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Effect of Laser Irradiation on Cell Cycle and Mitosis

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Abstract

Introduction: In this research, low-level helium-neon (He-Ne) laser irradiation effects on monkey kidney cells (Vero cell line) mitosis were studied.

Methods: The experiment was carried out on a monkey kidney cell line "Vero (CCL-81)". This is a lineage of cells used in cell cultures and can be used for efficacy and media testing. The monolayer cells were formed on coating glass in a spectral cuvette (20×20×30 mm). The samples divided into two groups. The first groups as irradiated monolayer cells were exposed by a He-Ne laser (PolyaronNPO, L'vov, Ukraine) with λ = 632.8 nm, max power density (P) = 10 mW/cm^2, generating linearly polarized and the second groups as the control monolayer cells were located in a cuvette protected by a lightproof screen from the first cuvette and also from the laser exposure. Then, changing functional activity of the monolayer cells, due to the radiation influence on some physical factors were measured.

Results: The results showed that low-intensity laser irradiation in the range of visible red could make meaningful changes in the cell division process (the mitosis activity). These changes depend on the power density, exposure time, the presence of a magnetic field, and the duration of time after exposure termination. The stimulatory effects on the cell division within the power density of 1-6 mW/(cm^2) and exposure time in the range of 1-10 minutes was studied. It is demonstrated that the increase in these parameters (power density and exposure time) leads to destructing the cell division process.

Conclusion: The results are useful to identify the molecular mechanisms caused by low-intensity laser effects on the biological activities of the cells. Thus, this study helps to optimize medical laser technology as well as achieving information on the therapeutic effects of low-intensity lasers.

Keywords: Low-intensity laser irradiation; Power density; Mitosis activity; Laser therapy.

Introduction

Laser radiation at power density of 1-100 mW/cm^2 with various wavelengths has come into the modern medicine market as an effective factor to treat wide range of diseases including healing wounds, bruises and ulcers; stomach and duodenal gut ulcer; teeth and oral mucosal diseases; rheumatoid arthritis; spinal and deforming osteochondroses; gynecologic diseases; dermatosis; acute and ortitis diseases of ear, throat, nose; burns, chronic prostatitises, pancreatic,cholecystitises;ischemic heart trouble and chronic kidney diseases.[1-5] This topic was first explored by the Cossack scientists, Injushin et al,[6] who showed that monochromatic radiation helium-neon (He-Ne) (λ = 632.8 nm) laser possesses essentially more medical effects in comparison with the red light of lamp sources. Therapeutic effects of sunlight and red radiation lamp sources were investigated before laser applications, but their practical efficiency has been studied only in recent years.[7]

The principal distributions about low-power laser treatment (laser biostimulation) as an argumentative topic has been studied since over 60 years.[8] During the last decade, a number of researchers demonstrated that laser radiation could influence specific physical factors in animal and human cells, tissues, organs, and human organism.[9,10] These factors may lead to changes in metabolism activity of major enzymes, permeability of cellular membranes, accelerate tissues synthesis, DNA and RNA synthesis, cell division, regeneration of tissues, reparations of the genetic damages, and immune systems.[11-13] But the essential mechanism of photophysical process of low-intensity laser radiation on biological and therapeutic actions structures of organism is not clear.[14-18] To answer such questions, it is necessary to set up different

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cellular systems, which low-intensity laser irradiation can affect the activity for a specific function.

In this research, changes in mitotic activity of monolayer cells caused by low-intensity laser parameters such as power density, and exposure time have been studied. The results of this study can help to specify the photophysical mechanism in biological activity and the therapeutic effects of low-intensity laser irradiation. Consequently, this research may lead to the optimization of laser medical technology.

Methods

The experiment was carried out on a monkey kidney cell line "Vero (CCL-81)". The cells was isolated from kidney epithelial cells extracted from an African green monkey. The cells were grown in DMEM (Dulbecco's Modification of Eagle's Medium) supplemented with 10% fetal bovine serum without antibiotics. At 24-48 hours after plating when cells were 50%–60% confluence, the medium was changed before starting the process. The monolayer cells were formed on coating glass in a spectral cuvette (20×20×30 mm). The following laser was used for irradiation: He-Ne laser with wavelength 632.8 nm, generating linearly polarized. Then, changing the mitotic activity of the cells was caused by a low–intensity laser radiation on the monolayer cells. The experimental set up is presented in Figure 1, in which the laser irradiation from a source passes through an optical filter to protect the ultraviolet radiation from the gas discharge tube (when using He-Ne laser), and passes through a short-focus lens (it was a homemade laser device). In this setup, the radiation is defocused and contacts cuvette containing monolayer cells. The short-focus lens provides an irradiation which is used to affect the cells. The control monolayer cells were located in a cuvette protected by a lightproof screen from the first cuvette and also from the laser exposure. In this study, except for the exposed monolayer cells to the laser beam, all other physical and chemical parameters (temperature 37°C, pH nutrient medium) were similar for both irradiated and control monolayer cells.

As a quantitative measure in changing functional activity of the monolayer cells, due to the radiation influence on some physical factors, the value η was selected. η is the relation of mitosis index in experiment monolayer (M_{ex}) to mitosis index in control monolayer (M_c):

$$\eta = (M_{ex}/M_c)\cdot 100\% \quad (1)$$

that M = (n/N)·1000, n and N – are divided cell number and total number of monolayer cells, respectively.

To determine the mitotic index, an optical microscope was used to record the number of cells in a monolayer. Data were analyzed by one-way ANOVA and all values are means ± standard error (SE). The reliability of the obtained results was assessed by the student's *t* test.

Results

Effect of Laser Power Density and Exposure Time on Mitotic Activity of Cells

The experimental data exhibited that the low-intensity laser radiation could significantly induce changes in mitotic activity in the irradiated monolayer cells. Photobiological effects of laser radiation depend on the exposure time and power density, as well as the duration of time after irradiation termination. The effect of exposure time and power density of He-Ne laser irradiation on the mitotic activity of monolayer cells are shown in Figures 2 and 3.

Correspondence of photobiological effect value η = f (t) and η = f (p), as is evident from figures 3 and 4 represents a bell-shaped curve with a maximum stimulating effect η= (126 * 4)% at t = 300 seconds (power density 3 mW/cm^2) (Figure 3) and at P = 3 mW/cm^2, exposure 300 seconds (Figure 4).

Effects of Low-Intensity Laser Radiation in the Presence of a Constant Magnetic Field

The experiment was conducted as follow: the monkey kidney monolayer cells were exposed: (*a*) low intensity

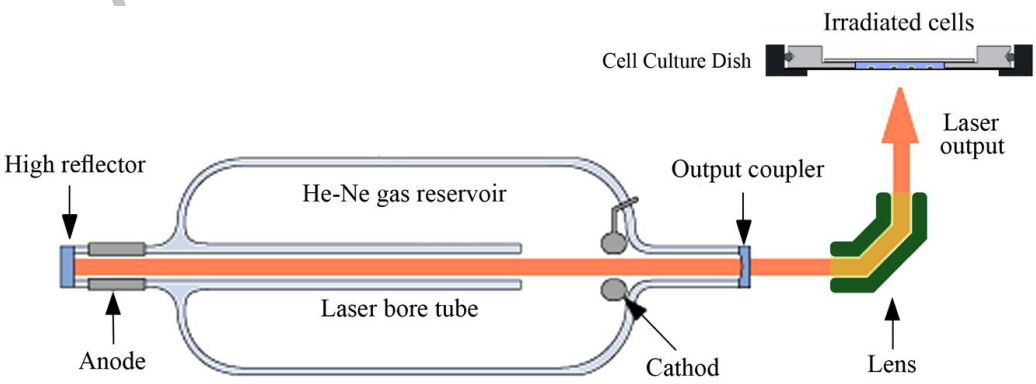

Figure 1. The Experiment He-Ne Laser Setup for the Irradiation of Monkey Kidney Cell (With Wavelength 632.8 Nm, Generating Linearly Polarized).

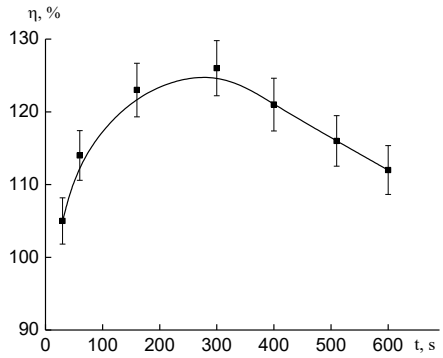

Figure 2. Effect of the Exposure Time With Linearly Polarized He-Ne Laser Radiation (λ = 632.8 nm, P = 3.0 mW/cm^2) on the Mitotic Activity of Cells (as Percentage of Control).

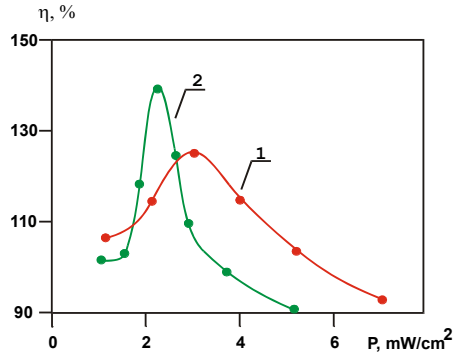

Figure 4. Dependence of Mitotic Activity of Cells (as Percentage of Control) on Power Density of Linearly Polarized HeNe Laser (λ = 632, 8 nm, t = 300 s) in the Absence of a Magnetic Field (1) and With a Magnetic Field Inductance 50 mT (2).

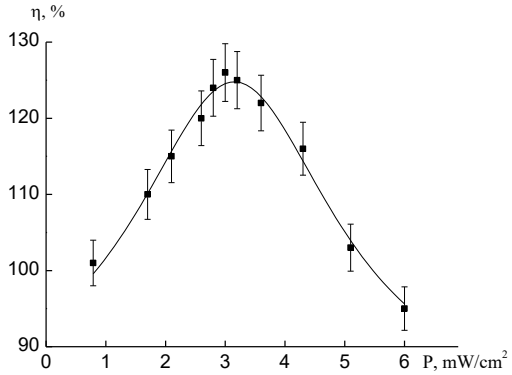

Figure 3. Effect of Power Density With Linearly Polarized He–Ne Laser Radiation (λ = 632, 8 nm, t = 300 s) on the Mitotic Activity of Cells (as Percentage of Control).

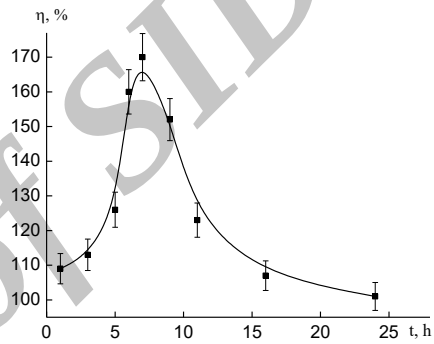

Figure 5. Effect of Mitotic Index (Percentage of Control) By-time After Stopping of He-Ne Laser Radiation on Monolayer Cells

laser radiation, and (*b*) a constant magnetic field, (*c*) low intensity laser radiation and a constant magnetic field together. The effect of the laser power density in the presence of magnetic field is shown in Figure 4. At the same time, the bell-shaped curve, which characterizes the correlation η = f(P) in the absence of a magnetic field, is preserved. As it can be seen from Figure 4, the application of magnetic field with laser irradiation leads to significant increase in mitotic activity of cells. The mitotic activity in the lower power density P=2.3 mW/cm^2 is about η= (140±4)%.

Effect of Time Duration After Radiation Termination on Mitotic Activity of Cells

Figure 5 shows the influence of mitotic index (percentage of control) by-time after stopping of laser radiation on monolayer cells. As a result, the maximum stimulating effect of He-Ne laser was observed in 7 hours after exposure termination. After 24 hours the mitotic index in the experiment and control samples were not different.

Discussion

The data showed that the low-intensity laser radiation could induce changes in mitotic activity in the irradiated monolayer cells. Given that light-induced Frederick transition is observed only in the field of polarized light.[19,20] The results are another important evidence showing the benefit put forward by the authors[14-16] hypothesis about the nature of the orientational mechanism of biological activity caused by low-intensity laser radiation.[21-23] As illustrated in following figures, stimulation of mitotic activity of cells were observed in exposure interval (60–600 seconds) (Figure 3), at constant power density (P = 3 mW/cm^2) and in power density interval (0.8–6 mW/cm^2) (Figure 4) (at constant exposure time t = 300 seconds). Decrease or increase in time or power density leads to reduction of photobiostimulation effect. Moreover (see Figure 4), increasing the power density more than 7-10 mW/cm^2 leads to the destruction of the mitotic sample in comparison with the control sample. It can be noted that alteration in the activity of the mitotic index (excess over the control) was observed in a fairly narrow range of laser intensity. This means that increasing the functional activity of cells only changes some conformation in macromolecules of the cell. Therefore, more conformational change (orientation) is not optimal

for intracellular processes that determine cell division (mitosis).[24]

The photobiological effect value induced by laser radiation is not determined by dose of incident beam, and depends on specific values of intensity at which this dose was intended.[25] Thus, if the impact on the monolayer of cells by radiation λ= 632.8 nm, P = 3.0 mW/cm^2, t = 300 seconds (the dose of 0.9 J/cm^2) has photobiological effect equal to η = (126 ± 4)% related to the control sample. On the other hand, if the sample is exposed to the same dose of radiation with less than intensity P = 0.6 mW/cm^2, but more than t = 1500 c (dose 0.9 J/cm^2), the effect value equals η = (106 ± 3)% (i.e. as the same of control level). Based on these results, the observed photobiological effects are not subject to the Bunsen-Roscoe law on the interchangeability of the intensity and irradiation time.[26]

The experiment results showed that the effect of a magnetic field with B = 50 mT for t = 300 seconds, on the monolayer cells caused stimulation and the value of their mitotic activity is η = (115 ± 5)%. When a constant magnetic field along with intensity P = 3.0 mW/cm^2 (i.e, at the maximum photobiological effect η= (126±4)% of the He-Ne laser) is applied there is an almost complete absence of biological effect. This fact serves as proof of nonadditively in the action of low-intensity laser radiation with a constant magnetic field.[27] This study shows that a weak magnetic field, as well as low-intensity laser radiation is an effective biostimulation. These results suggest the equivalence of the biological effect of low-intensity laser radiation and constant magnetic field. The accompanying action of a constant magnetic field and electric field of laser radiation on monolayer cell membranes have orienting effects of two physical factors.[28] Therefore, in the lower power densities, definite biological effects are achieved. So, improving the sensitivity of cells to He-Ne laser in the presence of a constant magnetic field confirms our conclusion; the basis of the molecular mechanism of high biological activity under the exposure of low-intensity laser radiation, along with the presence of a weak magnetic field, corresponds to the effect of Fredericks transition. Furthermore, as shown in Figure 5, for the effect time after termination of radiation on monolayer cells on the mitotic index, the maximum stimulating effect of He-Ne laser could be observed in 7 hours after exposure termination. After 24 hours the mitotic index in the experiment and control samples are not different.

Taking together, the present study by providing valuable information on the use of laser for cell cycle and mitosis manipulation could suggest further developments in the clinical applications of laser for potential use in cancer therapy and regenerative medicine. Although there are some wake signals toward the successful use of laser for medical applications, further studies and developments are needed prior to consideration for daily clinical applications, since the observed cellular manipulations should be first fully controlled and predicted and then applied to humans.

Conclusion

In this research, quantity experiments were performed on the effect of laser irradiation on Vero cell lines using a He-Ne laser. The obtained results indicate that enhanced mitosis activity cells vary under the induction of a constant magnetic field and with changes in exposure conditions of low-intensity He-Ne laser (power density, time exposure, and duration of time after termination of irradiation). The outcome of this study can be used for optimization of the molecular activity caused by low-intensity laser on the biological activities of the cells.

Ethical Considerations

Not applicable.

Conflict of Interests

The authors declare no conflict of interest.

References

1. Yumura S. A novel low-power laser-mediated transfer of foreign molecules into cells. *Sci Rep.* 2016;6:22055. doi:10.1038/srep22055
2. Akhavan O, Ghaderi E, Shirazian SA. Near infrared laser stimulation of human neural stem cells into neurons on graphene nanomesh semiconductors. *Colloids Surf B Biointerfaces.* 2015;126:313-321. doi:10.1016/j.colsurfb.2014.12.027
3. Moon BJ, Lee HY, Kim KN, et al. Experimental Evaluation of Percutaneous Lumbar Laser Disc Decompression Using a 1414 nm Nd:YAG Laser. *Pain Physician.* 2015;18(6):E1091-1099.
4. Terhorst D, Fossum E, Baranska A, et al. Laser-assisted intradermal delivery of adjuvant-free vaccines targeting XCR1+ dendritic cells induces potent antitumoral responses. *J Immunol.* 2015;194(12):5895-5902. doi:10.4049/jimmunol.1500564
5. Tuner J, Hode L, Nobel A. Laser therapy: clinical practice and scientific background: a guide for research scientists, doctors, dentists, veterinarians and other interested parties within the medical field. Prima Books; 1999.
6. Injushin YM, Chekurov PR. *Biostimulation of the bioplasma by laser beams*. Alma Ata, Kazakhatan; 1975:117.
7. Aronowitz JN. The Other Radiation Therapy. *Int J Radiat Oncol Biol Phys.* 2015;93(2):227-228. doi:10.1016/j.ijrobp.2015.04.041
8. Mester A. Laser Biostimulation. *Photomed Laser Surg* 2013;31(6):237-239. doi:10.1089/pho.2013.9876
9. Wu JY, Chen CH, Wang CZ, Ho ML, Yeh ML, Wang YH. Low-power laser irradiation suppresses inflammatory response of human adipose-derived stem cells by modulating intracellular cyclic AMP level and NF-kappaB activity. *PLoS One.* 2013;8(1):e54067. doi:10.1371/journal.pone.0054067
10. Wu JY, Chen CH, Yeh LY, Yeh ML, Ting CC, Wang YH. Low-power laser irradiation promotes the proliferation

and osteogenic differentiation of human periodontal ligament cells via cyclic adenosine monophosphate. *Int J Oral Sci.* 2013;5(2):85-91. doi:10.1038/ijos.2013.38
11. Karu T. Photobiology of low-power laser effects. *Health Phys.* 1989;56(5):691-704.
12. Gao X, Xing D. Molecular mechanisms of cell proliferation induced by low power laser irradiation. *J Biomed Sci.* 2009;16:4. doi:10.1186/1423-0127-16-4
13. Hawkins D, Abrahamse H. Effect of multiple exposures of low-level laser therapy on the cellular responses of wounded human skin fibroblasts. *Photomed Laser Surg.* 2006;24(6):705-714. doi:10.1089/pho.2006.24.705
14. Hawkins DH, Abrahamse H. The role of laser fluence in cell viability, proliferation, and membrane integrity of wounded human skin fibroblasts following helium-neon laser irradiation. *Lasers Surg Med*. 2006;38(1):74-83. doi:10.1002/lsm.20271
15. Smith K. The photobiological basis of low level laser radiation therapy. *Laser Ther.* 1991;3(1):19-24. doi:10.5978/islsm.91-OR-03
16. Ohshiro T, Calderhead RG. *Low level laser therapy: A practical introduction.* John Wiley & Sons; 1988. doi:10.1002/bjs.1800760445
17. Boulnois JL. Photophysical processes in recent medical laser developments: A review. *Lasers Med Sci*. 1986;1(1):47-66. doi:10.1007/bf02030737
18. Fenyo M. Theoretical and experimental basis of biostimulation by laser irradiation. *Opt Laser Technol.* 1984;16(4):209-215. doi:10.1016/0030-3992(84)90029-X
19. Khoo IC. *Cholesteric, Smectic, and Ferroelectric Liquid Crystals, in Liquid Crystals.* 2nd ed. Wiley; 2007. doi:10.1002/9780470084038.ch4
20. Simoni F. Nonlinear optical properties of liquid crystals and polymer dispersed liquid crystals. *World Scientific Pub Co Inc*; 1997. doi:10.1142/2343
21. Brill G. Molecular and cellular basis of therapeutic action of low - intensity laser radiation. Acad Saratov Med Univer; 2000.
22. Sullins KE. Lasers and wound healing: Practical uses. *Clinical Techniques in Equine Practice.* 2004;3(2):182-187. doi:10.1053/j.ctep.2004.10.001
23. KaruTI. *Low-Power Laser Effects, in Lasers in Medicine.* CRC Press; 2001.
24. Kondo T, Hayashi S. Mitotic cell rounding accelerates epithelial invagination. *Nature*. 2013;494(7435):125-129. doi:10.1038/nature11792
25. Hillenkamp F. Lasers in biology and medicine. *Springer Science & Business Media*; 2013.
26. Parvin P, Dehghanpour HR, Moghadam MS, Daneshafrooz V. Validity of reciprocity rule on mouse skin thermal damage due to CO2 laser irradiation. *Opt Spectrosc.* 2013;115(1):147-154. doi:10.1134/s0030400x13070138
27. Wang X, Zgadzaj R, Fazel N, et al. Quasi-monoenergetic laser-plasma acceleration of electrons to 2 GeV. *Nat Commun*. 2013;4:1988. doi:10.1038/ncomms2988
28. Wang L, Li J, Pan J, et al. Revealing the binding structure of the protein corona on gold nanorods using synchrotron radiation-based techniques: understanding the reduced damage in cell membranes. *J Am Chem Soc.* 2013;135(46):17359-17368. doi:10.1021/ja406924v