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INFLUENCE OF BIOACTIVATION ON THE BLUE PUS BACILLUS (INFORMATION 2)

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The article contains the discussion of the results of the influence of bioactivation (BA) on the blue pus bacillus (Bacillus pynocyaneus). It has been proven that the usage of the current of minor intensity in complex therapy is effective and available non-pharmacological method of influence on the micro flora of thermal wound.

Key words: burn, bioactivation, blue pus bacillus.

У статті наведені результати впливу біоактивації (БА) на культуру синьо гнійної палички. Доведено, що використання струму малої інтенсивності в комплексній терапії є ефективним і доступним безмедикаментозним засобом впливу на мікрофлору опікових ран.

Ключові слова: опік, біоактивація, ксеношкіра, синьо гнійна паличка.

В статье приводятся результаты влияния биоактивации (БА) на культуру синегнойной палочки. Доказано, что использование токов малой интенсивности без внешнего источника энергии целесообразно в комплексной терапии ожоговых поверхностей.

Ключевые слова: ожог, биоактивация, ксеношкіра, синегнойная палочка.

Introduction

Nosocomial (clinical) infection has been one of the unresolved issues of combustiology. Though, the existing antibacterial drugs improve the results of treatment [4], the infection still remains the main reason of complications [2,3] and fatal outcomes [3]. Hence, the search for non-pharmacological, effective and available means and methods of influence on the micro flora of thermal wounds is an important task of contemporary combustiology.

Materials and methods. In order to study the sensitivity rate of the blue pus bacillus to antibiotics under durable influence of BA, we have developed and patented a special device (fig. 1; patent of Ukraine N 43358).

The change in the sensitivity rate of the blue pus bacillus (strain ATCC 27853) to antibiotics under durable influence of BA was being observed in a series of experiments. In each series, we conducted 12 researches on the development of the colonies of blue pus bacillus, which was test tubes with saline solution between the electrodes DE-AE with the factor of influence of current strength up to 40mcA, and the voltage 0,03V. The test tubes with bacteria were in the conditions of thermostat under the temperature +360C during 24 hours. In 24 hours, from every series of the test tubes, we took 0,1 ml of blue pus bacillus, added saline solution (1:1000) and seeded on meat-peptonic agar in the Petri dishes.



Fig.1 The device for the study of the BA influence on the cultures microorganisms: support 1, test tubes with microorganisms 2, electrode-donor of electrons (DE) 3, electrode-acceptor of electrons (AE) 4, measuring device 5, conductor 6.

On separate bacteria-inhabited areas we placed standard discs with antibiotics and put the dishes again in the thermostat with the temperature of +360C for 24 hours. In 24 hours we assessed the sensitivity of the blue pus bacillus to antibiotics according to the diameter of its

growth inhibition. Analogical culture of blue pus bacillus, which was not under the influence of BA served as the control sample.

Antibacterial efficiency of BA was studied according to its influence on the development of microbe colonies. For this purpose, on the previously seeded, according to the optical standard (500 thousands of microbe bodies in 1 ml) microbe suspension, we placed bioactivated and non-activated xenoskin, standard discs with ceftriaxonum and electrodes DE and AE, modeling various conditions of BA influence on the microbe testobjects. For the electrodes DE we used highly cleaned copper plates, and for the electrodes AE – specific aluminum-magnesium-zinc allows. The BA influence of the blue pus bacillus was studied in the conditions of closed and disconnected electric circuit. A standard disc with ceftriaxonum and hemolytic staphylococcus bacteria served as the control sample.

Results and discussion

The results of 12 research series showed that the number of colonies of blue pus bacillus in the research and control samples was analogical. At the same time, after BA the sensitivity rave of the microbes to antibiotics grew (fig. 2) from 14,3% to 50,0%. Thus, after 24 hours' BA their sensitivity to norfloxacinum grew in 14,3% times, gentamicinum and in 14,8%, amikacinum in 16,7%, cephasolinum in 30,0%, cephoperasonum in 30,8%, ceftriaxonum in 33,3%, cefuroxim in 27,8% and refampicin in 50,0%.

The research allowed to identify that the diameter of blue pus bacillus growth inhibition zone under bioactivated xenoskin (fig. 3) was $19,0\pm2,1$ mm, which was in 52,6% bigger than the diameter of growth inhibition under non-activated xenoskin (P<0,05). At the same time, the character of influence was more close to bacteriostatic action.

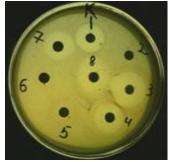


Fig.2 Sensitivity of blue pus bacillus to antibiotics of the research (1) and control (2) samples.



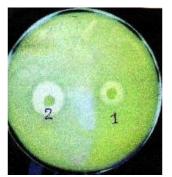


Fig. 3. Diameter of blue pus bacillus growth inhibition (1) hemolytic staphylococcus (2) under activated (a) and non-activated (b) xenoskin.

The antibacterial influence of bioactivated xenoskin on the bacteria of hemolytic staphylococcus possesses bactericidal character, was in 10,4% stronger in comparison with antibacterial influence on blue pus bacillus (21,2 \pm 1,9 mm versus 19,0 \pm 2,1 mm; P<0,055) and in 41,5% stronger in comparison with non-activated xenoskin (21,2 \pm 1,9 mm versus 12,4 \pm 0,9

mm, P<0,01). Throughout a number of experiments we were discovering that the blue pus bacillus growth inhibition diameter under standard discs with ceftriaxonum was $17,0\pm0,1$ mm (P<0,05), which was in 23,1% smaller than the diameter of growth inhibition of hemolytic staphylococcus under analogical discs (fig. 4).

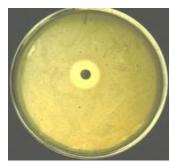
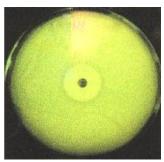


Fig.4 Diameter of growth inhibition of blue pus bacillus (1) and hemolytic staphylococcus (2) on the agar under standard disks with ceftriaxonum.



The antibacterial influence of BA on the blue pus bacillus in the conditions of closed circuit was insignificant (fig. 5), depended on the nature of the electrodes DE and AE and was in 1.5 times stronger under the electrode AE ($15,0\pm0.6$ mm versus $10,0\pm0.4$ mm, P<0,05).

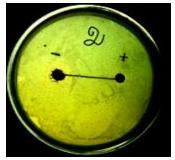
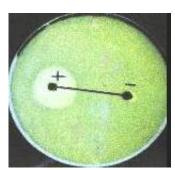
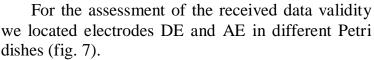


Fig.5 Diameter of growth inhibition of blue pus bacillus (1) and hemolytic staphylococcus (2) on the agar under the electrodes in the conditions of closed circuit.



At the same time analogical influence on the culture of hemolytic staphylococcus showed strong dependency of the lysogenic phenomenon of BA on the nature of the electrodes DE and AE. It was fixed, that the zone of lysis on meat-peptonic agar under the electrode DE was in 3,2 times stronger than under the electrode AE $(27,2\pm2,4 \text{ mm versus})$ 8,5±0,7 mm; P<0,001), and bactericidal effect under DE was in 1,2 times stronger in comparison with the disc with ceftriaxonum, and in 2,7 times stronger in comparison with bactericidal effect against blue pus bacillus. Under the negative electrode AE antibacterial influence on the blue pus bacillus was in 43,3% stronger than the influence on hemolytic staphylococcus.

Antibacterial influence of BA on blue pus bacillus in the conditions of disconnected circuit (fig. 6) was insignificant, depended on the nature of electrodes and was in 1,5 times stronger under the negative electrode AE $(12,1\pm0,3 \text{ mm versus } 8,0\pm0,1 \text{ mm})$ P<0,001). At the same time, the influence on the culture of blue pus bacillus under the electrode AE was in 1,1 times stronger than DE $(8,0\pm0,1 \text{ mm versus})$ 7.4 ± 1.2 mm; P>0.05) and in 2.1-1.4 times weaker than the disc with ceftriaxonum (12,1±0,3 mm -8,0±0,1 mm versus 17,0±0,1 mm; P<0,05).



circuit.

The antibacterial influence of BA on the culture of blue pus bacillus in the different Petri dishes was analogical and more substantial. Bactericidal action under the electrode AE in the first dish was only in 1,2 times stronger than under DE in the second one $(7,4\pm1,3 \text{ mm versus } 6,1\pm0,9 \text{ mm; } P>0,05)$. In other words, antibacterial influence under



Fig.6 The diameter of growth inhibition of blue pus bacillus (1) and hemolytic staphylococcus (2) on the agar under the electrodes in the conditions of disconnected

the electrode AE in different dishes was in 1,6 times weaker than the analogical influence of analogical pair in a single Petri dish (7,4 \pm 1,3 mm versus 12,1 \pm 0,3 mm; P<0,05). Under the electrode DE it was also weaker in 1,3 times (6,1 \pm 0,6 mm versus 8,0 \pm 0,1 mm; P>0,05).

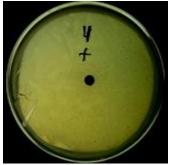
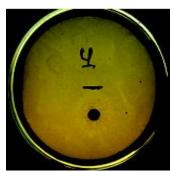


Fig. 7. Diameter of the growth inhibition zone of the blue pus bacillus on the agar under the electrodes DE (+) and AE (-) in different Petri dishes.

The antibacterial influence of the electrode pair on the culture of blue pus bacillus was also less apparent in comparison with the influence on the culture of hemolytic staphylococcus (fig. 8). Thus, under the electrode DE it was weaker in 1,2 times ($6,1\pm0,9$ mm versus $7,4\pm1,2$ mm; P>0,05), and under AE in 1,6 times ($7,4\pm1,3$ mm versus 12,1±1,4 mm; P<0,05). The efficiency was also smaller in 2,3-2,8 times in comparison with the antibacterial action of a standard disc with ceftriaxonum ($7,4\pm1,3$ mm – $6,1\pm0,9$ mm versus 17,0±0,1 mm; P<0,001). There was a significant decrease of the antibacterial influence of BA under disconnected circuit and the presence of electrodes



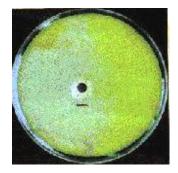


Fig. 8. *Diameter of growth inhibition* of hemolytic staphylococcus on the agar under the electrodes DE and AE in different Petri dishes.

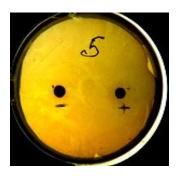
in different Petri dishes. This testifies to the formation of specific circuits of BA between electrodes and the agar.

There was conducted a series of experiments of the bactericidal influence of xenoskin on the culture of blue pus bacillus in the conditions of disconnected circuit. For this purpose, standard discs of xenoskin that had been placed on the culture of blue pus bacillus, were covered with the electrodes DE and AE (fig. 9). At the same time, in the conditions of disconnected circuit, the bactericidal influence of xenoskin on the culture of blue pus bacillus was insignificant (under DE 7,2±1,5 mm, and under AE 6,1±0,4 mm; P>0,05) and in 2,2 times weaker in comparison with the influence on hemolytic staphylococcus (7,2±1,5 mm versus 15,8±1,9 mm; P<0,01).

The antibacterial influence of xenoskin of the blue pus bacillus in the conditions of the disconnected circuit was also weaker than the influence of the standard disc with ceftriaxonum (7,2 \pm 1,5 mm – 6,1 \pm 0,4 mm versus 17,0 \pm 0,1mm; P<0,01).



Fig.9 Diameter of growth inhibition of blue pus bacillus (1) and hemolytic staphylococcus (2) on the agar under xenoskin in the conditions of disconnected circuit.



The results of the research of antibacterial action of xenoskin in the conditions of closed circuit (fig. 10) testified that under the electrode DE it was in 3,1 times $(7,2\pm1,1\text{mm versus } 22,5\pm1,3\text{ mm})$, and under the electrode AE in 1,5 times $(6,1\pm0,4\text{ mm versus } 16,6\pm1,5\text{ mm})$ weaker than the influence of BA on hemolytic staphylococcus.



Fig.10 Diameter of growth inhibition of blue pus bacillus (1) and hemolytic staphylococcus (2) under xenoskin in the conditions of closed circuit.



And finally, the bactericidal influence of xenoskin under the electrode DE in the conditions of closed and disconnected circuit did not change and was 7,2 mm. The influence grew simultaneously in 2,0 times under the electrode AE ($6,1\pm0,4$ mm versus 12,3 $\pm1,2$ mm; P<0,001). At the same time, in comparison with the antibacterial influence of a standard disc with ceftriaxonum it was weaker in 1,4-2,4 times (7,2 $\pm1,1$ mm – 12,3 $\pm1,2$ mm versus 17,0 $\pm0,1$ mm; P<0,05). The growth of sensitivity of blue pus bacillus to antibiotics in 14,3-50,0% under durable BA and its high antibacterial activity in relation to hemolytic staphylococcus in the conditions of closed circuit condition the necessity of BA for the clinical treatment of thermal injuries.

Conclusions and perspectives of further research

1. The antibacterial influence of the electrodes DE and AE, and xenoskin on the culture of blue pus bacillus in the conditions of the closed and disconnected circuit is moderate, which testifies to its resistance to the currents of minor intensity.

2.Lysogenic phenomenon of BA cultures of the blue pus bacillus depends on the nature of electrodes and it in 27,2% more apparent under the electrode AE.

3. The durable influence of BA increases microbes' sensitivity to antibiotics in 14,3-50,0% and conditions moderate bactericidal and bacteriostatic influence.

4. Bioactivation can be recommended for the usage in complex therapy of pyoinflammatory diseases. The complete research of the antibacterial efficiency of BA needs thorough studies of its influence on gram-negative micro flora.

References

- Belikov Yu. N., Iashvili B. P., Tsutskiridze N., Sanashvili K. I. Problema nozokomialnoy infektsii u tyazhelo obozhzhYonnyih. Podhodyi k antibakterialnoy terapii // Vestnik neotlozhnoy i vosstanovitelnoy meditsinyi. – 2005. – T.6, № 2. – P. 253–257.
- 2. Makats V.G. Biogalvanizatsiya v fizio i refleksoterapii//Vinnitsa. 1992. 236 p.
- 3. Nagaychuk V.I., Makats V.G., Povstyanoy N.E. Biogalvanizatsiya v kombustiologii // Vinnitsa .- 1993. 330 p.
- Teoriya i praktika mestnogo lecheniya gnoynyih ran / Bezugla O. P., Belov S. G., Gunko V. G. i dr.; Pod red. B. M. Datsenka. – K.: Zdorovya. 1995. – 384 p.
- 5. Usenko L.V. Sovremennyie podhodyi k ratsionalnoy antibakterialnoy terapii v usloviyah ORIT. Dnepropetrovsk, 2002. 34 p.
- De Jonge E., Schultz M., Spanjaard L. et al. Effects of selective decontamination of digestive tract on mortality and acquisition of resistant bacteria in intensive care: a randomized controlled trial // Lancet. – 2003. – Vol. 362. – P. 1011–1016.