V. V. NAGAYCHUK INFLUENCE OF BIOACTIVATION ON THE BACTERIA E.COLI AND HEMOLYTIC STAPHYLOCOCCUS (INFORMATION 3)

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The article contains the research results of the influence of the currents of minor intensity without external sources of power on E. coli bacteria and hemolytic staphylococcus. It has been proved that the use of bioactivation in complex therapy is effective and available non-pharmacological method of influence on the micro flora of thermal wounds.

Key words: burns, bioactivation, xenoskin.

У статті викладені результати вивчення впливу струму малої інтенсивності без зовнішніх джерел на культуру E.coli та гемолітичного стафілококу. Доведено, що використання біоактивації в комплексній терапії є ефективним і доступним безмедикаментозним методом впливу на мікрофлору опікових ран.

Ключові слова: опіки, біоактивація, ксеношкіра.

В статье изложены результаты изучения влияния токов малой интенсивности без внешних источников на культуру E.coli и гемолитического стафилококка. Показано, что использование биоактивации в комплексной терапии есть эффективным и доступным без медикаментозным методом влияния на микрофлору ожоговых ран.

Ключевые слова: ожоги, биоактивация, ксенокожа.

Introduction

Nosocomial infection has always been one of unresolved issues of combustiology [1]. The existing antibacterial pharmaceuticals do improve the outcomes of thermal injury treatment [4], but infection was and remains the main reason of complications and lethal outcomes [3]. That is why the search for non-pharmacological, efficient and available means and methods of influence on the micro flora of thermal wound still remains a very important task of contemporary combustiology [2].

Materials and methods. Antibacterial action of bioactivation (BA) was studied in the series of researches of 12 experiments. In accordance with the requirements of microbiological experiment, we studied the character of influence of bioactivation on the development of E. coli bacteria on meat-peptonic agar and compared it with already known influence of bioactivation on hemolytic staphylococcus. For this purpose, we located standardized discs with antibiotics (including ceftriaxonum) and standardized discs with electrodes donors (DE) and electrodes acceptors (AE) of electrons on the surface of the agar of Petri dish, modeling various conditions of influence of BA on bacterial test-objects. The agar had been previously seeded from the standardized, according to optical standard of transparency (500 thousands of bacteria in 1 ml.), suspension with E. coli bacteria. For the electrode DE we used ultra clean copper plate, and for the electrode AE – specific aluminum-magnesium-zinc alloy.

The action of BE on E. coli was studied in the conditions of closed and disconnected electric circuit. Antibacterial efficiency of a standard disc with ceftriaxonum and hemolytic staphylococcus served as the control for the antibacterial efficiency of BA influence on E. coli. In order to evaluate sensitivity of E. coli to antibiotics during durable influence of BA, we made and patented the device (fig. 1, Ukrainian patent N_{2} 43358).

The essence of the research. E.Coli bacteria were in the test tubes with saline solution between the electrode pair DE-AE, which conditioned influence of BA with the strength up to 40 mcA, and voltage 0,03 V under conditions within thermostat with the temperature 36^{0} C during 24 hours. Analogical strains of E. coli without bioactivation

served as the control factors. In 24 hours, from each series of test tubes we took 0,1 ml. of E. coli, diluted with saline solution to 1000 times, seeded in the Petri dishes on meatpeptonic agar, where we located standardized discs with antibiotics and placed back into thermostat for 24 hours with the temperature 36° C.



Fig.1 The device for the research of the influence of bioactivation on the cultures of various microorganisms: support -1, test tubes with a culture of microorganisms -2, electrode-donor of electrons -3, electrode-acceptor of electrons -4, measuring device -5, conductor -6.

At the end of observation, we counted the number of cultivated colonies in investigated and control test tubes and their sensitivity to antibiotics with the diameter of culture growth inhibition.

Discussion. The results of the research indicated that the diameter of growth inhibition of E. coli under standard discs with ceftriaxonum was $25,3\pm 0,1$ mm (P<0,05), that by 12,6% exceeded the diameter of growth inhibition of hemolytic staphylococcus under analogical discs (fig. 2). Antibacterial influence of BA on E. coli in the conditions of closed circuit was insignificant and practically did not depend on functional activity of the electrode pair.



Fig.2. Diameter of growth inhibition of E. coli (1) and hemolytic staphylococcus (2) on the agar under standard discs with ceftriaxonum.



Fig.3. Diameter of growth inhibition of E.coli (1) and hemolytic staphylococcus (2) on the agar under the electrodes DE and AE under conditions of closed circuit.

Under these conditions, the diameter of growth inhibition was $9,3\pm0,4$ mm under the electrode DE and $10,2\pm0,6$ mm under the electrode AE was in 2,7-2,5 times weaker, in comparison with antibacterial influence of the standard disc with ceftriaxonum (fig. 3).

Analogical influence on hemolytic staphylococcus points to the dependency of lysogenic phenomenon of BA on the nature of the electrodes DE-AE that form it. It was identified that the zone of growth inhibition on meatpeptonic agar under the electrode AE was in 3,2 times smaller than under the electrode DE ($8,5\pm0,7$ mm against $27,2\pm2,4$ mm; (P < 0,001). Bactericidal influence under the electrode DE was in 1,2 times stronger, in comparison with the standard disc with ceftriaxonum and in 2,9 stronger, in comparison with the antibacterial influence on E. coli. Under the electrode AE the antibacterial action against E. coli was in 16,7% stronger in comparison with the antibacterial influence on hemolytic staphylococcus.



Fig. 4. Diameter of E. coli (1) and hemolytic staphylococcus (2) growth inhibition on the agar under the electrodes DE and AE under the conditions of disconnected circuit.



Antibacterial influence of BA on E. Coli under the conditions of disconnected circuit (fig. 4) was insignificant, did not depend on the nature of bioactivational electrodes $(8,1\pm0,1 \text{ i } 9,2\pm0,3 \text{ mm})$ and in 3,1–2,8 times smaller in comparison with antibacterial influence of the standard disc with ceftriaxonum.

Another picture was being observed during the study of antibacterial influence on hemolytic staphylococcus of the electrode pair DE-AE under conditions of disconnected circuit. Under the electrode DE antibacterial influence on E. coli was stronger only in 1,1 times in comparison with the bacteria of hemolytic staphylococcus, while under the electrode AE it was weaker in 2,2 times (P<0,05). In order to verify the validity of the received results we located the electrode pair DE-AE on different Petri dishes (fig. 5).

Antibacterial influence of BA on the E. coli bacteria $(7,4 \pm 0,9 \text{ mm})$ in different Petri dishes under the electrode DE was analogous to its influence on hemolytic staphylococcus (fig. 6).



Fig.5. Diameter of E. Coli growth on the agar under the electrodes in different Petri dishes.



Fig.6. Diameter of hemolytic staphylococcus growth on the agar under the electrodes DE and AE in different Petri dishes.

Under the electrode AE, antibacterial action of BA was in 27, % stronger in comparison with ceftriaxonum (7,4±0,9 mm counter 25,3±0,1mm; P< 0,001). Antibacterial influence on the bacteria of hemolytic staphylococcus under the electrode DE did not change and reached 7,4±0,6 mm, while under the electrode AE decreased in 41,0% (12,1±1,4 mm counter 20,5±3,1mm; P< 0,05). The change of bactericidal efficiency under the electrode AE in the conditions of disconnected circuit in one Petri dish testifies to the possibility of the formation of separate specific circuits through (over) the agar.

The simultaneously conducted series of researches on the study of bactericidal efficiency of xenoskin on E. coli bacteria under the conditions of disconnected circuit. For this purpose, separate areas of previously seeded E. coli on meat-peptonic agar were covered with standard discs of xenoskin with standard discs of the electrodes DE and AE on them (fig. 7).



Fig.7 Diameter of E. Coli (1) and hemolytic staphylococcus (2) growth inhibition on the agar under xenoskin in the conditions of disconnected circuit.



Bactericidal influence of xenoskin on E. coli bacteria under disconnected circuit was insignificant. Thus, the zone of influence under the electrode DE was $12,3\pm1,7$ mm, which in 1,2 times stronger in comparison with the electrode AE ($12,3\pm1,7$ mm counter $10,0\pm0,7$ mm; P>0,05), but in 28% weaker in comparison with antibacterial action against hemolytic staphylococcus under the electrode DE ($12,3\pm1,7$ mm counter $15,8\pm1,9$ mm; P>0,05). Under the electrode AE, the bactericidal influence on E. coli bacteria was

in 1,7 times weaker in comparison with bactericidal influence on the bacteria of hemolytic staphylococcus ($10,0\pm0,7$ mm counter $16,6\pm1,5$ mm; P<0,01).

In addition, antibacterial action of xenoskin against E. coli grew under the positive potential of DE in 34,1% in comparison with antibacterial action of the electrode DE in the conditions of disconnected circuit (12,3 \pm 1,7 mm counter 8,1 \pm 0,1 mm; P>0,05), while antibacterial action of xenoskin against E. coli under the negative potential of AE grew only in 8,0% in comparison with antibacterial influence of the electrode AE in the conditions of disconnected circuit (10,0 \pm 0,7 mm counter 9,2 \pm 0,3 mm; P>0,05).

In the conditions of disconnected circuit, antibacterial influence on the bacteria of hemolytic staphylococcus under xenoskin was significant and comparatively equivalent (15,8±1,9 mm under the electrode ΔE and 16,6±1,5 mm under the electrode AE). However, antibacterial influence on the hemolytic staphylococcus under xenoskin with DE was in 2,1 times stronger in comparison with the electrode DE (15,8±1,9 mm counter 7,4±1,2 mm; P<0,01) in the conditions of disconnected circuit. Under xenoskin with the negative potential of AE it decreased in comparison with the electrode AE in 1,3 times and was 16,6±1,5 mm counter 20,5±3,1mm; (P>0,05).

The results of the study of antibacterial action of xenoskin against E. coli bacteria in the conditions of closed circuit (fig. 8) in comparison with the hemolytic staphylococcus under the electrode DE testify, that it was in 1,9 weaker (11,8±1,3 mm counter 22,5±1,3 mm; P<0,001), and under the electrode AE in 1,7 times (10,9±1,1 mm counter 18,7±0,8 mm; P < 0,001).



Fig.8 Diameter of E. coli (1) and hemolytic staphylococcus (2) growth inhibition on the agar under xenoskin in the conditions of closed circuit.

High antibacterial influence of xenoskin on the bacteria of hemolytic staphylococcus under the electrode AE (18,7 \pm 0,8 mm) comes close to the influence of a standard disc with ceftriaxonum (22,1 \pm 0,2 mm), while under the electrode DE (22,5 \pm 1,3 mm) is equal to it, which conditions the appropriateness of BA

for the treatment of thermal wounds. In addition, after the BA, the sensitivity of E. coli to antibiotics grew (fig. 9) from 0 to 31,6%.

Thus, after the 24 hour's influence of BA, the sensitivity of E. coli to amikacinum grew in 8,7%, cefutaxinum in 13,3%, gentamicinum and norfloxacinum in 20,0%, ceftriaxonum in 27,8% and cefoperazonum in 31,6%. However, there were no changes found in sensitivity of E. coli after BA.



Fig.9 Sensitivity of E.coli to antibiotics in the experiment (1) and control (2) samples



Conclusions and the perspectives of further researches

1. Antibacterial efficiency of the electrode pair DE-AE and xenoskin on E. coli ba-

cteria in the conditions of closed and disconnected circuits significant, which points to na tural resistance of to the currents of minor intensity.

2. Lysogenic influence of BA on E. coli bacteria does not depend on the nature of bioactivational electrodes. 3. Durable influence of BA increases the sensitivity of E. coli to antibiotics in 8,7-31,6%. That is why, the method can be recommended for the usage in complex therapy of pyoinflammatory diseases. The researches of BA influence on gram-negative micro flora of thermal wounds in strongly required for the complete research of its antibacterial efficiency.

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