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THE EFFECT OF SUBINHIBITORY CONCENTRATIONS OF ETHYLMETHYLHYDROXYPYRIDINE SUCCINATE ON BIOFILMS OF MICROORGANISMS

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Recently, biofilms of microorganisms have been attracting the attention of scientists. Antibiotics are not effective enough to treat biofilm infections due to high minimum inhibitory concentrations (MIC) against biofilm forms of microorganisms, which can cause toxic reactions. The potential antibiofilm properties of other compounds, in particular antioxidants, such as ethylmethylhydroxypyridine succinate (mexidol), are being studied. The aim of the work is to determine the influence of mexidol on the formation of biofilms by test strains of microorganisms. Clinical strains of E. coli 311, P. aeruginosa 449, S. aureus 222, and C. albicans 1486 were used in the research. The antibacterial and antifungal activity of mexidol was determined by the micromethod of serial dilutions in the liquid nutrient media. The ability of microorganisms to form biofilms was studied according to the O'Toole method. The effect of mexidol on the biofilm formation by clinical test strains of bacteria was studied at concentrations of 5-200 μg/ml. Statistical processing of the results was performed using the Newman-Keuls test by the software StatSoft Statistica 6.0. It has been shown that for planktonic microorganisms E. coli, P. aeruginosa, S. aureus, and C. albicans the MIC of mexidol is more than 200 μg/ml. Gram-negative bacteria (E. coli 311, P. aeruginosa 449) had a strong ability to form biofilms, gram-positive (S. aureus 222) – a medium ability. Fungi C. albicans 1486 did not adhere to the abiotic surface and were no longer used. Mexidol in the studied concentrations stimulates the biofilm formation by E. coli 311, increasing the biomass of biofilms by 12-15%. It causes an increase in the biomass of P. aeruginosa biofilms by 33-110% depending on the concentration. The drug also stimulates the formation of S. aureus 222 biofilms in 2.1-2.7 times compared to the control. The ability of mexidol in low concentrations to stimulate biofilm formation indicates the need for further in-depth studies to assess the feasibility of this antioxidant use in patients with infectious diseases.

Key words: *ethylmethylhydroxypyridine succinate, mexidol, microorganisms, clinical strains, biofilm.*

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Introduction. Recently, biofilms have attracted the attention of researchers as a syntrophic consortium of microorganisms in which cells are embedded in the matrix consisting of extracellular polymeric substances, a conglomerate of polysaccharides, proteins, lipids, and DNA [1]. Microbes form biofilm in response to various factors, which may include the recognition of specific and nonspecific sites of attachment on the surface or subinhibitory concentrations of antibiotics [2]. One of the advantages of biofilm for microorganisms is the increased resistance to detergents and antibiotics, as a dense extracellular matrix and outer layer of cells protect the internal environment of the community, as well as facilitated gene transfer [3].

Biofilm microorganisms cause about 80% of chronic microbial infections in humans, which leads to increased morbidity and mortality, increased hospital stays, and, consequently, increased costs for medical care [4]. Biofilms can also develop on abiotic surfaces, including orthopedic prostheses, artificial heart valves, coronary stents, intravascular and urinary catheters [4]. In addition, currently available antibiotics are not effective enough to treat biofilm infections due to higher values of minimum inhibitory concentration (MIC) and minimum bactericidal concentration against biofilm forms of pathogens, which can cause *in vivo* toxicity [5, 6].

It is extremely important to develop medicinal drugs that can actively combat biofilm infections. Attention is paid to plant components, chelating agents, peptide antibiotics, and synthetic chemical compounds [7, 8]. Antibiofilm action is found in antioxidants, for example acetylcysteine, quercetin, etc [9, 10, 11].

Synthetic antioxidant ethylmethylhydroxypyridine succinate (mexidol) is widely used in clinic, both for noninfectious diseases including cerebrovascular disorders, trauma of the brain, neurosis, withdrawal syndrome, intoxication with neurotropic poisons, acute myocardial infarction, and infectious pathology such as peritonitis or acute necrotic pancreatitis [12, 13]. Since the drug is administered intravenously and by infusion, and is also prescribed for purulent-inflammatory diseases of the abdominal cavity, it is of interest to study its antimicrobial properties, in particular the effect on biofilms of microorganisms.

The aim of the study was to determine the effect of mexidol on the formation of biofilms by test strains of microorganisms.

Object and methods of research. Clinical strains of bacteria (*E. coli* 311, *P. aeruginosa* 449, *S. aureus* 222) and fungi (*C. albicans* 1486) were used in studies of the antimicrobial activity of mexidol. The antibacterial and

Table 1 – Biofilm formation by clinical strains of microorganisms

Microorganisms	Ability to biofilm formation
E. coli 311 (n=3)	Strong
P. aeruginosa 449 (n=3)	Strong
S. aureus 222 (n=3)	Medium
C. albicans 1486 (n=3)	Absent

Footnotes: 1 – quantitative parameters of biofilm formation met the criteria specified in the reference [17], and therefore were not given in the table. 2 – n – a number of observations.

antifungal activity of mexidol was determined by serial dilutions in liquid nutrient media Tryptone Soya Broth (TSB) (for bacteria) and Saburo (for fungi) (HiMedia, India) in polystyrene deep well plates according to [14, 15]. The range of mexidol concentrations was 1.5-200 μg/ml. The substance of the drug was obtained from the manufacturer (TOV NVF Microchem, Ukraine). The microbial inoculum was prepared according to McFarland. The density of the inoculum was 10^6 colony-forming units/ml (bacteria) or 10^5 fungal elements/ml of nutrient medium. The incubation period was 24 h at 35-37°C for bacteria and 24-48 h at 35°C for fungi. The MIC was taken to be the minimum concentration of mexidol at which the growth of microorganisms was visually absent.

Determination of the ability of microorganisms to form biofilms was performed by microtiter dish biofilm formation assay described by O'Toole [16]. Biofilms were grown for 24 h at 37°C, after which the contents of the wells were removed. They were washed with distilled water and stained with 0.1% gentian violet, followed by extraction of the dye with ethanol. The degree of intensity of biofilm formation was determined according to the criteria developed by Stepanovic S. et al. [17].

The potential antibiofilm activity of mexidol against clinical test strains of bacteria was studied at subinhibitory concentrations of 5 μg/ml, 50 μg/ml and 200 μg/ml. Concentrations of the drug were selected on the basis of data on its maximum concentration in blood plasma (3.5-4.0 μg/ml) when administered intravenously at a dose of 400–500 mg [13]. The ability of mexidol to affect the biofilm formation by clinical isolates of bacteria was detected according to [16]. The study used a night culture of microorganisms diluted 1:100 in nutrient medium TSB. The incubation period of the culture with mexidol was 24 h at 37°C. The formed biofilms were stained with 0.1% gentian violet solution. The optical density was recorded by Adsorbance Microplate Reader ELx×800 (VioTek, USA) at a wavelength of 630 nm. Each experiment was performed in at least 3 replicates.

The research results are presented as M±m, where M is a mean value and m is a standard error of the mean. Statistical processing of the results was performed using the Newman-Keuls test by standard computer software StatSoft Statistica 6.0. The difference between groups was considered significant at $p<0.05$.

Research results and their discussion. The obtained data on the antimicrobial activity of mexidol showed that for planktonic forms of microorganisms *E. coli*, *P. aeruginosa*, *S. aureus*, and *C. albicans* MIC of the drug is more 200 μg/ml.

At the initial stage of the study of potential antibiofilm activity of mexidol, the ability of clinical isolates to form biofilms on the abiotic surface was determined. According to the obtained data, gram-negative bacteria (*E. coli* 311, *P. aeruginosa* 449) demonstrate a strong ability to form biofilms, gram-positive ones (*S. aureus* 222) – a medium ability (**table 1**).

Yeast-like fungi *C. albicans* 1486 do not adhere to the abiotic surface, so in further studies, this test strain was not used.

The results on the mexidol's action on the biofilm formation by gram-negative bacteria *E. coli* 311 and *P. aeruginosa* 449 are presented in the **table 2** and **table 3**.

Table 2 – Mexidol's influence on the biofilm formation by *E. coli* 311 (M±m)

Conditions of the experiment	Optical density, units	Formed biofilm, %
Test culture control (n=3)	0,049±0,002	100±6
Mexidol, 5 μg/ml (n=5)	0,058±0,002*	112±4*
Mexidol, 50 μg/ml (n=5)	0,058±0,001*	112±3*
Mexidol, 200 μg/ml (n=5)	0,060±0,003*	115±7*

Footnotes: 1 – * – differences significant as compared to the test culture control. 2 – n – a number of observations.

It was found that mexidol in the studied concentrations stimulates the formation of *E. coli* 311 biofilms, as evidenced by an increase in biofilm biomass by 12–15% ($p<0.05$). In the range of drug concentrations of 5–200 μg/ml, this increase is equally pronounced.

According to the results of the study, mexidol does not disrupt the formation of biofilms by *P. aeruginosa* (**table 3**). This is evidenced by the increase in biomass of *P. aeruginosa* biofilms under the action of the drug at concentrations of 200 μg/ml by 33% and 50 μg/ml – by 79% ($p<0,05$). At reduction of mexidol's concentration to 5 μg/ml, stimulation of biofilm formation by *P. aeruginosa* 449 is noted with a 2.1-fold increase in biomass compared to the control ($p<0.05$). The biofilm formation of this test strain of microorganisms under the influence of the highest concentration of mexidol is probably lower than under the influence of both two other concentrations of this remedy, and under the influence of medium concentration is lower than under the action of minimum one ($p<0,05$). This means that in the concentration range of mexidol 5-200 μg/ml, there is an inverse dependence of the detected effect on the concentration of the investigated agent.

Table 3 – Mexidol's influence on the biofilm formation by *P. aeruginosa* 449 (M±m)

Conditions of the experiment	Optical density, units	Formed biofilm, %
Test culture control (n=3)	0,059±0,002	100±4
Mexidol, 5 μg/ml (n=5)	0,128±0,005*	210±9*
Mexidol, 50 μg/ml (n=5)	0,105±0,001*,#	179±6*,#
Mexidol, 200 μg/ml (n=3)	0,081±0,003*,#,##	133±5*,#,##

Footnotes: 1 – differences which are significant as compared: * – to the test culture control; # – to mexidol's concentration 5 μg/ml; ## – to mexidol's concentration 50 μg/ml. 2 – n – a number of observations.

Data on the effect of mexidol on the formation of biofilms of *S. aureus* 222 are presented in the **table 4**.

Mexidol has been shown to stimulate *S. aureus* 222 biofilm production.

In this case, the biomass of *S. aureus* biofilms increases 2.1-2.7 times ($p<0.05$) compared to the control.

There are no significant differences in the intensity of the biofilm formation at different concentrations of the drug.

Therefore, the MIC value of mexidol against clinical strains of bacteria and fungi exceeds 200 μg/ml. Stimulation of the biofilm formation by gram-negative (*E. coli* 311, *P. aeruginosa* 449) and gram-positive bacteria (*S. aureus* 222) is observed under the action of the drug in subinhibitory concentrations.

The absence of specific antimicrobial action of mexidol (MIC is more 200 μg/ml) is quite natural for the antioxidant and is consistent with literature data showing that the MIC of the drug for reference strains of microorganisms is in the range of 1250-10000 μg/ml [18]. Another known antioxidant, ascorbic acid, has antimicrobial activity in the near range [19].

At the same time, we have not found reports in the literature on the ability of antioxidants to induce the development of biofilms in both subinhibitory concentrations and concentrations exceeding MIC. The antioxidant activity of mexidol has been confirmed by numerous studies [12], and therefore the ability of the drug to enhance the biofilm formation may be due to other properties of its molecule, which allow this substance to stimulate biofilm development in other way than antioxidant effect. In particular, it may be the delivery of iron, which can enhance the formation of biofilms [20]. It is also possible that metabolic processes in the bacterial cell with the participation of 3-hydroxypyridine structure analogous to pyridoxine or succinate play a role in stimulating of biofilm formation [21].

Of course, the discovered ability of mexidol in low concentrations to stimulate biofilm formation indicates the need for further in-depth studies to assess the feasibility of its use in patients with infectious diseases. However, at this stage, given ability should draw the attention of clinicians who prescribe this drug to the most careful care of catheters and other medical devices, as the use of mexidol may increase the formation of biofilms on them. Also, using this antioxidant in the complex treatment of purulent-inflammatory diseases of the abdominal cavity, we should simultaneously choose a regimen of antibiotic therapy that prevents the development of biofilms of microorganisms or eradicates of biobilm-associated pathogens, providing high doses of antibiotics, combining them with each other and with adjuvants [22].

Table 4 – Mexidol's influence on the biofilm formation by *S. aureus* 222 (M±m)

Conditions of the experiment	Optical density, units	Formed biofilm, %
Test culture control (n=5)	0,034±0,002	100±5
Mexidol, 5 μg/ml (n=5)	0,083±0,007*	249±21*
Mexidol, 50 μg/ml (n=5)	0,092±0,015*	274±46*
Mexidol, 200 μg/ml (n=3)	0,072±0,009*	212±30*

Footnotes: 1 – * – differences significant as compared to the test culture control. 2 – n – a number of observations.

Conclusions.

1. The MIC of ethylmethylhydroxypyridine succinate (mexidol) for planktonic cells of clinical strains of bacteria and fungi (*E. coli* 311, *P. aeruginosa* 449, *S. aureus* 222, *C. albicans* 1486) exceeds 200 μg/ml, indicating the absence of potent antimicrobial activity.

2. Stimulation of biofilm formation by gram-negative (*E. coli* 311, *P. aeruginosa* 449) and gram-positive bacteria (*S. aureus* 222) is observed under the action of the drug in subinhibitory concentrations (5-200 μg/ml).

Prospects for further research. The study of the effect of high concentrations of mexidol on the biofilms of microorganisms and the investigation of the mechanisms of such effect will be the direction of further work.

References

1. Kostakioti M, Hadjifrangiskou M, Hultgren SJ. Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the postantibiotic era. Cold Spring Harb Perspect Med. 2013 Apr;3(4):a010306. DOI: 10.1101/cshperspect.a010306.
2. Kaplan JB. Antibiotic-induced biofilm formation. Int J Artif Organs. 2011 Sep;34(9):737-51. DOI: 10.5301/ijao.5000027.
3. Chimileski S, Franklin MJ, Papke RT. Biofilms formed by the archaeon Haloferax volcanii exhibit cellular differentiation and social motility, and facilitate horizontal gene transfer. BMC Biology. 2014 Aug;12:65. DOI: 10.1186/s12915-014-0065-5.
4. Magana M, Sereti C, Ioannidis A, Mitchell CA, Ball AR, Magiorkinis E, et al. Options and limitations in clinical investigation of bacterial biofilms. Clin Microbiol Rev. 2018 Jul;31(3):e00084-16. DOI: 10.1128/CMR.00084-16.
5. Yan J, Bassler BL. Surviving as a community: antibiotic tolerance and persistence in bacterial biofilms. Cell Host Microbe. 2019 Jul;26(1):15-21. DOI: 10.1016/j.chom.2019.06.002.
6. Sharma D, Misba L, Khan AU. Antibiotics versus biofilm: an emerging battleground in microbial communities. Antimicrob Resist Infect Control . 2019 May 16;8:76. DOI: 10.1186/s13756-019-0533-3.
7. Roy R, Tiwari M, Donelli G, Virulence VT. Strategies for combating bacterial biofilms: a focus on anti-biofilm agents and their mechanisms of action. Virulence. 2018 Jan;9(1):522-54. DOI: 10.1080/21505594.2017. 1313372.
8. Hrynchuk NI, Vrynchanu NO, Bukhtiarova TA, Dudikova DM, Korotkyi YuV, Bondarenko LB. Antybioplivkova diia pokhidnoho adamantana shchodo Staphylococcus aureus. Mikrobiol. Z. 2021;83(1):58-67. DOI: 10.15407/microbiolj83.01.058. [in Ukrainian].
9. Francolini I, Piozzi A. Role of antioxidant molecules and polymers in prevention of bacterial growth and biofilm formation. Curr Med Chem. 2020;27(29):4882-904. DOI: 10.2174/0929867326666190409120409.
10. Dinicola S, De Grazia S, Carlomagno G, Pintucci JP. N-acetylcysteine as powerful molecule to destroy bacterial biofilms. A systematic review. Eur Rev Med Pharmacol Sci. 2014 Oct;18(19):2942-8.
11. Memariani H, Memariani M, Ghasemian A. An overview on anti-biofilm properties of quercetin against bacterial pathogens. World J Microbiol Biotechnol. 2019 Sep;35(9):143. DOI: 10.1007/s11274-019-2719-5.
12. Voronina TA, Ivanova EA. Kombinirovannoe primenenie meksidola s izvestnyimi lekarstvennyimi sredstvami. Zhurnal nevrologii i psihiatrii. 2019;4:115-124. DOI: 10.17116/jnevro2019119041115. [in Russian].
13. Schulkin AV. Meksidol: sovremennyie aspektyi farmakokinetiki i farmakodinamiki. Farmateka. 2016;4(16):65-71. Dostupno: article/33771. [in Russian].
14. Semina SA, Sidorenko SV, Rezvan SP, Grudinina SA, Strachunskiy LS, Stetsyuk OU, et al. Opredelenie chuvstvitelnosti mikroorganizmov k antibakterialnyim preparatam (Metodicheskie ukazaniya MUK 4.2.1890-04). Klin mikrobiol antimikrob himioter. 2004;6(4):306-59. [in Russian].
15. Nekrasova LS, Svyta VM, Hlushkevych TH, Tomchuk VV, Zherebko NM, Pokas OV. Vyznachennia chutlyvosti mikroorhanizmiv do antybakterialnykh preparativ: Metodychni vkazivky MV 9.9.5-143-2007. Kyiv: MOZ Ukrainy; 2007. 73 s. [in Ukrainian].
16. O'Toole GA. Microtiter dish biofilm formation assay. J Vis Exp. 2011 Jan 30;(47):2437. DOI: 10.3791/2437.
17. Stepanovic S, Vukovic D, Hola V, Di Bonaventura G, Djukic S, Cirkovic I. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. APMIS. 2007 Aug;115(8):891-9. DOI: 10.1111/j.1600-0463.2007.apm_630.x.

18. Vazhnichaya EM, Bobrova NA, Devyatkina TA, Loban GA, Devyatkina NN. Vliyanie emoksipina i meksidola na razvitie kultur etalonnyih shtammov mikroorganizmov i ih chuvstvitelnost k antibiotikam. Eksperimentalnaya i klinicheskaya farmakologiya. 2019;2:58-62. [in Russian].
19. Verghese RJ, Mathew SK, David A. Antimicrobial activity of vitamin C demonstrated on uropathogenic Escherichia coli and Klebsiella pneumoniae. J Curr Res Sci Med. 2017;3:88-93. DOI: 10.1038/s41598-020-74977-y.
20. Oh E, Andrews KJ, Jeon B. Enhanced biofilm formation by ferrous and ferric iron through oxidative stress in Campylobacter jejuni. Front Microbiol. 2018 Jun;9:1204. DOI: 10.3389/fmicb.2018.01204.
21. El Qaidi S, Yang J, Zhang JR, Metzger DW, Bai G. The vitamin B6 biosynthesis pathway in Streptococcus pneumoniae is controlled by pyridoxal 5′-phosphate and the transcription factor PdxR and has an impact on ear infection. J Bacteriol. 2013 May;195(10):2187-96. DOI: 10.1128/JB.00041-1.
22. Ciofu O, Rojo-Molinero E, Macià MD, Oliver A. Antibiotic treatment of biofilm infections. APMIS. 2017 Apr;125(4):304-19. DOI: 10.1111/apm. 12673.

ВПЛИВ СУБІНГІБУЮЧИХ КОНЦЕНТРАЦІЙ ЕТИЛМЕТИЛГІДРОКСИПІРИДИНУ СУКЦИНАТУ НА БІОПЛІВКИ МІКРООРГАНІЗМІВ

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Резюме. Біоплівкові мікроорганізми спричиняють близько 80% хронічних інфекцій, що призводить до підвищення рівня захворюваності, збільшення випадків госпіталізації і зростання витрат на медичне обслуговування. Біоплівки також можуть розвиватися на абіотичних поверхнях, включаючи ортопедичні протези, стенти та катетери. Антибіотики не достатньо ефективні для лікування інфекцій, пов'язаних з біоплівкою, і пошук антибіоплівкових агентів ведеться серед інших класів сполук, зокрема серед антиоксидантів, до яких належить відомий препарат етилметилгідроксипіридину сукцинат (мексидол). Мета дослідження – визначити вплив мексидолу на формування біоплівок тест-штамами мікроорганізмів. Використано клінічні штами бактерій (*E. coli* 311, *P. aeruginosa* 449, *S. aureus* 222) та грибів (*C. albicans* 1486). Антимікробну активність мексидолу визначали методом серійних розведень у рідких поживних середовищах у діапазоні концентрацій 1,5-200 мкг/мл. Визначення здатності мікроорганізмів до плівкоутворення проводили за відомою методикою O'Toole. Ступінь інтенсивності утворення біоплівки визначали, як описано Stepanovic S. et al. Потенційну антибіоплівкову активність мексидолу досліджували у концентраціях 5-200 мкг/мл. Статистичну обробку результатів проводили з використанням критерію Ньюмена-Кейлса за допомогою програм «StatSoft Statistica 6.0». Показано, що для планктонних форм *E. coli, P. aeruginosa, S. aureus* та *C. albicans* мінімальна інгібуюча концентрація мексидолу більша 200 мкг/мл. *E. Coli* 311, *P. aeruginosa* 449 виявляють сильну здатність до плівкоутворення, *S. aureus* 222 – середню. *C. albicans* 1486 не адгезуються до абіотичної поверхні і надалі не використовувалися. Встановлено, що мексидол посилює плівкоутворення *E. coli* 311, збільшуючи біомасу біоплівок на 12–15%. Препарат збільшує біомасу біоплівок *P. aeruginosa* на 33–110% проти контролю, що найбільш виразне за мінімальної концентрації препарату. Мексидол також стимулює плівкоутворення *S. aureus* 222 у 2,1-2,7 разу порівняно з контролем.

Отже, за дії препарату у субінгібуючих концентраціях відмічена стимуляція плівкоутворення грамнегативними (*E. coli* 311, *P. Aeruginosa* 449) та грампозитивними бактеріями (*S. aureus* 222). Виявлена здатність мексидолу стимулювати плівкоутворення потребує подальших досліджень, щоб оцінити доцільність його застосування в комплексній терапії гнійно-запальних захворювань.

Ключові слова: етилметилгідроксипіридину сукцинат, мексидол, мікроорганізми, клінічні штами, біоплівка.

THE EFFECT OF SUBINHIBITORY CONCENTRATIONS OF ETHYLMETHYLHYDROXYPYRIDINE SUCCINATE ON BIOFILMS OF MICROORGANISMS

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Abstract. Biofilm microorganisms cause about 80% of chronic infections, leading to increased morbidity, increased hospital stays and enhanced health care costs. Biofilms can also grow on abiotic surfaces, including orthopedic prostheses, stents, and catheters. Antibiotics are not effective enough to treat biofilm infections, and antibiofilm agents are being sought among other classes of compounds, in particular antioxidants, including the well-known drug ethylmethylhydroxypyridine succinate (mexidol). The aim of the research was to study the effect of mexidol on the formation of biofilms by test strains of microorganisms. Clinical strains of bacteria (*E. coli* 311, *P. aeruginosa* 449, *S. aureus* 222) and fungi (*C. albicans* 1486) were used. The antimicrobial activity of mexidol was determined by serial dilutions in liquid media in the concentration range of 1.5-200 μg/ml. Determination of the microorganisms ability to form biofilm was performed according to the known O'Toole method. A degree of the biofilm formation intensity was determined as described by Stepanovic S. et al. The potential antibiofilm activity of mexidol was studied at concentrations of 5–200 μg/ml. Statistical processing of the results was performed using Newman-Keuls criterion by the software StatSoft Statistica 6.0. It was shown that for planktonic forms of *E. coli, P. aeruginosa, S. aureus*, and *C. albicans*, the minimum inhibitory concentration of mexidol is more 200 μg/ml. *E. coli* 311 and *P. aeruginosa* 449 show a strong ability to form biofilms, *S. aureus* 222 – the medium ability. *C. albicans* 1486 does not adhere to the abiotic surface and was no longer used. Mexidol enhances the biofilm production by *E. coli* 311, increasing the biomass of biofilms by 12–15%. The drug increases the biomass of *P. aeruginosa* biofilms by 33–110% against control, and this is most pronounced at the minimum concentration of the drug. Mexidol also stimulates the formation of *S. aureus* 222 biofilms by 2.1-2.7 times compared to the control.

Therefore, under the action of the drug at subinhibitory concentrations, stimulation of the biofilm formation by gram-negative (*E. coli* 311, *P. aeruginosa* 449) and gram-positive bacteria (*S. aureus* 222) is observed. The identified

ability of mexidol to stimulate biofilm formation requires further research to assess the feasibility of its use in the complex therapy of purulent-inflammatory diseases.

Key words: ethylmethylhydroxypyridine succinate, mexidol, microorganisms, clinical strains, biofilm.

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The authors declare no conflict of interest.

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