Control under pharmacological media activity with the laser liquid nanoclusterization

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Abstract: *Influence of a nutrient medium laser irradiation on microorganism's population development dynamics and on nystatin activity is experimentally established. On the basis of the received results the microbiological quantitative analysis technique of bioorganic solutions laser nanoclusterization processes can be developed. In the practical plan similar technologies presume to lower a therapeutic dose of preparations, having reduced by-effects, not reducing medical result and not changing a medicine chemical compound.*

Keywords: *laser, activity, cluster, microbiologic, nystatin, energy, doze, solution, nutrient, dynamics, population.*

INTRODUCTION

In [1] it has been established, that at an intravenous laser irradiation of blood at surgical intervention the anaesthetizing preparations dose can be considerably lowered.

On the other hand, by a crystallographic method it has been established, that under the influence of a laser irradiation there can be a destruction of macromolecules clusters (associations) in a solution that leads to increase of their physical and chemical activity [2]. Therefore laser activation technology of medical products and possibility definition of their activation is interested.

In the present work for biological effect definition of the laser nanoclusterizated solutions microbiological systems are used.

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ABOUT THE MECHANISM OF LASER ACTION ON BIOORGANIC STRUCTURES

Possibility of electromagnetic radiation action on organic and colloidal solutions is connected with specific behaviors of boundary monomolecular layers of polar solvent at a surface of macromolecules or particles. The monomolecular film of polar water molecules densely filling a macromolecule surface and turned to it by one sign of a dipole represents a double electric layer. Though these double layer facings are in balance, but it is unstable. The instability reason is necessity of regular dipoles arrangement infringement on the macromolecule ends for preservation of a dipoles arrangement self-coordination on all closed surface as a whole. Therefore the double layer is spontaneously polarized in that more degrees, than it is more size of a particle or macromolecule. As a result there is a spontaneous polarization of a two-dimensional layer of polar molecules on a particle surface and the "gigant" dipole [3] which provides a bioorganic solutions photosensitivity is formed.

The dipole moment of a water molecule is equal to 1,855 D (1 Debay = $3{,}33564\times10^{-30}$ Q×m) with relaxation time 10^{-11} nsec, and for gelatin solution

(collagen) – $1,5\times10^4$ D with relaxation time equal a few minutes. Still big the dipole moment has microorganisms - so possess, at a tobacco mosaic virus it equal $10^4 – 10^5$ D, and at T-2 bacteriophage – to 64400 D for a particle in the size of 350 nanometers.

Considering, that electric field ***E*** energy of interaction with a dipole ***d*** is estimated by ***(d,E)*** value, it is possible to assert, that sensitivity to laser radiation action is more than macromolecule or them associations (clusters), and the action result can remain a macroscopically big time.

EXPERIMENTAL RESULTS

Researches on influence studying a antimicobacterum preparation – nystatin - on growth and development of periodic yeast culture Y-355 strain of *Saccharomyces cerevisiae* have been carried out. This culture has normal a reparation system and is characterized by low spontaneous mutation frequency.

The liquid nutrient Saburo medium, which is necessary for yeast culture development, was exposed to the laser semi-conductor diode irradiation (100 mW, a wave length about 532 nanometers) in a «a light copper» mode. In liquid dynamic holographic structures at the expense of a multibeam wave fronts interference were formed. Exposition doses nearby 1,5 kJ/mole and 5 kJ/mole were used.

After that in a nutrient medium the microbic suspension was located. Similar manipulations have been spent with the liquid environment with the nystatin dissolved in it (24 ed/ml). Crops were spent on Saburo agar on Gold's method. *S. cerevisiae* Culture preliminary grown up on a dense nutrient medium, washed off by a physiological solution, microbes concentration in suspension lead up to size of 10^5 cells/ml.

The account of results was carried out within 10 days. For a growth curve construction defined dynamics of microorganisms number by periodic inoculate crops on nutrient Saburo medium. A growth phases duration defined by a graphic method by of the colonies number estimate, which have grown on a nutrient medium in Petry cups. Change of cultures growth in the periodic cultivation conditions was estimated in dynamics with an interval 24,0 hours, since a zero point. Control variant was *S. cerevisiae*, which grown up in a nutrient medium without addition of a antimicobacterum preparation therapeutic dose or without a laser irradiation.

It is established, that at development of microbic culture in normal physiological conditions (control) the graphic has typical S-shaped character of growth (Fig. 1), in which it is possible to allocate an adaptation phase, the exponential period and stationary growth, and also a phase of its destruction.

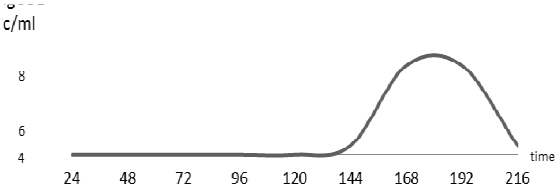

Fig. 1. Dynamics of a microbe's colony development in the physiological norm conditions. On a vertical axis – the logarithm of concentration of cells per a milliliter, horizontal – time in hours.

The adaptation stage in the physiological norm conditions makes 120 hours, and then it slowly passes during the exponential period growth which 24 hours last. Thus concentration of microbe cells increases on four order at average specific growth rate 0,1 $hour^{-1}$. Within 48 hours the culture is in the stationary growth period and then starts to perish slowly.

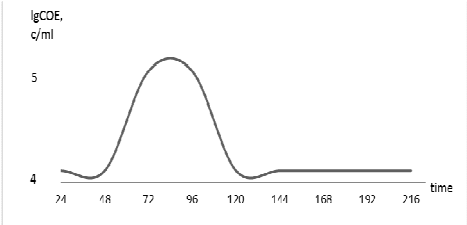

Fig. 2. Dynamics of a yeast culture development in a nutrient medium which has been irradiated by green semi-conductor laser radiation. A wavelength was equal 532 nanometers, a dose nearby 1,5 kJ/mole. On a vertical a[is – the logarithm of concentration of cells her a milliliter, across axis – time in hours.

Character of growth and development of yeast culture in the nutritious liquid which were exposed with a laser energy dose nearby 1,5 kJ/mole (Fig. 2), shows decrease an adaptation time at 72 hours. At the same time, specific growth rate of culture in a exponentional stage does not differ from the control. And if in the control the culture destruction in a dying off stage has exponential dependence in the given conditions it still for a long time remains viable.

At increase in a laser exposition doze for nutrient liquids to 5 kJ/mole (Fig. 3) are observed features, namely:

- duration of a stationary growth stage is prolonged at 48 hours in comparison with the control and a laser irradiation to 1,5 kJ/mole;
- specific growth rate of microbes culture in a exponentional growth stage increases twice

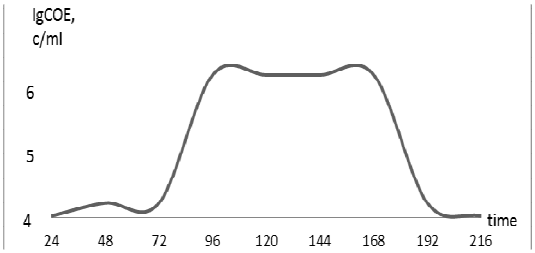

Fig. 3. Dynamics of a yeast culture development in a nutrient medium, which at an irradiation a dose near 5 kJ/mole. A wavelength is 532 nanometers. On a vertical a[is – the logarithm of concentration of cells per a milliliter, a horizontal a[is – time in hours.

It is known, that under the action of a laser irradiation in organic substances solutions macromolecules clusters can be crushed to smaller molecular structures, that can provide with it higher permeability in a live cell and by that to raise their biological activity. Experimental researches on action studying antimicrobiotic preparation – nystatin - on test cultures have been spent. Nystatin contained in a nutritious liquid which was exposed to a laser irradiation with doses 1,5 and 5 kJ/mole.

At action on microbic culture a antimicrobiotic preparation – nystatin – (a control variant, Fig. 4), character of its development does not differ from conditions of physiological growth, but the adaptation period decreases at 24 hours. The maximum increase in microbe cells concentration during the exponentional growth period makes only two order that specifies in decrease in their viability. Besides, if in the phase beginning decrease in number of microbe populations with high speed since cultivation 168 hours, stabilization of its density is observed, and is marked, that, apparently, is connected with formation at it the adaptive mechanism. Nystatin possesses the expressed biological activity concerning test yeast culture, but at long cultivation time in population structure resistance to a preparation is formed.

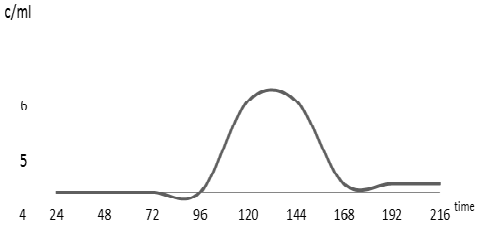

Fig. 4. Dynamics of a microbes colony development in the conditions of a nutrient medium with nystatin. On a vertical axis – the logarithm of cells per a milliliter concentration, horizontal axis across – time in hours.

At a laser action with energy doze 1,5 kJ/mole on a nutrient liquid containing nystatin, it has been established, that character of a curve does not differ from control variants, but has the few features (Fig. 5). First of all, a logarithmic growth stage time is reduced accordingly to 24 and 48 hours in comparison with a control variant. The maximum development in a exponentional growth stage the culture receives of 10^7 COE cells/ml that corresponds to the control at concentration. Besides, specific growth rate increases in these conditions in 3 times and makes 0,3 hours^{-1} to the relation of physiological norm.

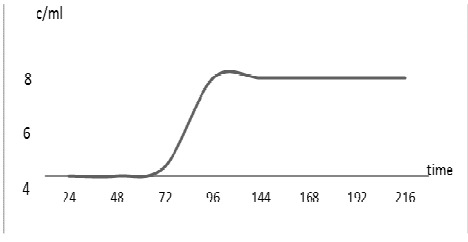

Fig. 5. Dynamics of a yeast culture development in a nutrient medium with nystatin which has been irradiated by the green semi-conductor laser radiation.

A wavelength was 532 nanometers, a dose near 1,5 kJ/mole. On a vertical axis – the logarithm of concentration of cells per a milliliter, horizontal axis – time in hours.

In case of action on a nutrient liquid containing nystatin, in a dose near 5 kJ/mole (Fig. 6) is observed full inhibition (suppression) of test culture growth.

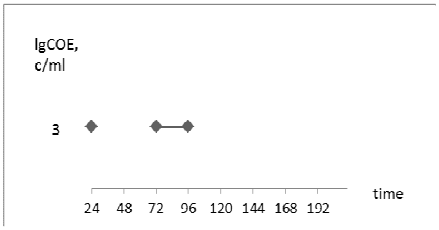

Fig. 6. Dynamics of a yeast culture development in a nutrient medium which has been irradiated by the green semi-conductor laser radiation. A wavelength was 532 nanometers, a energy dose near 5 kJ/mole. On a vertical axis – the logarithm of concentration of cells per a milliliter, horizontal axis – time in hours.

CONCLUSION

Thus, results of pilot researches have shown, that laser action with doses in a energy range less than energy of hydrogen bonds (10 – 40 kJ/mole), crushes cluster nystatin structure in a solution that leads to its biochemical activization. It will allow lowering a therapeutic dose of preparations, thereby to reduce by-effects, not reducing medical result.

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