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Biological Effect of Continuous, Quasi-Continuous and Pulsed Laser Radiation

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Abstract

In this work, for the first time, comparative studies of biological activity of low intensity continuous, quasi-continuous and pulsed laser radiation of nano- and picosecond time ranges with the same average power density are carried out. It is shown, that, despite the significant differences in peak values of intensity of acting factor, both continuous and quasi-continuous radiation and radiation of nano- and picosecond ranges are able to have both stimulating and inhibiting effects on all investigated parameters of functional activity of biological systems in a certain range of dose rates. The ability of laser radiation of near infra-red spectral region (800 - 1340 nm) located out the absorption bands of main chromophores of cells to have regulatory effect on biochemical processes that control the hatching of branchiopod crustaceans *Artemia salina L*. upon irradiation of their cysts is revealed. The role of molecular oxygen and water as acceptors of laser radiation is discussed.

Keywords: Low intensity laser radiation, Laser activation, Biological activity, Zooplankton *Artemia salina L.,* Sturgeon sperm.

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1. Introduction

Despite the progress in the practical use of low-intensity laser radiation in medicine and agriculture [1-6], question about mechanism of biological activity of mentioned physical factor is still open [2, 4]. As a rule, the basic studies are carried out using a continuous radiation; biological effect of pulsed radiation of nano- and picosecond ranges is studied poorly. The main reasons of this are the lack of available sources of radiation with stable parameters as well as well-established biological models providing reception of statistically significant, well-reproducible results upon exposure to low-intensity optical radiation. It is commonly accepted to carry out the studies of regularities of biological action of physical factors at organism level using experimental

animals (rats, rabbits) comparing the effect of physical fileds with 3-4 parameters which are important, in opinion of the experimentalist, for a reasonable conclusion. However, due to complexity of multiple experiments on laboratory animals, researcher in comparative experiments has to be limited to any single fixed value of the radiation dose that causes change in controlled biological parameters. It does not allow finding out the general regularities in the action of that or another physical factor and can even lead to false conclusion. Furthermore, due to heterogeneity of biological species, reliability of the results is often insufficient to make a reasonable conclusion. For this reason, taking into account the complexity of experiments on animals (requiring 3-5 replicates per experimental point), quantitative studies at organism level with parameters of acting factors varying in a wide range are practically absent in the literature. It is not coincidence that the main conclusions about the mechanism of biological effect of laser radiation were made in the experiments with cell cultures or blood cells. In this regard, we proposed to use fish embryos (fertilized eggs), zooplankton, and fish sperm as a model to study the mechanisms and regularities of action of physical fields at organism level. In our opinion, the attractiveness of the use of mentioned objects is determined by, on the one hand, possibility of choice of a large number of similar species (obtained from one manufacturer), on the other hand, – good sensitivity of mentioned biological objects to the action of physical fields, reproducibility of results, their high reliability, and correspondence to the data, obtained upon exposure of experimental animals to radiation.

The aim of the present work is comparative studies of biological activity of continuous, quasi-continuous and pulsed laser radiation of nano- and picosecond time ranges of low intensity with equal average power density (3,0 mW/cm^2).

2. Materials and methods

Zooplankton (branchiopod crustaceans) *Artemia salina L.* and sturgeon sperm were used as objects. As a test, to check the action of laser radiation, percentage of nauplii hatched from cysts (protective shells) after activation of eggs in salt water in a stable thermal regime was chosen. The indicators of biological action on fish sperm were the data on duration of sperm motility as well as their curvilinear velocity after activation with water. Analysis of motility parameters was performed programmatically based on an assessment of their trajectory. The value of photobiological effect (dose curve) was evaluated in comparison with control intact objects.

The exposure was realized using the second-harmonic radiation (wavelength – 532 nm, average output power ~30 mW) of Nd:YAG-lasers working in continuous and quasi-continuous (pulse repetition rate – F = 1 kHz, pulse duration – τ = 100 ns) modes, as well as in pulsed mode with generation of nanosecond (τ = 15 ns, F = 10 Hz) and picosecond (τ = 60 ps, F = 20 Hz) pulses.

Comparative studies upon exposure to radiation of red (632.8 nm, He-Ne laser) and near IR (808 and 976 nm – diode lasers; 1064 and 1342 nm – diode pumped Nd:YVO_4 laser; 1176 nm – diode pumped Nd:YVO_4 laser (1064 nm) with intracavity Raman self-frequency conversion) spectral region were also carried out. Power density (P) – 3 mW/cm^2.

3. Results and Discussion

It is shown, for the first time, that, despite the significant differences in peak values of intensity of acting factor, both continuous and quasi-continuous radiation and radiation of nano- and picosecond ranges are able to have both stimulating and inhibiting effects on all investigated parameters of functional activity of biological systems in a certain range of dose rates.

As an example, Figures 1 and 2 show the effect of continuous, quasi-continuous and pulsed laser radiation of nano- and picosecond time ranges with λ = 532 nm at equal average power density 3 mW/cm^2 on the hatching of nauplii from cysts (γ, %, Fig.1) as well as the duration of the motility of sturgeon sperm after activation with water (η, %, Fig.2) in percentage to control. Data show that the optimal dose of optical radiation which initiates the stimulation of functional characteristics of biosystems is strongly dependent on the mode of acting radiation. For example, using the aforementioned parameters of acting factors, the optimal stimulating dose when controlling the sperm motility is 135 mJ/cm^2 for continuous radiation; 90 mJ/cm^2 - for quasi-continuous and nanosecond and 60 mJ/cm^2 – for picosecond radiation. At the same time, maximal stimulating effect (compared to the control) is 140±6 % for continuous; 163±9 % – for quasi-continuous; 122±6 % – for nanosecond and 115±7 % – for picosecond modes. Even more pronounced stimulating effect (180±9 %) has a continuous radiation of red spectral region.

It is typical that stimulating effect in the case of nano- and picosecond modes is observed in a very narrow dose interval: 30–60 mJ/cm^2. The rapid suppression of functional characteristics of biological systems is observed upon increasing the dose: at

a dose of 1.8 J/cm^2 duration of sperm motility reduced more than two times compared to the control.

Similar bell-shaped dose curves are registered when controlling the curvilinear sperm velocity and percentage of nauplii hatched from cysts after activation of eggs in salt. This type of dose curves testifies to "soft" regulatory nature of biological action of laser radiation. On the other hand, similar nature of dose curves upon control of functional characteristics differing in their structural organization of biological systems (zooplankton and fish sperm) is evidence of biological significance of the results obtained.

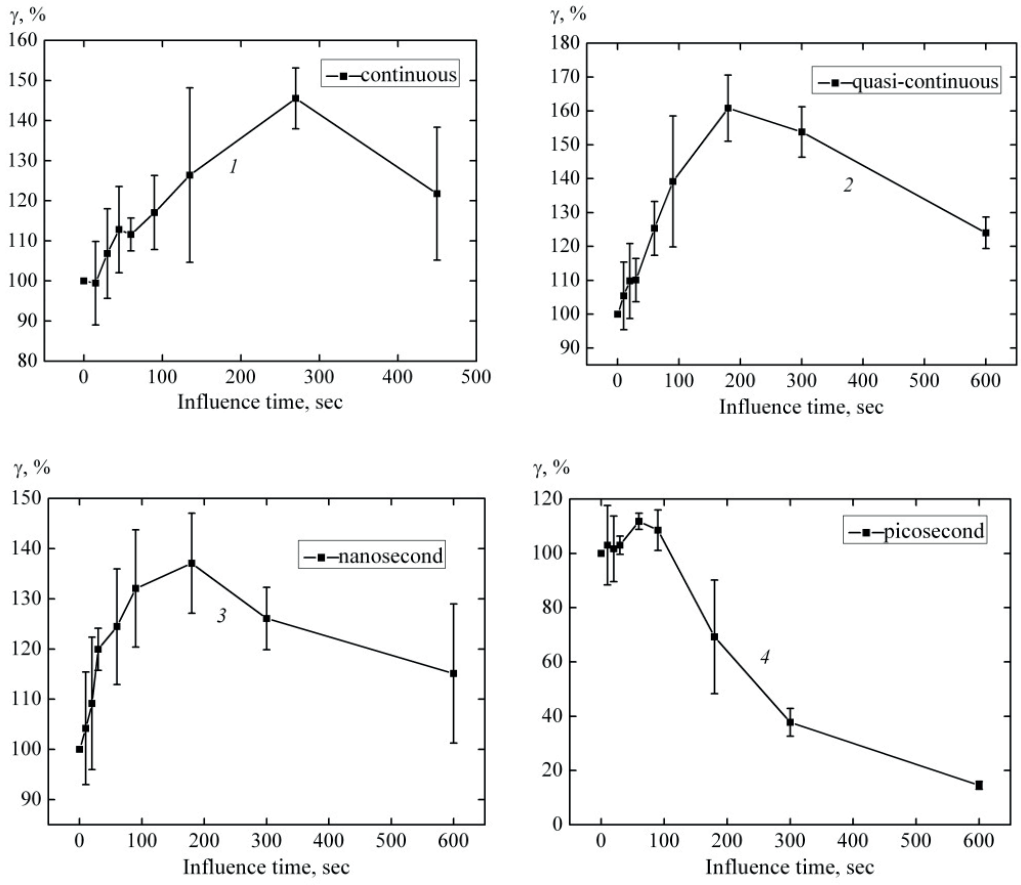

Figure 1: Effect of continuous (1), quasi-continuous (2) and pulsed laser radiation of nano- (3) and picosecond (4) time ranges (P = 3,0 mW/cm^2) with wavelength 532 nm on the hatching of branchiopods crustacean *Artemia salina L*.

Studies have also shown that photobiological effect initiated by laser radiation (the hatching of the nauplii *Artemia salina L*.) is strongly dependent on the wavelength of incident radiation.

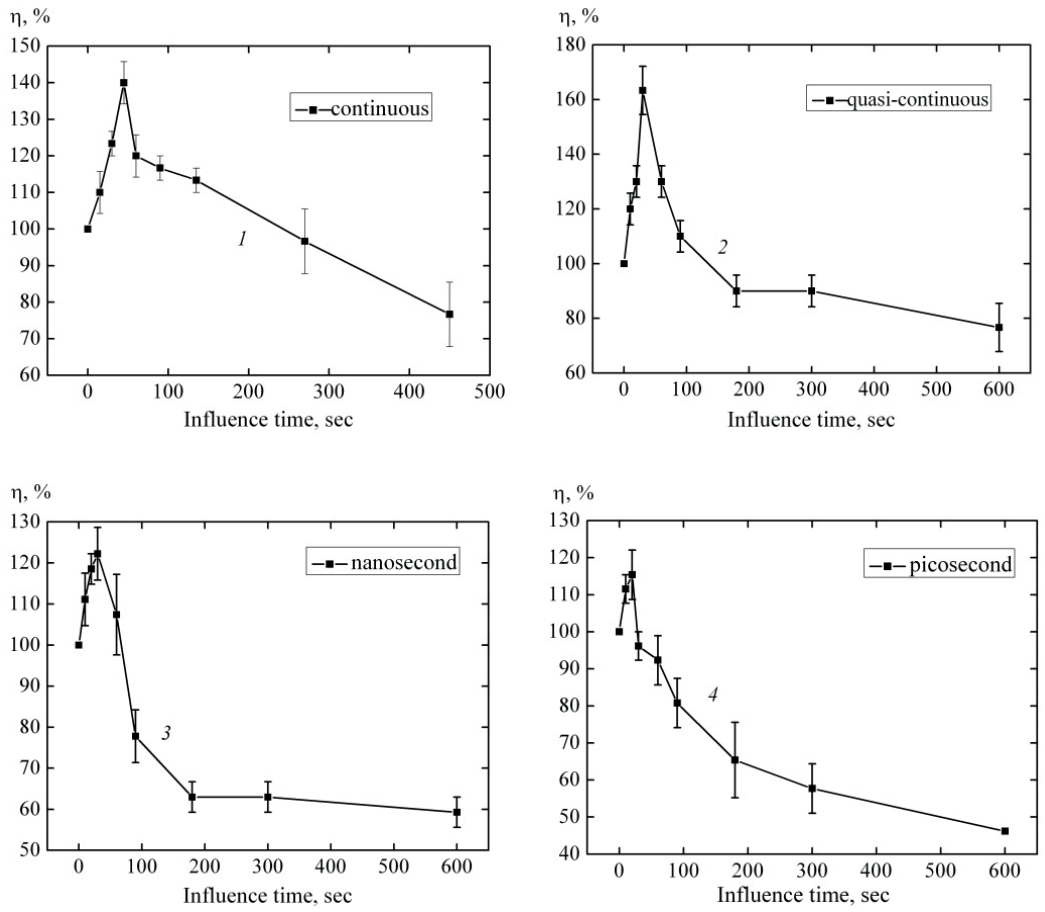

Figure 2: Effect of continuous (1), quasi-continuous (2) and pulsed laser radiation of nano- (3) and picosecond (4) time ranges (P = 3,0 mW/cm^2) with wavelength 532 nm on the sperm motility.

Figure 3 shows the dependence of efficiency of nauplii hatching on influence time of laser radiation (power density - P = 3,0 mW/cm^2) with λ = 632,8; 976; 1176 and 1342 nm on cysts.

It is noteworthy that under optimal conditions if radiation with λ = 632,8 nm, λ = 976 and λ = 1064 nm has an inhibitory effect on the hatching of the nauplii, the radiation with λ = 808 nm, λ = 1176 nm and λ = 1342 nm – stimulating effect. The results obtained by the action of laser radiation on the hatching of the nauplii *Artemia salina L*. allow making some conclusions about bioactivity of optical radiation of the near IR spectral range. Since the laser light with λ = 808, 976, 1064, 1176, 1342 nm is outside the absorption band of the porphyrins, the possible role of photosensitized reactions involving them should be excluded. Severe photobiological effect when exposed to radiation λ = 1176, 1342 nm can also questioned the role of the direct photochemical reactions of oxyhemoglobin (and other macromolecules containing the prosthetic groups), as the impact of a powerful pulsed laser radiation with a wavelength λ = 1060 nm for its solutions does not cause any reversible or irreversible spectral changes.

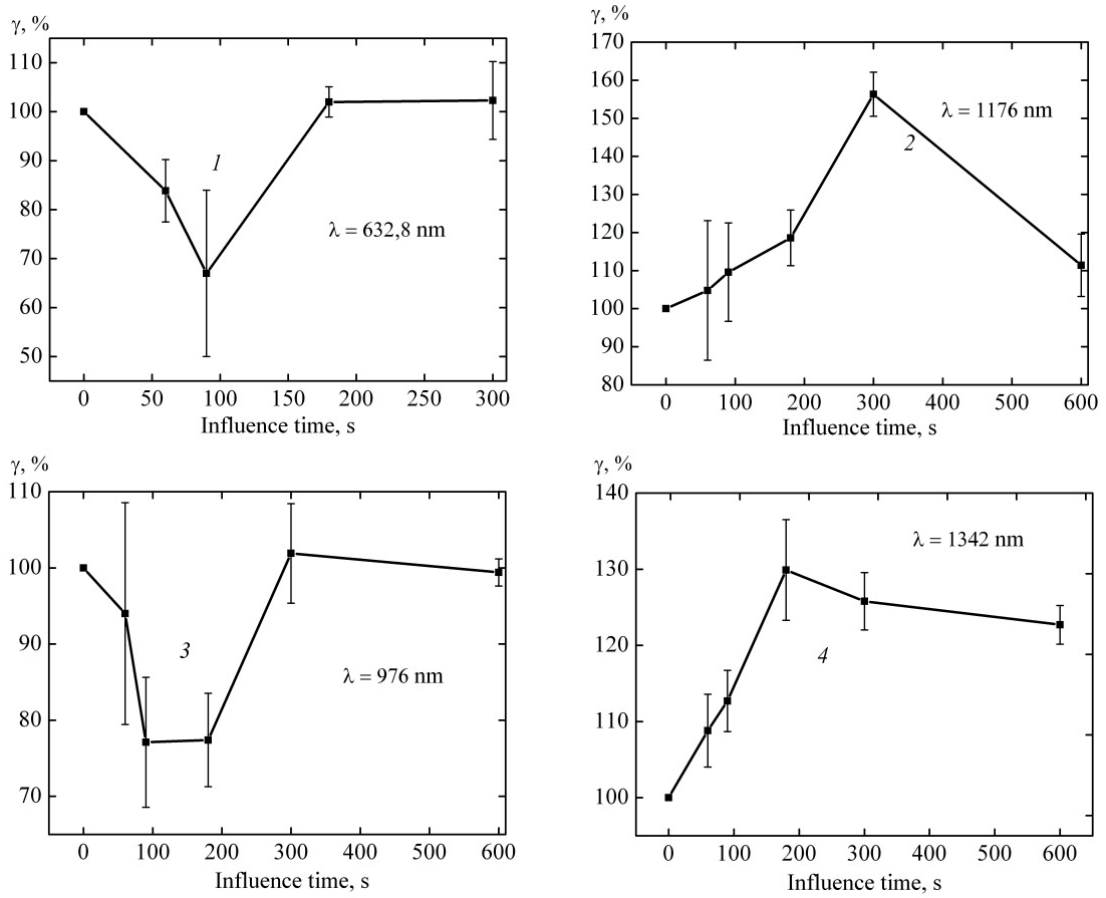

Figure 3: Effect of laser radiation (P = 3,0 mW/cm^2) with wavelengths 632,8 (1), 1176 (2), 976 (3) and 1342 nm (4) on the hatching of nauplii upon irradiation of cysts.

Note also that the presence of extreme points in the absorption spectrum of aqueous solutions of macromolecules in λ = 1100 - 2500 nm is almost completely determined by the solvent.

In our opinion, most likely that dissolved molecular oxygen and water can act as acceptors of laser radiation of near infrared spectral region, determining its biological effects. Despite the small molar extinction coefficient there are data confirming the possibility of direct triplet-singlet excitation of molecular oxygen. Accounting low value of molar extinction coefficient of such transition and relatively low power density of exciting radiation (3 mW/cm^2) the concentration of singlet oxygen in biological system is sufficiently small to generate noticeable destructive action. Most likely that its formation in biological system can play signal (trigger) function that influences the bio-chemical and physiological processes in organism, for example, such as starting apoptosis. However, the presence of biological effects when exposed to radiation cysts nauplii, out of the corresponding bands for direct excitation of singlet oxygen, suggests that in addition to molecular oxygen a water - an universal biological environment,

which plays a leading role in the maintenance and regulation of homeostasis in living systems can be an acceptor of laser radiation near-infrared region of the spectrum.

4. Conclusions

It is found that biological effect of laser radiation controlled on functional activity of zooplankton and sturgeon sperm is strongly dependent on the mode of acting radiation under conditions with similar average power density. We showed for the first time that, despite the significant differences in the peak intensities of acting factor, both continuous and quasi-continuous radiation, as well as radiation of nano- and picosecond time ranges was able in a certain range of dose rates to have both stimulating and inhibiting effect on all the studied parameters of functional activity of biological systems.

The ability of laser radiation of near infra-red spectral region (800 - 1340 nm) located out the absorption bands of main chromophores of cells to have regulatory effect on biochemical processes that control the hatching of branchiopod crustaceans *Artemia salina L*. upon irradiation of their cysts is revealed. Molecular oxygen can be potential acceptor of radiation of near infra-red spectral region and observed photobiological effect can be caused by direct excitation of singlet oxygen and its further influence as signal (trigger) molecule on the physiological processes. In addition to oxygen, water can also be the acceptor of laser radiation since the absorption of aqueous solutions of biological molecules in the spectral region of $\lambda = 1200 - 2500$ nm is fully due to solvent.

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