Complex Effects of Pulsed Infrared Laser and Asparaginase on *Escherichia coli* Strains Isolated from Patients with Urinary Diseases

T. V. Kolganova, T. Dzharadat, A. V. Ermolaev, I. G. Osipova, E. A. Vasil'eva, M. V. Dalin, and R. J. Doyle***

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Treatment with L-asparaginase and low-frequency laser decreased adhesion of uropathogenic *Escherichia coli* to human erythrocytes. The maximum effect was observed after combined treatment (laser exposure followed by enzyme treatment).

Key Words: *adhesion; L-asparaginase; laser exposure; adhesion inhibition; uropathogenic Escherichia coli*

Adhesion is a specific ligand-receptor interaction between the protein lectins of the microorganism and glycoconjugates on the surface of host cells [1]. This is the first stage of interactions between the macro- and microorganism in the worst case eventuating in infectious process. *Escherichia coli* is isolated from 95% patients with urinary tract inflammation; it is often present in bacterial associations with staphylococcus, proteus, and other opportunistic bacteria.

The tropism of *E. coli* to uroepithelial cells is determined by P fimbria, but the role of types I and IC pili, S fimbria, X adhesins, *etc.* in the pathogenesis of inflammatory diseases of the urinary tract was also demonstrated [11,12]. Blockade of the infectious process at the early stage can be achieved via prevention of bacterial adhesion to target host cells. L-asparaginase (ASG) and polyphenoloxidase prevent adhesion of microorganisms to eukaryotic cells [8,9]. We previously confirmed this fact for pathogenic *E. coli* and *Candida albicans* [4]. The effects of low-frequency infrared laser on the microorganism are poorly studied, but rapid cleansing of the urinary tract after laser irradiation attests to a direct effect of laser on not only host cells, but also on microorganisms [5]. The mechanisms underlying the effect of laser on bacterial adhesion remain uclear. Conformational modification of fimbrial structure or modulation of the spatial structure of target cell receptors can play a role.

We investigated the effects of complex treatment with ASG and laser on the adhesive activity of *E. coli* isolated from patients with urinary diseases. Erythrocytes were selected as a universal model for studying adhesion, because they carry glycophorin (a substance identical to the epitheliocyte glycocalyx) on their surface, where receptors for bacterial adhesin are located, but in contrast to epithelial cells, erythrocytes are more standard [3].

MATERIALS AND METHODS

Seven clinical strains of *E. coli* were isolated from patients with diseases of the urinary system. Strains 407, 88, 391, JJP 160 were isolated from the urine of patients with pyelonephritis, strain NU14 from the urine of a patient with cystitis, strain JJP 10 was isolated from feces, and strain O157:H7(212) was isolated from the urine of a patient with the hemolytic uremic syndrome. We also used strain NU14/pPKL9 (laboratory variant of strain NU14) with a plasmid carrying *fimB* gene responsible for the expression of

Russian University of Peoples' Friendship, Moscow; *Department of Microbiology and Immunology, Medical Center, Louisville University, Kentucky; **L. A. Tarasevich State Institute of Standardization and Control of Biological Preparations, Moscow

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type I pili [10]. Cell viability was evaluated routinely. Adhesive activity of strains was studied *in vitro* on human erythrocytes O/I(Rh+) by a previously described method [2] in our modification [3,4,6]. Treatment with ASG in a final concentration of 6 U/ml was carried out as described previously [4]. The effect of laser exposure on the suspension of live microorganisms was studied on a culture of uropathogenic *E. coli* at a concentration of 2×10^9 CFU/ml. Milta magnetic infrared laser device for quantum therapy (State Venture for Production and Designing of Humanitarian Information Technologies) served as the generator of low-frequency infrared radiation [7]. Bacterial cultures (250 μl) were exposed to laser at 1000 Hz for 1 or 10 min. Adhesion was evaluated on at least 100 erythrocytes in 25 visual fields. Adhesive activity was estimated as the mean number of microorganisms per erythrocyte. Four experimental series were carried out: I) ASG treatment; II) laser exposure; III) ASG+laser; IV) laser+ASG.

The results were processed statistically using parametrical methods with estimation of the mean values.

RESULTS

Low-frequency infrared laser irradiation had no significant effects on viability of clinical strains of *E. coli* (Table 1).

Effect of 1- and 10-min laser exposure (1000 Hz) on the adhesive activity of the studied strains showed different levels of adhesive activity of different strains. Laser exposure significantly decreased the adhesive activity of *E. coli* (Fig. 1). These changes were different in different strains, which can be explained by the presence of different types of adhesin molecules (more or less sensitive to laser) on the cell surface. The fact that laser exposure did not reduce the viability of microorganisms and decreased their adhesive activity indicates that laser modified bacterial surface structures (adhesins). On the other hand, 1- and 10-min exposure produced similar decrease in adhesive activity. Therefore we used 1-min exposure at 1000 Hz in further experiments.

TABLE 1. Effect of Laser Exposure on Viability of Uropathogenic *E. coli* Strains (*M*±*m*)

E. coli strain	Concentration of bacteria ($CFU\times10^9$/ml)		
	without laser irradiation	duration of laser exposure, min	
		1	10
407	6.9±0.28	6.7±0.14	6.27±0.32
88	4.6±0.42	3.99±0.95	3.6±0.14
NU14/pPKL9	14.76±1.8	11.3±2.77	13.05±2.21
NU14	3.76±0.82	3.38±0.45	5.05±1.12

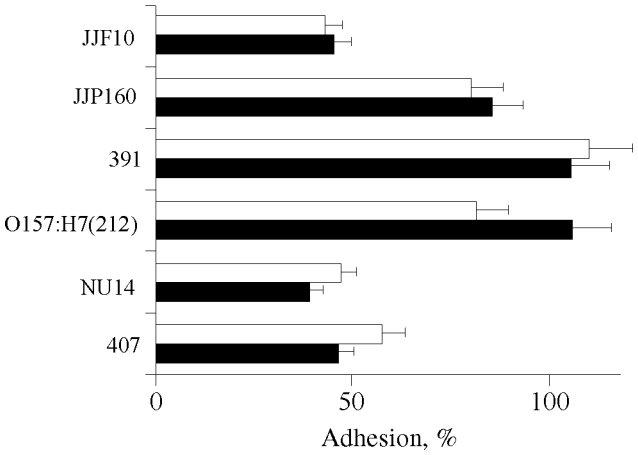

Fig. 1. Effect of laser exposure (1000 Hz) on adhesive activity of *E. coli* compared the initial level (100%). Dark bars: 10-min exposure; light bars: 1-min exposure.

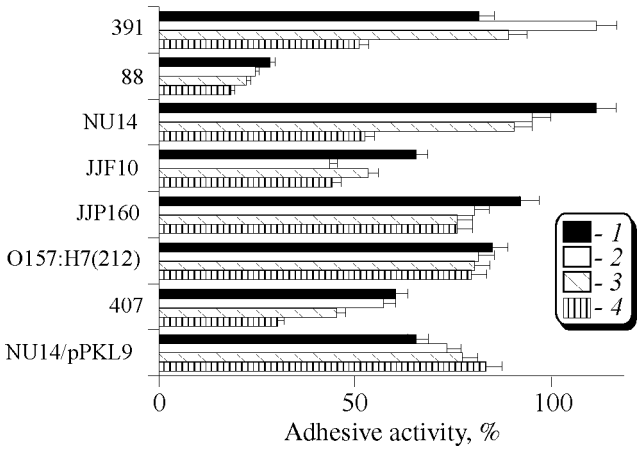

Fig. 2. Changes in adhesive activity of *E. coli* compared the initial level (100%) after 1-min complex treatment with L-asparaginase and laser (1000 Hz). *1*) L-asparaginase, 6 U/ml; *2*) laser exposure; *3*) laser exposure followed by L-asparaginase; *4*) L-asparaginase followed by laser exposure.

When investigating the complex effect of laser and ASG treatment on the adhesive activity of microorganisms, we expected a summation of their effects, but this result was obtained only when enzyme treatment was preceded by laser irradiation and only for 5 strains (407, 88, NU14, 391, JJF 10) (Fig. 2). Presumably, initial laser treatment modulated the conformation of adhesin and made asparagine residues more accessible for the enzyme. When laser was applied after enzyme treatment the additive effect was not observed.

Hence, combination of ASG treatment with laser irradiation effectively reduced adhesive activity of uropathogenic *E. coli*. If adhesion of pathogenic microorganisms is the first stage in development of infectious process, combined laser exposure and local

ASG treatment can be effective in the treatment of infectious inflammatory processes of the urinary tract.

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