

SUSCEPTIBILITY OF S.AUREUS TO METHYLENE BLUE HAEMATOPORPHYRIN, PHTALOCYANINES PHOTODYNAMIC EFFECTS

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SUMMARY

Introduction: Many of oral diseases are caused by microorganisms. The emergence of antibiotic-resistant strains, such as methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecalis*, stimulated a search for alternative treatments. Such an alternative method is photo-activated disinfection. The aim of this study is to compare the antimicrobial properties of four new photosensitizers-methylene blue, haematoporphyrin and new Ga, Zn- phtalocyanines after the laser radiation (630 nm) against *Staphylococcus aureus*.

Materials and methods: For the study gram-positive Methicillin-resistant *Staphylococcus aureus* 1337 from collection of the Institute of Microbiology, Bulgarian Academy of Science is used. For PAD is used source of light which activates photosensitizer through emission of visible light at a specific wavelength. Suspensions of *Staphylococcus aureus* was irradiated with diode laser (630 nm) in the presence of methylene blue, haematoporphyrin and new Ga, Zn- phtalocyanines. There were two control groups: samples that were only treated with dye and samples that were exposed neither to the laser light nor to dye.

Results: Reduction in the viability of *Staphylococcus aureus* was achieved with some of the photosensitizers and light associations.

Conclusions: Photoactivated disinfection appears as an effective method of *Staphylococcus aureus* inactivation.

Key words: photodynamic therapy, photo-activated disinfection, microbial resistance, photosensitizer, antimicrobial action .

INTRODUCTION

Many of oral diseases are caused by microorganisms. Traditionally impact on aerobic and anaerobic oral cavity microorganisms is being performed through mechanical cleaning and chemical treatment by agents with various

antimicrobial activities. In order to affect the pathogens in radical-canal system sodium hypochlorite, chlorhexidine gluconate, hydrogen peroxide, 15-17% EDTA, 3-10% citric acid, calcium hydroxide, metronidazole, are being applied, as well as combinations of these [1]. In recent years, the emergence of antibiotic-resistant strains, such as methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecalis*, stimulated a search for alternative treatments. Such an alternative method is photodynamic therapy - PDT. [8, 14]. Photodynamic therapy (PDT) is basically applied in medicine for treatment of cancer [1]. PDT involves three components: light, a photosensitizer, and oxygen. A photosensitizer or its metabolic precursor is administered to the patient. Upon irradiation with light of a specific wavelength, the photosensitizer undergoes a transition from a low-energy ground state to an excited singlet state. Subsequently, the photosensitizer may decay back to its ground state, with emission of fluorescence, or may undergo a transition to a higher-energy triplet state. [7, 9]. The triplet state can react with endogenous oxygen to produce singlet oxygen and other radical species, causing a rapid and selective destruction of the target tissue. There are two mechanisms by which the triplet-state photosensitizer can react with biomolecules. Type I involves electron/hydrogen transfer directly from the photosensitizer, producing ions, or electron/hydrogen removal from a substrate molecule to form free radicals. [1, 7, 9, 14]. These radicals react rapidly with oxygen, resulting in the production of highly reactive oxygen species (superoxide, hydroxyl radicals, hydrogen peroxide). Type II reactions produce the electronically excited and highly reactive state of oxygen known as singlet oxygen. [1, 5, 7, 9, 14].

In PDT, it is difficult to distinguish between the two reaction mechanisms. PDT requires a source of light that activates the photosensitizer by exposure to low-power visible light at a specific wavelength. [1, 5, 7, 9, 14]. Most photosensitizers are activated by red light between 630 and

700 nm, corresponding to a light penetration depth from 0.5 cm (at 630 nm) to 1.5 cm (at ~ 700 nm). /6/. At present, diode laser systems that are easy to handle, portable, and cost-effective are used predominantly.

The principle of photodynamic therapy lies in the heart of photo-activated disinfection - PAD. Photosensitizer molecules attach to the membrane of the bacteria. Irradiation with light at a specific wavelength matched to the peak absorption of the photosensitizer leads to the production of singlet oxygen, which causes the bacterial cell wall to rupture killing the bacteria. /7, 13, 14/. Microorganisms that are killed by PAD include bacteria, fungi, viruses, and protozoa. The development of resistance to PAD appears to be unlikely, since, in microbial cells, singlet oxygen and free radicals interact with several cell structures and different metabolic pathways. /3, 7/.

Many natural and synthetic photoactive compounds have photosensitizing potential. Photosensitizer should be non-toxic, and should display local toxicity only after activation by illumination. The majority of the sensitizers used clinically belong to dyes. Initially antimicrobials dyes included tricyclic dyes such as acridine orange, methylene blue, fluorescein, Rose Bengal /4/. An inherent problem in using tricyclic dyes is that they might be toxic or may act as mutagens. Our project is directed to the development of new biomaterial for PAD (the so called photosensitizer), which is based on the pairing between a photoactive tetracyclic photosensitizer, e.g. phthalocyanine with the second photosensitizer as fullerene incorporated in liposome vehicles. The new biomolecule conjugates are expected to be selective towards bacterial cells, i.e. increasing microbial phototoxicity.

The aim of this study is to compare the antimicrobial properties of four photosensitizers - methylene blue, haematoporphyrin and new Ga, Zn- phtalocyanines after the laser radiation (630 nm) against *Staphylococcus aureus*. *Staphylococcus aureus* is etiological factor of periodontitis, refractory endodontic lesions and takes part in infectious endocarditis after bacteraemia, caused by dental manipulations /2, 10, 12/.

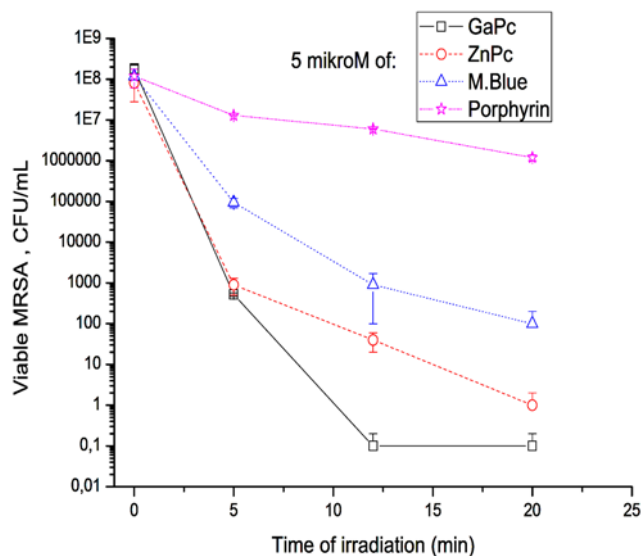
MATERIALS AND METHODS

For the study gram-positive Methicillin-resistant *Staphylococcus aureus* 1337 from collection of the Institute of Microbiology, Bulgarian Academy of Science is used. For PAD is used source of light which activates photosensitizer trough emission of visible light at a specific wavelength. Suspensions of *Staphylococcus aureus* was irradiated with diode laser (630 nm) in the presence of methylene blue, haematoporphyrin and new Ga, Zn-phtalocyanines. There were two control groups: samples that were only treated with dye and samples that were exposed neither to the laser light nor to dye. From each sample, serial tenfold dilutions were prepared and aliquots of 0.1 ml of

each dilution were plated in duplicate on Muller Hinton agar. After incubation at 37°C for 24 h, the number of colony-forming units (CFU/ml) was obtained.

RESULTS

Reduction in the viability of *Staphylococcus aureus* was achieved with some of the photosensitizers and light associations. In order to determine the optimal concentration of photosensitizer required to effectively kill the oral pathogen *Staphylococcus aureus*, 5 mikrom of each photosensitizer and times of irradiation were tested. For *S. aureus* the irradiation with diode laser in presence of Ga-phtalocyanines for 12 and 20 minutes lead to - 0,1 CFU/ml viable MRSA; for 5 minutes - 10i CFU/ml viable MRSA. In presence of Zn-phtalocyanines - for 5 minutes there were - 10i CFU/ml viable MRSA; for 12 and 20 min respectively 10I and 1 CFU/ml viable MRSA. The use of Methylene blue for 12 and 20 minutes gave 10i CFU/ml viable MRSA; for 5 minutes there was poor reduction of *S. aureus* - 105 CFU/ml viable MRSA. The irradiation in presence of Porphyrin leads to - 107 CFU/ml viable MRSA.



Photodynamic effect of GaPc, ZnPC, MB and Porphyrin on MRSA depending on time of irradiation

From obtained results we can conclude that the irradiation of *Staphylococcus aureus* with diode laser in presence of Ga-phtalocyanines more then 12 minutes and in presence of Zn-phtalocyanines for 20 min. is sufficient to kill the bacteria and lead to complete yeast destruction. In presence of Zn-phtalocyanines and of Methylene blue for 12 and 20 minutes, there were a great reduction of *S. aureus*. The use of Methylene blue for 5 minutes and of Porphyrin for 5, 12 and 20 minutes weren't very effective to destroy the microorganism.

CONCLUSIONS

Photoactivated disinfection appears as an effective method of *Staphylococcus aureus* inactivation. The application of new phthalocyanines leads to complete yeast destruction. The proper combination of drug-light doses can lead to a full photodynamic response without bacteria survival. The proposed method could become a promising alternative treatment of *Staphylococcus aureus* infections in dental medicine.

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