

# Assessment of Antimicrobial Activity of Photosensitized Quantum Therapy in Purulous Inflammatory Diseases of the Upper Respiratory Tract (Experimental Study)

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## Abstract

The article studies the antimicrobial activity of photodynamic therapy (PDT) in an experiment against the most significant causative agents of pyoinflammatory processes in the upper respiratory tract and determines the optimal modes of its implementation. It was found that the optimal mode of PDT action occurs at a concentration of the photosensitizer methylene blue of 0.05 - 0.1% and at irradiation exposures of 3 - 5 minutes, at which all tested microorganisms are sufficiently sensitive.

**Keywords:** Purulent-Inflammatory Diseases; Upper Respiratory Tract; Microbe; Antimicrobial Photodynamic Therapy; Photosensitizer; Methylene Blue; Experiment

## Introduction

The growth of antibiotic resistance of pathogens of purulent-inflammatory processes is one of the most serious problems of modern medicine [2,4,8]. The role of microflora in maintaining purulent-inflammatory processes in the upper respiratory tract is great, which aggravates the course of the underlying disease and complicates their rehabilitation [3,5,9].

Photodynamic therapy (PDT) has recently attracted close attention of researchers in various fields due to its high efficiency. In this case, antimicrobial PDT occupies a special place, characterized by a wide spectrum of antimicrobial action and, in contrast to antibiotics, with an almost complete absence of side effects and the inability of microorganisms to develop resistance to this factor. Its essence lies in the selective oxidative destruction of pathogenic microorganisms with simultaneous exposure to a photosensitizer (PS) (dye) and optical radiation of the corresponding wavelength range. In this case, being in an excited state, PS molecules generate reactive oxygen species (singlet oxygen), which induce damage to various cellular structures of microorganisms and their death [6,7].

According to the results of preliminary studies and literature data, the following microorganisms most often act as pathogens in patients with concomitant inflammatory diseases of the upper respiratory tract: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Candida albicans* [5,14]. Laboratory strains of the listed microorganisms were used as test cultures when testing the antimicrobial activity of PDT in an *in vitro* study. When isolating and identifying microorganisms, as well as determining their sensitivity to antibiotics, traditional methods were used [12].

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### Purpose of the Study

To study the antimicrobial activity of PDT in an experiment against the most significant pathogens of purulent-inflammatory processes in the upper respiratory tract, and to determine the optimal modes of its implementation.

### Materials and Research Methods

We used the laboratory strains of microorganisms most often found in inflammatory diseases of the respiratory tract as test cultures when testing the antimicrobial activity of PDT in an experiment on the Mueller-Hinton medium (HiMedia, India). In this case, aqueous solutions of methylene blue (MS) at concentrations of 0.01, 0.05 and 0.1% were used as PS. The source of light (red) radiation was an FDU-1 LED setup with a special attachment, which allows focusing radiation on an area of 2 cm<sup>2</sup> with radiation in the spectral range of 600 - 660 nm, a power of 5 W and a power density of 200 mW/cm<sup>2</sup> [1].

The tests were carried out as follows: a daily test culture was inoculated with a lawn on a Petri dish with a nutrient medium, then a solution of PS was dropped on the surface of the lawn (2 drops each), after a 10-minute exposure, the contact zone of microorganisms and PS was irradiated with red light (1, 3 and 5 min). The distance from the emitter to the surface of the nutrient medium was 2.0 cm. Then the crops were placed in a thermostat at 37°C for 18 - 24 hours. The results were taken into account by determining the presence or absence of microbial growth in the irradiation zone of the test strain [10,11,13].

The results obtained were processed by the generally accepted methods of variation statistics.

### Research Results and their Discussion

A preliminary study of the antimicrobial activity of these microorganisms showed their high resistance to antibiotics widely used in the clinic and the absence of drugs among them that are active against the entire spectrum of potential causative agents of upper respiratory tract diseases in the observed contingent of patients.

The results of studying the antimicrobial activity of PDT against test strains are given in the table.

Microorganism name	Concentration walkie-talkie FS, %	Irradiation exposure (min)		
		1	2	3
		Zone of inhibition of microbial growth, mm (M ± m)		
<i>Staphylococcus aureus</i> 835	0,01	14 ± 21,2	14 ± 1,1	14 ± 1,1
	0,05	14 ± 1,1	14 ± 1,3	14 ± 1,0
	0,1	14 ± 1,2	14 ± 1,1	15 ± 1,4
<i>Staphylococcus epidermidis</i> 834	0,01	12 ± 1,1	14 ± 1,4	14 ± 1,21
	0,05	14 ± 1,3	14 ± 1,2	14 ± 1,1
	0,1	14 ± 1,4	14 ± 1,2	14 ± 1,3
<i>Streptococcus pneumonia</i> 38	0,01	18 ± 1,5	18 ± 1,3	18 ± 1,1
	0,05	24 ± 2,1*	24 ± 1,8*	24 ± 1,6*
	0,1	25 ± 1,9*	25 ± 2,2*	25 ± 2,1*
<i>Moraxella catarrhalis</i> 01	0,01	23 ± 1,5	25 ± 1,3	25 ± 1,4
	0,05	35 ± 2,1*	35 ± 1,2*	35 ± 1,8*
	0,1	35 ± 1,6*	35 ± 1,3*	35 ± 1,5*
<i>Candida albicans</i> 271	0,01	13 ± 1,1	13 ± 1,0	13 ± 1,3
	0,05	15 ± 1,2	15 ± 1,1	15 ± 1,4
	0,1	17 ± 1,2*	17 ± 1,3*	18 ± 1,1*

**Table:** Antimicrobial activity of PDT against the leading causative agents of inflammatory processes in the upper respiratory tract.

As follows from the table, the zones of inhibition of microbial growth of *S. aureus* under photodynamic exposure at all used PS concentrations and exposure exposures were 14 mm or more, while separately both PDT factors (PS and irradiation) were indifferent, which indicates antimicrobial activity specifically PDT for the tested microorganism. In these modes, the values of the zones of inhibition practically did not depend on the concentration of PS and the duration of light exposure ( $P > 0.05$ ).

The sizes of the zones of inhibition of microbial growth of *S. epidermidis* were within 12 - 15 mm and as in relation to *S. aureus*, practically did not depend on the PS concentration and the duration of light exposure ( $P > 0.05$ ). Zones of inhibition of microbial growth of *S. pneumonia* under photodynamic exposure were observed in all tested modes. At the same time, they tended to grow with an increase in the PS concentration (in the range from 0.01% to 0.05%) ( $P \leq 0.05$ ) and practically did not depend on the used exposure exposures. That is, the zones of inhibition reached a maximum already at an exposure of 1 min.

The diameters of the zones of inhibition of the microbial growth of *M. catarrhalis* were recorded in the range from 23 to 35 mm. They also depended on the PS concentration (in the range from 0.01% to 0.1%) ( $P \leq 0.05$ ) and practically did not depend on the radiation exposure. The results obtained indicate a very high antimicrobial activity of PDT against *M. catarrhalis*. The zones of inhibition of microbial growth of *C. albicans* occurred in all tested regimes and ranged from 13 to 18 mm. At the same time, they tended to grow with an increase in the PS concentration (in the range from 0.01% to 0.05%) ( $P \leq 0.05$ ) and practically did not depend on the used exposure exposures. This suggests that the zones of inhibition reached a maximum already at an exposure of 1 min.

Analyzing the results of the studies performed, we can state the following. The study of the effectiveness of various modes of antimicrobial PDT in an experiment with respect to the most significant causative agents of pyoinflammatory processes of the upper respiratory tract was carried out. The test cultures were represented by both gram-positive (*S. aureus*, *S. epidermidis*, *S. pneumonia*) and gram-negative (*M. catarrhalis*) microorganisms, as well as fungi of the genus *Candida* (*C. albicans*). It was found that PDT had a pronounced antimicrobial effect against all tested microorganisms. The most sensitive were *M. catarrhalis* and *S. pneumonia*, followed by *C. albicans* and *staphylococci* (*S. aureus*, *S. epidermidis*). The most optimal regimes should be considered the performance of PDT at a PS (MS) concentration of 0.05 - 0.1% at irradiation exposures of 3 - 5 minutes, at which all tested microorganisms are sufficiently sensitive.

The results obtained indicate a high antimicrobial activity of PDT against all the most significant pathogens of purulent-inflammatory processes of the upper respiratory tract. On this basis, it seems appropriate to use antimicrobial PDT in the proposed modes in the treatment of purulent-inflammatory processes of the upper respiratory tract.

## **Conclusion**

1. Antimicrobial photodynamic therapy is characterized by high activity against all the most significant pathogens of purulent-inflammatory processes of the upper respiratory tract and can be recommended for their treatment.
2. The most optimal regimes should be considered the performance of PDT at a PS (MS) concentration of 0.05 - 0.1% and at irradiation exposures of 3 - 5 minutes, at which all tested microorganisms are sufficiently sensitive.

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