Asian Journal of Scientific and Educational Research

№1(19), January-June, 2016

"Seoul National University Press"
2016

Asian Journal of Scientific and Educational Research

No.1. (19), January - June, 2016

VOLUME IX

"Seoul National University Press"

2016

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The effect of low-intensity ultrasound in combination with antimicrobial agents on biofilms of the pathogens of pyo-inflammatory diseases in children

Abstract: There was investigated the combined influence of low-intensity ultrasound and antimicrobials on biofilms of clinical strains of E. coli and S. aureus to assess the degree of disruption of daily biofilms, and to study their ability to produce plankton cells as a factor of dissemination of the pathogen. The breach of the integrity of daily biofilms of clinical strains of E. coli and S. aureus and decreased ability of the plankton cells production after the action of low-intensity ultrasound within 10-min processing combined with application of antimicrobial agents were determined. It was shown that only the action of low-intensity ultrasound during 10 min with subsequent use of antimicrobials led to the complete inactivation of plankton cells and disorganization of the biofilm of E. coli and S. aureus isolates.

Keywords: biofilm, E. coli, S. aureus, ultrasound, antimicrobials.

Relevance. The complex of curative measures aimed at the suppression of pathogens, stimulation of reparative processes, improvement of blood circulation etc. is used for the treatment of pyo-inflammatory diseases in modern pediatric surgery. At the same time, antibacterial agents that are widely used, greatly suppress the immune protecting mechanisms of a macroorganism and contribute to the activation of microflora mechanisms of adaptation, resulting in an emergence of poly-resistant pathogens. An acquired drug resistance of clinical strains of microorganisms occurs, on the one hand, due to acquisition of new genetic information or altering in the level of expression of own genes, and, on the other hand, is associated with the formation of biofilms [1, 2]. At present, it is proved that the basic condition of existence for bacteria is the biofilm form and the plankton form is considered to be a way to transfer microbial cells from the primary site of localization of the biofilm to another surface on which a new (secondary) biofilm is formed. Despite active scientific efforts to elaborate optimal schemes of antimicrobial therapy of pyo-inflammatory diseases, the problem of effective combating bacterial biofilms still remains relevant. Many scientists around the world are conducting studies concerning the mechanisms of formation of biofilms and means that will be able to block the formation of biofilms and suppress the production of plankton cells [3,4].

In clinical and experimental practice, the perspectives of the use of ultrasonic cavitation for the prevention and treatment of many different diseases, in particular inflammatory, have been determined. It is of particular interest to determine the associated effect of continuous low-intensity ultrasound and antimicrobials on the daily

biofilms disruption and the inhibition of the production of plankton cells of pathogenic microorganisms.

Despite the development of various schemes of antimicrobial therapy and implementation of new technologies to suppress pathogenic microorganisms, the problem of effective therapy of pyo-inflammatory processes remains urgent.

The objective of the study was to assess the degree of disruption of daily biofilms and ability to produce plankton cells of E. coli and S. aureus, which is as a factor of dissemination of the pathogen.

Materials and methods. Preparations of suspensions of E. coli and S. aureus with a certain concentration of microbial cells were carried out using an electronic appliance Densi-La-Meter (PLIVA-Lachema, Czech Republic) by a scale of McFarland in compliance with the instructions for the device. Suspension cultures of isolates were inoculated in 96-well polystyrene plates and thermostated over 1 day in order to receive biofilms, which then were disposed in the zone of action of ultrasound (ultrasonic waves of low intensity from 2 to 3 W/cm^2; operating frequency – 26,5 kHz; oscillation amplitude - from 50 to 80 μm) during 10 minutes [5] followed by the addition of antimicrobials, and then a conclusion about the degree of disorganization of biofilms and ability to produce plankton cells was made grounded on comparison of optical density of experimental and control series of biofilms formed, evaluated on the spectrophotometer "Multiskan EX 355" with 540 nm wave-length [6]. The result was expressed in standard units of optical density (absorbance units - AU). Statistical processing of the results was performed using the Excel program and Biostat [7].

Results of the research. Where was determined that after an exposure of E. coli daily biofilms to 10-min ultrasound handling the decrease of the optical density of the daily biofilm of 8 times in comparison with the optical density of the biofilm before the influence (0,185±0,04 and 1,473±0,18 AU, respectively) was found (see Fig. 1). Similar results were obtained after the manipulation over daily biofilms of S. aureus: 9-fold reduction of optical density in comparison with values before the exposure (0,252±0,04 against 2,26±0,62 AU, respectively) was noted.

Analyzing the results concerning the susceptibility of formed daily biofilms of poly-resistant S. aureus and E. coli to disruption after combined exposure to 10 min ultrasound handling and further antimicrobials application, it was found that the ad-

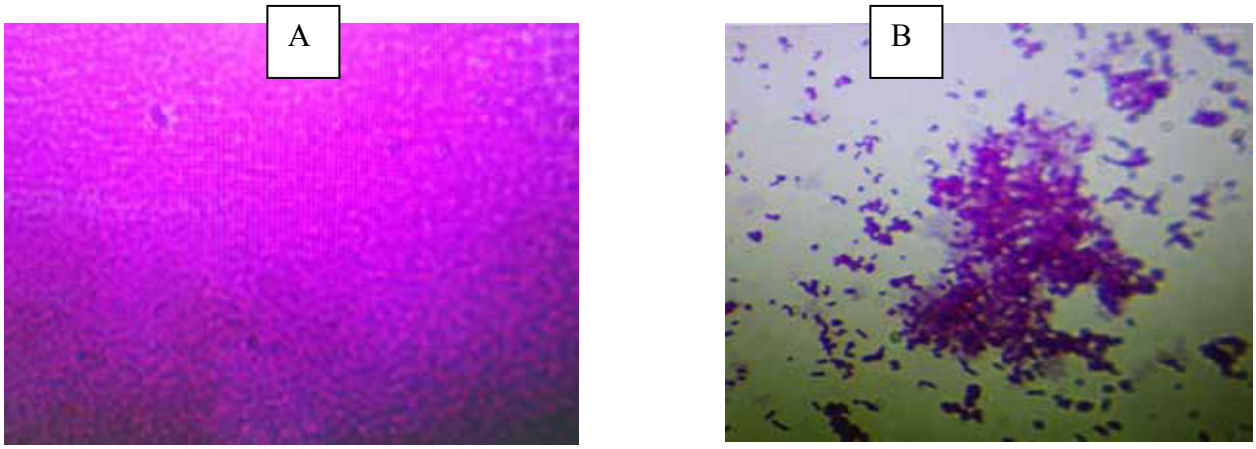

Fig. 1. Disruption of daily biofilms of E. coli after ultrasound processing during 10 min (B) in comparison with control biofilms without treatment (A)

ding of β-lactam antimicrobial agents after exposure the daily biofilms of S. aureus and E. coli to ultrasound caused a significant decrease in the optical density of the biofilms, both S. aureus (Amoxiclav - 0,088±0,002 and 2,35±1,91 AU in control; Ceftriaxone – 0,051±0,0037 and 2.24±0,29 AU respectively), and E.coli strains (Amoxiclav - 0,082±0,004 and 1,469±0,16 AU; Ceftriaxone – 0,079±0,004 and 1,475±0,12 AU as compared with the control).

In case of the complex application of levofloxacin and 10-min ultrasound there was determined the reduction of the optical density of the formed biofilms: S.aureus 30,4 times (0.076±0,008 AU), and E. coli 25,4 times (0,058±0,006 AU) less than in the control series.

Integrated bacteriophage and ultrasound use significantly inhibited the optical density of S. aureus biofilms - 44.4 times (0,033±0,002 AU), E. coli -68,5 times (0,039±0,002 AU) as compared with the control, which led to the complete disorganization of biofilms examined.

The determination of the ability of daily biofilms of poly-resistant strains of S. aureus and E. coli to form planktonic cells revealed that after their exposure to ultrasound and β – lactam antimicrobials there was observed an inhibition of production of plankton cells by daily poly-resistant clinical strains of S. aureus - 4,3 times less than in the control series (for Amoksiklav application – 0,167±0,02 as compared with 0,824±0,08 AU in the control). With the use of Ceftriaxone the production decreased 5,1 times - (0,162±0,08 and 0,832±0,09 AU respectively). The rate of formation of plankton cells by poly-resistant strains of E. coli was also suppressed as after the ap

Table 1. The effect of antimicrobials and ultrasound application on the daily biofilms of microorganisms and subsequent production of plankton cells

Isolates	life-form	Control series	Antimicrobials			
			Amoxiclav	Levofloxacin	Ceftriaxone	Bacteriophage
E. coli	biofilms	No US 1,473±0,18	1,469±0,16	1,472±0,18	1,475±0,12	0,234±0,021
		US 0,185±0,04	0,082±0,004	0,058±0,006	0,079±0,004	0,039±0,002
	Plankton cells	No US 0,707±0,05	0,702±0,08	0,706±0,04	0,705±0,09	0,258±0,04
		US 0,259±0,08	0,139±0,03	0,118±0,07	0,126±0,06	0,034±0,002
S. aureus	biofilms	No US 2,26±0,62	2,35±1,91	2,31±0,26	2,24±0,29	0,316±0,028
		US 0,252±0,04	0,088±0,002	0,076±0,008	0,051±0,0037	0,033±0,002
	Plankton cells	No US 0,827±0,06	0,824±0,08	0,819±0,06	0,832±0,09	0,315±0,03
		US 0,485±0,07	0,167±0,02	0,159±0,06	0,162±0,08	0,033±0,004

plication of US and Amoksiklav it was 5.1 times less than in the control (0,139±0,03 and 0,702±0,08 AU respectively), when using Ceftriaxone – 5,6 times (0,126±0,06 against 0,705±0,09 AU). In series with the complex use of fluoroquinolones (levofloxacin) and ultrasound there was noted the inhibition of the formation of planktonic cells of S. aureus 5.2 times (0,159±0,06 and 0,819±0,06 AU), and in case of E. coli – 6 times (0.118±0,07 against 0,706±0,04 AU respectively).

The study showed that the use of ultrasound combined with bacteriophage suppressed production of plankton cells by daily E. coli biofilms 20,8 times

(0,034±0,002 and 0,707±0,05 AU respectively) and by S. aureus - 25,1 times (0,033±0,002 and 0,827±0,06 AU) as compared to the control group.

The microscopy of E. coli biofilms after 10 min ultrasound exposition followed by the addition of the bacteriophage discovered that in place of formed and then destroyed by ultrasonic cavitation biofilms spheroplasts and shadows, that had the shape of the control biofilm, were subsequently formed.

This can be explained by the fact that the influence of ultrasound followed by specific action of the bacteriophage lead to significant changes in the mechanical properties of the cell membranes of E. coli, as well as to disruption of the internal structure of cells and varying concentrations of the substances dissolved in the cytoplasm. The low-intensity ultrasound changes mechanically the structure of the walls of plankton cells and biofilms, that reside in the matrix, which causes the violation of the integrity and disintegration of biofilms and the majority of planktonic cells, acting as inhibitor of a factor of dissemination and colonization by an infectious agent.

Summary. There was established the disruption of the integrity of daily biofilms after the action of low-intensity ultrasound on poly-resistant clinical strains of E. coli and S. aureus within 10 minutes of processing and decrease in the ability to produce plankton cells after combined application with antimicrobial agents. It was shown that only the action of ultrasound for 10 minutes with subsequent use of antimicrobials led to the complete inactivation of plankton cells and disorganization of the biofilm of E. coli and S. aureus isolates.

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