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DRUG DEVELOPMENT MODEL WITH ANTI-BACTERIAL AND IMMUNE PROTECTIVE EFFECT FOR THE TREATMENT OF PURULENT AND SEPTIC DISEASES

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The article studied the effect mechanisms of the antibiotics vancomycin and gentamicin combined which with purified cell biopolymer of *Staphylococcus aureus* (EAP), that like as it was shown in previous studies is an inducer of the production of protective cytokines by cells of the immune system. The obtained results shown the synergistic effect of the drug combination with adherence protein - an inducer of the production of protective cytokines. It was shown that the drug combination results in enhancement of antimicrobial activity, as well as an increase of phagocytic activity and cytotoxicity index. The research results may form the design basis of the combined drugs which have antibacterial and immunoprotective effects that can be used in treatment purulent and septic diseases.

Keywords: gentamicin, vancomycin, antibiotic sensitivity assay, *Staphylococcus aureus,* cytokins, cellular biopolymer (EAP), phagocytic activity.

РОЗРОБКА МОДЕЛІ ЛІКАРСЬКОГО ЗАСОБУ З АНТИБАКТЕРІАЛЬНИМИ ТА ІМУНОКОРЕГУЮЧИМИ ВЛАСТИВОСТЯМИ ДЛЯ ЛІКУВАННЯ ГНІЙНО-СЕПТИЧНИХ ЗАХВОРЮВАНЬ

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У статті вивчаються механізми поєднаної дії антибіотиків ванкоміцину або гентаміцину з очищеним біополімером природного походження (ЕАР) *Staphylococcus aureus*, що як показано в попередніх дослідженнях, є індуктором продукції захисних цитокінів клітинами імунної системи. Отримані результати свідчать про синергізм дії досліджуваних комбінованих препаратів з індуктором продукції захисних цитокінів - білком адгезином. Встановлено, що застосування комбінації препаратів посилювало

антибактеріальну дію, фагоцитарну активность та індекс цитотоксичності. Результати досліджень можуть ввійти в основу розробки комбінованих препаратів з антибактеріальною дією та імунокорегуючими впливами, що можуть бути застосовані для лікування гнійно-септичних захворювань.

Ключові слова: гентаміцин, ванкоміцин, антибіотикограма, *Staphylococcus aureus,* цитокіни*,* клітинний біополімер ЕАР, фагоцитарна активність.

Today it is relevant to create pharmaceuticals and technologies to struggle against purulent-inflammatory processes in conditions of immunodeficiency and immunosuppression of the organism. Increasing drug resistance among microbial pathogens has led to the exploration of novel methods to enhance the efficacy of existing drugs. Also, due to development of the industry and the need to provide the market with new pharmaceuticals, it is relevant to study the development of the production of the new drugs, including antibiotics-combined with other substances for the strengthening of antibacterial action and to ensure the stability of the body.

Increasing drug resistance among microbial pathogens has led to the exploration of novel methods to enhance the efficacy of existing drugs. [1, 2].

Staphylococcus aureus is a widespread and persistent human pathogen that causes a remarkable range of community-acquired and nosocomial diseases in humans and animals [7].

The evolution of *Staphylococcus aureus* during the modern antibiotic era has been delineated by distinct strain emergence events, many of which include acquisition of antibiotic resistance. The relative high burden of methicillin-resistant *S. aureus* (MRSA) in healthcare and community settings is a major concern worldwide [9].

S. aureus infections range from mild skin and soft-tissue infections to life-threatening endocarditis, chronic osteomyelitis, pneumonia, or bacteremia, which are associated with significant morbidity and mortality [11]. The advent and use of antibiotics such as penicillin and methicillin in the mid-20th century initially proved effective against *S. aureus*. However, *S. aureus* rapidly acquired resistance to these antibiotics and infections. For example, *S. aureus* isolates represent 29 percent of all

reported bacterial isolates in Europe [12]. Importantly, the glycopeptide antibiotic vancomycin has proven effective in treating severe MRSA infections [10]. However, *S. aureus* clinical isolates with reduced susceptibility to vancomycin, and less commonly, with complete resistance to vancomycin have emerged within the past 20 years [13].

The protein components of the staphylococcal cell wall play an extremely important role in regulating the resistance of the microorganism to antibiotics and providing protective mechanisms of influence on the body [6].

The extracellular adherence protein (Eap) of *Staphylococcus aureus* participates in a wide range of protein–protein interactions that facilitate the initiation and dissemination of *Staphylococcal* disease. Depending on the *S. aureus* strain, the mature Eap molecule is ∼50–70 kDa. The SERAM extracellular adherence protein (Eap) is nearly ubiquitously distributed among *S. aureus* strains and appears to function as a virulence determinant in animal models of chronic *S. aureus* infection. In addition to its well-established roles in promoting adhesion-based processes such as bacterial aggregation and invasion of eukaryotic cells, Eap has also been shown to interfere directly with complex, signaling-dependent events such as leukocyte recruitment and both wound healing and angiogenesis. Eap has also been shown to interfere directly with complex, signaling-dependent events such as leukocyte recruitment [14, 15] and both wound healing and angiogenesis. Current evidence suggests that this remarkable diversity in Eap functions is related to its unique ability to form protein–protein interactions with an array of nearly a dozen ligands, including a bacterial cell surface-retained phosphatase, host extracellular matrix molecules such as collagen, fibronectin, and laminin, and the pro-inflammatory mammalian surface adhesin ICAM-1. However, the structural adaptations and biochemical features of Eap that allow specific interactions with so many different ligands remain largely unexplored [8]. It is important to establish previously discovered signaling mechanisms of molecular interaction of EAP with cells of the immune system and launching the production of protective cytokines (G.A. Liubchenko, 2012).

Nowadays are widely used antibacterials with unique properties - vancomycin and gentamicin. Vancomycin is a tricyclic glycopeptide natural antibiotic obtained from *Amycolatopsis orientalis* the spectrum of action of which applies to gram positive bacteria: *Staphylococcus spp*., including *Staphylococcus aureus* and *Staphylococcus epidermidis*, incl. methicillin-resistant strains; *Streptococcus spp*., incl. *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Streptococcus agalactiae*; *Enterococcus faecalis*, *Listeria spp*., *Clostridium spp*., *Corynebacterium diphtheriae*, *Actinomyces spp*., *Bacillus spp*., *Lactobacillus spp*. The drug is resistant to gram negative bacteria, mycobacteria, fungi, viruses, protozoa [9, 10]. The bactericidal action is to inhibit bacterial wall synthesis by inhibiting the polymerization of glycopeptides and selectively inhibiting bacterial RNA synthesis. The drug is effective for antibacterial therapy in the surgical treatment of purulent processes caused by staphylococci. Vancomycin is successfully used for the treatment of patients with diphtheroid endocarditis, and in combination with aminoglycosides or rifampicin - for the treatment of early endocarditis caused by *S. epidermidis* or diphtheroids, after prosthetic heart valve. In combination with aminoglycoside antibiotics, vancomycin exhibits a synergistic effect against many *S. aureus* strains [10]. Also used in the treatment of sepsis, bone and joint infections, lower respiratory tract infections, skin and soft tissue infections.

Changes have been shown in vancomycin resistance, however it remains a drug of choice for treatment of severe MRSA infections. *S. aureus* strains exhibiting increased resistance to vancomycin, known as vancomycin intermediate-resistant *S. aureus* (VISA) (MIC=4-8 μg/mL), were discovered in the 1990s. The molecular basis of resistance in VISA is polygenic and involves stepwise mutations in genes encoding molecules predominantly involved in cell envelope biosynthesis. *S. aureus* isolates with complete resistance to vancomycin (MIC≥16 μg/mL) are termed vancomycin-resistant *S. aureus* (VRSA) – they were first reported in the U.S. in 2002. Resistance in VRSA is conferred by the *vanA* gene and operon, which is present on a plasmid. Although treatment of VRSA infections is challenging. By comparison, the burden of VISA is relatively high and the molecular mechanisms of resistance are less

well-defined. VISA are associated with persistent infections, vancomycin treatment failure [9].

Gentamicin is an aminoglycoside obtained from *Micromonospora purpurea* with broad bacteriocidal activity against many aerobic gram negative and some aerobic gram positive organisms: *Escherichia coli*, *Proteus spp.*, *Pseudomonas aeruginosa*, *Klebsiella spp., Enterobacter spp., Serratia spp.*, *Citrobacter spp.*, *Salmonella spp.*, *Shigella spp., Staphylococcus spp*. The following microorganisms are generally resistant to gentamicin: *Streptococcus pneumoniae*, most other types of streptococci, enterococci, *Neisseria meningitidis*, *Treponema pallidum* and anaerobic microorganisms such as *Bacteroides spp.* or *Clostridium pp.* Like other aminoglycosides, gentamicin is thought to act by binding to bacterial ribosomes (30S) and inhibiting protein synthesis [10]. Nevertheless, gentamicin is considered bactericidal as well as bacteriostatic. Gentamycin sulfate is used to treat infections caused by sensitive pathogens, such as lower respiratory tract infections, complicated urogenital infections, bone and joint infections, including osteomyelitis, skin and soft tissue infections, infected burn wounds, abdominal infections (peritonitis), central nervous system infections, including meningitis in combination with β-lactam antibiotics, septicemia, bacterial endocarditis, pelvic inflammatory disease and pneumonia.

Study aim: the combined effect of Vancomycin or Gentamicin antibiotic with the inducer of the production of protective cytokines by cells of the immune system was studied. Potential effects were carried out using a biopolymer of natural origin (EAP), highly purified by ion exchange chromatography and gel filtration and a biochemically characterized staphylococcal adhesive protein EAP (mM 70 kDa), as described by Liubchenko G.A. (Taras Shevchenko Kiev National University).

Materials and methods.

The studies were conducted on mice of the CBA line (weighing 18-22 g), which were kept under standard vivarium conditions. Experimental animals were divided into groups depending on the active ingredient used. Antibiotics to animals were injected

according to the instructions, and the EAP was administered intramuscularly at a dose of 1 mg/kg. The bactericidal activity of the antibioticogram was evaluated by diffusion method using standard disks. Microorganisms were grown on meat-peptone agar (MPA) and after 20-24 hours the diameter of the staphylococcal growth suppression zones was determined. Phagocytic activity (FA), cytotoxicity index (IC) of phagocytic cells was also used, using methicillin-resistant *Staphylococcus aureus* (MRSA) Cowan-1 as the object of phagocytosis, producing the main pathogenicity factor of staphylococcal protein A and *Staphylococcus aureus* strain 209 (Collection of microorganisms of the Department of Microbiology and Immunology, Taras Shevchenko Kyiv National University).

Results.

In the current study, we have determined whether combination therapy, which includes nanosized staphylococcal biopolymer with previously determined immunobiological activity and the used antibiotic, can be effective against methicillin-resistant *Staphylococcus aureus* (MRSA). Impact on the production of protective cytokines was also evaluated [5, 6].

Combination of Vancomycin or Gentamicin with biopolymer significantly increased the bactericidal effect of antibiotics in relation to the isolated staphylococcus. Indeed, it has been shown that the biofilm of the cell wall of *Staphylococcus* and gentamicine, are acting synergistically, with the effect being more pronounced when there is a lower concentration of gentamicine. The obtained results testify to the possibility of using combined antibacterial therapy with the use of staphylococci biopolymer for treatment.

By assessing bactericidal activity by antibiotic sensitivity assay performed using a diffusion method [6], has demonstrated a dose-dependent growth inhibition of both strains of staphylococci, with the diameter of bacterial growth zone inhibition between 10 to 25 mm. Establishing experimental conditions for the bacterial growth inhibition beyond this range requires further studies.

The dynamics of changes in phagocytic activity (FA) and the index of cytotoxicity (IC) of macrophages and neutrophils in animal groups after the introduction of combined medications was also studied. There was observed a dose-proportional increase in FA and IC in the groups of animals receiving an antibiotic in combination with protective cytokine inducer. Such a dosing effect is obviously due to the effect of investigated cytokines on membrane processes in lymphocytes and phagocytic cells, which leads to their activation, signaling of cytokine production, as shown in earlier studies. When combined introduction of antibiotic and staphylococcus biopolymer EAP intramuscularly, the results were also evaluated by FA and IC. The dose-dependent changes of the investigated parameters, indicating the potentiating effects of this biopolymer, the launch of mechanisms of immune-regulating influence, stimulation of FA and its regulation by immunocompetent cells, have been established, which leads to immunocorrectional effects. The results are summarized in Fig.1 and Fig.2.

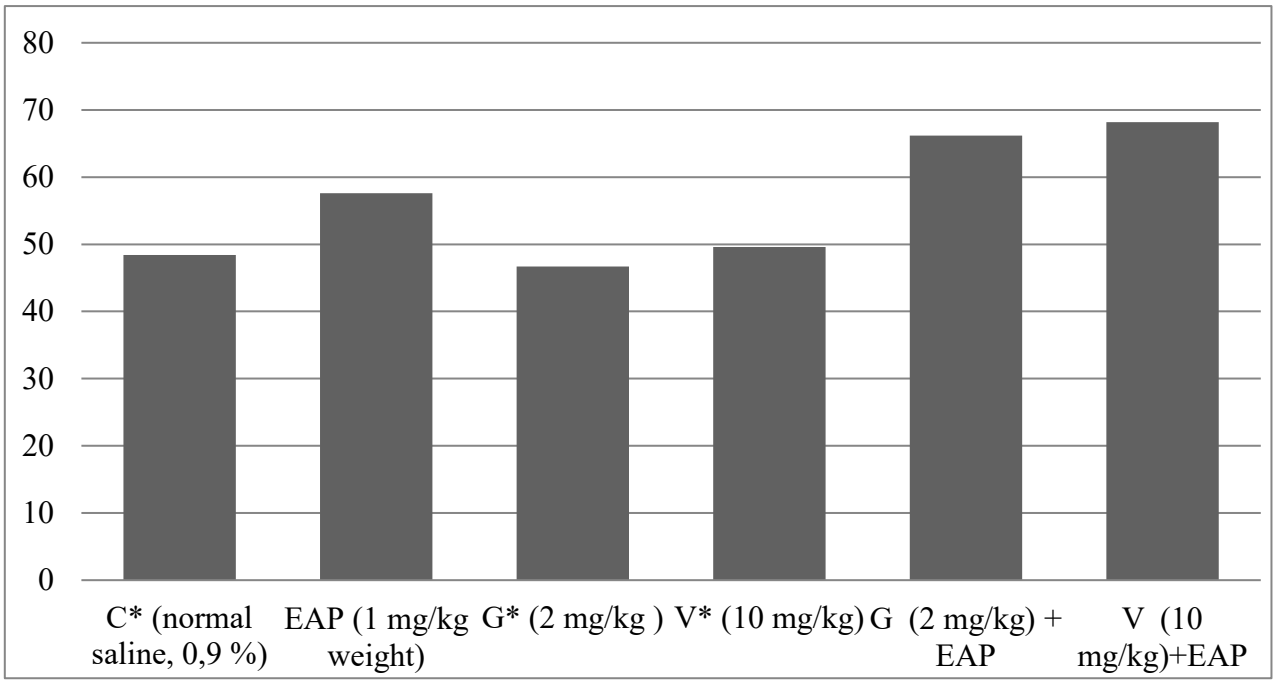

Figure 1. Percentage of phagocytosis of neutrophils in peripheral blood of experimental mice, %.

Note: C - control; G - Gentamicine; V - Vancomicine

**p < 0,05*

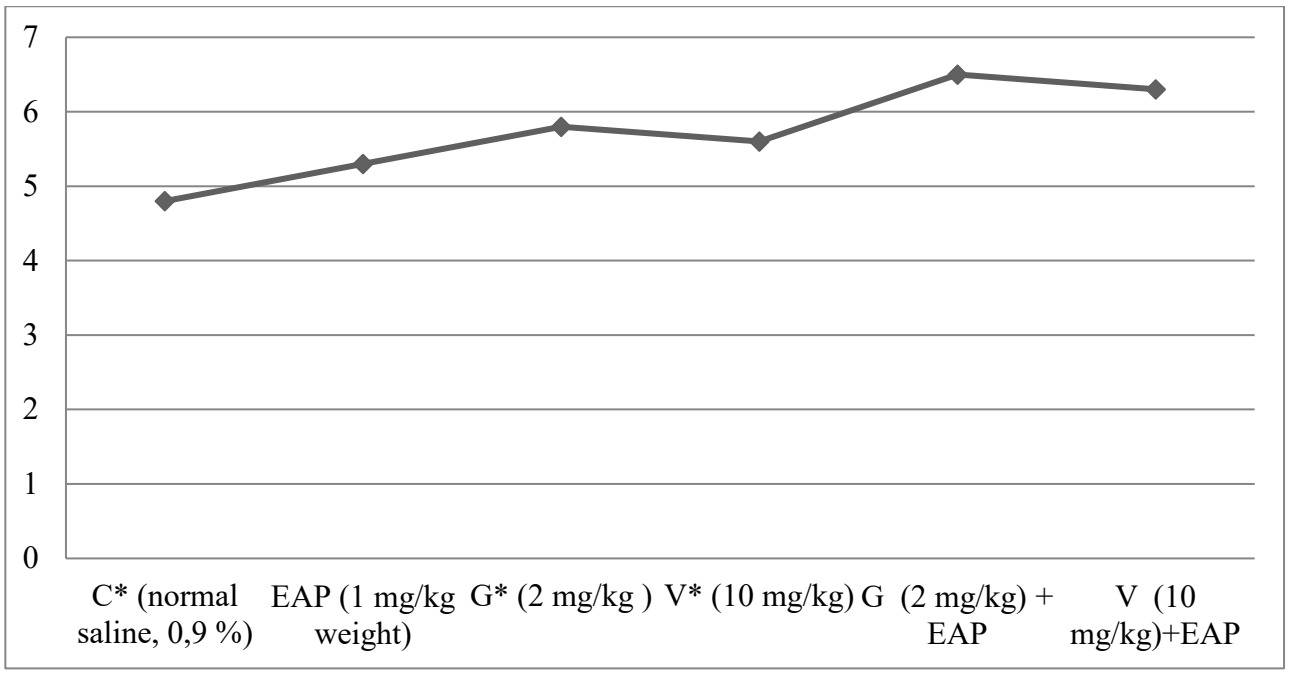

Figure 2. Phagocyte number of neutrophils in peripheral blood of experimental mice, c.u.

Note: C - control; G - Gentamicine; V - Vancomicine

**p < 0,05*

Conclusions.

1. The combination of vancomicine or gentanicine with biopolimer significantly increased the bactericidal activity of antibiotics against isolated *Staphylococcus aureus*.

2. The conducted studies have demonstrated the synergy of action in the combined antibiotics with the inducer of production of protective cytokines - adherence protein.

3. Dose-dependent changes in the potentiating effect of the investigated biopolymer have been established, as well as the initiation of mechanisms of immunocorrective influence, stimulation of FA, IC, and involved immunocompetent cells.

4. The obtained results may form the basis of the development of combined antibacterial drugs with adherence protein and immunocorrective effects that can be used to treat purulent and septic diseases.

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