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In vitro photodynamic properties of methylene blue in a combination with laser illumination at 630 nm concerning Candida albicans

Oddziaływanie na Candida albicans fotodynamicznych własności błękitu metylenowego w połączeniu z promieniowaniem laserowym o długości fali 630 nm – badanie in vitro

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Summary: The study had to define influence of combined applications of laser radiation and methylene blue (MB) on pathogenic culture of Candida albicans in vitro. The experimental study was done at standard techniques of method of cultivations in a broth. The laser irradiation of cultures was done at once after addition of MB in concentration 0.05%, 0.1% and 0.2%. During studying action of MB in dark, influence of MB to the growth of test-shtam without laser radiation, were prepared fluid Gissa's broth with glucose without Andrede's indicator. Activation of MB was done by laser with wave length 630 nm during 3 or 5 min. All experiments were passed in 4 parallels and 3 repeats. Maximal suppression of growth of microorganisms was noted in group with using 0.1% MB with laser radiation 3 minutes without centrifugation after 24 hours. Maximal suppression of growth was noted in group after centrifugation with 0.05% MB with exposure of laser 3 min. after 48 hours. Sensitivity of pathogenic culture of Candida albicans to application of B and laser raises accordingly to increase of concentration of MB.

Key words: methylene blue, photosensitizer, Candida albicans.
Słowa kluczowe: błękit metylenu, substancja uczulająca na światło, Candida albicans.

Currently, new antibacterial preparations are developed. But occurrence of new generations of antibiotics is accompanied by occurrence of new cultures of microorganisms with stability to these medicines (1). It is continuing active development of photodynamic antimicrobic chemical therapy (PACT) (2). At the some time usage of PACT is at the initial stage of development, despite of the knowledge since 19 century about abilities of some chemical substances as photosensitizers, in particular aniline dyes (3,4). Absence of effective antimycotic agents for the local use, makes mycotic keratitis an important problem in ophthalmology (5,6,7).

The purpose

Of the study was to define influence of combined applications of laser radiation and methylene blue (MB) on pathogenic culture of Candida albicans in vitro.

Design

Pilot, openlabel, comparative laboratory study.

Methods

Study was conducted with a culture of pathogenic test-shtam of Candida albicans. Test-shtam were kept and conducted to the surface of meat-pepton oblique agar at temperature 4°C. For experiment were used daily cultures which were grown up in test-tubes on meat-pepton oblique agar at 37°C. Initial solution of MB in distilled water was done.

For **studying of action of MB in dark,** influence of MB to the growth of test-shtam without laser radiation, were prepared fluid Gissa's broth with glucose without Andrede's indicator. Broth was spilled into test-tubes for 1 ml. Concentrations of MB were 0.05%, 0.1% and 0.2%. A number of parallels for each variant were 4. Test-tubes with the broth were sterilized in autoclave at 0.5 atm. Culture of test-microorganisms was swabbed by sterile physiological solution. Suspension were diluted by sterile physiological solution until concentration $2 \cdot 10^4$ cells/ ml. From this inoculum were selected 50 mcl and were added into the each test-tube. Terminal concentration of cells in 1 ml of the broth were $1 \cdot 10^3$ cells/ ml.

Culture with MB was incubated in a thermostat at temperature 37°C during 24 and 48 hours. Intensity of growth of testshtam carried out by optic density of culture by spectrophotometer "Spekol-10" at wave length 540 nm. For control were used same cultures of microorganisms without adding of MB. Estimation of results – intensity of the growth of culture by optical density of solution, was done after 24 and 48 hours after added of MB in test-tubes with culture.

Defining of photoinducted influence of methylene blue to the microorganisms

Culture of test-microorganisms was swabbed by sterile physiological solution. Into the test-tubes with 1 ml of sterile physiological solution, which contained 0.05%, 0.1% and 0.2% MB, was added 50 mcl of this suspension with final concentration $1 \cdot 10^7$ cells/ ml in test-tubes. Suspension with MB was incubated 30 min. at room temperature, for binding substance with the cells. Laser with wave length 630 nm during 3 or 5 min was used for activation of MB. Radiated suspensions were passed for 1 hour at room temperature, and diluted by sterile physiological solution until concentration $1 \cdot 10^3$ cells/ ml. From the last dilution selected 50 mcl and added into the sterile Gissa's broth with glucose without indicator (variant without centrifugation). In parallel the initial suspension were centrifuged (1200 circles/ min, 20 min), after this supernatant liquide was poured with added sterile physiological solution, and than selected 50 mcl into sterile broth (variant with centrifugation), with final concentration $1 \cdot 10^3$ cells/ ml. The culture after radiation without MB was used as control. A number of repeats, conditions of incubating and estimation of results were done in the same way with the previous technique. All experiments were passed in 3 repeats.

Results

Maximal suppression of growth of microorganisms was noted in group with 0.05% solution of MB with exposure of laser 3 min with centrifugation after 24 hours (Tab. I). In groups with 0.1% and 0.2% solution of MB were noted stimulation of growth of microorganisms with exposure of laser radiation 3 min. at 1.2 and 5 times accordingly. In groups with exposure of laser 5 min. increasing of growth of microorganisms were: MB 0.1% – in 1.5 times, 0.2% – in 3 times.

After 24 hours without centrifugation maximal suppression was noted with 0.1% solution of MB with exposure of laser 3 min (Tab. II). In group with concentration of MB 0.05% was noted stimulation of growth, 0.2% – suppression of growth in 1.3 times. In all groups with radiation 5 min. were noted stimulation of growth of cells. After 48 hours suppression of growth was significant in groups with 0.2% solution of MB with duration of radiation 3 and 5 min, and also in groups with 0.1% concentration of MB with duration of radiation 5 min. After 48 hours maximal suppression of growth was noted in group after centrifugation with 0.05% MB and exposure of laser 3 min.

Concentration of methylene blue, %	Time of influence by the laser, min			
	3		5	
	M ±m	δ	M ±m	δ
24 hours				
0.05	0.075 ± 0.005	0.015	0.334 ± 0.004	0.012
0.1	0.232 ± 0.008	0.023	0.306 ± 0.008	0.0024
0.2	0.586 ± 0.008	0.024	0.300 ± 0.011	0.033
48 hours				
0.05	0.275 ± 0.021	0.061	0.304 ± 0.019	0.054
0.1	0.433 ± 0.026	0.074	0.329 ± 0.018	0.052
0.2	0.388 ± 0.039	0.111	0.305 ± 0.019	0.055

Tab. I. Growth of Candida albicans with presence of methylene blue after laser influence after centrifugation.

Tab. I. Wzrost Candida albicans w obecności błękitu metylenowego po laserowaniu i wirowaniu.

Concentration of methylene blue, %	Time of influence by the laser, min			
	3		5	
	M ±m	δ	M ±m	δ
24 hours				
0.05	0.377 ± 0.005	0.015	0.300 ± 0.009	0.024
0.1	0.161 ± 0.003	0.008	0.366 ± 0.004	0.011
0.2	0.282 ± 0.005	0.014	0.434 ± 0.005	0.013
48 hours				
0.05	0.470 ± 0.055	0.155	0.333 ± 0.018	0.051
0.1	0.633 ± 0.021	0.062	0.321 ± 0.022	0.063
0.2	0.308 ± 0.007	0.021	0.329 ± 0.008	0.023

Tab. II. Growth of Candida albicans with presence of methylene blue after laser influence without centrifugation.

Tab. II. Wzrost Candida albicans w obecności błękitu metylenowego po laserowaniu bez wirowania.

Concentration of methylene blue, %	M ±m	δ
24 hours		
0.05	0.160 ± 0.016	0.031
0.1	0.189 ± 0.016	0.046
0.2	0.102 ± 0.005	0.015
48 hours		
0.05	0.314 ± 0.008	0.016
0.1	0.389 ± 0.014	0.041
0.2	0.328 ± 0.011	0.032

Tab. III. Growth of Candida albicans with presence of methylene blue after centrifugation.

Tab. III. Wzrost Candida albicans w obecności błękitu metylenowego po wirowaniu.

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Concentration of methylene blue, %	M ±m	δ
24 hours		
0.05	0.170 ± 0.021	0.042
0.1	0.231 ± 0.006	0.016
0.2	0.394 ± 0.007	0.019
48 hours		
0.05	0.257 ± 0.021	0.042
0.1	0.353 ± 0.012	0.035
0.2	0.396 ± 0.023	0.067

Tab. IV. Growth of Candida albicans with presence of methylene blue without centrifugation.
Tab. IV. Wzrost Candida albicans w obecności błękitu metylenowego bez wirowania.

Significant suppression of growth of fungoid flora was not observed after centrifugation after 24 and 48 hours with action of MB in a dark (Tab. III, IV). Obtained results prove significant suppression of Candida albicans by using methylene blue as photosensitizer.

Discussion

After 24 hours the maximal suppression was noted in group after centrifugation with 0.05% MB and exposition of laser 3 min. However, in group without centrifugation the maximal suppression was noted in test-tubes with 0.1% MB and laser 3 min. After 48 hours the maximal suppression of microorganisms was noted in group after centrifugation with 0.05% MB and exposition of laser 3min. It is possible to explain these results, most likely, by the expressed thermal effects at increase of duration of laser. This condition could stimulate growth of Candida albicans. MB is distributed in tissues of eye with gradual full removing from structures of an eye after 24 hours (8). So it is necessary to take into account the results of group without centrifugation after 24 hours. In our next study we shall research efficiency of MB as photosensitizer in the treatment of fungoid keratitis.

Conclusions

1. Growth of Candida albicans may be suppressed by methylene blue as photosensitizer in a combination with laser radiation with wave length 630 nm.
2. After 24 hours maximal suppression of growth of microorganisms was noted in group without centrifugation with using of 0.1% MB and duration of laser radiation 3 minutes.
3. After 48 hours maximal suppression of growth of microorganisms was noted in group after centrifugation with using of 0.05% MB and exposure of laser radiation 3 minutes.

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