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Antibacterial effect of semiconductor laser radiation against the strains of *S. aureus* and *P. aeruginosa*, leading pathogens in osteomyelitis

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Abstract

Introduction The study of the antibacterial effect of photodynamic therapy against the leading pathogens of chronic osteomyelitis is one of the promising directions today.

Purpose of the work was to evaluate the antibacterial effect against the strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa* with the ALOD-01 laser system in the presence of photodithazine.

Materials and methods The object of the study was 24-hour archival cultures of gram-positive and gram-negative microorganisms belonging to two taxa: *Staphylococcus aureus* (25923), *Pseudomonas aeruginosa* (27853). The antibacterial effect after the exposure to laser radiation in the presence of photodithazine on the microbial cells of the studied cultures was assessed by the absence of microorganism growth in the area of the light beam.

Results Laser exposure in combination with photodithazine (concentration 0.5–1.0 mg/ml) on *S. aureus* for two minutes at 200–300 J achieved a bactericidal effect in the beam area. A bactericidal effect on the entire surface of the Petri dish was achieved with light exposure of 400 J for 5 minutes and a photodithazine concentration of 1.0 mg/ml. Laser exposure for 2 minutes in the presence of photodithazine at a concentration of 0.5 mg/ml and 1 mg/ml did not have an antibacterial effect on *P. aeruginosa* strains. Continuous growth of microorganisms was observed on the dish. Increasing the light dose and exposure time contributed to a decrease in the growth of microbial cells. A bactericidal effect was obtained only in the center of the dish in treating the bacterial suspension with photodithazine at a concentration of 5 mg/ml.

Discussion The effectiveness of PDT depends on the type of microorganisms, the anatomical location of the infection site, as well as the properties of the photosensitizer and the laser used. Depending on the structure of the cell wall, different susceptibility of bacteria to photodynamic effects is observed.

Conclusion *S. aureus* strains can be successfully photoinactivated using photodithazine. For *P. aeruginosa* strains, it was not possible to find a regime in which microbial cell growth was absent throughout the dish. The photodynamic reaction occurs only when adequate doses of light energy act on the photosentisizer in the presence of oxygen in the medium, while the photodynamic damage is local and the bactericidal effect is limited by the zone of light exposure.

Keywords: photodynamic therapy, photodithazine, chronic osteomyelitis, antimicrobial effect

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INTRODUCTION

The generally accepted method of treating chronic osteomyelitis is surgical. However, according to a number of authors, poor results in the treatment of patients with bone and joint pathology complicated by purulent infection are observed in 25–30 % of patients, relapses of the disease develop in 25–68 % of cases [1–4].

Bacterial infection plays a major role in the development of chronic osteomyelitis. Upon admission to hospital, gram-positive microorganisms, mainly *Staphylococcus aureus*, are most often isolated from the wounds of patients with chronic osteomyelitis. The joining of hospital microflora (*Pseudomonas aeruginosa, Enterobacter cloacae, Klebsiella pneumoniae*, and others) during treatment aggravates the course of the pathological process [4]. Standard antibacterial therapy is not always able to achieve complete elimination of the pathogen from the focus. In this regard, the search for new methods and means to achieve positive results in the treatment of this category of patients continues.

Currently, the photodynamic therapy (PDT) has been widely used in clinical practice. It is based on the use of photosensitizers (PS) and low-frequency laser radiation [5–8]. Singlet oxygen and free radicals are formed in microbial cells, which have a toxic effect on them [6]. The method is minimally invasive and non-toxic to healthy cells, which allows it to be used in various fields of medicine: oncology, gynecology, otolaryngology, and others. [9–22].

Since the most common agents of the wound microflora in patients with chronic osteomyelitis are *S. aureus* and *P. aeruginosa*, the study of the possibilities of using PDT as an alternative method to standard antibiotic therapy in the treatment of this category of patients can be considered relevant.

Purpose: to evaluate the antibacterial effect of ALOD-01 laser radiation in the presence of photoditazine against the strains of Staphylococcus aureus and Pseudomonas aeruginosa.

MATERIAL AND METHODS

The material for the study was 24-hour archival cultures of gram-positive and gram-negative microorganisms belonging to two taxa: *Staphylococcus aureus (No 25923), Pseudomonas aeruginosa (No 27853)*.

The experiment was conducted in two stages. In the first stage, the effect of light radiation using the ALOD-01 laser system (ALKOM Medica, Russia) (wavelength (λ) 660 nm, output power up to 3 W) on the viability of microbial cells in the absence of the drug was assessed. For this purpose, the surface of nutrient agar (Müller-Hinton Agar) in Petri dishes seeded with a lawn of 24-hour old cultures of the studied microorganisms with a certain concentration of microbial cells per 1 ml of meat-peptone broth (MPB) was exposed to a semiconductor laser for a time determined by the experiment. The parameters of laser radiation and the initial concentrations of cultures of the microorganisms are presented in Table 1. The result was assessed after 24 hours by the presence or absence of growth in the area of laser exposure.

At the second stage, a solution of photosensitizer (PS) with a known concentration was added to the suspension of 24-hourh cultures of the studied microorganisms. After 30 minutes, a lawn was made on the surface of the nutrient agar (Muller-Hinton Agar) and exposed to a semiconductor laser light for a period of time determined by the experiment with specified radiation parameters (Table 2). Photoditzhaine is a second-generation photosensitizer intended for fluorescence diagnostics (FD) and PDT of malignant tumors.

Table 1

Laser radiation parameters

Time of exposure (t), min	Height of light guide (h), cm	Power of radiation (P), W	Targeted beam, %	Light dosage, J	Concentration of microbial cells per 1 ml	Volume of introduced suspension (V), ml
2	18	1.7	25	200	5×10^7	50
2	18	2.4	25	300	5×10^7	50
5	18	2.5	90	400	2×10^7	20
5	5	2.5	90	400	1×10^6	20

Table 2

Characteristics of study stages

Photodithazine concentration, mg/ml	Time of exposure (t), min	Volume ratio (V)	Light dosage, J	Height (h), cm	Radiation power (P), W	Targeted beam, %	Volume of introduced suspension (V), mcl	Concentration of microbial cells per 1 ml
0.5	2	1:1	300	18	1.7	25	50	5×10^7
1.0	2	1:1	200	18	2.4	25	50	5×10^7
1.0	2	1:1	200	18	1.7	25	50	5×10^7
1.0	5	1:1	300	5	2.5	90	50	2×10^7
1.0	5	1:1	400	18	2.5	90	50	1×10^6
1.0	5	1:2	400	18	2.5	90	50	1×10^6
1.0	5	1:3	400	18	2.5	90	50	1×10^6
5.0	5	1:3	400	5	2.5	90	50	1×10^6
1.0	5	1:3	400	5	2.5	90	50	1×10^6

The analysis of the obtained data was carried out with Gnumeric 1.12.17 software.

RESULTS

The light of the laser system targeted hon microbial cells of the studied cultures witout photoditazine did not have a bactericidal effect. Continuous growth of microorganisms was observed on Petri dishes (Table 3).

Exposure to laser light of *S. aureus* combined with photoditazine (concentration 0.5–1.0 mg/ml) for 2 min. at 200–300 J, achieved a bactericidal effect n the beam action zone (Table 4). The laser action was local. A slight growth of microbial cells was observed along the edges of the dish. A bactericidal effect on the entire surface of the Petri dish was achieved with a light exposure of 400 J for 5 min and a PS concentration of 1.0 mg/ml.

Table 3

Effect of the ALOD-01 semiconductor laser on microbial cells in the absence of PS

Laser effect without photodithazine	Time (t), min	Light dosage, J	Height (h), cm	Power (P), W	Targeted beam, %	Volume of introduced suspension (V), mcl	CFU/ml (MFar)	Results	
								S. aureus	*P. aeruginosa*
L –, PS –	–	–	–	–	–	50	0.5	Continuous growth	
L +, PS –	2	200	18	1.7	25	50	0.5		
L +, PS –	2	300	18	2.4	25	50	0.5		
L +, PS –	5	400	18	2.5	90	20	0.2		
L +, PS –	5	400	5	2.5	90	20	0.01		

Note: L — laser, PS — photosensitizer

Table 4

Effect of the ALOD-01 semiconductor laser radiation on archival cultures of *S. aureus* in the presence of photoditazine

Laser effect with photodithazine	Time (t), min	Light dosage, J	Height (h), cm	Power (P), W	Targeted beam, %	Suspension volume (V), mcl	CFU/ml (MFar)	Result
L+, PS+ 0.5 mg/ml (1:1)	2	200	18	1.7	25	50	0.5	No growth in the center, solitary colonies on the edges
L+, PS+ 1.0 mg/ml (1:1)	2	300	18	2.4	25	50	0.5	No growth in the zone of beam action, partial growth represented by several colonies
L+, PS+ 1.0 mg/ml(1:1)	2	200	18	1.7	25	50	0.5	
L+, PS+ 1.0 mg/ml (1:1)	5	300	5	2.5	90	50	0.02	
L+, PS+ 1.0 mg/ml (1:1)	5	400	18	2.5	90	50	0.01	Bactericidal effect

Note: L — laser, PS — photosensitizer

When the laser and photoditazine acted on microbial cultures of *P. aeruginosa*, the results were mixed depending on the radiation mode. Thus, laser exposure for two minutes in the presence of photoditazine at a concentration of 0.5 mg/ml and 1 mg/ml did not have any antibacterial effect. A continuous growth of microorganisms was observed on the dish. An increase in the light dose and exposure time contributed to a decrease in the growth of microbial cells (Table 5). A bactericidal effect was obtained in the center of the dish by treating the bacterial suspension with photodithazine at a concentration of 5 mg/ml. Single colonies were observed at the edges.

It was found that the ALOD-01 semiconductor laser exposure, regardless of the selected mode, did not have any antibacterial effect by itself. The use of the laser in combination with phhotoditazine significantly reduced the number of microbial cells, and in relation to *Staphylococcus aureus* strains contributed to a pronounced bactericidal effect (photoditazine concentration of 1.0 mg/ml and radiation dose of 400 J, exposure time of 2 min). For *Pseudomonas aeruginosa* strains, it was not possible to find a mode in which microbial cell growth was absent throughout the dish. However, the use of photoditazine at the maximum concentration (5 mg/ml), laser exposure time of 5 min and radiation dose of 400 J contributed to the pointed death of the microorganisms.

Table 5

Impact of the ALOD-01 semiconductor laser on archival cultures *P. aeruginosa* in the presence of photodithamine

Laser exposure in the presence of photodithazine	Time (t), min	Energy quantity, J	Height (h), cm	Power (P), W	Targeted beam, %	Volume of introduced suspension(V), mcl	CFU /ml (MFar)	Result
L+, PS+ 0.5 mg/ml (1:1)	2	200	18	1,7	25	50	0.5	Continuous growth
L+, PS+ 1.0 mg/ml (1:1)	2	300	18	2,4	25	50	0.5	Partial growth inhibition in the center
L+, PS+ 1.0 mg/ml (1:1)	2	200	18	1,7	25	50	0.5	
L+, PS+ 1.0 mg/ml (1:1)	5	300	5	2,5	90	50	0.02	Significant growth inhibition in the area of the beam action
L+, PS+ 1.0 mg/ml (1:1)	5	400	18	2,5	90	50	0.01	No growth in the area of highest drug concentration (diameter 10 mm)
L+, PS+ 1.0 mg/ml (1:2)	5	400	18	2,5	90	50	0.01	Sterile zone, diameter 12 mm
L+, PS+ 1.0 mg/ml (1:3)	5	400	18	2,5	90	50	0.01	
L+, PS+ 1.0 mg/ml (1:3)	5	400	5	2,5	90	50	0.01	Partial growth, single colonies in the center, continuous growth at the edges
L+, PS+ 5.0 mg/ml (1:1)	5	400	5	2,5	90	50	0.01	Sterile zone in the center, single colonies at the edges

Note: L — laser, PS — photosensitizer

DISCUSSION

The current problem of the spread of antibiotic-resistant strains contributes to the search for new methods and drugs for the treatment of purulent infections. Currently, PDT is one of the promising areas [9, 12, 14–16, 20–24]. An important advantage of this method over antibiotic therapy is the absence of toxicity of photosensitizers in relation to healthy tissues [5, 12, 20].

It has been established that the effectiveness of PDT depends on the type of microorganism, the anatomical location of the infection site, as well as on the properties of the photosensitizer and the laser system used [8, 13–17, 24–30]. The mechanisms of the impact of laser radiation on bacterial strains have not been fully studied [5, 25]. The different susceptibility of gram-negative and gram-positive bacteria to photodynamic effects is associated with the structure of their cell walls. The peptidoglycan layer of the bacterial cell wall of S. aureus has a much higher permeability (namely, for antibiotics) than the outer membrane of gram-negative bacteria.

In one of the works, the authors studied the effect of laser exposure on the growth of methicillin-resistant Staphylococcus aureus along with the use of dimegine. It was shown that with an increase in the dose of photoexposure, there was a bacteriostatic effect [31]. Other authors proved the effectiveness of PDT using photodithazine as a photosensitizer in the treatment of inflammatory joint diseases in children and adolescents [9]. Chepurnaya et al used PDT in the treatment of purulent diseases of the hand. The authors noted a noticeable healing of postoperative wounds in the patients treated with PDT [15]. A technique for combined antimicrobial photodynamic therapy in surgery of purulent wounds has also been developed and its effectiveness has been proven [8, 14, 16, 32].

CONCLUSION

The study showed that archival strains of *S. aureus* can be successfully photoinactivated using photodithazine. The photodynamic reaction occurs only when adequate doses of light energy act on photosensitizers in the presence of oxygen in the environment. The photodynamic damage is local in nature, and the bactericidal effect is limited to the zone of light exposure.

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