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POSSIBILITIES OF METHYLENE BLUE IN PHOTODYNAMIC INACTIVATION OF BACTERIA IN VITRO EXPERIMENT

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✓ ***Resume***

The paper presents the results of bench microbiological studies on the study of the antibacterial properties of red spectrum LED radiation with a wavelength of 630 ± 20 nm, the photosensitizer methylene blue (MB) in various concentrations. The objects of exposure were bacteria sown from hernial water in strangulated hernia and abdominal exudate in peritonitis (Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli). It was found that LED radiation and MB, separately, do not possess bactericidal properties. Photodynamic effect (PDT) (LED radiation in combination with 0.05% MB solution) has a pronounced bactericidal effect. The obtained results are discussed from the point of view of the possibility of using PDT for the prevention of postoperative proinflammatory complications.

Key words: methylene blue, infection, photodynamic effect

ОБОСНОВАНИЕ ВОЗМОЖНОСТИ УСИЛЕНИЯ БАКТЕРИЦИДНЫХ СВОЙСТВ МЕТИЛЕНОВОЙ СИНИ В ФОТОДИНАМИЧЕСКОЙ ИНАКТИВАЦИИ БАКТЕРИЙ В ЭКСПЕРИМЕНТЕ IN VITRO

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✓ ***Резюме***

В статье представлены результаты лабораторных микробиологических исследований по изучению антибактериальных свойств светодиодного излучения красного спектра с длиной волны 630 ± 20 нм, фотосенсибилизатора метиленового синего (МБ) в различных концентрациях. Объектами воздействия были бактерии, высеянные из грыжевой воды при ущемленной грыже и брюшной экссудат при перитоните (Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli). Установлено, что светодиодное излучение и МБ по отдельности не обладают бактерицидными свойствами. Фотодинамический эффект (ФДТ) (светодиодное излучение в сочетании с 0,05% раствором МБ) оказывает выраженное бактерицидное действие. Полученные результаты обсуждаются с точки зрения возможности использования ФДТ для профилактики послеоперационных гнойно-воспалительных осложнений.

Ключевые слова: метиленовая синь, инфекция, фотодинамическое воздействие.

IN VITRO TAJRIBADA BAKTERIYALARNI FOTODINAMIK INAKTIVATSIYASIDA METILEN KO'KNING IMKONIYATLARI.

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✓ ***Rezyume***

Ilmiy ishda 630 ± 20 nm to'lqin uzunligi bo'lgan qizil spektrli LED nurlanishining antibakterial xususiyatlarini, turli konsentrasiyalardagi metilen ko'k (MK) fotosensibilizatorini o'rganish bo'yicha mikrobiologik tadqiqotlar natijalari keltirilgan. Qisilgan churra suvidan aniqlanilgan bakteriyalar va peritonitda qorin ekssudati (Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli) ta'sir qilish ob'ekti hisoblanadi. LED nurlanishi va MK alohida-alohida bakteritsid xususiyatiga ega emasligi aniqlandi. Fotodinamik ta'sir (PDT) (0,05% MK eritmasi bilan birgalikda LED nurlanishi) aniq bakteritsid ta'siriga ega. Olingan natijalar operatsiyadan keyingi yiringli-yallig'lanishli asoratlarning oldini olish uchun PDT dan foydalanish imkoniyati nuqtai nazaridan muhokama qilinadi.

Kalit so'zlar: metilen ko'k, infektsiya, fotodinamik ta'sir

Relevance

Methylene blue (MS) is a thiazine dye belonging to the class of basic dyes. MS is used as an external and internal antiseptic, an antidote for cyanide, carbon monoxide, hydrogen sulfide poisoning [2]. Figure 1 shows the structural formula of the MS. Based on the structural formula of the arrangement of atoms (Fig. 1), it can be seen that the ML molecule is large and differs significantly from the strength of the bond between atoms. MS can play the role of a donor and acceptor of hydrogen ions. Its antiseptic properties are associated with the ability to selectively accumulate in microorganisms and viruses, causing damage to the cytoplasmic membrane, organelles and nuclear elements.

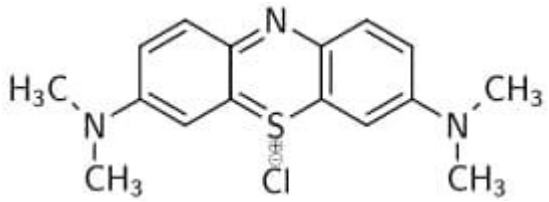


Fig. 1. MS structural formula

The emergence of modern antibiotics and antiseptics with high activity against a wide range of pathogenic microorganisms has led to a decrease in the frequency of clinical use of MS as an antiseptic. Recently, there has been an increase in antibiotic resistance among pathogenic bacteria [1,3,9] and dictates the need to search for new alternative methods of treating localized infections. One of these methods is photodynamic therapy (PDT) with the use of photosensitizers (PS), which was previously successfully used in oncology [4,5,7], and was recently proposed for the treatment of bacterial infections [5,6,8].

It has been proven that MS is a PS and can be effectively used in PDT. When illuminated with light that coincides with its absorption spectrum, a photodynamic (photochemical) reaction occurs with the formation of reactive oxygen species, which play a significant role in damage to bacterial cells. The absorption spectrum of MS is in the red part of the spectrum (lambda = 588-714 nm, lambda max = 660 nm) [phload]. The advantages of antibacterial PDT are its high efficiency against most pathogenic microorganisms, which persists during long-term treatment of chronic infections; the local nature of the antibacterial effect; reducing the pharmacological load on the body.

It should be noted that various concentrations and exposure times are given in

the literature to obtain an antibacterial effect, which was the basis for our own research.

Purpose of the study. Study of the bactericidal properties of red spectrum LED radiation (630 ± 20 nm) and the photosensitizer methylene blue in various concentrations. Determine the optimal radiation parameters, exposure time, effective concentration of methylene blue (MS) in the in vitto experiment.

Materials and methods

Clinical and laboratory studies carried out in patients with strangulated hernias, peritonitis, postoperative purulent endo bronchitis during prolonged mechanical ventilation after abdominal operations have shown that the main culprits of postoperative pyoinflammatory complications are several microbes, among which the frequency of occurrence is priority:

1. Staphylococcus aureus – 28%
2. Staphylococcus epidermidis – 6%
3. Escherichia coli – 46%

Taking this into account, for microbiological research we used museum strains of these microbes obtained from the national collection of microorganisms for human infection, Research Institute EMIZ, Ministry of Health of the Republic of Uzbekistan with passport data.

To carry out these studies, we initially prepared inoculations of three microbial cultures taken in the experiment, while taking into account the methodological recommendations, we prepared broth cultures of microbes at a concentration of 5 × 105 CFU / ml using the US turbidity standards (Fig. 1).

Sowing of microbial cultures was carried out by the "Lawn" method on a special medium by Mueller Hinton, produced by the Indian company HeiMidia.

In parallel with this, MS dilutions were prepared in separate sterile vials at concentrations: 0.001%; 0.005%; 0.01% and 0.05%. After that, sterile discs (from filtered paper) were taken, soaked in methylene blue and placed in Petri dishes with microbial cultures. In this way, 5 sets of cups were prepared, with 1 set of cups being processed i.e. exposed to red color (PDT), in the following exposures: 1-3-5-10 min. Then all cups were closed and transferred to a thermostat at 37 ° C for 18-24 hours.

The source of LED radiation was a certified installation of domestic production "FDU-1" with a radiation range of 630 ± 20 nm, corresponding to the absorption spectrum of methylene blue.

Result and discussion

In the first set of microbial culture dishes: Staph. aureus, Staph. epidermidis and Escher. coli, on which discs soaked in methylene blue in different concentrations were applied: 0.001% - 0.005% - 0.01% - 0.05%, after which the dishes were closed and placed in a thermostat at 37 ° C for 24 hours. After the end of the exposure, the cups were removed from the thermostat and the data obtained were analyzed. In these groups of microbiological studies, we noted a negative result, i.e. methylene blue at these concentrations did not have an antibacterial effect on the tested microbes (Table 1)

Table 1

The results of the sensitivity of microbes to various concentrations of methylene blue.

(M ± m) mm

№	Microbes	Mytilene Blue Concentration				
		0,001%	0,005%	0,01%	0,05%	Физ р-р (К)
1	Staph. aureus	0	0	0	0	0
2	Staph. epidermis	0	0	0	0	0
3	Esch. coli	0	0	0	0	0

In the second series of studies in sets of Petri dishes with nutrient media, the cultures used were inoculated: Staph. aureus, Staph. Epidermis and Esch. coli. Then the crops were exposed to red light at various exposures: 1-3-5-10 min (Fig. 2).

Fig. 2. Analysis of the results obtained after treatment with red light for 1-3 minutes at Sowing St. aureus.

Then the cups were closed and placed in a thermostat for 24 hours at a temperature of 37 ° C. Upon completion, the dishes were removed from the thermostat and the results were analyzed.

During these studies, we found that no antibacterial effect was noted in any dish, i.e. mono-exposure to red light (PDT), regardless of exposure, red light has no antibacterial effect (Table 2).

Table 2

Evaluation of the effect of LED radiation on microbes - with mono-exposure

№	Types of microbes	Exposure action (min)	Mytilene Blue Concentration			
			0,001%	0,005%	0,01%	0,05%
1	Staph. aureus	1	0	0	0	0
		3	0	0	0	0
		5	0	0	2,0±0,1	3,0±0,1
		10	0	0	2,0±0,1	3,0±0,1
2	Staph. epidermis	1	0	0	0	0
		3	0	0	0	0
		5	0	0	1,0±0,1	2,0±0,1
		10	0	0	2,0±0,1	1,0±0,1
3	Esch. coli	1	0	0	0	0
		3	0	0	0	0
		5	0	0	2,0±0,1	3,0±0,1
		10	0	0	3,0±0,1	3,0±0,1

Note: units are in mm of microbial growth inhibition zone. (M ± m) mm

The most interesting data we obtained in the third group of microbiological studies. In these experiments, microbial cultures (3 species) were inoculated in Petri dishes with Mueller Hinton's nutrient medium, after which disks were introduced into them, which were

saturated with methylene blue solutions in various concentrations (0.001% - 0.005% - 0.01% - 0.05 %), after which all these cups were exposed to red light in different exposures (1-3-5-10 min) (Fig. 3, 4).

Fig. 3 Analysis of the received results after processing1-3 min when sowing St. Epidermidis.

Fig. 4. Analysis of the obtained results after inserting discs with MS and red light treatments 1-3 min for E. coli.

Then the cups were closed and placed in a thermostat at 37 ° C.

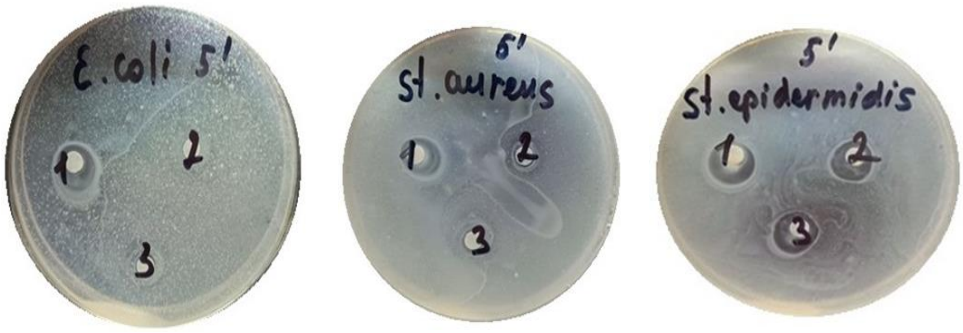
After the expiry of the exposure period, the cups were removed from the thermostat, and the analysis of the data obtained was carried out (Table 3).

The table shows that the microbial culture Staph. aureus with the introduction of methylene blue in dilutions of 0.001% and 0.005%, and the treatment with red light was not antibacterial. Although in dilutions of methylene blue in 0.01% and 0.05%, when exposed to red light in an exposure of 1-3 min, traces of antibacterial activity appeared (5.2 ± 0.1 and 7.0 ± 0.2 mm), but they not reliable (Fig. 5).

Fig. 5. Analysis of the received results and after processing red light – 3 minutes after sowing E. coli, antibacterial no action.

However, in Petri dishes where the dilutions of methylene blue were 0.01% -0.05% after exposure to red light for 5 and 10 minutes, the antibacterial effect significantly increased, so after 5 minutes of exposure to red light methylene blue at a concentration of 0.01%, it was 20.0 ± 0.4 mm, and with a methylene blue concentration of 0.05% and exposure to red

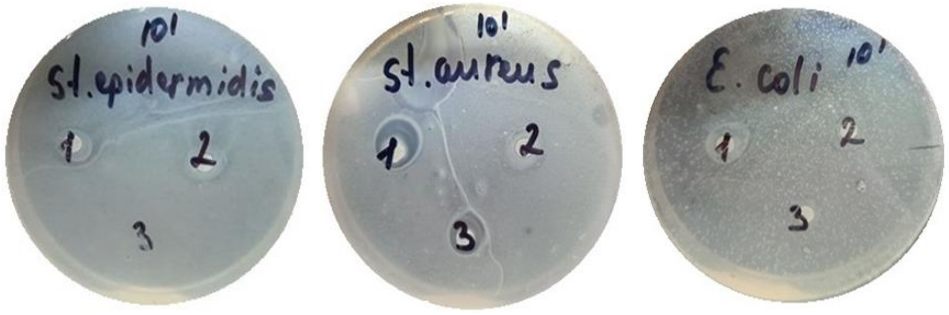
light for 10 minutes, it increased even more and amounted to 23.3 ± 0.5 mm (Fig. 6-7).



A B C

Fig. 6. Antibacterial activity of MS at a concentration of 0.01 and 0.05 and treatment with red light for 5 min

a) E. coli b) St. aureus c) St. epidermidis.



A B C

Fig. 7. Antibacterial activity of MS at a concentration of 0.01 and 0.05 and red light treatment for 10 min.

a) St. epidermidis b) St. aureus c) E. Coli

The next group of studies was devoted to the effect of methylene blue at various concentrations and treatment with red light when inoculating a microbial culture of Staph. epidermis. The data obtained from these studies are presented in tables 3 and 4.

Table 3 Characteristics of PDT Effect on Sensitivity microbes to methylene blue.

№	Types of microbes	Timing of light action (min)	Mytilene Blue Concentration				
			0,001%	0,005%	0,01%	0,05%	Физ р-р (К)
1	Staph. aureus	1	0	0	3,0±0,1	4,0±0,1	2
		3	0	0	5,0±0,2	4,0±0,1	1
		5	0	0	12,0±0,3	18,0±0,4	0
		10	0	0	15,0±0,2	22,0±0,5	1
2	Staph. epidermis	1	0	0	2,0±0,1	4,0±0,1	2
		3	0	0	5,0±0,1	6,0±0,1	1
		5	0	0	17,0±0,5	19,0±0,5	1
		10	0	0	16,0±0,3	20,0±0,5	1
3	Esch. coli	1	0	0	2,0±0,1	2,0±0,1	2
		3	0	0	3,0±0,1	4,0±0,1	1
		5	0	0	10,0±0,2	11,0±0,2	1
		10	0	0	11,0±0,2	13,0±0,2	1

Note: units are in mm of microbial growth inhibition zone. (M ± m) mm.

Table 4 Microbial sensitivity to methylene blue after exposure to red light.

№	Types of microbes	Mytilene Blue Concentration		
		Exposure k/c (min)	0,01%	0,05%
1	Staph. aureus	5	14,0±0,3	16,0±0,2
		10	13,0±0,2	18,0±0,3
2	Staph. epidermis	5	17,0±0,3	18,0±0,3
		10	11,0±0,2	15,0±0,2
3	Esch. coli	5	10,0±0,1	11,0±0,1
		10	10,0±0,1	10,0±0,1

Note: units are in mm of microbial growth inhibition zone. (M ± m) mm.

It can be seen from the table that methylene blue after treatment of a plate with crops with red light in an exposure of 1-3 min, has some antibacterial activity, but these data are not reliable.

At the same time, 0.01% methylene blue on Staph. epidermis after treatment with red light for 5 minutes significantly increased its antibacterial activity, which was 17.2 ± 0.2 mm, and at a methylene blue concentration of 0.05%, these data increased even more and amounted to 19.1 ± 0.3 mm.

When conducting the same research, when studying the culture of Esch. coli has been installed. These data are presented in table 3, from which it can be seen that methylene blue at a dilution of 0.01% and 0.05% and treatment with red light in an exposure of 1-3 minutes does not show its antibacterial effect, and the traces that show are unreliable.

However, in the case when methylene blue at a concentration of 0.01% and treatment of crops with red light, the antibacterial effect slightly increased and amounted to 11.3 ± 0.2 mm. At the same time, methylene blue at a concentration of 0.05% and processing of crops of microbes of the Esch culture. coli with red light in an exposure of 10 min slightly increased the antibacterial effect, which was 13.1 ± 0.2 mm.

FINDINGS

1. Thus, the analysis of the conducted microbiological studies dedicated to the assessment of the antibacterial activity of methylene blue in various dilutions (0.001% -0.005% -0.01% -0.05%) after treatment with red light at various exposures (1-3-5-10 min) when sowing: Staph. aureus, Staph. Epidermis, Esch. coli isolated from the contents of the hernial sac and peritoneal exudate was found:
2. At microbiological crops of hernial waters in patients with strangulated abdominal hernias and peritonitis, about ten species of various microbes were isolated. Moreover, the bulk of these microbes is made up of the families: Micrococcaceae and Enterolacteriaceae. At the same time, staphylococci and Escherichia constitute the largest sowing in quantitative terms
3. It is quite obvious that one of the most serious problems of modern medicine is a significant increase in resistance to many antibiotics, while this resistance is especially high in staphylococci to β-lactam antibiotics. Taking these positions into account, it is quite advisable to develop new approaches to the creation of drugs (antiseptics) that would have a wide range of antibacterial activity.
4. The conducted microbiological studies showed that the concentration of methylene

blue solutions: 0.001% - 0.005% - 0.01% - 0.05% in the study of sensitivity, taken in the experiment: Staph. aureus, Staph.

5. When exposed to microbes in Petri dishes and treated with red light at exposure 1-3-5-10 minutes, there is also no positive effect, that is, the growth of microbes is not delayed.
6. However, when inoculating the same microbes in Petri dishes, applying methylene blue in the form of discs at a concentration of 0.01% and 0.05% and

epidermis, Esch. coli did not have an antibacterial effect, that is, they do not have an antimicrobial effect.

treating them with red light at an exposure of 5 and 10 minutes has a completely positive antibacterial effect when the indicator of the growth inhibition zone microbes is 20.3 ± 0.5 mm. It should be noted that this complex has a more pronounced antibacterial effect on gram-positive microbes than on gram-negative ones.

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