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GRADIENT PHOTO-ELECTRIC MODEL OF LASER IRRADIATION BIOLOGICAL ACTION

A new model of low intensity laser irradiation action (LILI) on biological objects on cell level was suggested. Up to this model the effect of LILI action is a result of that fact that laser light irradiation due to it coherence can obtain periodical character (interference picture or speckle-structure) with small enough periods of dark and light parts changing comparable with cell or cell organelles sizes. In this conditions of high gradient illumination an electrical field can appear (Dember effect), which is able to change the character of photostimulated reactions and membrane charge conditions either of cell itself or of its organelles, that leads to cell life cycle shifts.

INTRODUCTION

Today the fact of low intensity laser irradiation action (LILI) on biological objects can be considered as real and well proved in numerous experiments on animals and humans. LILI action is widely used in clinical practice for more rapid wound healing, scar and chronicle inflammation processes decreasing, etc. There are many works devoted to LILI stimulation of metabolic processes in tissues and organs, blood microcirculation improvement, ferment systems activation, tissue regeneration. Antioxidant, antithrombus, analgetic, immune correcting action of LILI was noted [1, 2].

From the other side many scientists show skeptic attitude to this practice, which may be approved because there are no common used theory of LILI action till now [3]. There are no even sufficient hypotheses, which can explain all sides of experimental effects.

There is even an opinion that there are no any LILI actions at all, and all results in humans can be explained by self-suggestibility (positive placebo effect). But this mechanism can't explain numerous and quite convincible results of such experiments in animals.

Another strange thing is that usually only positive LILI action on various diseases is mentioned.

If exclude self-suggestibility effect, such positive reaction can be explained in such way. LILI has a «non specific» action and through different mechanisms can activate immune reactions of organism and in such way normalize it's state. This point of view have a grate experimental base, but it is valid only in case of multysell organisms and it can't explain the main — describe physical and chemical mechanisms of LILI action on cell level and give methodic base to works in this direction.

Another explanation of positive LILI action is that LILI action is weak or absent on normal sells and destroy only injured, defect ones. Thus LILI biological action can be described in the same way as classical methods of chemistry and radiotherapy. But in this case we also must describe physical mechanisms of LILI, and answer the main question: why we must use the very laser light. That is, what is the difference between laser light and light from other sources in relation to biological objects.

CONTRADICTIONS OF PRESENT HYPOTHESES

Two main hypotheses of LILI biological action exist.

Hypotheses of thermal effect. It is considered that laser irradiation can lead to sufficient thermal gradient appearance in tissues especially for separate sell or its organelles — that is local sell or its organelles heating if they electively better absorb light on laser wavelength. As a result it can influence biochemical reaction rates.

Hypotheses of resonance action. It is suggested that monochromatic laser irradiation is selectively absorbed by molecular chromophores that causes the activation of some biochemical reactions and that chromophores absorption bands are very narrow and very clearly match with used laser wavelength.

Pointed above hypotheses are not able to explain such very important facts:

1. Low intensity of often used laser irradiation (several mWatt per sm^2) practically excludes the first (thermal) hypotheses. Except this it can't explain why only laser irradiation have biological action, but not the light from other sources, even monochromatised with filter help. There are known works on so called quasi-laser irradiation more often obtained from light diodes, but the level of noted laser action and reliability of results of this works are sufficiently less then in the case of real laser use.

2. The necessity of laser use can be explained by the second hypotheses (the resonance one). But any such narrow absorption bands in biological objects weren't shown in experimental

results and their existence in condensed medium at room temperature is very problematic. But if we even suggest that such narrow absorption bands can exist, there is very little probability that they will occasionally clearly match with used laser wavelength. Such matching becomes absolutely impossible if we'll remember that wavelength of semiconductor lasers (which are widely used in therapeutic practice now) is not fixed and depends strongly on temperature and excitation current. On the other hand, it is known, that same biological action give lasers with different wavelength (from near infrared to almost ultraviolet). The suggestion that different chromophores groups can be included in work while wavelength changes is quite correct. But in this case to explain the absence of white light biological action, one must suggest that different chromophores groups initiate opposite biological reactions, which annihilate each other. But this suggestion looks artificial.

3. It is strange that while there are many experimental works on LILI action on animals or human organisms, the works on LILI action in vitro on sell cultures and microbiological objects are much more rear. But these last types of experiments are meaningly simpler, not expensive and give much information. Known from literature experiments of such kind as a rule showed less expressed results (reliable LILI action was weak or absent). It was explained by LILI action on nervous system of animals and man, and as a result biological immune reactions were initiated. But mechanism of direct action on nervous sell was not explained either.

PROPOSED MODEL OF LILI BIOLOGICAL ACTION

In this work we tried to fill in this empty place — to offer physical model of LILI action, which can without contradiction explain all pointed above facts. It was also important to develop an experimental methodic of LILI action on alive cell in vitro or on microorganisms (and here on this step whether this action will be stimulating or depressive is absolutely unimportant). The methodic will give an opportunity to check the accuracy of proposed theoretical hypotheses.

Our model of LILI biological action on cell level is based on coherent properties of laser light.

As it was shown in our previous works [4—6] the sufficient influence on photoinduced processes can cause space displacement of light in irradiated medium. Homogeneous irradiation (which is used often) and irradiation with high space frequency interference picture can lead to different results.

The mechanism of this phenomenon is rather general and such effect we observed in very different media — crystals [5, 6], semiconductor glasses [4] and liquid crystals. That's why it can appear also in biological systems.

This effect is linked with photo-stimulated diffusion and drift processes in high gradient light fields. The absorption of light with appearance of free charge carriers leads to electric field appearance between light minimums and maximums (Dember effect). This can not only change the performance of photo stimulated reactions but change the localization either of photo-stimulated reactions products or substances, which where initially present in media. That is especially important for biological objects. The most important processes are connected with diffusion and drift of molecules through sell membranes and sell organelle membranes. Changing of membrane charge conditions can lead to great shift in sell life cycle [2, 3].

Besides molecules of biological active substances (toxins, antibiotics, etc.) can also have electrical charge or dipole moment, thus electric high gradient field presence in the media can stimulate (or decrease) their penetration through membranes, and thus increase (or decrease) their action. So according to proposed model LILI effect is very much alike with local electrophoresis.

Thus, the key moment of this model is in the presence of dark and light places in illuminated biological media, and space period of their alteration must be near the size of cells or cells' organelles.

In proposed model the contradictions 1 and 2 can be lightly explained. There is no need in either resonance absorption, or in illumination of large power (neither total, nor spectral).

There also is no need in light monochromacy (by itself), which has been discussed till now as the main sigh of laser irradiation and LILI action, and coherent properties of laser light come on first place.

More over coherence is needed only for spot irradiation creation with little size of spots. In general this picture can be provided by other methods. For example, one can use white light and absorptive mask with correspondent size of spots, but in this case for needed space frequencies ($\sim$ 1000 lines/mm) the spot character of illumination will be preserved only on very short distance under mask, which can be compared with the size of spots. Probably this method can be used only in experiments to check the model correctness. But to get the appropriate spotty (speckle) picture in big enough volume only the coherent (laser) light must be used.

From this point of view the contradiction 3 can also be lightly explained. As till now the irradiation was fulfilled by space homogenous laser light, which by our model is not able to influence alive cells in vitro if they exist as monolayer cultures. The main difference of in vivo experiments on animals is in that fact that the laser light remains homogenous only in upper thin layer. In further layers speckle-picture develops as the result of not homogenous absorption and light refraction in upper layers of cells and illumination becomes spotty. While

usual depth of light penetration in alive tissues is up to 2—4 sm, speckle picture becomes frequent enough on the depth of several millimeters, and its period can be compared with cells sizes and biological action begins. In experiments on monolayer cell cultures conditions for light periodicy must be made artificially. It is interesting to note that in the experiments on cell cultures, when LILI action was seen, laser light was used to illuminate the emulsion of cells in rather big volume of several sm^3 (in this condition light penetration through muddy media can develop frequent enough speckle picture), and then emulsion was placed on solid media of Petry dishes. In the works, when illumination was fulfilled in Petry dishes directly, LILY action was often not noted. Though even in this case spotty picture can appear, which is connected with interference in meeting direct and reflected from Petry dishes' down glass beams of laser irradiation. But in this case interference picture contrast is weak, and space frequency of interference picture is too large (at about 6.000 lines/mm), that's why biological action mustn't be large, but it can be present. It's a pity that irradiation conditions are not described properly in many works, that's why the level of light not homogeneity can't be evaluated.

MATERIALS AND METHODS

For practical confirmation of suggested model we fulfilled several series of experiments to study LILY action in vitro on microorganisms cultures, which were grown on solid nutrition medium in Petry dishes. As microorganism cultures we used pure culture of Staphylococcus aureus (hospital strain, Gram positive) and Pseudomonas aeruginosa (hospital strain, Gram negative). Bacterial suspension was prepared according muddy standard counting 10^3—10^4 of microbes in 1 ml and were put on Petry dishes in 0.1 ml dose in lawn manner, that is 1 dish got about 10^2—10^3 microbes. To diluted in physiological solution culture antibiotics were added in several doses 1000, 100 and 30 Units per 1 ml directly before inoculation of medium and further irradiation. As a control irradiated, not irradiated intact and with added antibiotics cultures were used. Cultures were incubated at 37 °C 24 hours. Results were registrated by direct counting of grown colonies, if the colonies were not possible to divide the results were taken by aria of growth. The most commonly used in the hospitals antibiotics were used: they are ampicillin, gentomycin, lincomycin, cefasolin, which have different mode of antibacterial action.

Cultures in Petry dishes were irradiated by homogeneous light and by interferention (periodical) light field created by two coming together on different angle laser beams of equal intensity (Fig. 1).

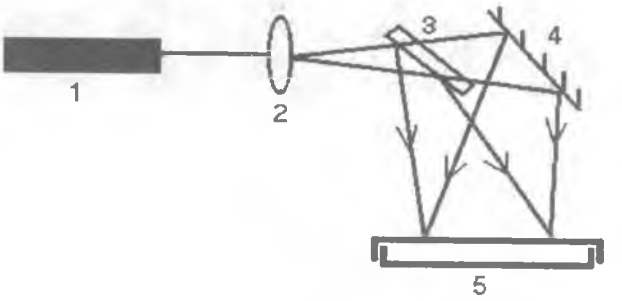

Fig. 1. Principle scheme of irradiation device:

1 — laser; *2* — lens; *3* — semitransparent mirror; *4* — mirror; *5* — irradiated object (Petry dish)

Invert rereflaction (parasite enlightening) was suppressed by special means. On beams coming together angle changing space frequency of interferention picture changed from 0 (homogeneous light) to 2000 lines per mm (coming together angle $\sim 45°$) and 6000 lines per mm (contrary beams). Irradiation was fulfilled by He—Ne laser red light (wave length 633 nm), irradiation power density was ~ 2 mWatt/sm^2, irradiation time — 1 min.

EXPERIMENTAL DATA

The result of experiments (Fig. 2) confirmed suggested models.

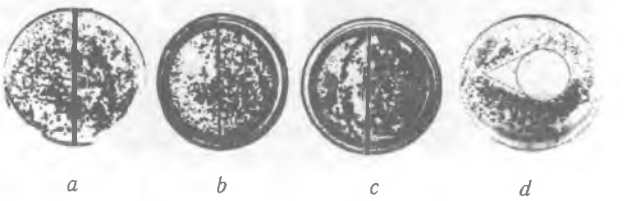

Fig. 2. *Staphylococcus aureus* + *gentomicine*:

a, *b*, *c* — the left half of the Petry dish was irradiated, right half was in darkness; *d* — irradiated aria is marked by a circle (the triangle to the left is the result of invert rereflection, as in this experiment it was not suppressed); irradiation was fulfilled by: *a* — homogeneous light; *b*, *c*, *d* — interferention field with space frequency 1000 lines/mm

We mast mark that:

1. No one experiment showed influence of homogeneous laser irradiation on microbe culture (fig 1, a).

2. Interferention field use (of the same to homogeneous irradiation total power in the same conditions) leaded to substantial decrease of survived quantity of microbes after antibiotic action (microbes quantity ratio in irradiated and not irradiated arias was about 6 and more, fig. 2, *b*, *c*, *d*).

3. Action intensity maximum was noted at on interferention picture space period near to the cells size, (space frequency ~ 1000 lines/mm, fig. 3, *d*).

Thus all three said above contradictions can be solved in proposed model. But as it was pointed out above this common model gives

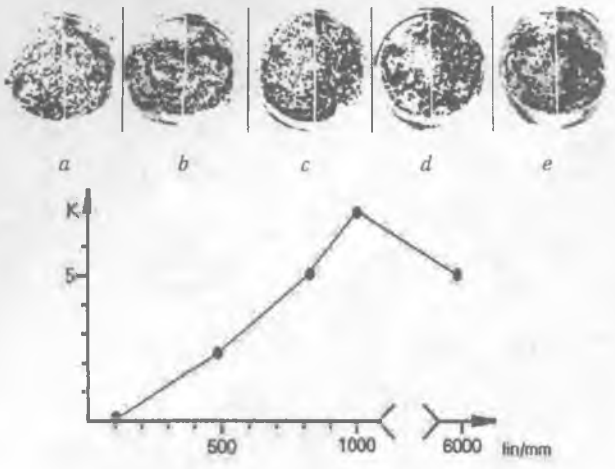

Fig. 3. *Staphylococcus aureus* + *gentomicine*:

Number colonies ratio dependence in irradiated and not irradiated parts of Petry dishes (K) on interferention picture space frequency: a — homogenious light; b, c, d, e — 100, 500, 1000 and 6000 lines per mm correspondingly

only conditions for LILI action possibility. It does not define whether this action will be positive or negative, and this problem has to be investigated in more detailed experiment. The complication of possible action is demonstrated on Fig. 4.

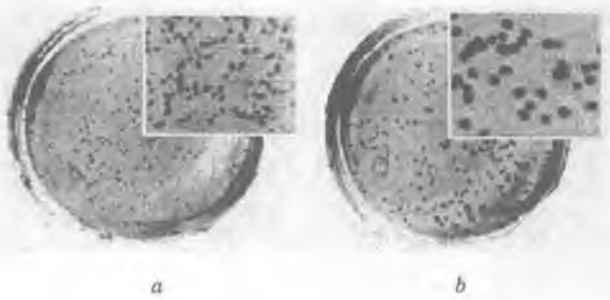

Fig. 4. *Pseudomonas aeruginosa* + cefasolin:

Fragments — multiplication with x5: a — light absence, b — irradiation with space frequency 1000 lines/mm

As a result of laser irradiation the common amount of colonies decreased, but the size of each survived colony has became larger. So we can watch in one time depressing and stimulating action.

CONCLUSION

Proposed physical model of LILI action on biological objects allowes to explain all observed experimental data and contradictions of previous models. The model gives clear criteria for selection of either irradiation parameters or accompanying chemical substances or medicines.

Offered nothomogeneous light irradiation method allows to increase level of LILI action and to perfect stability of results.

In case of successful clinical test fulfillment this method can be used in clinical practice for LILI effect increase and accompanying medicines dose decreasing.

Except this method can be used for aimed modification of separate cell with the help of specially formed optical fields.

The method have been patented in Ukraine [7].

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