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Combined Effect of Low-Frequency Laser and Polyphenol Oxidase on Adhesive Activity of *Escherichia coli* Strains Isolated from Patients with Urinary Diseases

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The effects of polyphenol oxidase and low-frequency laser irradiation on adhesion of pathogenic *Escherichia coli* to human erythrocytes and buccal epithelial cells were studied. The maximum decrease in adhesive activity of these strains was observed after complex exposure to laser and enzyme.

Key Words: *inhibition of adhesion; polyphenol oxidase; low-frequency laser exposure; uropathogenic Escherichia coli*

Adhesion is a common property of microorganisms largely determining their behavior in microbiocenosis. Bacterial adhesion is a factor determining the first contact of pathogenic microorganisms with target cells during the development of infectious process. Adhesion can be modulated by different methods, including exposure to L-asparaginase and polyphenol oxidase (PPO). These enzymes cleave the corresponding peptide bonds in adhesin molecules and inhibit their binding to receptors on target cells [9,10]. Another approach is exposure to low-frequency infrared laser. It induces conformation changes in adhesin molecules and receptors on the surface of target cells [6].

We previously demonstrated the possibility of blocking adhesion of uropathogenic *Escherichia coli* to human erythrocytes by laser irradiation combined with L-asparaginase treatment. The maximum inhibition was observed when laser irradiation was followed by L-asparaginase treatment [4].

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Here we studied the effect of laser irradiation combined with PPO treatment on adhesion of *E. coli* strains isolated from patients with urinary diseases to human erythrocytes and buccal epithelial cells (BEC).

MATERIALS AND METHODS

Seven clinical isolates of *E. coli* were used in the study. Six isolates were obtained from patients with urinary diseases. Strains 407, 88, 391, and JJP160 were isolated from the urine of patients with pyelonephritis and strain NU14 from the urine of a patient with cystitis. Strain O157:H7(212) isolated from the urine of a patient with the hemolytic uremic syndrome and chymeric NU14 strain NU14/pPKL9 with a pPKL9 plasmid carrying *fimB* gene responsible for type I pile expression [12] served as the reference strains. JJF10 culture was isolated from feces. The strains were treated with PPO in final concentrations of 50, 150, and 400 U/ml as described previously [7]. A Milta infrared laser for quantum therapy was used [8]. Bacterial cultures were exposed to laser as described previously [4].

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Adhesive activity of test strains was studied *in vitro* on O/I (Rh^+) human erythrocytes as described previously [1] with some modifications [3,5,7]. Erythrocytes were chosen as a universal model for evaluation of adhesion. The erythrocyte membrane contains glycophorine, a substance identical to epitheliocyte glycocalyx carrying adhesin receptors, but erythrocytes are more standard than epithelial cells [3]. However, in some cases *in vitro* adhesion of bacterial cells to tissue cultures does not correlate with their adhesion to erythrocytes [11]. Erythrocyte agglutination is associated with the presence of mannose-sensitive pili on the bacterium. However no correlation between adhesive and agglutinating activities was observed in uropathogenic strains possessing a great variety of adhesins and virtually obligatory mannose-sensitive pili [13].

Adhesive activity of bacteria towards BEC was evaluated as described previously [5]. BEC were obtained *ex temporo* from the same donors with blood groups O/I (Rh^-), O/I (Rh^+), A/II (RH^+), and A/II (Rh^-).

Adhesive activity was evaluated by examining at least 100 erythrocytes and 50 BEC in 25 visual fields under a microscope. Adhesive activity was estimated as the mean number of microorganisms per eukaryotic cell.

In series I the microorganisms were treated with enzyme, in series II the bacteria were exposed to laser, and in series III laser irradiation was followed by treatment with PPO. Low efficiency of laser irradiation preceded by PPO treatment was demonstrated previously [4].

The results were statistically processed using Microsoft Excel software.

RESULTS

In our previous study on uroepithelial cells PPO in concentrations of 70 and 140 U/ml was most effective against pathogenic *E. coli* expressing types I or P pili, respectively [5]. The maximum decrease of *Candida albicans* adhesion was observed after treatment with PPO in a concentration of 140 U/ml. The maximum inhibition of adhesion of uropathogenic *E. coli* was observed after treatment with 400 U/ml PPO (Fig. 1). This concentration was used in further studies of the effect of combined (laser+enzyme) treatment on bacterial adhesion to erythrocytes and BEC. The higher effective concentration of PPO in these experiments can be explained by the presence of different types of adhesins in the studied strains [2,14].

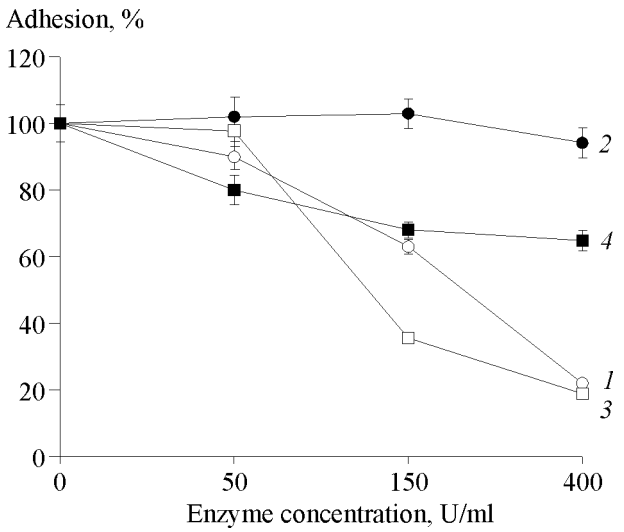

Fig. 1. Effects of polyphenol oxidase in different concentrations on *E. coli* adhesion to erythrocytes. Strains: *1*) NU14; *2*) NU14/pPKL9; *3*) JJP160; *4*) 88.

Combined treatment with laser and PPO inhibited adhesion of strains NU14/pPKL9, 407, O157 (212), JJP160, JJF10, NU14, 88, and 391 to erythrocytes. Laser irradiation markedly reduced adhesion of strains

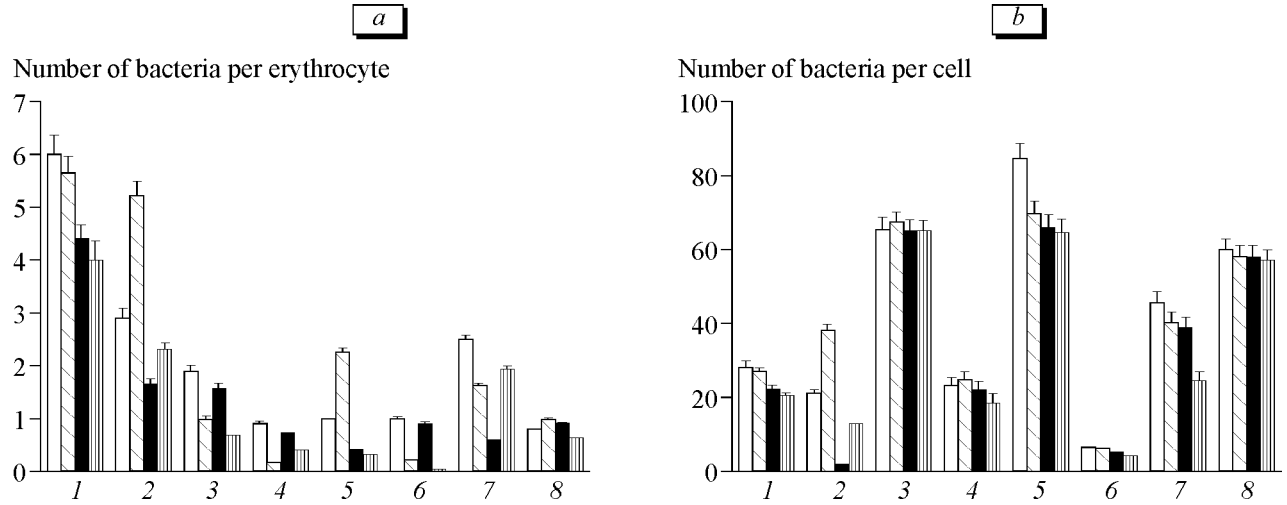

Fig. 2. Effects of laser exposure and PPO treatment on *E. coli* adhesion to erythrocytes (*a*) and buccal epithelial cells (*b*). Light bars: without treatment; cross-hatched bars: PPO, 400 U/ml; dark bars: laser, 1000 Hz, 1 min; vertical hatching: complex treatment. Strains: *1*) NU14/pPKL9; *2*) 407; *3*) O157:H7(212); *4*) JJP160; *5*) JJF10; *6*) NU14; *7*) 88; *8*) 391.

NU14/pPKL9, 407, JJF10, and 88. Treatment with PPO suppressed adhesion in only 4 strains (O157 (212), JJP160, NU14, and 88), while in strains 407, JJF10, and 391 adhesion increased (Fig. 2, *a*).

The majority of uropathogenic *E. coli* adhere to BEC [2], and hence, this model can be considered as universal. Maximum inhibition of *E. coli* adhesion to BEC was observed after combined exposure to laser and PPO (Fig. 2, *b*). However this effect was less pronounced than in experiments on erythrocytes, which can be explained by the involvement of different types of pili in the adhesive process.

Hence, combined treatment with laser and PPO reduced adhesive activity of uropathogenic *E. coli* variants. Bearing in mind that adhesion of pathogenic microorganisms is the first stage of the infectious process, we can conclude that combined treatment with laser and PPO can be effective in the therapy of infections and inflammations of the urogenital tract.

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