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Study of Complex Immunoglobulin Preparation Effect on Staphylococcus Aureus Biofilms

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ABSTRACT

The immunoglobulins are the promising alternative for surface molecules blocking and support the inhibition of biofilm formation. Complex immunoglobulin preparation has been developed for enteral application, which is an immunologically active protein fraction separated by fractionation of donor blood sera. The purpose of the research is to study the effect of complex immunoglobulin preparation on Staphylococcus aureus biofilming. We performed the sorption of immunoglobulin preparation on plates for enzyme-linked immunoassay. After adsorption of immunoglobulins, we cultured S. aureus. Biofilm stained with 1% alcoholic solution of basic fuchsin. Detection of stained biofilm extractions was performed at a wavelength of 492 nm by the method O'Toole (2011). Statistical analysis was performed using a paired version of Student's t-test. It has been found that the complex immunoglobulin preparation at concentrations of 60 and 20 mg/ml significantly reduced the intensity of S. aureus strains biofilm formation within 24 hours co-incubation. By increasing the incubation period to 48 hours it was found that all studied concentrations of complex immunoglobulin preparation significantly reduced the intensity of S. aureus biofilm formation. The complex immunoglobulin preparation is highly effective in reducing of S. aureus strains biofilm formation.

KEYWORDS

Bacterial Adhesion, Staphylococcus aureus, Antibodies

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It is known that intercellular interaction, as well as bacteria interaction with different surfaces, are important in biofilm formation [2]. As shown previously, the immunoglobulins are the promising alternative for surface molecules blocking and support the inhibition of biofilm formation [8, 11]. Human monoclonal antibodies specific to poly-β-1.6-N-acetylglucosamine (PNAG) effectively stimulate the deletion of staphylococci in opsonocytophagic reaction *in vitro*, and also provide the mice with protection against infection caused by PNAG-producing pathogens [4]. Currently, complex immunoglobulin preparation (CIP) has been developed for enteral application, which is an immunologically active protein fraction separated by fractionation of donor blood sera. Complex immunoglobulin preparation differs from all other immunoglobulin preparations used in Russia by the high content of IgA and IgM. In turn, IgM has a bactericidal effect on pathogens [5], IgA complicates their attachment to the mucosal epithelium, reproduction and provides rapid removal from the intestine [12], IgG neutralizes microbial toxins and viruses, mediates the "sticking" of bacteria to macrophages and their subsequent phagocytosis [13].

The purpose of the research is to study the effect of complex immunoglobulin preparation on *Staphylococcus aureus* biofilming.

Firstly, we performed the sorption of immunoglobulin preparation on plates for enzyme-linked immunoassay at 4°C for 1 hour. The study used the following preparation concentrations - 60, 20 and 6 mg/ml through protein. Not less than 97% of the preparation consisted of immunoglobulins A, M, G. Allocation of individual immunoglobulin in the preparation was the following: IgA 15-25%, IgM 15-25%, IgG 50-70%. The preparation does not contain preservatives and antibiotics. The use of preparation at a concentration of 20 mg/ml corresponds to the distribution of main classes of immunoglobulins in the blood serum of healthy individuals [7]. Immunoglobulin preparations containing 60 mg/ml through protein corresponds to

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CIP concentration through protein, mg/ml	Biofilm biomass in the presence of CIP	Samples only with CIP	*p* between groups
60	0.394±0.008*	0.365±0.009	<0.05
20	0.428±0.013*	0.439±0.011	>0.05
6	0.443±0.027	0.454±0.026	>0.05
0	0.490±0.018	0.431±0.014	<0.05

Table 1. S. aureus film biomass formed in the presence of complex immunoglobulin preparation within 24 hours

Note. * – p<0.05 when compared to the samples that do not contain CIP

CIP concentration through protein, mg/ml	Biofilm biomass in the presence of CIP	Samples only with CIP	*p* between groups
60	0.378±0.006*	0.346±0.008	<0.05
20	0.410±0.010*	0.384±0.006	<0.05
6	0.420±0.015*	0.491±0.040*	>0.05
0	0.475±0.020	0.361±0.017	<0.05

Table 1. S. aureus film biomass formed in the presence of complex immunoglobulin preparation within 48 hours

Note. * – *p*<0.05 when compared to the samples that do not contain CIP

the dose recommended by the manufacturer for the treatment of acute intestinal infections. The maximum dilution of the preparation up to 6 mg/ml of protein coincides with the concentration of immunoglobulins registered at their deficit [15].

After adsorption of immunoglobulins, 150 ml suspension of tested *S. aureus* cultures (bacterial concentration of 10^8 CFU/ml) was added to plate wells. Saline solution of *NaCl* were added to control wells instead of the immunoglobulin preparation. Plates were incubated with the samples for 24 and 48 hours at 37°C. Then the wells were washed and stained with 1% alcoholic solution of basic fuchsin followed by alcohol extraction of unbound dye. Detection of stained biofilm extractions was performed on ChroMate reader (Awareness Technology Inc., USA) at a wavelength of 492 nm [14]. The results were expressed in absorbance units.

Statistical analysis was performed using a paired version of Student's *t*-test. The threshold of significance was the value of $p<0.05$.

In the course of the research it has been found that the complex immunoglobulin preparation at concentrations of 60 and 20 mg/ml significantly reduced the intensity of *S. aureus* strains biofilm formation within 24 hours co-incubation (Table. 1).

By increasing the incubation period to 48 hours it was found that all studied concentrations of CIP significantly reduced the intensity of *S. aureus* biofilm formation (Table. 2).

Studies have shown the high efficacy of the complex immunoglobulin preparation for inhibition of *S. aureus* strains biofilm formation. Since the preparation contains a large number of different specific immunoglobulins, the strictly specific interaction with microorganisms is observed when used, which leads to blockage of their "*quorum sensing*" [6, 9] and facilitates disruption of biofilm formation [9, 10]. On the other side, bacteria complexes with antibodies are convenient targets for phagocytes [1] and point for the activation of the complement system [3].

Thus, the complex immunoglobulin preparation is highly effective in reducing of *S. aureus* strains biofilm formation.

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