UDC: 612.017.1.579.861.097.2

EXPERIMENTAL TECHNOLOGICAL MODEL FOR THE COMBINATION OF ANTIBACTERIAL DRUGS THAT PROVIDE ANTI-MICROBIAL EFFECTS FOR TREATMENT OF STAPHYLOCOCCAL INFECTIONS

Liubchenko G.A., Brovarets K.M., Yaschenko L.P.

Kyiv National University of Technologies and Design, Department of Industrial Pharmacy, Kyiv, Ukraine, e-mail: gannalb@yahoo.com

The article discusses the design of an experimental model system which combines natural (Benzylpenicillin) and semi-synthetic (Ampiox) antibiotics with calcium gluconate as an auxiliary substance and their potentiation by addition of cell biopolymer of *Staphylococcus aureus* (EAP). The combination of antibiotics, calcium gluconate and *staphylococcal biopolymer* had a synergistic effect. It was shown that the drug combination results in enhancement of antimicrobial activity, as well as an increase of phagocytic activity and cytotoxicity index in the model system. The obtained results may form the design basis of the combined drugs which have antibacterial and immunocorrective effects that can be used in treatment staphylococcal caused diseases.

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

ЕКСПЕРИМЕНТАЛЬНА МОДЕЛЬ ТЕХНОЛОГІЇ КОМБІНУВАННЯ АНТИБАКТЕРІАЛЬНИХ ПРЕПАРАТІВ, ЩО ЗАБЕЗПЕЧУЮТЬ АНТИМІКРОБНУ ДІЮ ДЛЯ ЛІКУВАННЯ ЗАХВОРЮВАНЬ СТАФІЛОКОКОВОЇ ЕТІОЛОГІЇ

Любченко Г.А., Броварець К.М., Ященко Л.П.

Київський національний університет технологій та дизайну, кафедра промислової фармації, м. Київ, Україна, e-mail: gannalb@yahoo.com

У статті розглядаються створена експериментальна модельна система комбінованої дії антибіотиків природного походження (бензилпеніциліну) та напівсинтетичного (ампіоксу) з додаванням в якості допоміжної речовини кальція глюконату потенціюючи дію додаванням біополімера (ЕАР) *Staphylococcus aureus*. Спостерігали синергізм дії в комбінованих препаратах антибіотиків, глюконату кальція та біополімеру стафілокока. Показано, що застосування комбінації препаратів призводило до посилення

антибактеріальної дії антибіотиків відносно *Staphylococcus aureus*, збільшення фагоцитарної активності та індексу цитотоксичності в модельній системі. Отримані результати можуть ввійти в основу розробки комбінованих препаратів з антибактеріальною дією та імунокорегуючими впливами, що можуть бути застосовані для лікування захворювань стафілококової етіології.

Ключові слова: антибіотики, антибіотикограма, *Staphylococcus aureus,* цитокіни*,* клітинний біополімер (ЕАР), фагоцитарна активність, стафілококова інфекція.

Increasing drug resistance among microbial pathogens has led to the exploration of novel methods to enhance the efficacy of existing drugs. Combination therapies that merge several existing approaches for antimicrobial defense, including nanoparticles, for combating the clinical infections associated with antibiotic resistance, have been of highest interest [7].

Industrial growth and urgency to supply market with pharmaceutical products justify the development of antibiotic preparations combined with other substances to potentiate their antibacterial action. One of the key discoveries of the twentieth century is the identification of antimicrobial properties of various molds, in particular from genus *Penicillium* [1]. The most widely used natural fungal preparation is benzylpenicillin. Benzylpenicillin has a broad-spectrum inhibitory effect against Streptococci groups A, B, C, G, H, L and M, *Streptococcus pneumoniae*, *Streptococcus viridans*, enterococci, penicillinase-producing strains of *Staphylococcus*, as well as *Neisseriae, Corynebacterium, Bacillus anthracis*, *Actinomycetes, Pasteurella multocida*, varieties of spirochetes, such as *Leptospira*, *Treponema, Borrelia*. At high concentrations, the drug is also active against other gram-negative microorganisms, such as *Escherichia coli, Proteus mirabilis, Salmonella, Shigella, Enterobacter aerogenes* and *Alcaligenes faecalis* [2]. For the infections caused by staphylococci, enterococci, *E. coli* or *E. aerogenes*, bacteriological studies, including antibiotic sensitivity tests, are recommended. Production of penicillinase by bacteria (i.e. staphylococci) causes resistance to the effects of this antibiotic [4]. Antibiotics are purified from the fungal cultures of *Penicillum chrysogenum* [1, 3]. It is highly effective against bacterial infections of various etiologies,

infections of the skin and soft tissues, burn infections, tonsillitis, urinary and biliary tract infections, meningitis, pneumococcal pneumonia [2, 4].

Ampiox is a complex antibacterial agent from the penicillin group, it includes semi-synthetic broad-spectrum antibiotics - ampicillin and oxacillin. The drug has a bactericidal effect. It interferes with the formation of peptide bond due to inhibition of transpeptidase, inhibits the final stages of the synthesis of peptidoglycan of the bacterial cell wall, this leads to the lysis of bacteria that are in the fission phase. Gram-positive (*Staphylococcus, Streptococcus, Pneumococcus*) and gram-negative (*Gonococcus, Meningococcus, E. coli, Haemophilus influenzae, Salmonella, Shigella*, etc.) microorganisms are susceptible to the drug [10]. Due to the presence of oxacillin in this preparation, the drug is active against penicillinase-forming staphylococci. *Pseudomonas aeruginosa*, most of the *P. vulgaris* strains, *P. rettgeri, P. morgànii*, viruses, protozoa, fungi are resistant to the effects of this drug. Ampiox has advantages over its individual components: a broad range of antimicrobial activity, a higher degree of bactericidal action, microbial flora does not develop or slowly develops resistance to the action of this antibiotic. The therapeutic concentration of this antibiotic is maintained for 4-6 hours. It is applicable for mixed infections that are no longer sensitive to benzylpenicillin caused by staphylococci or streptococci, gram negative bacteria; burn disease; for the treatment of various purulent-inflammatory processes [3].

The aim of the study: in this study we investigated the effects of natural benzylpenicillin or the semisynthetic antibiotic Ampiox in combination with calcium gluconate as an auxiliary substance, and natural biopolymer staphylococcal adhesive protein EAP (70 kDa). The later was purified from Staphylococcal cultures using ion exchange chromatography and gel filtration and biochemically characterized by us earlier [9].

Materials and methods.

CBA mice (18-23 g) were used in this study. Experimental animals were kept in the animal facility following standard rules and regulations. Animals were subdivided into several treatment groups in accordance with the experimental substances that were

used in this study. Antibiotics combined with calcium gluconate were injected into the animals following the instructions for the administration of these substances, and the EAP was administered intramuscularly at a dose of 1 mg/kg weight. The bactericidal activity of the antibiotics was evaluated by diffusion method using standard disks [9]. Microorganisms were grown on meat-peptone agar (MPA) in the presence of discs infused with antibiotics and after 20-24 hours the diameters of the staphylococcal growth suppression zones were determined. Phagocytic activity (FA) and cytotoxicity index (IC) of phagocytic cells were assessed. Methicillin-resistant *Staphylococcus aureus* (MRSA) Cowan-1 strain that produces protein A and *Staphylococcus aureus* strain 209 (collection of microorganisms of the Department of Microbiology and Immunology, Taras Shevchenko Kyiv National University) were used in this study for the evaluation of cellular phagocytic activity.

Results.

In the current study, we have determined whether combination therapy, which includes nanosized staphylococcal biopolymer and the widely used antibiotic Ampiox, can be effective against methicillin-resistant *Staphylococcus aureus* (MRSA). Staphylococcal biopolymer has been previously evaluated by our research group and its immunobiological activity and its effect on the production of protective cytokines were recently established [8]. Indeed, it has been shown that the biofilm of the cell wall of *Staphylococcus* and Ampiox act synergistically, with the effect being more pronounced when there is a lower concentration of ampicillin. The obtained results testify to the possibility of using combined antibacterial therapy with the use of staphylococci biopolymer for treatment.

Combination of Ampiox or Benzylpenicillin with calcium gluconate significantly increased the bactericidal effect of antibiotics against *Staphylococcus aureus*. Antibiotic sensitivity assay performed using a diffusion method [9] 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. A dose-dependent increase in FA and IC in the groups of animals receiving an antibiotic in combination with calcium gluconate at the highest concentration (100 mg/ml) was found. Such potentiation is likely due to the effect of Ca^{2+} ions on membrane processes in lymphocytes and phagocytic cells, which leads to their activation, cytokine production, as shown in earlier studies [8]. The results are summarized in the table.

Table 1. Phagocytic activity of neutrophils in peripheral blood of experimental mice.

Animal groups (per os, I/M)	Index	
	PF, %	FN c.u.
Control (normal saline, 0,9%)	52,4±6,0	4,8±0,1
EAP (1 mg/kg weight)	63,6±3,0*	5,3±0,2
Ampiox + Calcium gluconate (50 mg/kg)	49,7±3,0*	5,8±0,3*
Benzylpenicillin + Calcium gluconate (50 000 units/kg)	46,6±2,0*	5,6±0,4*
Ampiox + Calcium gluconate + EAP (50 mg/kg)	68,2±3,6*	6,5±0,4*
Benzylpenicillin + Calcium gluconate + EAP (50 000 units/kg)	68,2±3,6*	6,3±0,2*

Note: PF is the percentage of phagocytosis
FN-phagocyte number
**p < 0,05 as compared to control.*

FA and IC were also assessed if antibiotics and staphylococcus biopolymer EAP were introduced intramuscularly. The dose-dependent changes in the assessed parameters were found, indicating the potentiating effects of this biopolymer, due to its immune regulating effects, stimulation of FA and its activation of immunocompetent cells, leading to immune correction.

Conclusions.

1. The study has demonstrated a synergistic action between combinatory preparations of antibiotics, calcium gluconate and staphylococcal biopolymer EAP.

2. The combination of Ampiox or Benzylpenicillin with calcium gluconate significantly increased the bactericidal action of antibiotics against *Staphylococcus aureus*.

3. The obtained results may form the basis of the development of drugs that combine agents with antibacterial and immunomodulatory activities for treatment of staphylococcal infections.

References

1. Pryschep T.P., Chuchalin V.S., etc. Fundamentals of Pharmaceutical Biotechnology: Textbook. - Rostov n / D: Phoenix; Tomsk: Publishing house NTL, 2006. – 256 p.
2. Timoschenko L.V., Chubik M.V. Fundamentals of Microbiology and Biotechnology: Tutorial. - Tomsk: Publishing house of Tomsk Polytechnic University, 2009. – 194 p.
3. Mashkovsky M.D. Medicines. - M., 16th ed.,: New wave, 2012. – 1216 p.
4. Zverev V.V., Boychenko M.N., Medical microbiology, virology and immunology in 2 volumes: Textbook. GEOTAR Media,. 2010. – 480 p.
5. Betrosian A.P., Douzinas E.E. Ampicillin-sulbactam: an update on the use of parenteral and oral forms in bacterial infections. *Expert Opin Drug Metab Toxicol*. 2009 Sep;5(9):1099-112.
6. Surwade P, Ghildyal C, Weikel C, Luxton T, Peloquin D, Fan X, Shah V. Augmented antibacterial activity of ampicillin with silver nanoparticles against

methicillin-resistant Staphylococcus aureus (MRSA). *J Antibiot (Tokyo).* 2018 Oct 25. doi: 10.1038/s41429-018-0111-6.

7. Chudobova D, et al. Effect of ampicillin, streptomycin, penicillin and tetracycline on metal resistant and non-resistant *Staphylococcus aureus*. *Int J Environ Res Public Health*. 2014;11:3.
8. Liubchenko GA, Moriev RM, Kholodna LS. Modern fluorescent techniques to investigate the mechanisms of lymphocyte activation. *Ukr Biochem J.* 2015,87(1):33-45.
9. Liubchenko H*A, Gritsenko O.V., Oleshko H.M., Kholodna L.S. Effect of p70 Staphylococcal protein adhesions on the absorption capacity of circulating neutrophils. *Bulletin of the Taras Shevchenko Kiev National University*-2008.- 52-53. -p.70-71.
10. Betrosian A.P., Douzinas E.E. Ampicillin-sulbactam: an update on the use of parenteral and oral forms in bacterial infections. *Expert Opin Drug Metab Toxicol*. 2009 Sep;5(9):1099-112.

Стаття надійшла до редакції в листопаді 2018 року.