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ePoster Viewing

Biofilm-related infections

Effect of ultrasound and ozonated saline on *Klebsiella pneumoniae*

Y. Mozgova¹, V. Myasoedov¹, M. Myshyna¹, N. Filimonova¹, V. Davydenko¹, N. Davydenko¹, Y. Myshyn¹

¹Kharkiv National Medical University, Kharkiv, Ukraine

Objectives. Ultrasonic radiation is widely used in complex therapy of bacterial inflammatory diseases and in physiotherapy. Antibacterial ultrasound therapy is an alternative to antibiotics and consists of selective oxidizing destruction of pathogenic microorganisms under combined influence of biological and physical factors. Controlled effect of ultrasonic radiation can be used to correct the number of bacteria in the microbial composition taken from foci of inflammation in septic complications after surgery, from catheters and drainage structures because only antimicrobial agents applying is ineffective due to formation of microbial biofilms. However, data about complex effect of ultrasound and ozonated saline on causative agents of inflammatory processes are rarely available, and such research is under scientific interest and may have practical significance.

Methods. *K. pneumoniae* suspension and culture medium after 10 minutes exposure to low-intensity ultrasonic waves (2 to 3 W/cm², the operating vibration frequency – 26.5 kHz, oscillations amplitude – from 50 to 80 μm) were inoculated in a well plates and incubated in a moist chamber overnight at temperature of 37 °C. The degree of aggregation of microbial cells was evaluated in units of optical density by spectrophotometer. Data were analyzed using «Statistica» program.

Results. During first experiment ozonated saline was added to *K. pneumoniae* suspension culture after its 10 minutes ultrasound exposure and plates were incubated. It was found that the optical density of biomass ($0,039 \pm 0,006$ units of optical density) decreased in 5.6 times compared with initial *K.pneumoniae* suspension ($0,22 \pm 0,02$ units of optical density) and in 13.3 times compared with controls ($0,52 \pm 0,05$ units of optical density), indicating a lack of ability to reproduce and marking a stage of bacteriolysis. Second experiment showed the complex action of ozonated saline and ultrasound on *K. pneumoniae* suspension culture. Obtained results were that optical density index of biomass ($0,18 \pm 0,09$ units of optical density) was little different from the initial concentration of the *K. pneumoniae* suspension ($0,22 \pm 0,02$ units of optical density), which could be explained by the simultaneous action of ultrasound and ozonated saline on cell wall of bacteria and release of lipopolisaccharide complexes that provide density of suspension (due to turbidity). When seeding the biomass on nutrient media was established the fact that CFU were not formed, indicating the non-viability of planktonic cells. Planktonic cells produced by formed *K. pneumoniae* biofilm, were unable to form new biofilms ($0,046 \pm 0,008$ units of optical density) compared with control values ($1,4 \pm 0,14$ units of optical density) and single influence of ultrasonic radiation ($0,813 \pm 0,02$ units of optical density).

Conclusion. Action of ultrasound in combination with ozonated saline is optimal for inhibition of the formation of *K. pneumoniae* biofilms.