Mishina Marina M., Burov Andrey N., Vekshin Vitaliy A., Grabovetskaya Evgeniya R. Antibacterial effect of nisin in vitro. Journal of Education, Health and Sport. 2020;10(12):332-338. eISSN 2391-8306. DOI http://dx.doi.org/10.12775/JEHS.2020.10.12.032
https://apcz.umk.pl/czasopisma/index.php/JEHS/article/view/JEHS.2020.10.12.032
https://zenodo.org/record/4651465

The journal has had 5 points in Ministry of Science and Higher Education parametric evaluation. § 8. 2) and § 12. 1. 2) 22.02.2019.

Received: 09.11.2020. Revised: 25.11.2020. Accepted: 29.12.2020.

ANTIBACTERIAL EFFECT OF NISIN IN VITRO

Marina M. Mishina[1], Andrey N. Burov[1], Vitaliy A. Vekshin[1]✉,
Evgeniya R. Grabovetskaya[2]

[1]Kharkiv National Medical University, Kharkiv, Ukraine,
[2]V.N. Karazin Kharkiv National University, Kharkiv, Ukraine

Abstract

Results of experimental studies of antimicrobial action of gel compositions containing active substance nisin are presented in the article. It is shown that increasing concentration of nisin at constant concentrations of the other components in the mixture inhibits growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa.* The optimal concentration of nisin at which the process of pathogens growth inhibition is the most effective was determined in the experiment.

Key words: nisin; carbopol; bacteriocins; antibiotics

Introduction

Nowadays, infections causing development of purulent-inflammatory diseases of open wounds are of great importance in surgical practice. Despite the sanitary and hygienic measures taken, the possibility of contamination of hospital premises remains quite high. The areas where patients with damaged skin are located, i.e. operating rooms, intensive care wards, burn departments, etc. are at risk. In 1–33% of cases, such patients are at risk of contamination with *Staphylococcus aureus*, however, infection with *Staphylococcus epidermidis, Staphylococcus saprophiticus*, and some *Klebsiella species*, etc. is not excluded. Contamination of open skin areas with the bacteria leads to the development of concomitant

severe purulent-inflammatory diseases which do not respond well to standard methods of treatment [1]. The majority of patients with purulent-inflammatory diseases of soft tissues are of working age [2].

The main problem in the treatment of diseases caused by staphylococcal infections is their high antibiotic resistance. The misuse of antibiotics combined with self-medication is believed to have led to the development of bacteria resistant to the most common drugs. Methicillin-resistant *Staphylococcus aureus, Klebsiella* and *E. coli* have arisen due to natural adaptability and uncontrolled use of antibiotics. In recent years more attention is paid to this problem [3].

Scientists are trying to create new types of antibiotics to control bacterial diseases. However, only 0.01% of all synthesized molecules have antimicrobial activity and the new compounds in many cases are highly toxic for humans and have a high cost [4].

Investigation [5] of etiology of the inflammatory process in differently located purulent wounds and antibiotic resistance of pathogens shown the gram-positive micro-flora represented mainly by staphylococci to be predominant in all samples. Yeast-like fungi have also been identified. The isolated *S. aureus* strains were found to be polyresistant to cephalexin, ceftazidime, and ceftriaxone; 90% of the strains were resistant to penicillin, 97.5% to ofloxacin, 95% to amikacin and cefotaxime, and 92.5% to ampicillin. At the same time, the isolates were 50% sensitive to gentamicin, ciprofloxacin, streptomycin, and 100% to vancomycin.

Antibiotics used along with systemic antibacterial therapy can cure a wound infection, however, the use of certain type of antibiotic can also be accompanied by increased antibiotic resistance of pathogens. For example, erythromycin, tetracycline, doxycycline, and streptomycin have a very narrow scope and are not recommended for the purulent wounds routine treatment [6, 7].

Thus, despite the large variety of antibiotics, the choice for local use is rather limited and effective treatment of purulent wounds is still an unresolved problem. Practical surgery specialists are tend to use antiseptics due to arising of resistance in pathogens and decrease in the effectiveness of traditional medicines, however, the aggressive influence of antiseptics on open wounds and pronounced cytotoxic effect makes specialists increasingly return to antibiotics.

Objective

Application of bacteriocins is one of the promising alternative methods of controlling pathogenic bacteria. Bacteriocins have a number of advantages allowing them to be used as an

alternative to antibiotics, namely they [8]:

- have antimicrobial activity (*in vitro* and *in vivo*);
- have low toxicity;
- have wide and narrow spectrum of different peptides action;
- can be used for probiotics production *in situ* and creating of bioengineering structures.

Probably, in the near future, new methods of controlling infectious bacterial diseases will appear as a result of successful study of the bacteriocins effects, which will become a worthy alternative to antibiotics.

There are publications devoted to the possibility of bacteriocins using in the treatment of cancer [9]. This could make them almost a modern time panacea if successfully introduced into medical practice.

Bacteriocins are a large family of peptides secreted by bacteria. They possess antimicrobial activity and act on other strains of the same species or closely related species [10]. Bacteriocins are synthesized by almost all known bacteria, both gram-positive and gram-negative ones. They both suppress closely related species and thereby stop their growth.

Bacteriocins are a complex of peptides with a varying molecular mass. They significantly differ from each other in their physicochemical characteristics and biological effects [11]. It was revealed that antagonistic activity of bacteriocins is influenced by temperature, electric field, pH, composition, consistency of the medium, the presence of Ca^{2+} and Mg^{2+} ions and other factors [12].

The general properties of bacteriocins include their sensitivity to temperature, although this property can also vary widely. Some of bacteriocins are destroyed at a temperature of 48-50 °C, others can withstand temperatures of 60-70 °C for a short time, and some remain active even at 100 °C. Bacteriocins are sensitive to proteases. Bacteriocin protein is associated with lipopolysaccharide of the cell wall, but only protein part of the molecule possesses antibacterial activity [13].

Bacteriocins are classified into three classes according to physicochemical properties, amino acid composition, methods of elimination, and antimicrobial spectrum of activity [14].

Nisin is the most famous representative of bacteriocins which are widely used as antimicrobial drugs.

Nisin is a natural, non-toxic peptide with an antibacterial effect, obtained using a special strain of food-grade lactic acid bacteria *Lactococcus lactis subsp. lactis* during fermentation. This substance was discovered over 50 years ago, long before the most other

bacteriocins. Nisin is considered to be safe for use in the food industry in more than 50 countries.

Nisin shows antibacterial action in respect with a wide range of gram-positive bacteria, including spore-forming bacteria such as *Bacillus cereus*, as well as *Listeria Monocytogenes*. The mechanism of antimicrobial action is well understood.

The main mechanism of nisin action is the interaction with negatively charged membranes (bilipid layer), preferably with those containing anionic lipids. Gram-positive bacteria have higher concentrations of anionic lipid in cytoplasmic membrane compared to gram-negative ones. Bacteriocin first binds C-terminal end with anionic lipids, then the N-terminal end dips into membrane lipid and the peptide takes a parallel position on the membrane surface. Ultimately, the whole peptide can move along the entire length of the pore [15]. Nisin inhibits the synthesis of cell wall murein. As a result of the antibiotic action, an intermediate lipid accumulates. Nisin forms a strong complex with the lipid, which interrupts further synthesis of bacterial wall peptidoglycan. Nisin can lyse cells by irreversible destruction of the cell membrane which regulates osmotic pressure in the cell. It changes the surface tension of the cytoplasmic membrane which leads to a violation of cell permeability and, as a consequence, to bacterial lysis. Thus, the antibacterial action of nisin is mainly bacteriostatic; it does not kill bacterial spores, but prevents the swelling of the spores after germination and inhibits their growth.

Nisin is rather stable: it retains its properties in a wide pH range (from 3.5 to 8.0) and withstands boiling at 120° C keeping activity. Due to the high stability nisin reliably retains its effectiveness in various processing methods.

Currently nisin is actively used in the food industry but its medical application does not go beyond the scope of experimental research.

Based on the above, the purpose of this work was to study the possibility of using the bacteriocin concerned as an antimicrobial agent for the treatment of purulent lesions of the skin both after household trauma and after surgery.

Materials and methods

Experimental mixture antimicrobial activity against the pathogenic strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa* was studied. Experimental mixture contained: 5 ml of 10% NH_4OH; 0.2 g of carbopol; $0,05 \div 0,3$ g of nisin; up to 10 ml of distilled H_2O. Pure daily cultures of microorganisms grown on a solid nutrient medium were used. Antimicrobial effect *in vitro* was studied by the agar-gel diffusion method. An automatic turbidimeter Densi-La-Meter was used for microbial suspensions preparation. The

microorganisms were grown in Petri dishes on Mueller-Hinton agar [16]. The results were analyzed according to the zone of growth inhibition measured with an accuracy of 1 mm, including the diameter of the wells.

Results and discussion

The antimicrobial effect of the studied compounds was recorded by measuring the population growth inhibition around the well in mm. The results are shown in Fig. 1.

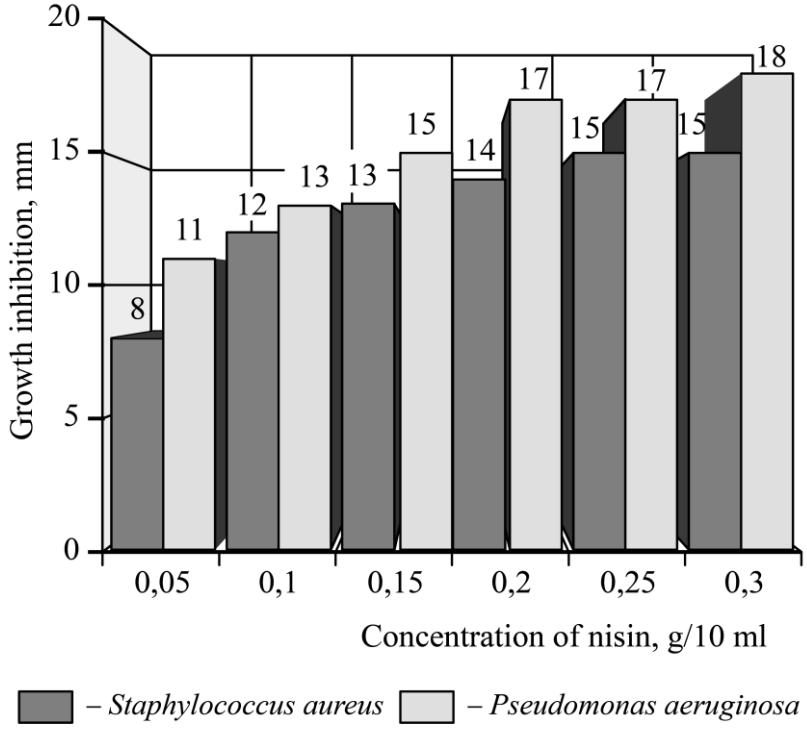

Fig. 1. Growth inhibition of *Staphylococcus aureus* and *Pseudomonas aeruginosa* versus nisin concentration

The results show that index of microorganisms' growth inhibition increases with an increase in the concentration of nisin. Exponential type of the dependences indicates the presence of a certain optimal value after which the increase in growth inhibition drops significantly. In this case, inhibition of both *Staphylococcus aureus* and *Pseudomonas aeruginosa* reproduction increases markedly under the action of $0{,}2 \div 0{,}25$ g of nisin. The following increase in nisin concentration does not influence the growth inhibition. This allows us to conclude that $0{,}2 \div 0{,}25$ g of nisin is sufficient for effective use as an antimicrobial agent against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. A further increase in its concentration, in this case, is not advisable.

References

1. Dowset, C. The management of surgical wounds in a community setting / C. Dowset // *Br. J. Communitu Nurs*. – 2002. – # 7. – P. 33–38.

2 . R.S. Sufriyarov, Z.G. Gabidullin, R.R. Sufriyarov, A.A. Akhtarieva, V.G. Tujgunova. Lechenie assocziirovanny`kh gnojny`kh ran czitrobakterno-stafilokokkovoj prirody` // *Vestnik YuUrGU*, #26, 2011, S.108–111. *(in Russian)*.

3. Kai Li, Lici A. Schurig-Briccio, Xinxin Feng, Ashutosh Upadhyay, Venugopal Pujari, et. al.. Multitarget Drug Discovery for Tuberculosis and Other lnfectious Diseases. // *J. Med. Chem*. – 2014.– #57. – P. 3126–3139.

4. Ville Ojala, Jarkko Laitalainen, Matti Jalasvuori. Fight evolution with evolution: plasmid-dependend phages with a wide host range prevent the spread of antibiotic resistance. // *Evol Appl*.– 2013.– #6.– P. 925-932.

5. A.V. Frolova, A.N. Kosinecz, V.K. Okulich. Ranevaya infekcziya. Sostoyanie problemy`. // *Vestnik VGMU. Mikrobiologiya*.– 2014.– T.13, #2. S.62–69. *(in Russian)*.

6. Golub A.V., Privol`nev V.V. Mestnaya antibakterial`naya terapiya khirurgicheskikh infekczij kozhi i myagkikh tkanej v ambulatorny`kh usloviyakh: slagaemy`e uspekha // *Rany` i ranevy`e infekczii*. – 2014. – # 1. – S. 33-39. *(in Russian)*.

7. Privol`nev V.V., Paskhalova Yu.S., Rodin A.V. Mestnoe lechenie ran i ranevoj infekczii po rezul`tatam anonimnogo anketirovaniya khirurgov Rossii // *Klinicheskaya mikrobiologiya i antimikrobnaya khimioterapiya*. – 2016. – T. 18, # 2. – S. 152-158. *(in Russian)*.

8. Paul D. Cotter, R. Paul Ross, Colin Hill. Bacteriocins – a viable alternative to antibiotics?. // *Nat Rev Micro*. – 2012.– #11.– P.95–105.

9. Sumanpreet Kaur, Sukhraj Kaur. Bacteriocins as Potential Anticancer Agents. // *Front. Pharmacol*.– 2015. – #6.– P.1–11.

10. Luc De Vuyst, Frederic Leroy. (). Bacteriocins from Lactic Acid Bacteria: Production, Purification, and Food Applications. // *J Mol Microblol Biotechnol*.– 2007.– #13.– P.194–199.

11. Minah C.J., Morero R.D. Inhibition of enterocin CRL35 antibiotic activity by mono- and divalent ions // *Lett. Appl. Microbiol*. – 2003. – Vol. 37, # 5. – P. 374-379.

12. De Vuyst L., Leroy F Bacteriocins from Lactic Acid Bacteria: Production, Purification, and Food Applications // *Mol. Microblol. Biotechnol*. – 2007. – Vol. 13. – P. 194–199.

13. Oscariz J.C., Pissabarro A.G. Classification and mode of action of membrane-

active bacteriocins produced by grampositive bacteria // *Int. Microblol*. – 2011. – #18. – P. 13–19.

14. Bittencourt E., Suzart S. Occurreonce of virulence-associated genes in clinical Enterococcus fae-calis strains isolated in Londrina, Brazil // *J. Med. Microblol*. – 2004. – Vol. 53. – P. 1069–1073.

15. Surugau L.N. Peptide separation by capillary electrophoresis with ultraviolet detection: some simple approaches to enhance detection sensitivity and resolution // *The Malaysian Journal of Analytical Sciences*. – 2011. – Vol. 15 (2). – P. 273-287.

16. Volyans`ky`j Yu.L. Metody`chni rekomendaciyi po vy`vchennyu specy`fichnoyi akty`vnosti proty`mikrobny`x likars`ky`x zasobiv / Yu.L. Volyans`ky`j, I.S. Gry`shhenko, V.N. Shy`robokov ta in.– K.: 2004.– S.21–22. *(in Ukrainian)*.