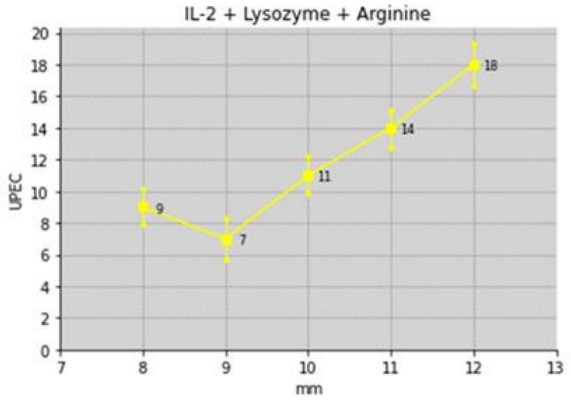


Fig. 2.

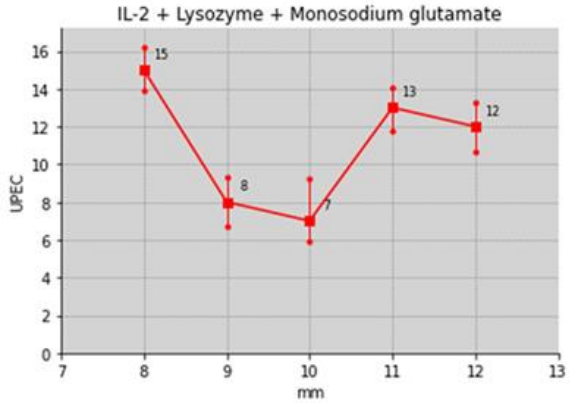
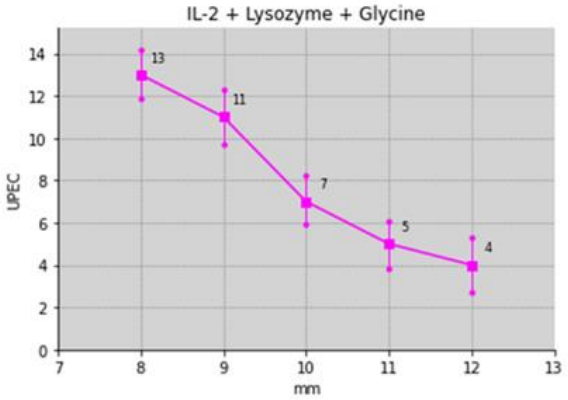


Fig. 3.



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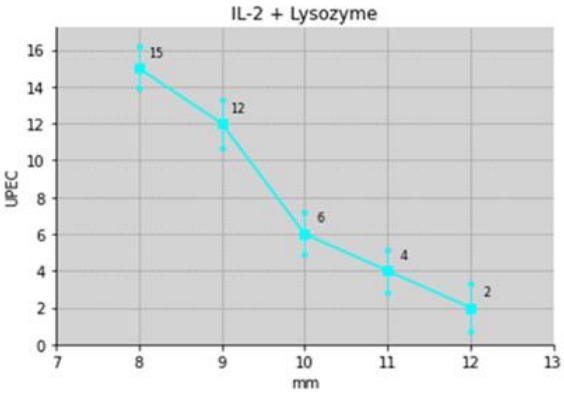

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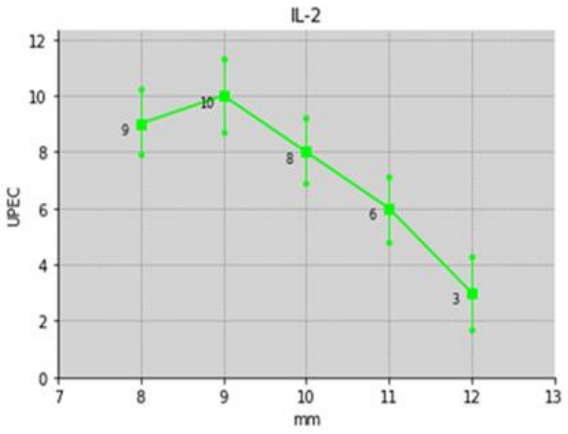

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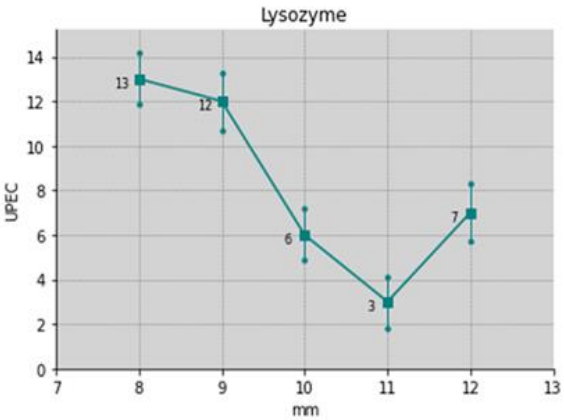

Fig. 7.

Fig. 2-7: Antibacterial activity against UPEC of the 5 objects of study is shown (IL-2 + lysozyme + L-arginine, IL-2, IL-2 + lysozyme, lysozyme, IL-2+lysozyme+glycine, IL-2+lysozyme+monosodium glutamate), ($P<0.05$).

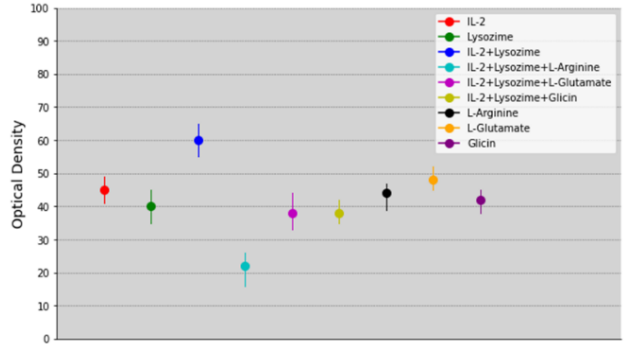

Fig. 8: The ability of UPEC to form biofilm in the presence of IL-2, lysozyme, IL-2 and lysozyme (lysozyme with arginine, glycine, MSG). Evaluation using the spectrophotometric method, wavelength 500nm.

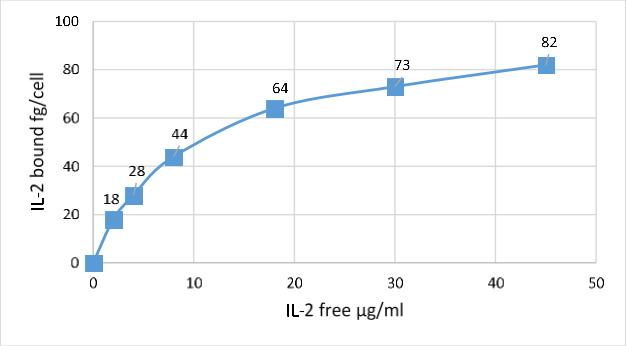

Fig. 9: Adsorption of IL-2 at the optimum of its pH activity on the surface of UPEC.

Inhibitory effect on biofilms of multi-resistant UPEC IL-2 and low-molecular compounds L - arginine, glycine, L-sodium glutamate ($P<0.05$), recorded using the spectrophotometric method. Thus, during the lysis of bacteria by interleukin 2 with the enzyme and low molecular weight compounds, part of the bacteriolytic factor can bind to the surface of *E. coli*. We conducted a study of the adsorption of IL-2 at the optimum of its pH activity on the surface of UPEC and presented the research results in the form of isotherms, Fig. 9. This fact may indicate that the productive binding of IL-2 to the cell of multi-resistant UPEC prevails in the pH optimum.

DISCUSSION

Essentially, we examined the influence of potential peptide effectors on the activity of bacteriolytic factors and a number of small molecular substances against E. coli. Firstly, the influence of those substances that can be found in human biological fluids and about the bactericidal effect of which there was previously unsystematized information was assessed. The enzyme lysozyme, the amino acids monosodium glutamate, L-glycine, and L-arginine were considered as potential effectors of the action of IL-2 (Marakhova et al., 2024). It has been established that human IL-2 exhibits bacteriolytic properties against bacteria containing diaminopimelic acid in the cell wall polymer. Such a microorganism is *E. coli*. There is information that *E. coli* can cause vaginitis of a nonspecific nature, and chronic endometritis, leading to reproductive losses. The second biologically active substance is the enzyme lysozyme. Lysozyme is present on mucous membranes and is one of the main humoral factors providing colonization resistance (Sedov et al., 2011; Levashov et al., 2019). In addition to lysozyme, the presence of which on mucous membranes has been proven, the amino acids L-arginine, monosodium glutamate, and L-glycine take part in the synthesis of metabolites that can potentially modify the activity of natural host resistance factors with varying degrees of activity. L-arginine, having its bactericidal activity, potentiates the effects of IL-2 and lysozyme in concentrations of more than 10mM against *E. coli*. L-arginine may inhibit protein aggregation. Such effects of arginine have been described previously and are called the "arginine paradox." The "L-arginine paradox" is that the addition of exogenous arginine, regardless of its intracellular concentration, leads to an increase in NO production (Vollmer and Born, 2010). This fact may explain the possible enhancement of the effect of arginine, lysozyme, and IL-2. Analysis of the bacteriolytic activity of IL-2, lysozyme, and low molecular weight amino acids against UPEC in vitro revealed the combination "IL-2 + lysozyme + L-arginine" with the highest bacteriolytic activity. It is known that nitric oxide has high permeability through cell membranes and subcellular structures due to the lack of charge and the small size of its molecule (Nathan and Shiloh, 2000; Tsikas et al., 2000; Asao, 2004). The NO molecules are active and can interact with other free radicals, low molecular weight compounds and proteins containing variable metal ions in the active center, and enter redox processes, forming numerous nitrogen-containing compounds (Radi, 2018; Stambulska and Bayliak, 2020). We demonstrated the toxic effect of the exogenous small molecular substance L-arginine in the mixture "IL-2 + lysozyme + L-arginine" in nanomolar concentrations, which negatively affected the formation of biofilm. It would be erroneous to explain the inhibitory effects of the mixture "IL-2 + lysozyme + L-arginine" on biofilm only by the presence of arginine, without considering the level of lysozyme and exogenous IL-2. Our study demonstrates the participation of several biologically active substances in the regulation of biofilm formation in *E. coli*, which established the toxic potential of nanomolar concentrations of the precursor of NO, the amino acid L-arginine, which stabilizes the enzyme-substrate complex and lysozyme and peptidoglycans of the cell wall of microorganisms. During the adsorption of the bacteriolytic factors we studied on the surface of UPEC, during the process of bacterial lysis, part of the bacteriolytic factor may be associated with the receptor apparatus on the cell surface (Minami et al., 2004; Levashov et al., 2012; Levashov et al., 2017).

The formation of *E. coli* resistant strains is the subject of attention of researchers and practitioners: gynecologists, urologists, surgeons, and specialists in other specialties (Baines et al., 2020; Fukuda et al., 2021). It is well known from modern and previous scientific studies in this area that the formation of resistance can be considered as a mechanism of various pathogens including bacteria that

increase their adaptive potential (Uddin et al., 2021; Abbas et al., 2023; Saeed and Alkheraije, 2023). The UPEC adaptation process with biofilm formation is a pathogenetic factor in the development of such pathological processes as vaginitis, urethritis, and cystitis, initiating complications after surgical interventions as surgical sepsis (Vestby et al., 2020). According to the World Health Organization, bacteria that are dangerous to humans and resistant to a wide range of antibiotics include all species belonging to the *Enterobacteriaceae spp.* (Hammes et al., 1973; Levashov et al., 2009; Senyagin et al., 2024). The search for ways to influence the processes of biofilm formation is a promising direction in the treatment of chronic inflammatory processes of the vulva and vagina, especially in women with a history of reproductive losses (Mohankumar et al., 2022).

The results of the study revealed the bacteriolytic activity of IL-2, lysozyme, monosodium glutamate, and arginine isolated from patients with vaginitis against UPEC. Lysozymes inhibit DNA and RNA synthesis and in contrast to the present view can damage the outer membrane of *E. coli* (Grabowski et al., 2021). Blockage of macromolecular synthesis, outer membrane damage, and inner membrane permeabilization bring about bacterial death (Michl et al., 2020; Grabowski et al., 2021).

Complex "IL-2+ lysozyme + L-monosodium glutamate" or "IL-2+ lysozyme+L-arginine" are bacteriolytic factors. The action of the above complexes on UPEC cells results in multiple cleavage of bonds in peptidoglycan, leading to the destruction of both single cells and their microbial associations. Previously, similar data on planktonic forms of *E. coli* was confirmed by studies of Sedov et al. (2011). The antibacterial activity of L-arginine can be explained by the interaction between the positively charged amino group and the negatively charged bacterial membrane UPEC, which contains lipopolysaccharide of the cell wall (Anguita et al., 2024; Mendes et al., 2024). This electrical interaction can lead to changes in the permeability of the *E. coli* wall, which can lead to an osmotic imbalance of the bacterial cell and ultimately inhibit the growth of the bacterium (Mendes et al., 2024).

Monosodium glutamate as part of the bacteriolytic complex, like arginine, increases the activity of IL-2, lysozyme at concentrations above 15mM (Levashov et al., 2010). The effect of glutamate, IL-2 and lysozyme may be associated with the formation of a complex between positively charged amino acids on the surface of proteins and glutamate, which reduces the unproductive sorption of factors on the surface of bacteria. An analysis of the literature data showed that the peptidoglycan of *E. coli* lysed by IL-2 contains a structural component, diaminopimelic acid (A2pm), which is the molecular target (Levashov et al., 2019). This mechanism underlies the inhibition of biofilm formation (80–90%) at an early stage of cultivation of a strain of UPEC. With the introduction of recombinant IL -2 and glutamine at the time of the experiment - 24 hours - 20-30%, at the time of 48 hours - 10-15%, at the time of 72 hours 5-7%.

Conclusion

The data obtained in this research makes it possible to expand knowledge about the pathogenetic mechanism of the influence of human enzymes and cytokines in combination with low-molecular compounds arginine and monosodium glutamate, as well as to evaluate the effectiveness of their action in real experimental conditions. The discovery of the effects of low-molecular amino acid effectors, arginine, and monosodium glutamate, which enhance the bacteriolytic effect of IL-2 against UPEC isolated from patients with nonspecific vaginitis, as part of a biofilm, allows the development of new highly effective drugs that can affect the internal bacteriolytic factors of the body, therefore, reduce the risk of biofilm formation in medical practice. Medical methods for dispersing biofilms using polysaccharide-degrading enzymes undoubtedly expands the arsenal of antibiofilm therapy for chronic and recurrent bacterial infections of the urogenital tract, especially those caused by antibiotic-resistant bacteria.

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