UDC 579.23, 615.281.9, 547.435

MORPHOLOGICAL ALTERATIONS OF *STAPHYLOCOCCUS AUREUS* CAUSED BY ARYL ALIPHATIC AMINOALCOHOL DERIVATIVE

Dronova M.L.

State Institution «Institute of pharmacology and toxicology of National academy of medical sciences of Ukraine »

Introduction

The emergence and spread of resistant strains of microorganisms, as well as reduction of the efficacy of current antimicrobial agents requires the development of novel antimicrobial compounds. However, in recent years, the marked decrease of attention of pharmaceutical companies to the development of new drugs, particularly among new chemical classes of antimicrobials was noted. Over the last decade only two new drugs – daptomycin and tigecycline were introduced into medical practice. But soon after their release to the market, resistance to these agents was reported [1, 2].

Development of new antimicrobial drugs involves significant amount of preclinical studies, including the determination of specific characteristics of the antimicrobial effect, e.g. bacteriostatic or bactericidal action and spectrum of activity. The mechanisms of action must also be determined, while this knowledge is required for the efficient drug usage.

One of the convenient and informative methods for studying the influence of antimicrobial agents on the bacterial morphology is electron microscopy. It provides an opportunity to assess some aspects of the compound's mechanism of action, namely their effect on the structure of the cell wall, membrane integrity, cell division, etc.

It is known that antibiotics, acting on the cell wall (penicillins, cephalosporins, glycopeptides), cause cell envelope alteration, affect the cell shape and size. Thus, gram-negative rods during exposure to subinhibitory concentrations of penicillins become elongated and form filaments [3, 4, 5, 6], whereas increase of antibiotic concentration resulted in spheroplast formation [4, 7, 8, 9, 10]. The impact of these drugs on gram-positive cocci is characterized by abnormal septation [7, 11] and formation of the mesosome-like structures [11, 12].

Treatment with membrane-acting antibiotics (gramicidin, polymyxin B, etc.) leads to blistering of the membrane and leakage of the cell content, cells rupture, formation of membrane structures within the cell and chains of incompletely divided cells [8, 13, 14, 15, 16].

Lincosamides, aminoglycosides, chloramphenicol and other drugs, are well-known as protein synthesis inhibitors, and cause alteration of the microbial cell wall and cell division [3, 7, 8, 12, 17].

One of the promising chemical classes for the development of new antimicrobial drugs are aryl aliphatic aminoalcohols. These compounds exhibit a wide range of pharmacological activities such as adrenoblocking [18, 19, 20], antiviral [21], antioxidant, membrane stabilizing [22], immunomodulatory [23, 24], and neuroprotective [25]. In addition, aryl aliphatic aminoalcohols show antimicrobial action against bacteria and fungi [26, 27]. According to the available data, some derivatives of aryl aliphatic aminoalcohols cause alterations of the protein synthesis [28], inhibit the activity of the fungal enzyme 1,3-β-D-glucansynthase [27]. New compounds of this class were synthesized at the Institute of organic chemistry NAS of Ukraine (Kiev, Ukraine), by Y. Korotkiy. The screening studies of the antimicrobial properties of the new aryl aliphatic aminoalcohol derivatives were carried out in our lab. The data obtained allowed us to select the compound KVM-194 as the potent antistaphylococcal agent [29]. The aim of the present study was to evaluate ultrastructural changes in the *S. aureus* cells under the influence of the compound KVM-194.

Materials and methods

Strain *S. aureus* ATCC 25923 was used in all experiments. The minimum inhibitory concentration (MIC) was determined by serial macrodilution method in Mueller-Hinton broth [29]. Inoculum density was 10^6 CFU/ml culture medium, 24 h culture was used for its preparation.

Bacteria for transmission electron microscopy samples were grown to exponential phase (6 h culture) and then were exposed to the 0,5 MIC (0,6 μg/ml) and 5 MICs (6.25 μg/ml) of the KVM-194 for 1 h and 24 h.

After the incubation, the cells were washed by 0.9 % sodium chloride solution and fixed in a mixture of glutaraldehyde and formaldehyde. Additionally, samples were stained by 1.0 % solution of osmium tetraoxide in phosphate buffer (pH 7.4), dehydrated in increasing concentration of acetone and placed in epoxy resin. Ultrathin sections were prepared using ultramicrotom LKB-8800, then contrasted by osmium tetraoxide and lead citrate. Sections were examined by electron microscope EM-125K at a voltage of 80 kV.

Results and Discussion

The data obtained suggest that the compound KVM-194 possesses a distinct antibacterial activity against *S. aureus*, the MIC is 1.25 μg/ml.

Morphology of the bacteria, exposed to the subinhibitory (0.5 MIC) and suprainhibitory (5 MICs) concentrations of KVM-194 during 1 h and 24 h was compared to the morphology of the untreated control cells.

Intact *S. aureus* cells (Fig. 1, a) were spherical or ellipsoidal, in various stages of division. Cell wall surface was smooth, a thick light outer layer and a thin inner electron-dense layer was observed. Cytoplasm was tightly covered with cytoplasmic membrane and filled with ribosomes and polyribosomes. Nucleoid was well distinguished on a background of cytoplasm.

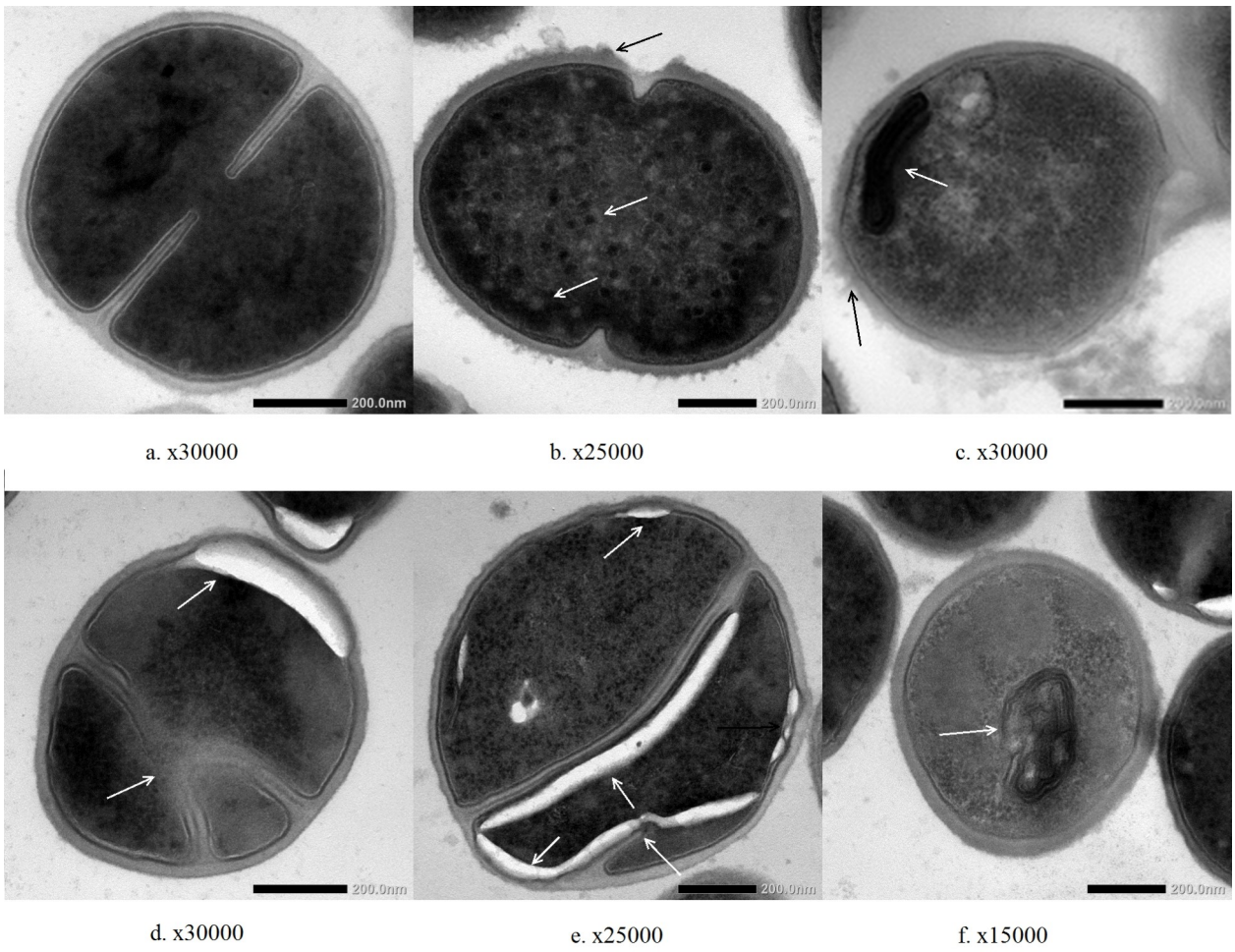

Fig. 1. Ultrastructure of *S. aureus* cells: a – intact cells; b, c - cells exposed to subinhibitory concentration (0.5 MIC) of the compound KVM-194 during 1 h; d, e, f - cells exposed to subinhibitory concentration (0.5 MIC) of the compound KVM-194 during 24 h. The arrows mark changes described in the text. Magnification is noted for each image separately. Transmission electron microscopy.

1 h exposition to KVM-194 at a concentration of 0.5 MIC (Fig. 1. b, c) resulted in the roughness of the cell surface and emerging of the intracellular particles of different electron density. However, despite the alterations, cells at different stages of division were observed, indicating normal reproduction.

We found that increase of the incubation time to 24 h (Fig. 1. d, e) resulted in formation of nonpolar septum. Additionally, detachment of membrane from cytoplasm (Fig. 1. d, e) and multi-membrane structures within cells were registered (Fig. 1. f).

For further investigation of the KVM-194 effect on the morphology of *S. aureus* we used increased concentration (5 MICs) of the compound. Results are shown in Fig. 2.

The exposure to suprainhibitory concentration of KVM-194 for 1 h resulted in nucleoid fragmentation with the formation of numerous separate zones (Fig. 2. a) and septum abnormalities (Fig. 2. b). These changes may indicate disturbances of the division process. Also, some cells showed signs of necrosis – enlightenment of the cytoplasm, detachment of the cytoplasm from the cell wall, deformation of the cell envelope (Fig. 2. c).

Increase of the incubation period to 24 h led to an alteration of cell division process (Fig. 2. d); cell wall changes exacerbated: a large number of lysed cells, cell wall rupture and leakage of cytoplasm were observed (Fig. 2. e, f).

Observed alterations of cell envelope resemble previously described effects of membrane-acting antibiotics. Therefore, we assume, that aryl aliphatic aminoalcohol derivative KVM-194 has a similar mechanism of action. In addition, we registered the violation of the genetic apparatus of cells, which could be due to compound's influence on the intracellular processes.

Conclusion

1. Antimicrobial activity of compound KVM-194 is realized by a complex mechanism of action: influence on cell membrane and genetic apparatus, confirmed by cell structure changes.
2. KVM-194 caused the alterations of ultrastructure of the *S. aureus* cells even after 1 hour of the exposure to compound and these changes were exacerbated with the extension of incubation period.

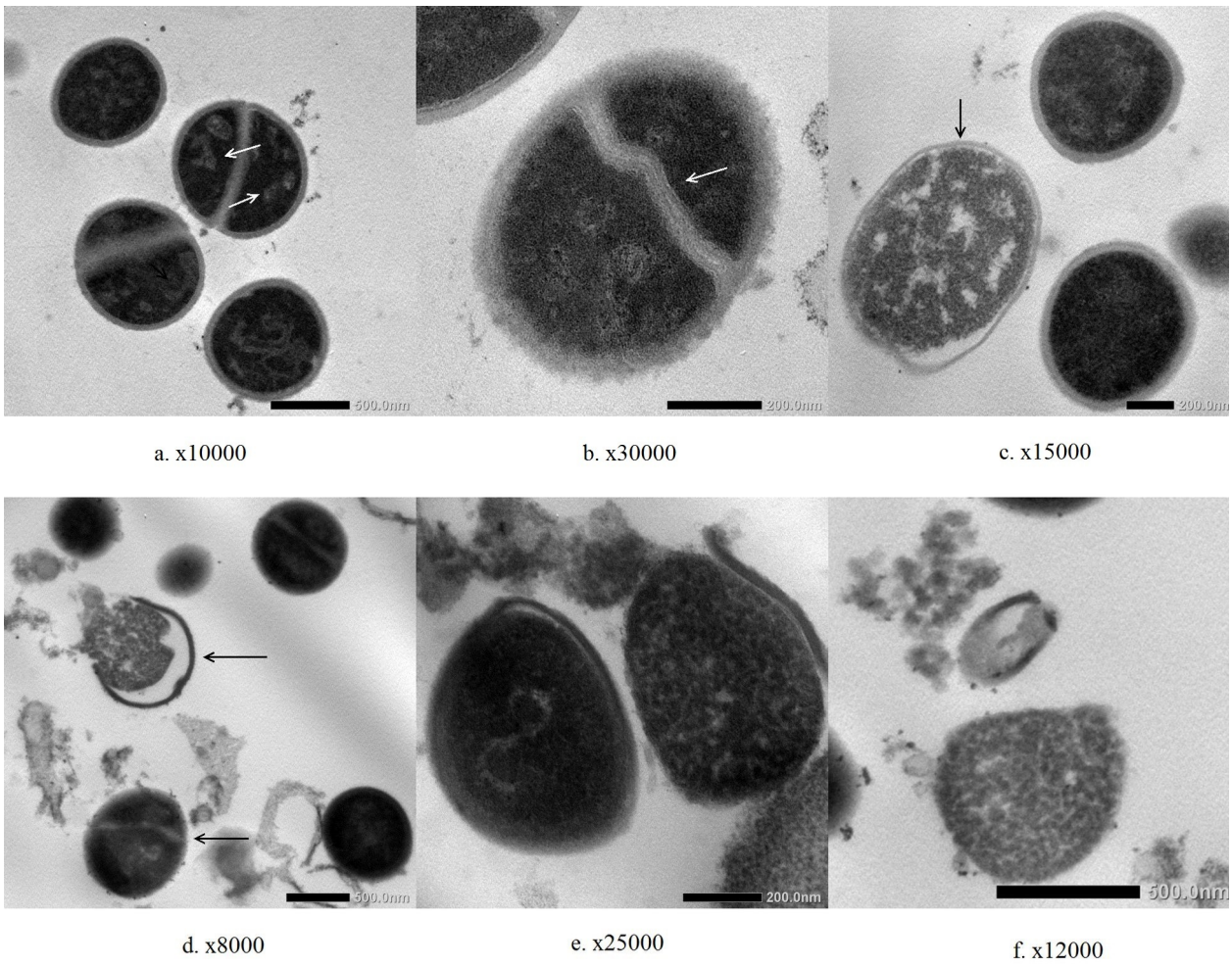

Fig. 2. Ultrastructure of *S. aureus* cells: a, b, c – cells exposed to suprainhibitory concentration (5 MICs) of the compound KVM-194 during 1 h; d, e, f – cells exposed to suprainhibitory concentration (5 MICs) of the compound KVM-194 during 24 h. The arrows mark changes described in the text. Magnification is noted for each image separately. Transmission electron microscopy.

Acknowledgement

The author expresses her deep gratitude to Voychuk S., «Zabolotnyi Institute of Microbiology and Virology of the NAS of Ukraine", for his assistance in the experiment.

References

1. Hentschke M. Combined ramR Mutation and Presence of a Tn1721-Associated tet(A) Variant in a Clinical Isolate of *Salmonella enterica* serovar hadar resistant to tigecycline [Text] / M. Hentschke [et al.] // Antimicrobial Agents Chemotherapy. – 2010. – Vol. 54, № 3. – P. 1319–1322.

2. Steed M. E. Characterizing vancomycin-resistant *Enterococcus* strains with various mechanisms of daptomycin resistance developed in an *in vitro* pharmacokinetic/ pharmacodynamic model [Text] / M. E. Steed [et al.] // Antimicrobial Agents Chemotherapy. – 2011. – Vol. 55, № 10. – P. 4748–4754.

3. Klainer A. S. Surface manifestations of antibiotic-induced alterations in protein synthesis in bacterial cells [Text] / Klainer A. S., Perkins R.L. // Antimicrobial Agents and Chemoterapy. – 1972. – Vol. 1, № 2. – P. 164–170.

4. Evaluation of antibiotic efficacy using electron microscopy: morphological effects of guanylureido cephalosporin, chlorobenzoylureido cephalosporin, bl-p1654, and carbenicillin on *Pseudomonas aeruginosa* [Text] / L. F. Ellis [et al.] // Antimicrobial Agents and Chemoterapy. – 1976. - Vol. 9, № 2. – P. 334–342.

5. Braga P. C. Atomic force microscopy: application to investigation of *Escherichia coli* morphology before and after exposure to cefodizime [Text] / P. C. Braga, D. Ricci // Antimicrobial Agents and Chemoterapy. – 1998. – Vol. 42, № 1. – P. 18–22.

6. Presslitz J. E. Mode of action of a structurally novel beta-lactam [Text] / J. E. Presslitz // Antimicrobial Agents and Chemoterapy. – 1978. – Vol. 14, № 1. – P. 144–150.

7. Lorian V. Effects of subinhibitory concentrations of antibiotics on cross walls of cocci [Text] / V. Lorian, B. Atkinson // Antimicrobial Agents and Chemoterapy. – 1976. – Vol. 9, № 6. – P. 1043–1055.

8. Waisbren S. J. Morphological expression of antibiotic synergism against Pseudomonas aeruginosa as observed by, scanning electron microscopy [Text] / S. J. Waisbren, D. J. Hurley, B. A. Waisbren // Antimicrobial Agents and Chemoterapy. – 1980. – Vol. 18, № 6. – P. 969–975.

9. Prior R. B. Morphological alterations of *Pseudomonas aeruginosa* by ticarcillin: a scanning electron microscope

study [Text] / R. B. Prior, J. F. Warner // Antimicrobial Agents and Chemoterapy. – 1974. – Vol. 6, № 6. – P. 853–855.
10. DeLoney C. R. Competition of various β-lactam antibiotics for the major penicillin-binding proteins of *Helicobacter pylori*: antibacterial activity and effects on bacterial morphology [Text] / C. R. DeLoney, N. L. Schiller // Antimicrobial Agents and Chemoterapy. – 1999. – Vol. 43, № 11. – P. 2702–2709.
11. Sanyal D. An electronmicroscope study of glycopeptide antibiotic-resistant strains of *Staphylococcus epidermidis* [Text] / D. Sanyal, D. Greenwood // Journal of Medical Microbiology. – 1993. – Vol. 39. – P. 204–210.
12. Santhana Raj L. Mesosomes are a definite event in antibiotic-treated Staphylococcus aureus ATCC 25923 [Text] / L. Santhana Raj [et al.] // Tropical Biomedicine. – 2007. – Vol. 24, № 1. – P. 105–109.
13. New insights into the antibacterial mechanism of action of squalamine [Text] / K. Alhanout [et al.] // Journal of Antimicrobial Chemotherapy. – 2010. – Vol. 65, № 8. – P. 1688–1693.
14. Damage of the bacterial cell envelope by antimicrobial peptides gramicidin S and PGLa as revealed by transmission and scanning electron microscopy [Text] / M. Hartmann [et al.] // Antimicrobial Agents and Chemoterapy. – 2010. – Vol. 54, № 8. – P. 3132–3142.
15. Daptomycin exerts bactericidal activity without lysis of *Staphylococcus aureus* [Text] / N. Cotroneo [et al.] // Antimicrobial agents and chemoterapy. – 2008. – Vol. 52, № 6. – P. 2223–2225.
16. Scanning electron microscopy of *Staphyococcus aureus* and *Enterococcus faecalis* exposed to daptomycin [Text] / L. J. Wale [et al.] // Journal of Medical Microbiology. – 1989. – Vol. 30. – P. 45–49.
17. Klainer A. S. Effect of the inhibition of protein synthesis on the *Escherichia coli* cell envelope [Text] / A. S. Klainer, R. R. B. Russell // Antimicrobial Agents and Chemoterapy. – 1974. – Vol. 6, № 2. – P. 216–224.
18. Pat. № EP1098642 B1 USA, IPC A61P17/02, A61P25/28, C07D295/12 Diamino-propanol-compounds for treating ischemia / Chatterjee S. ; applicant Johns Hopkins University. – №: EP 19990937409 ; fil. 22.07.99 ; pub. 04.04.07.
19. Vanillylamide-based propanolamine derivative displays alpha/beta-adrenoceptor blocking and vasodilating properties [Text] / J. L. Yeh [et al.] // J. Cardiovasc. Pharmacol. – 2002. – Vol. 39, № 6. – P. 803–813.
20. Vartanyan R. S. Synthesis of new aminopropanol derivatives as potencial β-adrenoblockers [Text] / R. S. Vartanyan, E. A. Adamyan, M. A. Sheyranyan // Chemical Journal of Armenia. – 2003. – Vol. 56, №3. – P. 87–91.
21. DiCuollo C. J. The metabolism of 3-((5-methyl-5H-as-triazino(5,6-b)-indol-3-yl)amino)-1-propanol (SK&F 21687). A potent antiviral [Text] / DiCuollo C. J., Zarembo J. E., Pagano J. F. // Xenobiotica. – 1973. – Vol. 3, № 3. – P. 171–178.
22. Antioxidant and antiradical properties of new aminoacid derivatives of aminoalcohols [Text] / M. H. Malakyan [et al.] // Khimiko-Farmatsevticheskii Zhurnal. – 2010. –Vol. 44. № 8. – P. 19–21.
23. Pat. 20110118210 USA, IPC A61 K 31/675 Aminopropanol derivatives [Text] / Rainer A., Francotte E., Zecri F., Zollinger M. – № US 2011/0118210 A1 ; fil. 28.01.11 ; pub. 19.05.2011.
24. Pat. 7625950 USA, IPC C07C 217/72 Amino-propanol derivatives as sphingosine-1-phosphate receptor modulator [Text] / Klaus H., Carsten S., Zecri F. ; applicant Novartis AG. – № US 10/554556 ; fil. 29.03.2004 ; pub. 01.12.2009.
25. Pat. 23451 UA, IPC C07C217/28 Agent for treating memory disorders [Text] / Zaycev L. M., Korotkiy Y. V, Krasavtsev I. I., Losinskiy M. O., Khromova I. V., Seredenin S. B., Voronina T. A. ; applicant Institute of organic chemistry NAS of Ukraine. – № 94063487 ; fil. 16.06.1994 ; pub. 29.12.1999, Bul. № 8, 1999.
26. Pat. WO 2007/058639 WO, IPC A61K31/135, A61K31/785, A01N33/02 Antibacterial agents [Text] / Yerramilli V. S. N. M. ; applicant Idexx Laboratories Inc. – № PCT/US2005/029602 ; fil. 23.08.2005 ; pub. 24.05.2007.
27. Piperazine propanol derivative as a novel antifungal targeting 1,3-beta-D-glucan synthase [Text] / O. Kondoh [et al.] // Biol. Pharm. Bull. – 2005. – Vol. 28, № 11. – P. 2138–2141.
28. Pat. 4361557 USA, IPC A61K31/22, C07C233/16 1-Aryl-2-acylamido-3-fluoro-1-propanol acylates, methods for their use as anti-bacterial agents and compositions useful therefore [Text] / Nagabbusha T. L. ; applicant Schering Corporation. – № US 291/663 ; fil. 10.08.1981 ; pub. 30.11.1982.
29. Guidelines for Susceptibility Testing of Microorganisms to Antibacterial Agents 4.2.1890-04 [Text] // Clinical Microbiology and Antimicrobial Chemotherapy. – 2004. – Vol. 6, № 4. – P. 306 – 359.

UDC 579.23, 615.281.9, 547.435

MORPHOLOGICAL ALTERATIONS OF *STAPHYLOCOCCUS AUREUS* CAUSED BY ARYL ALIPHATIC AMINOALCOHOL DERIVATIVE

Dronova M.

Increasing of antimicrobial resistance has created a critical need of the novel antimicrobial agents. One of the promising chemical classes for its development are aryl aliphatic aminoalcohols. New compounds of this class were synthesized at the Institute of organic chemistry (Kiev, Ukraine), by Y. Korotkiy. After the screening studies compound KVM-194 was selected as the potent antistaphylococcal agent. The aim of the study was to examine ultrastructural changes in the bacterial cells under the influence of the compound KVM-194.

Materials and methods. *Staphylococcus aureus* ATCC 25923 was used in all experiments. The minimum inhibitory concentration was determined by serial macrodilution method in Mueller-Hinton broth. Bacteria were exposed to the 0,5 MIC and 5 MICs of the KVM-194 for 1 h and 24 h. Ultrastructure of intact and treated *Staphylococcus aureus* cells was examined by transmission electron microscopy after contrasting by osmium tetraoxide and lead citrate.

Results and Discussion. The compound KVM-194 possesses a distinct antibacterial activity against *Staphylococcus aureus*, the minimum inhibitory concentration is 1.25 μg/ml. . We found that exposure to

KVM-194 at a subinhibitory concentration resulted in alterations of the cell morphology even after 1 h of treatment. The roughness of the cell surface and emerging of the intracellular particles of different electron density were observed. Increase of the incubation time to 24 h led to detachment of membrane from cytoplasm, multi-membrane structures within cells emergence and formation of nonpolar septum. 1 h exposition to suprainhibitory concentration of KVM-194 resulted in nucleoid fragmentation, septum abnormalities and necrosis of some cells. We found that increasing of the incubation period to 24 h led to exacerbation of alterations: cell wall rupture, leakage of cytoplasm and a large number of lysed cells were registered.
Conclusion. Observed alterations, suggest the possible mechanism of action of KVM-194, due to its influence on the cell membrane and intracellular processes.
Key words: *Staphylococcus aureus*, antibiotics, mechanism of action, aryl aliphatic aminoalcohols.