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Abstract. The effects observed under near-infrared laser irradiation of mammalian cells have been demonstrated to depend to a large extent on the type of irradiation source. In our experiments, we have measured concentration of reactive oxygen species in cell cultures of different origin (rodent and human, cancerous and non-cancerous) exposed to the radiation of low-level lasers at 1265 nm. Surprisingly, the radiation effects of narrowband laser occur to be more pronounced compared with those provided by the lasers of broader linewidth. Also, we have found that the aggressive types of cancer require a more accurate selection of irradiation parameters and laser operation regime.

1. Introduction

In recent years, the laser sources emitting in the near infrared range have been enthusiastically exploited in medical applications. An infrared light exhibits ability essential for medical needs to penetrate tissues deeper than the visible light [1-3].

The laser sources operating 1265-1270 nm range are of particular interest. Such sources enable singlet oxygen generation in inhomogeneous media and, in particular, in biological objects [4-8].

Within these wavelengths, the effects in biological objects have been observed at very low power. Therefore, the 1265-1270 nm radiation can be used in low-level laser therapy or in the so-called photobiomodulation therapy (PBMT) allowing minimal tissue damages [9-11].

Photobiomodulation applications range from regenerative medicine to cancer treatment [12-16]. It is very important to study the radiation-induced effects provided by different laser sources. The radiation emitted by different sources may possess different physical parameters thus having different effects on the biological objects.

In our study, the 1265 nm laser effects on mammalian cell cultures are explored. The performed in vitro studies have provided simple and convenient preclinical models that allow eliminating involvement of laboratory animals.

2. Methods

2.1. Cell cultures

The research has been performed on mammalian cell cultures.

1. CHO-K1 (ATCC® CCL-61™) is the Chinese hamster ovary epithelial cell line. The cells are not cancerous but immortalized.
2. HCT116 (ATCC® CCL-247™) is the human colon cancer cell line.
3. B16-F10 (ATCC® CRL-6475™) is the murine melanoma cell line. This transplantable cell culture is the most commonly exploited to study melanoma and to develop the laboratory animal models.
4. A875 (ATCC) is the human melanoma cell line.

2.2. Laser Sources

A semiconductor narrow-band (highly coherent) laser (Yenista Optics (Osics T100)) and a laser diode (Innolume, ThorLabs) operating at the wavelength of 1265 nm are used in the experiments. To adjust the diameter of the laser beam, the two collimators with waists of 3.5 and 7 mm are used.

2.3. Experiment

Twenty-four hours before the experiment, the cells were seeded in a slide chamber for adhesion and growth to the exponential phase. During the experiment, the cells were kept in a general or benchtop incubator in a humidified atmosphere, with 5% CO_2 and at a temperature of 37˚C.

Since the laser irradiation at a higher power is accompanied by the heating effect, the culture medium temperature was measured in the well of the slide chamber before the experiment in order to keep the temperature inside the incubator at 37˚C.

To avoid heating under the irradiation at higher powers, the control well was placed in the general incubator at 37˚C to provide the same temperature. The slide-chamber was placed on the stand; the collimator was fixed at the bottom at a distance of 1 cm from the sample. During the irradiation of the experimental well, the control well was shielded with a metal foil.

The energy density of laser radiation is calculated as follows:

$$E = \frac{\mathrm{P}\times\mathrm{t}}{\mathrm{S}}, \quad (1)$$

where P is the average output power (W), t is the exposure time (sec), S is the laser spot area on the cell culture (cm^2) [4].

The irradiation time in all experiments was 30 minutes, unless otherwise indicated in Results and Discussion. Each experiment was performed in triplicate.

2.4. Fluorescent staining of cells

After irradiation, the cells were stained for fluorescence microscopy with dichloro-dihydro-fluorescein diacetate to evaluate intracellular concentration of the reactive oxygen species as described [17].

3. Results and Discussion

Figure 1 shows the concentration of reactive oxygen species in cell cultures immediately after irradiation with a low-power narrowband laser. The dotted line is the baseline (hereinafter) where the experimental and control values are nearly the same.

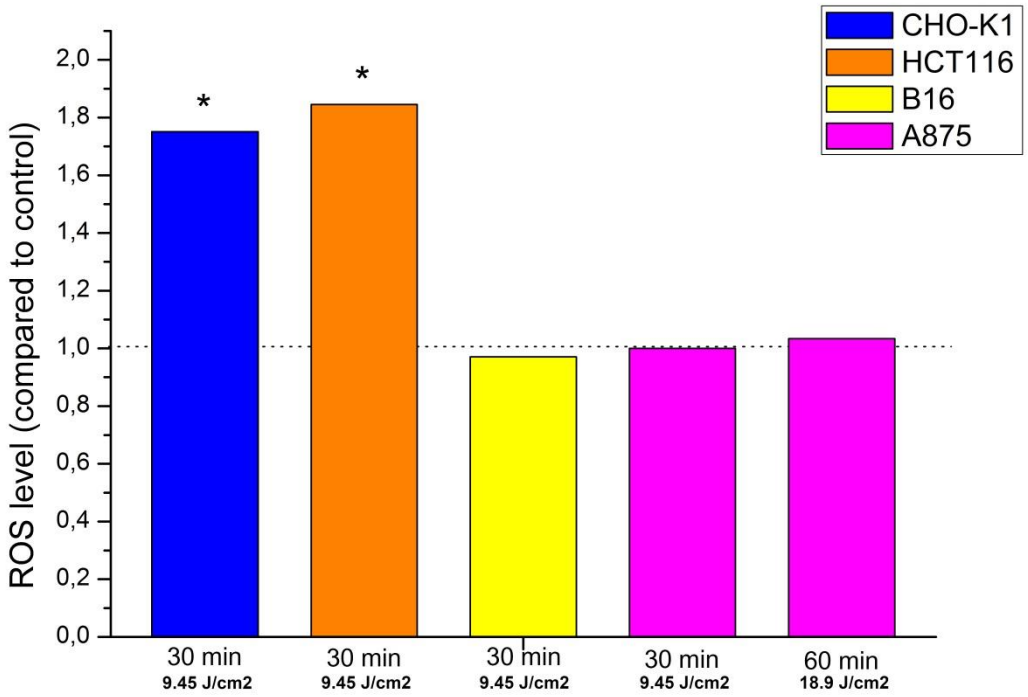

Figure 1. Level of ROS in the CHO-K1 (Chinese hamster ovary), HCT116 (human colon cancer), B16/F10 (murine melanoma), and A875 (human melanoma) cell lines exposed to 1265 nm narrowband laser irradiation (t=30min); P=4.2 mW; S=0.8 cm^2; E=9.45-18.9 J/cm^2. * - statistically significant difference between experiment and control.

The ROS level in the Chinese hamster ovary and human colon cancer cells significantly increases after the laser irradiation. However, in melanoma cells, no increase of the oxidative stress is registered even after the exposure for 60 minutes (energy density is 18.9 J/cm^2). It could be explained by the different intracellular mechanisms.

Figure 2 shows the ROS concentration in Chinese hamster ovary cells and human colon cancer cells after the exposure to laser sources of different linewidths. One can see that the effects of narrowband laser irradiation are more pronounced. Noteworthy, the power of the laser diode was more than twice as high in these experiments than the power of narrowband laser.

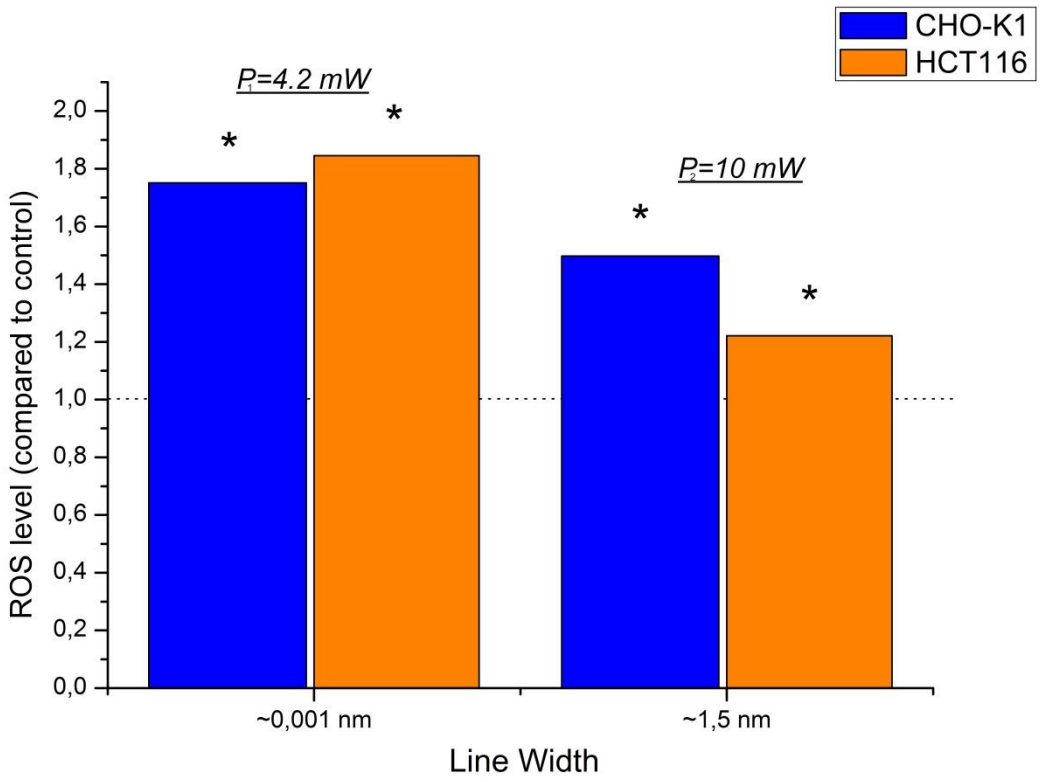

Figure 2. Level of ROS in the CHO-K1 (Chinese hamster ovary) and HCT116 (human colon cancer) cell lines exposed to 1265 nm narrowband laser and broadband laser diode irradiation (t=30min); P_1=4.2 mW, P_2=10 mW; S=0.8 cm^2; E_1=9.45 J/cm^2, E_2=18.9 J/cm^2. * - statistically significant difference between experiment and control.

It can be explained by the fact that the power spectral density of the narrowband low-power laser radiation at the point of its maximum is higher than that of the radiation provided by the high-power (broadband) laser diode: A ratio describing the power spectral densities for these laser radiation sources is as follows:

$$\frac{P_{\lambda l}}{P_{\lambda h}} \sim \frac{\Delta\lambda_h}{\Delta\lambda_l}\frac{E_l}{E_h} \sim 2\times10^3\frac{E_l}{E_h}\ , \qquad (2)$$

where $P_{\lambda l}$ is the power spectral density of the narrowband source (*W*), $P_{\lambda h}$ is the power spectral density of the broadbsand source (*W*), E_l is the surface energy density of PBM (*J/cm2*), E_h is the surface energy density of high-power laser irradiation (*J/cm*^{2}), $\Delta\lambda_l$ is the linewidth of PBMT source (*nm*), $\Delta\lambda_h$ is the linewidth of high-power laser source (*nm*).

Low-power and high-power laser linewidths are 0.001 nm and 1.5 nm, respectively. Therefore, in the present study, the power spectral density of the low-power laser radiation at the point of its maximum is higher than that of the radiation provided by the high-power laser. All this allows us to conclude existence of the narrowband resonance effect presumably on the surface of mitochondrial membranes [18-19].

Further experiments were performed with the 1265 nm laser diode to provide higher powers.

Figure 3 demonstrates resistance of the human melanoma cell line to laser irradiation. The power of 10 mW is required for normal cells or non-aggressive cancer to develop oxidative stress (Fig. 2), whereas melanoma demonstrates this effect at 250 mW.

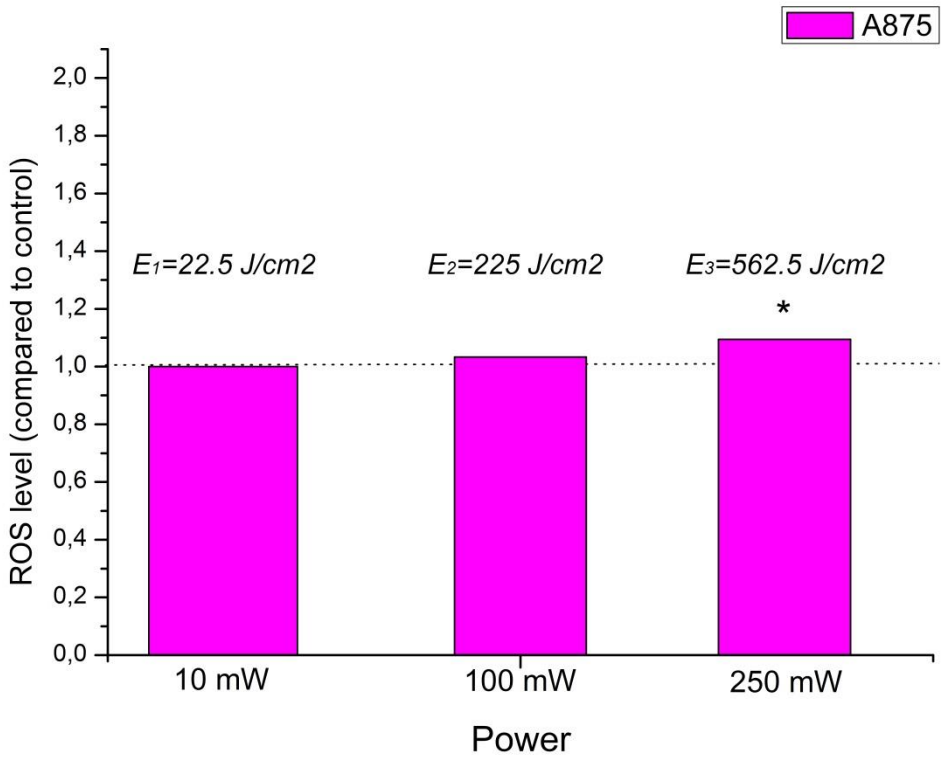

Figure 3. Level of ROS in the A875 (human melanoma) cell line exposed to 1265 nm laser diode irradiation (t=30min); P=10 mW, 100 mW, 250 mW; S=0.8 cm^2; E_1=22.5 J/cm^2, E_2=225 J/cm^2, E_3= 562.5 J/cm^2.* - statistically significant difference between experiment and control.

Figure 4 shows the ROS concentration at different intensities of laser irradiation. A significant difference is registered in the oxidative stress level of human and murine melanoma cells. This can be explained by different origin of species (human and murine) or mutation and requires further research.

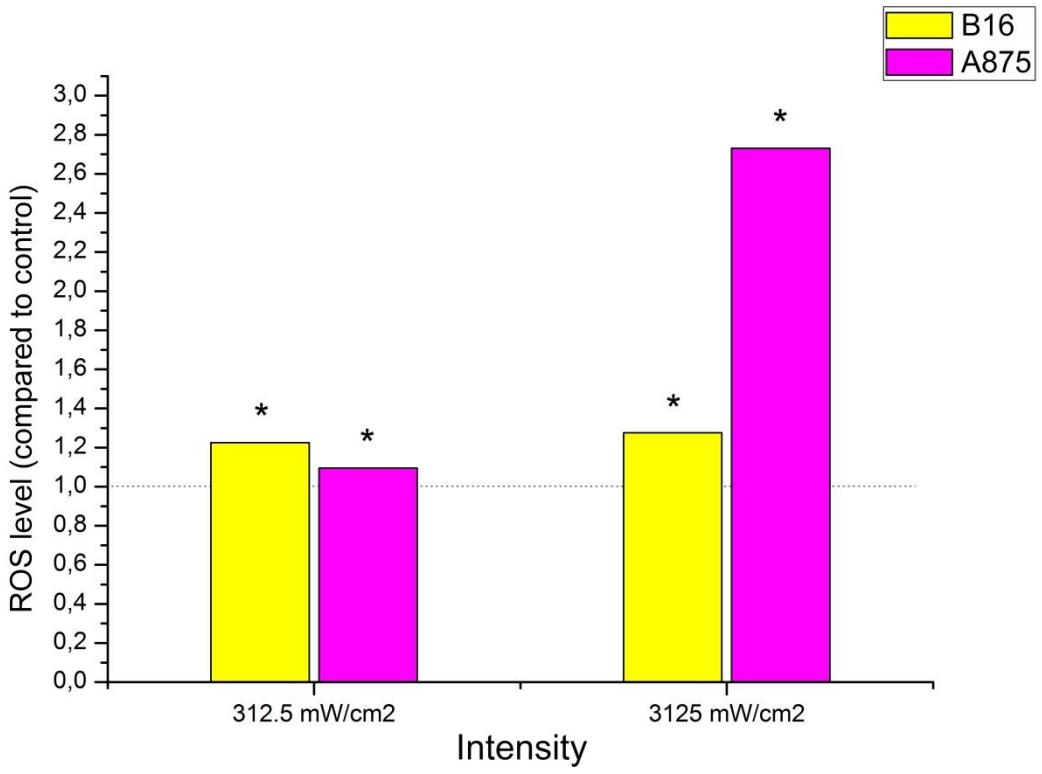

Figure 4. Level of ROS in the A875 (human melanoma) and B16/F10 (murine melanoma) cell lines exposed to 1265 nm laser diode irradiation (t=30min); P=250 mW; S=0.8 cm^2, 0.0812 cm^2; E_1=562.5 J/cm^2, E_2=5625 J/cm^2.* - statistically significant difference between experiment and control.

Figure 5 shows the concentration of reactive oxygen species 1 hour after irradiation. One can see that in the Chinese hamster ovary cells the oxidative stress is still increasing. It may indicate the antioxidant system failure leading to cell death. In melanoma cells, the level of oxidative stress decreases. Moreover, in the murine melanoma cells 1 hour after laser irradiation exposure there is no statistical significance between experiment and control groups.

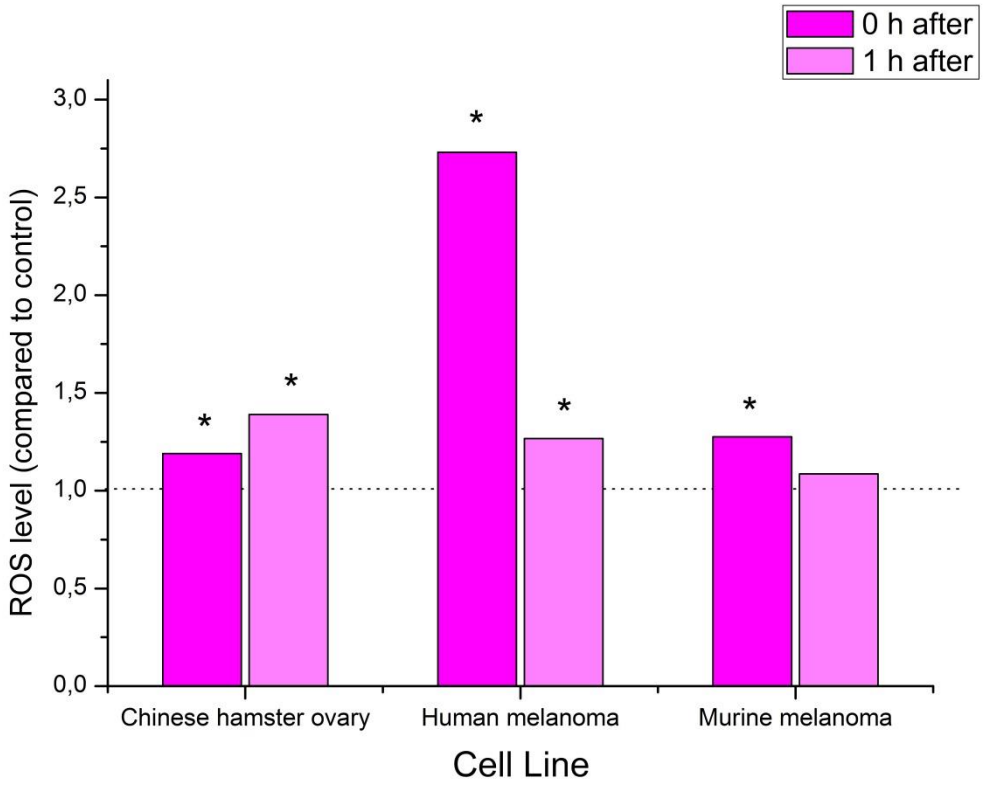

Figure 5. Level of ROS in the CHO-K1 (Chinese hamster ovary), A875 (human melanoma), and B16/F10 (murine melanoma) cell lines exposed to 1265 nm laser diode irradiation (t=30min); P=250 mW; S=0.8 cm^2; E=562.5 J/cm^2.* - statistically significant difference between experiment and control.

4. Conclusions

Our experiments have shown that the effects of the 1265 nm laser irradiation on biological cells depend not only on the irradiation dose but also on the laser linewidth, power, and intensity, as well as on the cell type.

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Conflict of interests

The authors declare no conflict of interests.

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