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IMPACT OF POLARIZED LOW-INTENSE RADIATION AND PHOTOSENSITIZERS ON GROWTH OF *STAPHYLOCOCCUS AUREUS*

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Due to the growing level of resistance to antimicrobials worldwide, it is important to study the effect of non-medicamentous means on some biological properties of infectious agents. Among those means physical factors, particularly low intense radiation occupies an important place. The influence of polarized incoherent low-intensity radiation of different wavelengths, photosensitizers (0.1% aqueous solutions of methylene blue, brilliant green, malachite green, methylene green, azure, crystal violet, fuchsin, and chrysoidin) and the combined effect of these factors on the growth intensity of clinical and collection strains of Staphylococcus aureus were studied. The obtained results were compared to control series. It was found that irradiation of the studied microorganisms caused a weak antimicrobial effect on the studied strains, compared to the control; the use of photosensitizers caused a decrease in the growth rate of investigated microorganisms by 16.6–42.5%. The most significant antimicrobial effect showed crystal violet, malachite green, and brilliant green. With the combined effect of photosensitizers and radiation, a decrease in the growth rate of the studied strains was observed – by 22.4–66.2%. Combinations of methylene blue with red spectrum and crystal violet with yellow spectrum showed the most pronounced antimicrobial activity. Obtained data can be used in treatment of inflammatory purulent processes, caused by Staphylococcus aureus.

Key words: *photosensitizers, polarized radiation, Staphylococcus aureus, photodynamic effect, antimicrobial action.*

Relationship between the publication and planned research work. The study is a fragment of the research project "Alternative methods of treatment of opportunistic infections with using medicamentous and non-medicamentous means", state registration № 0121U110174.

Introduction. In recent years it has been observed that more and more microorganisms are becoming resistant to frequently used antibiotics. Drug resistance occurrence and spread is a global and unsolved healthcare concern that leads to the increased morbidity and mortality in hospitals, time of hospitalization, and huge financial loss [1]. *Staphylococcus aureus* along with other bacteria has an extraordinary ability to develop resistance to any antibiotic to which it has been exposed regardless of the mechanism of their action [2].

Due to this, the investigation of novel approaches to combat microorganisms is of great relevance. Among those approaches, the most advanced are antibodies, probiotics, vaccines, and developing advanced materials with novel antimicrobial properties [1, 3]. Lack of physical factors, such as phototreatment, and photodynamic therapy (PDT) in this list determines their more detail investigation, especially in aspects of their impact on biological features of microorganisms.

PDT was studied more than 100 years ago and has since become a reliable and effective treatment for cancer as well as infectious diseases. [4]. Antimicrobial photodynamic therapy (aPDT) involves the use of low power radiation with appropriate wavelength (typically from the visible to near-infrared spectrum) treated with a photosensitizer (PS), which results in the generation of reactive oxygen species that kill bacteria unselectively via an oxidative burst [5, 6]. Thus, the coincidence of the spectral characteristics of the compounds used and the radiation sources is a necessary condition for the implementation of the principle of this method [7].

This method has reemerged recently as a non-invasive therapeutic option and alternate treatment of drug-resistant bacteria, fungi, and viruses [6]. Unlike antibiotics, aPDT acts on several structures of bacterial cell, such as external components, cytoplasmic membrane, and cell wall. Main molecular targets in bacteria are proteins, lipids, and nucleic acids [7, 8]. Thus the consequence of the multi-target nature of aPDT is a low probability of resistance development by microorganisms.

Nowadays three main sources of light are used for activation of PSs: lasers, light emitting diodes (LED), and gas-discharge lamps [5]. At the same time there is too little information about light-activation ability of PSs by polarized incoherent low-energy radiation (PILER).

The purpose of the study was to investigate combine impact of polarized incoherent low-energy radiation with different wavelengths and photosensitizers on growth rate of *Staphylococcus aureus*.

Object and methods of research. We studied direct impact of polarized light with different light filters, photosensitizers (PS), and complex of these factors on growth rate of clinical strain of *Sthaphylococcus aureus* isolated from patient with chronic generalized periodontitis who was treated at the University Dental Clinic of Uzhhorod National University, and reference strain *S. aureus* ATCC 25923. For identification of clinical strain bacterioscopic and bacteriological methods were used. Definitive identification was provided by the use of test-system Staphytest 16 by Erba Lachema. The source of PILER light was Bioptron Med All device with set of color filters (Bioptron light therapy system by Zepter Group) with the power density 40 mV/cm^2

For investigation were taken following PSs: 0.1% aqueous solutions of methylene blue, brilliant green, malachite green, methylene green, azure, crystal violet, fuchsin, and chrysoidin. Literature data about the absorption peaks of widely used photosensitizers vary [7, 9], so we defined their optical absorption spectra in the visible and near infra-red diapasons. For this purpose, the UV-VIS spectra in the range from 200 nm to 1000 nm were recorded using optical quality quartz cuvettes and

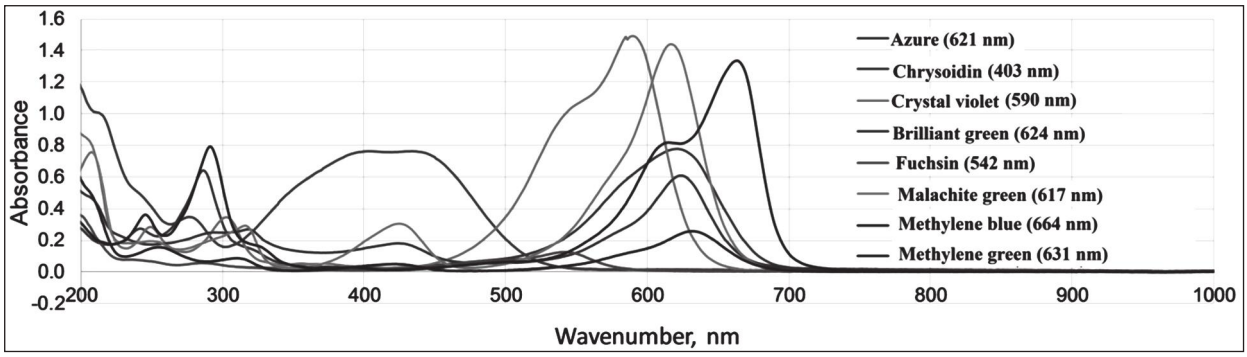

Figure – Absorption peaks of photosensitizers.

the InSpect spectrophotometer. To fit the device's working diapason, the dyes' solutions were additionally diluted with water, so the final sample solutions were examined at a concentration of about 5–10 ppm. Distilled water was used as a reference in all measurements.

To examine impact of radiation, PSs, and their combined action on the growth intensity of microorganisms, latter were divided on four groups [7, 10]. We prepared 24-hour agar culture of microorganisms with subsequent their standardization to 0.5 according to McFarland using densitometer (DEN-1, Biosan, Latvia). Then microbial inoculum was additionally diluted 1.25×10^5 fold. After this 1st control group of microorganisms were simply inoculated on solid nutrient media (meat-peptone agar) and cultivated in thermostat with 37°C for 24 hours. 2nd group of microorganisms was irradiated by PILER light for 10 min with appropriate wavelength followed by inoculation on nutrition agar. Irradiation of standard inoculum was conducted in sterile Petri dish with diameter 50 mm from the distance 50 mm, using appropriate color filter. To the inoculum of the 3rd group of microorganisms were added PS in ratio 1:10 with the subsequent reseeding obtained solution into the Petri dishes. Finally, microorganisms of the 4th group were treated with PSs, according to described above method with subsequent irradiation by PILER light with appropriate light filter. Each experiment has been conducted in fivefold frequency.

It should be noted that to avoid extraneous light, irradiation of 2nd and 4th groups of microorganisms and adding of PS to microbial inoculum of 3rd and 4th groups were conducted in the darkness.

Obtained data were statistically processed with the definition of arithmetic mean and standard deviation using Microsoft Office Excel The difference between control and experimental groups was assessed using Student's t-test. The difference was considered statistically significant at $p<0.05$.

Results of the study and their discussion. Examination of PSs optical absorption shoved, that their maximums were in the visible diapason **(fig.)**.

Thus, methylene blue and methylene green peaks were in the red spectrum. Absorbance maximum of azure, malachite green and brilliant green were in the border of red and orange diapasons, so to define their photodynamic activity we made separate series with light filters of specified colors. Crystal violet, fuchsin, and chrysoidin maximums were in yellow, green, and violet diapasons respectively. Subsequently, according to obtained data, to define the complex antimicrobial effect of PSs and PILER light, we used appropriate light filters.

It should be noted that malachite green, and brilliant green when used in the concentration described in methods (0.1% aqueous solution added to microbial inoculum in ratio 1:10) showed a significant antimicrobial effect and totally inhibited the growth of investigated strains of *S. aureus*. That is why to evaluate their photodynamic activity we additionally diluted these PSs tenfold (0.01% aqueous solution).

Table 1 displays antimicrobial effect of complex impact of investigated PSs and PILER radiation on clinical strain *S. aureus*.

Obtained data showed that irradiation of microbial suspension by PILER light (2nd group) caused either no effect or barely noticeable inhibition of clinical isolate *S. aureus* growth, compared to the control (1st group). The use of PSs (3rd group) induced a decrease in colony count on average on 16.7–42.5%, compared to the 1st group. Given the fact that the concentration of malachite green and brilliant green was 10 times lower, compared to other PSs, the most significant antimicrobial effect showed crystal violet ($t=5.73$, $p<0.001$). Finally, complex use of PSs and PILER light (4th group) led to decrease in bacterial colonies count on 22.4–60%, compared to control group. The biggest bactericidal effect was observed for crystal violet and PILER light with

Table 1 – Number of colonies of clinical isolate *S. aureus* on solid media

Photosensitizer	I group (control)	II group (irradiation)	III group (adding PS)	IV group (PS+irradiation)
Methylene blue	60.4±4.77	58.4±5.07	42±3.87	25±4.94
Methylene green	65.0±9.3	62.0±7.9	50.4±8.0	37.8±5.26
Azure (with red diapason)	64.6±9.6	61.2±6.1	53.0±8.0	44.0±8.2
Azure (with orange diapason)	64.6±7.8	61.0±10.6	53.0±9.2	46.0±10.1
Malachite green (with red diapason)	71.2±8.3	67.4±8.8	58.0±7.5	48.6±8.6
Malachite green (with orange diapason)	64.0±8.4	66.0±10.0	53.0±9.1	45±9.1
Brilliant green (with red diapason)	72.8±9.7	66.8±8.9	55.0±8.7	45.4±7.5
Brilliant green (with orange diapason)	72.6±9.8	71.6±9.9	59.8±8.7	52.0±6.9
Crystal violet	71.0±8.4	67.4±4.7	40.8±8.2	28.4±8.1
Fuchsin	66.0±6.8	61.6±5.0	55.0±5.7	49.4±6.2
Chrysoidin	63.2±6.6	57.2±6.1	51.2±7.6	44.2±7.9

Table 2 – Number of colonies of test strain *S. aureus* ATCC 25923 on solid media

Photosensitizer	I group (control)	II group (irradiation)	III group (adding PS)	IV group (PS + irradiation)
Methylene blue	68.8±8.3	64.8±8.0	44.8±5.9	24.6±5.0
Methylene green	70.6±8.4	67.4±6.7	55.2±7.5	41.8±7.5
Azure (with red diapason)	73.4±5.8	79.8±5.5	60.4±4.1	50.2±8.1
Azure (with orange diapason)	68.6±6.2	65.4±6.6	57.2±5.8	48.8±5.3
Malachite green (with red diapason)	66.4±7.5	63.4±7.4	54.0±6.3	42.8±4.4
Malachite green (with orange diapason)	72.2±6.8	68.6±6.2	57.2±5.8	48.8±7.1
Brilliant green (with red diapason)	64.0±7.0	61.4±7.0	48.8±6.6	39.0±6.3
Brilliant green (with orange diapason)	68.6±7.5	67.0±8.1	53.8±5.5	45.4±4.3
Crystal violet	68.8±8.6	65.0±8.7	43.2±6.5	27.6±6.8
Fuchsin	74.0±6.9	70.4±5.2	61.0±5.8	53.2±4.3
Chrysoidin	67.8±8.2	61.6±7.8	52.6±6.9	44.0±6.8

yellow filter (t=8.16, p<0.001), and methylene blue and PILER light with red filter (t=11.52, p<0.001).

Table 2 displays antimicrobial effect of complex impact of investigated PSs and PILER radiation on test strain *S. aureus* ATCC 25923.

As in case of clinical isolate, irradiation of collection test strain *S. aureus* ATCC 25923 by PILER light (2nd group) showed either no effect, or non-significant antimicrobial effect. The most pronounced antimicrobial effect showed light with violet filter. PSs decreased growth rate of *S. aureus* ATCC 25923 (3rd group) on 16.6–37.2%, comparing to the 1st control group. Complex use of PSs and PILER light (4th group) caused decrease in colony count on 28.1–66.2%. This time the most significant effect showed methylene blue and PILER light with red filter (t=10.2, p<0.001).

Comparing results of combined impact of azure, malachite green, and brilliant green and PILER light with red and orange light filters, we can state slightly more pronounced bactericidal effect in case of using red spectrum radiation.

Obtained results are corresponding with similar research by a number of scientists [7, 11, 12] as well as our previous investigations [10]. Peculiarity of present research is the study of complex impact of polarized light and PSs on the growth rate of clinical and collection strains of *S. aureus*. Also should be noted that combined use of PSs and radiation in 4th group of microorganisms led to 10–45% bigger decrease in number of bacterial colonies comparing to simple use of PSs in 3rd group indicating that combined effect of PSs and PILER radiation possesses much more pronounced bactericidal effect comparing to separate action of PSs. According to our previous investigations [10] 5 minutes irradiation of microorganisms by low-power radiation caused slight stimulating effect on the growth rate of microorganisms. In current research we used 10 minute application of PILER light for both 2nd and 4th groups of microorganisms what caused mild antimicrobial effect instead of stimulation in 2nd group and more significant bactericidal effect in 4th group. Thus, increase of irradiation duration and hence the dose density leads to the increase of antimicrobial properties of light by itself or in combination with PSs.

We can state that PILER did not show significantly different impact neither by itself nor in combination with PSs, comparing to other sources of light with similar parameters of power density and wavelength like LED or laser [7, 9, 10, 11]. Specified proves that such parameters of light as polarization and coherence do not have a determining influence on biological properties of microorganisms while the most important are wavelength and power.

Conclusions.

1. Combined impact of photosensitizers and polarized incoherent low-energy radiation have pronounced bactericidal effect on both clinical isolate of *S. aureus* and collection test strain *S. aureus* ATCC 25923.

2. The use of photosensitizers caused decrease in number of bacterial colonies on 16-42.5%, compairing to the control group, while complex impact of photosensitizers and polarized light led to much more pronounced reduction in growth intensity of investigated strains – on 22.4-66.2%.

3. The most significant antimicrobial effect showed combination of methylene blue and radiation with red spectrum, and crystal violet with yellow spectrum light.

4. Obtained data can be used in treatment of inflammatory-purulent processes caused by *Staphylococcus aureus*, particularly periodontal tissue diseases.

Prospects of further research. Investigation of complex impact of different sources of low-power light like laser, LED, PILER and photosensitizers on biological properties of planktonic and biofilm forms of bacteria and yeast fungi.

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ВПЛИВ ПОЛЯРИЗОВАНОГО НИЗЬКОІНТЕНСИВНОГО ВИПРОМІНЮВАННЯ ТА ФОТОСЕНСИБІЛІЗАТОРІВ НА РІСТ *STAPHYLOCOCCUS AUREUS*

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Резюме. Виникнення та поширення резистентності до антибіотиків – глобальна та невирішена проблема в галузі охорони здоров'я, яка призводить до підвищення рівня важкості перебігу захворювань та смертності, часу госпіталізації та зумовлює значні фінансові збитки. Внаслідок цього актуальними є новітні шляхи боротьби з інфекційними агентами, зокрема використання немедикаментозних засобів, одним з яких є антимікробна фотодинамічна терапія. Остання полягає у використанні низькоінтенсивного випромінювання певної довжини хвилі та фотосенсибілізатора, внаслідок чого утворюються реактивні форми оксисену. Наслідком цього є неселективне знищення бактеріальних клітин шляхом окислювального вибуху. Особливістю методу є дія на численні структури бактеріальної клітини, внаслідок чого імовірність розвитку стійкості з боку мікроорганізмів є низькою.

Мета роботи – дослідити комбінований вплив поляризованого некогерентного низькоінтенсивного випромінювання та фотосенсибілізаторів на інтенсивність росту *Staphylococcus aureus*.

Окремими серіями вивчали вплив поляризованого випромінювання відповідних довжин хвиль при експозиції 10 хв та щільністю потужності 40 мВт/см^2 (друга група), фотосенсибілізаторів: метиленовий синій, метиленовий зелений, діамантовий зелений, малахітовий зелений, азур, генціанвіолет, фуксин та хризоїдин (третя група) та комбінований ефект вказаних факторів (четверта група) на інтенсивність росту клінічного ізоляту *Staphylococcus aureus* та колекційного штаму *Staphylococcus aureus* ATCC 25923 на щільних поживних середовищах. Результати визначали шляхом підрахунку кількості бактеріальних колоній на чашках Петрі після 24-годинного інкубування у термостаті при 37°С та порівнювали з першою (контрольною) групою, на яку не впливали вищеперерахованими факторами. Спектри поглинання фотосенсибілізаторів визначали спектрофотометрично в діапазоні 200-1000 нм.

Встановлено, що опромінення досліджуваних штамів поляризованим випромінюванням зумовлює незначне зниження інтенсивності їх росту. Фотосенсибілізатори знижували кількість бактеріальних колоній на чашках Петрі на 16-42,5 %, порівняно з контролем. Комплексний вплив фотосенсибілізаторів та поляризованого випромінювання зумовлював зниження інтенсивності росту мікроорганізмів на 22,4-62,5 %. При цьому найбільш виражений протимікробний ефект відзначали при використанні метиленового синього та випромінювання червоного спектру, а також генціанвіолету та випромінювання жовтого спектру.

Слід відзначити, що комплексний вплив фотосенсибілізаторів та поляризованого випромінювання зумовлював в середньому на 10-45 % більш виражене зниження інтенсивності росту досліджуваних мікроорганізмів четвертої групи, порівняно з використанням лише фотосенсибілізаторів для мікроорганізмів третьої групи.

Отримані результати можуть бути використані при лікуванні гнійно-запальних процесів, зумовлених *Staphylococcus aureus*, зокрема захворювань тканин пародонту.

Ключові слова: фотосенсибілізатори, фотодинамічний вплив, поляризоване випромінювання, *Staphylococcus aureus*, протимікробна дія.

IMPACT OF POLARIZED LOW-INTENSE RADIATION AND PHOTOSENSITIZERS ON GROWTH OF *Staphylococcus aureus*

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Abstract. Drug resistance occurrence and spread is a global and unsolved healthcare concern that leads to the increased morbidity and mortality in hospitals, time of hospitalization, and huge financial loss. Due to this, the investigation of novel approaches to combat microorganisms is of great relevance. One of such approach is the use of non-medicamentous means, one of which is antimicrobial photodynamic therapy. Latter involves the use of low power radiation with appropriate wavelength treated with a photosensitizer, which results in the generation of reactive oxygen species that kill bacteria unselectively via an oxidative burst. The peculiarity of the method is the effect on numerous structures of the bacterial cell, as a result of which the probability of developing resistance by microorganisms is low.

The purpose of the study was to investigate combine impact of polarized incoherent low-energy radiation with different wavelengths and photosensitizers on growth rate of *Staphylococcus aureus*.

We studied direct impact of polarized light with different light filters (second group), photosensitizers –methylene blue, brilliant green, malachite green, methylene green, azure, crystal violet, fuchsin, and chrysoidin (third group), and complex of these factors (fourth group) on growth rate of clinical strain of *Staphylococcus aureus* and collection test strain *Staphylococcus aureus* ATCC 25923 on solid nutrient media. The results were determined by counting the number of bacterial colonies on Petri dishes after 24 hours of incubation in a thermostat at 37 ⍰ and compared

with the first (control) group, which was affected by neither irradiation nor photosensitizers nor their complex. The absorption spectra of photosensitizers were determined spectrophotometrically in the range of 200-1000 nm.

It is established that irradiation of the studied strains with polarized radiation causes a slight decrease in the intensity of their growth. Photosensitizers reduced the number of bacterial colonies on Petri dishes by 16-42.5% compared with controls. The complex effect of photosensitizers and polarized radiation caused a decrease in the growth rate of microorganisms by 22.4-62.5%. The most pronounced antimicrobial effect was observed when using methylene blue and red spectrum, as well as gentian violet and yellow spectrum.

It should be noted that the complex effect of photosensitizers and polarized radiation led to an average of 10-45% more pronounced decrease in the growth rate of the studied microorganisms of the fourth group, compared with only photosensitizers for microorganisms of the third group.

The results obtained can be used in the treatment of purulent-inflammatory processes caused by Staphylococcus aureus, in particular periodontal diseases.

Key words: photosensitizers, polarized radiation, *Staphylococcus aureus*, photodynamic effect, antimicrobial action.

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The authors of the paper confirm the absence of conflict of interest.

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