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PECULIARITIES OF METABOLIC PARAMETERS IN THE HEALING OF WOUNDS COMPLICATED BY EXPERIMENTAL CHRONIC KIDNEY DISEASE

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The work is devoted to the study of the features of changes in the main indicators of metabolic processes during the healing of experimental wounds complicated by chronic kidney disease. Histological examination showed a significant violation of the morphological and functional state of the kidneys in rats with chronic kidney disease. There was a decrease in the levels of glucose, urea, uric acid in the blood serum of experimental animals 1 week after the infliction of wounds in comparison with similar indicators of animals in the control group. In rats, 4 weeks after surgery, the concentration of urea and uric acid remained reduced, while the glucose level almost recovered to the level of the control group, and the creatinine content significantly increased in comparison with the control. Inflammatory processes in the renal tissue lead to metabolic disorders during the healing of wounds complicated by chronic kidney disease, which is reflected by statistically significant changes in the levels of biochemical parameters.

Key words: wound healing, concomitant diseases, kidney damage, biochemical indicators, metabolic disorders.

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ОСОБЛИВОСТІ ПОКАЗНИКІВ ОБМІНУ ПРИ ЗАГОЄННІ РАН, УСКЛАДНЕНИХ ЕКСПЕРИМЕНТАЛЬНОЮ ХРОНІЧНОЮ ХВОРОБОЮ НИРОК

Робота присвячена вивченню особливостей змін основних показників обмінних процесів при загоєнні експериментальних ран, ускладнених хронічною хворобою нирок. Гістологічне дослідження показало значне порушення морфологічного та функціонального стану нирок у щурів із хронічною хворобою нирок. Відзначено зниження рівнів глюкози, сечовини, сечової кислоти в сироватці крові експериментальних тварин через 1 тиждень після нанесення ран в порівнянні з аналогічними показниками тварин контрольної групи. У щурів через 4 тижні після моделювання ран концентрація сечовини і сечової кислоти залишилася зниженою, в той час як рівень глюкози практично відновився до рівня аналогічних показників контрольної групи, а вміст креатиніну підвищувався в порівнянні з контролем. Показано, що запальні процеси у нирковій тканині призводять до порушень обміну при загоєнні ран, ускладнених хронічною хворобою нирок, що відображаються статистично достовірними змінами рівнів біохімічних показників.

Ключові слова: загоєння ран, супутні захворювання, ураження нирок, біохімічні показники, порушення обміну речовин.

The study is a fragment of the research project "Peculiarities of cellular-molecular mechanisms of activation of reparative processes in case of tissue damage against the background of a decrease in adaptation reserves characteristic of emergency situations", state registration No. 0120U101408.

The treatment of wounds of various etiologies is a serious public health problem. Molecular-cellular mechanisms, leading to the deterioration of wound healing, have not been sufficiently studied. Wound healing is a complex process of restoring the structure and function of damaged tissue. It is based on cooperation, which covers many types of cells, both locally, at the wound site, and also systemically. At the same time, in this field of research, there are very few animal models that can provide a targeted approach to the mechanisms underlying the formation of chronic wounds. This presents a certain complexity in the transition from experiment to clinical trials.

The normal process of wound healing is dynamic, complex, and consists of several stages: inflammation, proliferation and remodeling. The phase of inflammation is characterized by an increase in blood levels of growth factors and pro-inflammatory cytokines, such as IL-1, IL-6, IL-8, TNF-α, etc. Events occurring during the proliferative phase include angiogenesis, collagen deposition, epithelialization, and reduction of the wound [6, 7]. Numerous factors, including systemic ones, caused, for example, by concomitant diseases, can cause disruption of the healing process and lead to the development of chronic wounds. For example, renal dysfunction has numerous consequences for wound healing [8]. In mice against a background of experimental chronic kidney disease (CKD), a significant reduction in re-epithelialization and the rate of precipitation of the granulation tissue was observed. A decrease in the intensity of cell proliferation and angiogenesis was observed. Which were accompanied by a simultaneous increase in the level of inflammation compared with control animals [13]. It should also be borne in mind that impaired renal tissue remodeling is caused by an imbalance between cell proliferation and apoptosis [10]. All this contributes to a significant distortion of the course of regenerative processes. The mechanisms for the development of such violations of reparative regeneration have not been investigated completely.

The purpose of the work was to study of the features of changes in the main indices of metabolic processes in the healing of experimental wounds complicated by chronic kidney disease.

Materials and methods. The experimental study was carried out in 3 groups (6 animals) of white rats weighing 250±30 grams at the age of 9 months. The experiments were carried out in accordance with

the principles of the "European Convention for the Protection of Vertebrates used for experimental and other scientific purposes" (Strasbourg, 1986) and the "General principles of animal experiments" approved by the First National Congress on Bioethics (Kyiv, 2001). The study was approved by the Committee on Ethical Animal Care and Use of the Kharkiv Medical Academy of Postgraduate Education, Ukraine (Protocol No. 5 dated November 12, 2019).

Comparison group (control group) – rats with CKD. CKD developed 8 weeks after a single administration of a 50 % solution of glycerin at a dose of 10 ml/kg of body weight of the animal [15]. Intramuscular administration of glycerol leads to toxic damage to both the glomerular and tubular renal apparatus [2].

The model of the chronic wound in the form of a circle with a diameter of 20 mm in the inter-blade area was reproduced in experimental groups 1 and 2 consisting of rats with CKD [3]. Wound healing throughout the experiment took place under the scab. Animals were removed from the experiment the first (for group 1) and fourth (for group 2) weeks after the wound was applied. The blood for study has been taken from the heart.

The histological examination included samples of the kidneys of experimental animals. The material was fixed in 10 % neutral formalin. Dehydrated in alcohols of increasing strength (50°, 70° and twice 96°), then in alcohol with chloroform, chloroform, was embedded in paraffin [1]. The prepared sections 5–7 microns thick were stained with hematoxylin and eosin, and according to Van Gieson. The preparations were analyzed in the field of view of the "PrimoStar" microscope (Zeiss). Imprints of the preparations were carried out using a Microocular digital camera.

Serum glucose, uric acid, creatinine, and urea levels were determined using diagnostic kits for clinical biochemistry SpineLab (Ukraine). Statistical processing of the results was carried out with the statistical analysis package Statistica 6.0 (StatSoft Inc., USA). To describe the results obtained, the data were presented in the form M±SE, where M is the arithmetic mean; SE is the standard error of the arithmetic mean. The reliability of differences between groups (statistical significance) was determined using the nonparametric ANOVA Kraskel–Wallis criterion for independent samples. Statistically significant differences were considered in cases that $p<0.05$. Histograms were plotted by GraphPad Prism 9 (GraphPad Software, USA).

Results of the study and their discussion. Histological examination of the kidneys of experimental rats revealed significant structural changes, indicating a violation of the excretory function of the organ. First of all, manifestations of impaired blood circulation in the organ and rheological properties of blood were noted. Thus, diffuse venous-capillary plethora is noted in all the specimens, blood separation into plasma and uniform elements, erythrostases are observed in the dilated vessels. (fig. 1).

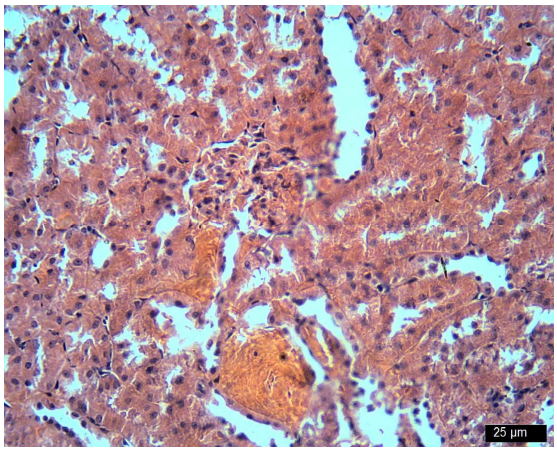

Fig. 1. The site of rat's kidney. Venous-capillary plethora. Dystrophy of the epithelium. Hematoxylin and eosin.

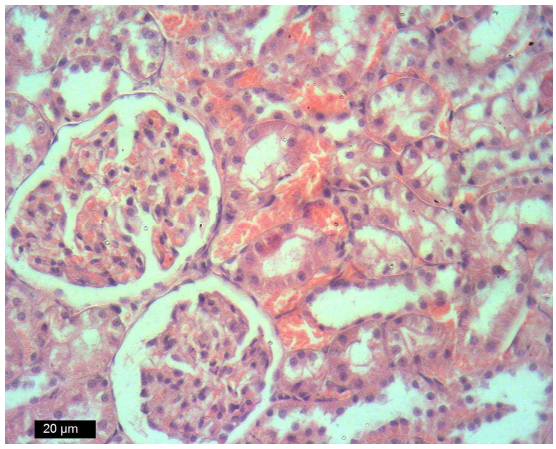

Fig. 2. The site of rat renal cortex Shape of glomeruli is "branches". Capillaries are full of blood. Dystrophy of the epithelium. Hematoxylin and eosin.

The structure and shape of most of the renal glomeruli are preserved, but on the sites, there are globules of a "branched" form, which is also a manifestation of microcirculatory disorders. Polygonal and wrinkled glomeruli with enlarged capsule lumen, indicative of edema and atrophy were observed. Nephrothelium of proximal and distal tubules with signs of destructive-dystrophic disorders was observed. In some tubules, the cytoplasm of epithelial cells is granular, edematous, the lumens of these tubules are narrowed. The lumens of others are expanded due to the rejection of the apical site of the cytoplasm of nephrothelial cells, which creates the effect of "bare cores" (fig. 2).

Furthermore, nephrosclerosis centers in the cortex were noted. Connective tissue with a large number of fibroblasts with large, brightly colored functionally active nuclei, grows in a destructively altered renal epithelium, gradually replacing it. In the lumens of the tubules of the cortical and medullar substance, there are weakly-granular masses, the remains of the ejaculated epithelium, a homogeneous pink colloid-like substance, and calcinates (fig. 3).

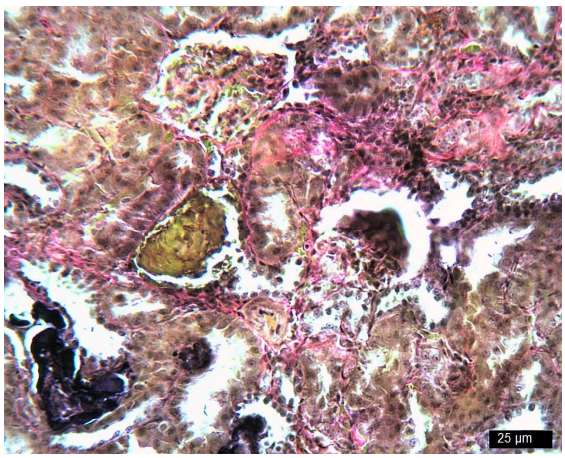

Fig. 3. The site of rat renal cortex. There are nephrosclerosis centers. The shape of the glomerulus is polygonal. Calcination and colloid-like substance in the lumens of the tubules. Van Gieson.

Thus, a histological study indicates a violation of metabolic processes in the tissues of the kidneys. Studies have shown that the healing of wounds complicated by chronic kidney disease led to a change in the biochemical parameters in the blood serum of experimental animals (fig. 4).

The study of the biochemical composition of the blood allows you to monitor the condition of the body in the process of wound healing to correct treatment tactics.

The glucose level in the rats of experimental group 1 decreased sharply in comparison with the control and practically recovered 4 weeks after the model wounds. One of the reasons for the decrease in this indicator after an injury is due to the increased absorption of glucose in the conditions of wound healing [14]. As the wounds healed, the glucose level in group 2 animals was practically restored to the initial concentration.

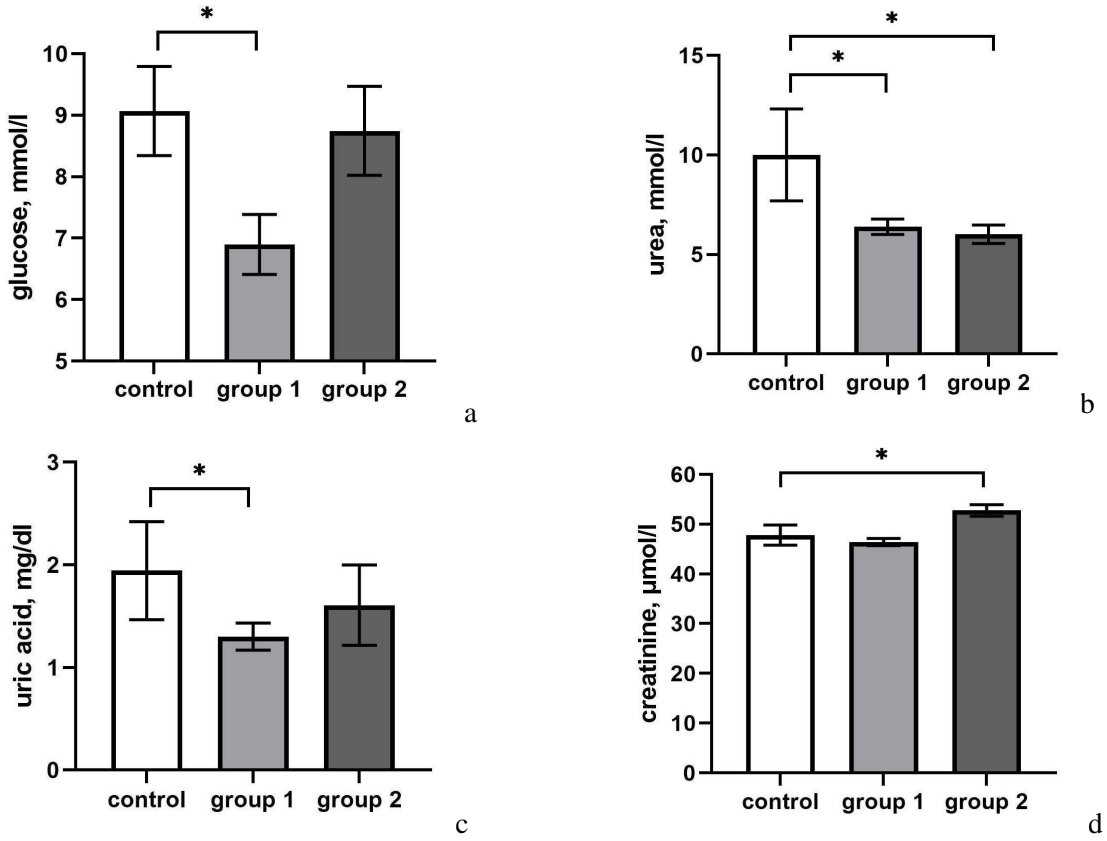

Fig. 4. Levels of the studied biochemical parameters in the blood serum of animals: (a) glucose, (b) urea, (c) uric acid, (d) creatinine (*$p < 0.05$). The error bars represent the standard error of the arithmetic mean for each indicator (n=6).

The level of urea in the rats of experimental groups 1 and 2 decreased compared with the animals of the control group. Similar trends were observed in the study of uric acid. One week after the wounds were made, the level of uric acid was significantly lower than that of the rats of the comparison group. Four weeks after the operation, a slight increase in the concentration of uric acid was observed. The concentration of urea in the blood depends on the rate of protein metabolism, liver and kidney function. Since the liver is the main place of urea formation, when its function decreases in response to damage, the urea level also falls. Uric acid plays an important role in maintaining the inflammatory process on surfaces where tissue necrosis is present and in triggering the inflammatory cascade [5]. Disturbances in the liver also lead to a lack of activity of enzymes involved in the formation of uric acid. This may be due to both the development of the inflammatory process initiated by CKD, and the decrease in the level of physiological reserves of body adaptation. On the other hand, the decrease in urea and uric acid in the blood serum of experimental animals can be explained by the enhanced synthesis of proteins necessary for wound healing. At the same time, the breakdown of proteins slows down, which leads to a decrease in the formation of final products of nitrogen metabolism. Such a significant decrease in the level of these

metabolites, combined with a decrease in the rate of wound healing, suggests that the primary cause of this phenomenon is CKD, while the other processes described above are a consequence. At the same time, uric acid is closely associated with the onset and development of oxidative stress, especially in patients with CKD. Decreased uric acid levels can annul and partially reverse cellular damage caused by oxidative stress [12].

The serum creatinine content of the animals decreased one week after the application of wounds in comparison with the animals of the control group. After 4 weeks, the level of creatinine significantly increased in comparison with the control. It should be borne in mind that creatinine is formed when protein compounds are destroyed, and its blood indices depend on the general condition of the muscular system and the adequacy of the excretory function of the kidneys. Since in our experiment the excretory function of the kidneys was reduced, a decrease in the level of serum creatinine in animals of group 1 indicates a disturbance in the metabolic processes accompanied by protein metabolism disorders and indicates a slowdown in the breakdown of proteins in the body. If in the case of a decrease in the level of creatinine, renal dysfunctions do not have a particular effect on the concentration of this metabolite in the blood, then the hypercreatinemia, which is observed in animals of group 2, mainly depends on the functional ability of the kidneys. Elevated serum creatinine levels in experimental animals in this group may be due to decreased renal function. While low serum creatinine levels in some clinical situations can be considered an indicator of protein-energy wasting [9]. These assumptions are also confirmed by a slight increase in the level of urea and uric acid in the serum of the rats of group 2. Probably, the decrease in the studied parameters in the blood serum of animals of group 1 is the compensatory reaction of the organism to damage. With wound healing (group 2), some recovery of carbohydrate and protein metabolism occurs, but there are signs of progression of kidney damage.

The healing of wounds passes through several overlapping phases: beginning with the migration and proliferation of cells, precipitation of the extracellular matrix, and ending with the remodeling of newly formed tissues. Slowing down one or more phases of the healing process leads to chronic wounds [4]. Wounds in CKD are characterized by a longer phase of inflammation and a slower rearrangement of the components of the extracellular matrix. These are disorders contributing chronization of the wound. CKD, which is based on the inflammatory process, leads to the depletion of reserves, used for the physiological course of the wound process. Chronic inflammation, leading to an imbalance between pro-inflammatory cytokines, chemokines, and their inhibitors, causes violations of the molecular and cellular mechanisms of repair at all its stages [11]. The normal process of wound healing is determined by the balance of the production processes and the degradation of protein structures, however, in our study, this balance is violated. The violation of carbohydrate metabolism detected by us can cause an increase in the level of matrix metalloproteinases (MMPs) and is the reason for the slowing down of extracellular matrix formation processes [4].

Conclusion

Histological examination showed a significant violation of the morphological and functional state of the kidneys in rats with CKD. The destructive changes in the epithelium, parenchymal sclerosis, vascular congestion, stromal edema, the presence of tissue detritus, colloidal masses, and calcifications in the tubules indicated in the histopreparations reflected impaired renal excretion.

Inflammatory processes in the renal tissue lead to metabolic disorders during the healing of wounds complicated by CKD, which is reflected by statistically significant changes in the levels of biochemical parameters. Namely, a decrease in glucose and uric acid levels one week after wound modeling, an increase in creatinine concentrations four weeks after surgery, and a decrease in urea levels at the study periods.

The findings will provide a better understanding of the relationship between CKD and wound healing. Further study of the repair mechanisms and the factors influencing them will greatly facilitate the development of new more effective methods of treating patients with chronic wounds.

References

1. Zinatullin RM, Gizatullin TR, Pavlov VN, Kataev VA, Farhutdinov RR, Baymurzina YuL i dr. izobretatel; Zinatullin RM, Gizatullin TR, patentoobladatel. Sposob modelirovaniya troficheskoy ranyi v eksperimente. Patent 2510083 RF. 2014 Mar 20. [in Russian]

2. Sarkisov DS, Perova YuL. Mikroskopicheskaya tehnika. M.: Meditsina; 1996. 542 s. [in Russian]

3. Tseboeva AA, Kokaev RI, Bibaeva LV, Oganesyan DH, Malikiev IE, Gutsaeva EA, et al. Vozmozhnosti primeneniya kletochnoy terapii na fone toksicheskogo nefrita. Fundamentalnyie issledovaniya. 2014; 10-7: 1394–1398. [in Russian]

4. Eming SA, Martin P, Tomic-Canic M. Wound repair and regeneration: Mechanisms, signaling, and translation. Sci Transl Med, 2014; 6(265): 265sr6. doi:10.1126/scitranslmed.3009337.

5. Fernandez ML, Upton Z, Shooter GK. Uric acid and xanthine oxidoreductase in wound healing. Curr Rheumatol Rep. 2014; 16(2): 396. doi: 10.1007/s11926-013-0396-1.

6. Hop MJ, Polinder S, Vlies CH, Middelkoop E, Baar ME. Costs of burn care. Wound Repair Regen. 2014; 22: 436–450. doi:10.1111/wrr.12189.

7. Lai HY, Lim YY, Kim KH. Potential dermal wound healing agent in Blechnum orientale Linn. BMC Complement Altern Med. 2011; 11: 62. doi:10.1186/1472-6882-11-62.

8. Maroz N, Simman R. Wound healing in patients with impaired kidney function. J Am Coll Clin Wound Spec. 2013; 5(1): 2–7. doi:10.1016/j.jccw.2014.05.002.

9. Park J, Mehrotra R, Rhee CM, Molnar MZ, Lukowsky LR, Patel SS, et al. Serum creatinine level, a surrogate of muscle mass, predicts mortality in peritoneal dialysis patients. Nephrol Dial Transplant. 2013; 28(8): 2146–55. doi: 10.1093/ndt/gft213.
10. Pavlov SB, Babenko NM, Kumetchko MV, Litvinova OB, Semko NG, Mikhaylusov RN. The influence of photobiomodulation therapy on chronic wound healing. Rom Rep Phys. 2020; 72: 609.
11. Pavlov S, Babenko N, Kumetchko M, Litvinova O, Semko N, Pavlova O. Intercellular mediators in bone remodeling regulation in the experimental renal pathology. Actual osteol. 2019; 15(3): 180–191.
12. Roumeliotis S, Roumeliotis A, Dounousi E, Eleftheriadis T, Liakopoulos V. Dietary Antioxidant Supplements and Uric Acid in Chronic Kidney Disease: A Review. Nutrients. 2019; 11(8): 1911. doi: 10.3390/nu11081911.
13. Seth AK, De la Garza M, Fang RC, Hong SJ, Galiano RD. Excisional wound healing is delayed in a murine model of chronic kidney disease. PLoS ONE. 2013; 8(3): e59979. doi:10.1371/journal.pone.0059979.
14. Vinaik R, Barayan D, Auger C, Abdullahi A, Jeschke MG. Regulation of glycolysis and the Warburg effect in wound healing. JCI Insight. 2020; 5(17): e138949. doi:10.1172/jci.insight.138949
15. Vlahović P, Cvetković T, Savić V, Stefanović V. Dietary curcumin does not protect kidney in glycerol-induced acute renal failure. Food Chem Toxicol. 2007; 45: 1777–1782. doi:10.1016/j.fct.2007.04.004.

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ANTIMICROBIAL ACTIVITY OF SURFACTANCES OF BACTERIA *NOCARDIA*, *RHODOCOCCUS* AND *ACINETOBACTER* GENERA

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It was found that the minimum inhibitory concentrations against bacteria and yeast of *Acinetobacter calcoaceticus* IMV B-7241, *Rhodococcus erythropolis* IMV Ac-5017 and *Nocardia vaccinii* IMV B-7405 surfactants, synthesized on traditional substrates, were 9–120 μg/ml and were within the limits defined for the surfactants known in the world. It was for the first time established that surfactants synthesized by the study strains on wastes of biodiesel production and fried sunflower oil were characterized by high antimicrobial activity against bacteria and yeast (minimum inhibitory concentrations 0.45–120 and 1.9–142 μg/ml respectively). It was found that the added of both live and inactivated *Escherichia coli* IEM-1 and *Bacillus subtilis* BT-2 cells in *R. erythropolis* IMV Ac-5017 and *N. vaccinii* IMV B-7405 medium cultivation was accompanied by synthesis of surfactants, minimum inhibitory concentrations of which were several times lower than those showed for surfactants synthesized without competitive microorganisms. The obtained results indicate the possibility of using the studied surfactants as effective antimicrobial agents.

Key words: microbial metabolites, antimicrobial agents, minimum inhibitory concentrations, wastes of biodiesel production, fried sunflower oil, competitive microorganisms.

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АНТИМІКРОБНА АКТИВНІСТЬ ПОВЕРХНЕВО-АКТИВНИХ РЕЧОВИН БАКТЕРІЙ РОДІВ *NOCARDIA*, *RHODOCOCCUS* ТА *ACINETOBACTER*

Встановлено, що мінімальні інгібуючі концентрації щодо бактерій і дріжджів поверхнево-активних речовин *Acinetobacter calcoaceticus* IMB B-7241, *Rhodococcus erythropolis* IMB Ac-5017 та *Nocardia vaccinii* IMB B-7405, синтезованих на традиційних субстратах, становили 9–120 мкг/мл і перебували в межах визначених для відомих у світі поверхнево-активні речовини. Вперше встановлено, що поверхнево-активні речовини, синтезовані досліджуваними штамами на відходах виробництва біодизелю та пересмаженій соняшниковій олії, характеризувалися високою антимікробною активністю щодо бактерій та дріжджів (мінімальна інгібуюча концентрація 0,45–120 та 1,9–142 мкг/мл відповідно). Встановлено, що внесення як живих, так і інактивованих клітин *Escherichia coli* IEM-1 та *Bacillus subtilis* у середовище культивування *R. erythropolis* IMB Ac-5017 та *N. vaccinii* IMB B-7405 супроводжувалося синтезом поверхнево-активних речовин, мінімальні інгібуючі концентраціїяких були у кілька разів нижчими порівняно з показниками, встановленими для поверхнево-активних речовин, синтезованими у середовищі без сторонніх мікроорганізмів. Одержані результати засвідчують можливість використання досліджуваних ПАР як ефективних антимікробних агентів.

Ключові слова: мікробні метаболіти, антимікробні агенти, мінімальні інгібуючі концентрації, відходи виробництва біодизелю, пересмажена соняшникова олія, конкурентні мікроорганізми.

The study is a fragment of the research project "Complex microbial preparations for multifunctional purposes: from the regulation of biosynthesis and properties to the prospects for practical use", state registration No. 0116U001530.

Over the past decades, the number of multidrug-resistant extremely antibiotic resistant pathogenic bacteria has been increasing, which is primarily due to the uncontrolled use of these drugs and non-compliance with therapeutic doses. [2]. Some experts predict that in 2050, the death rate from infectious diseases caused by antibiotic-resistant pathogens may reach 10 millions people. [8]. This situation creates