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Effects of high and low level 1265 nm laser irradiation on HCT116 cancer cells

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ABSTRACT

The mechanism responsible for the oxidative stress due to photobiomodulation induced by 1265 nm laser is still unclear. Mitochondria are assumed to be the most probable acceptors of the 1265 nm laser irradiation. We study oxidative stress, mitochondrial potential, GSH, cell viability, DNA damage. We demonstrated that narrowband (high-coherent) and wideband lasers employed at the doses of 9.45 and 66.6-400 J/cm2, respectively, induce a dose-dependent cell death, increase ROS level, disturb mitochondrial functioning and can damage DNA. Thus, the 1265 nm lasers can affect the HCT116 cells through mitochondrial damage. Energy density increase contributes to cell damaging without heating effects.

KEYWORDS

1265 nm laser irradiation, cancer cells, DNA damage, near-infrared lasers, oxidative stress, photobiomodulation therapy, reactive oxygen species, singlet oxygen

INTRODUCTION

In recent decades, the range of biomedical application of lasers has extended significantly and shifted from surgical instruments [1] and physiotherapeutic techniques [2, 3] towards the treatment of neoplastic diseases [4, 5] and manipulations in cellular and molecular medicine [6]. The latter requires a deeper understanding of mechanisms of laser radiation interaction with biological structures. Nowadays, laser therapy is widely used in dentistry [7, 8], photodynamic therapy [9, 10], treatment of skin diseases and cosmetic defects [11, 12] with or without photosensitizers. Different wavelength ranges are known to cause diametrically opposite cellular effects - from damage [13, 14] to regeneration [15, 16] opening great prospects for both treatment and rehabilitation after it.

Laser radiation emitted in certain wavelength ranges is attractive due to its ability to induce damage in individual biomolecules inside the cell and to initiate programmed cell death. Thus, it has been shown that laser radiation in the near-infrared range induces oxidative stress that is responsible for further damage of DNA and proteins, cell dysfunction [17, 18] leading to cell death through apoptosis [19, 20]. Noteworthy, this persists at doses typical for low-level light therapy (LLLT) [14].

One of the most demanding area of the laser use is photobiomodulation therapy (PBMT) widely applied in modern medicine. The main mechanism of PBM action is associated with the effect of low-level laser or light therapy (LLLT) on intracellular processes by activating intracellular signaling pathways through their interaction with endogenous photosensitizers [21, 22, 23, 24], which can be endogenous porphyrins with absorption spectra at 400-900 nm and 1000-1550 nm [25-27]. Noteworthy, most of intracellular endogenous photosensitizers are localized in mitochondria making them main acceptors of laser radiation [28, 29].

PBM at the wavelengths in the range 600–1070 nm can increase superoxide anion radical (O2∗-) production inducing intracellular oxidative stress [18, 30]. Besides, the wavelengths 1264-1270 nm corresponding to the absorption line of molecular oxygen are one of the PBMT ranges where intracellular oxidative stress is induced [17, 19, 20, 31, 32,].

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However, the mechanism responsible for the oxidative stress due to photobiomodulation induced by 1264-1270 nm laser is still unclear. It is assumed that laser irradiation of living objects at these wavelengths can induce intracellular generation of the singlet oxygen [31]. Although, in the isolated molecule direct $3O2 \rightarrow 1O2$ transition is forbidden according to the selection rules of spin and orbital symmetry highlighting small chance of singlet oxygen generation without photosensitizers [33], such a transition has been recently observed in inhomogeneous media [34].

If endogenous photosensitizer sensitive to the 1264-1270 nm laser radiation presents in the cell, the mitochondria, due to their structure and functional features, can be assumed as the most probable place of its localization. A linearity of intracellular response to the absorbed laser radiation dose should be studied. Here, we report the effect of narrowband (high-coherent) laser radiation at a wavelength of 1265 nm and energy density of 9.45 J/cm2 and wideband laser irradiation at a wavelength of 1265 nm and energy density of 66.6-400 J/cm2 on the cancer cells parameters characterizing mitochondria functioning: oxidative stress, mitochondrial potential, level of reduced glutathione, DNA damage and survival.

MATERIALS AND METHODS

Laser parameters

1. The source of PBMT.

Semiconductor narrowband (highly coherent) laser (Yenista Optics, OSICS T100 Tunable Laser Module T100 1310) with a tuning range from 1260–1360 has been used as the irradiation source. The average output power is 4 mW with a linewidth of $\Delta\lambda_l \sim 10^{-3}\ nm$ and the wavelength stability of 0.1 nm/h. The irradiation output is made with the help of fiber patch cord with an air-spaced doublet collimator at the end. The fiber collimator has Non-Magnetic Stainless Steel Housing. It is pre-aligned to collimate a laser beam propagating from the tip of an FC/PC-connectorized fiber with diffraction limited performance at the design wavelength.

2. The source of high-intensity laser radiation.

To study the effect of high dose radiation, we employed the fiber laser with a linewidth of $\Delta\lambda_h \sim 2\ nm$, that provides the maximum energy density of 400 J/cm2 avoiding heating of the irradiated object. [17]

3. Radiation dose calculations.

The surface dose (energy density) of laser radiation absorbed by a biological tissue (E, J/cm2) is calculated as follows:

$$E = P \times t / S, \quad (1)$$

where P is the average output power (W), t is the exposure time(sec), S is the laser spot area on the cell culture (cm2). [17]

4. Ratio of power spectral densities for the respective laser radiation sources:

$$\frac{P_{\lambda l}}{P_{\lambda h}} \sim \frac{\Delta\lambda_h}{\Delta\lambda_l}\frac{E_l}{E_h} \sim 2\times 10^3 \frac{E_l}{E_h}, \quad (2)$$

where $P\lambda l$ – power spectral density of the narrowband source (W), $P\lambda h$ - power spectral density of the wideband source (W), E_l – surface energy density of PBMT (J/cm2), E_h - surface energy density of relatively high-power laser irradiation (J/cm2), $\Delta\lambda_l$ – linewidth of PBMT source (nm), $\Delta\lambda_h$ – linewidth of relatively high-power laser source (nm).

Cell culture and Chemicals

Experiments have been performed with colorectal cancer HCT116 cells (ACCT CCL-247TM) obtained from American Type Culture Collection (Manassas, VA, USA). The cells are maintained in DMEM/F12 medium, supplemented with 10%fetal bovine serum and gentamycin at a final concentration of 50 μg/ml at 37°C, 95% and 5% CO2, at the CO2-incubator MCO-5AC (Sanyo, Japan). Twenty four hours before irradiation the cells are made a passage in the 8-well slide chamber (SPL LifeSciences) at a concentration of 10^5 cells/ml. The volume in the slide chamber is 500 μl.

DMEM/F12 medium and fetal bovine serum have been obtained from Paneco Ltd (Russia, Moscow) and PAA Laboratories (Austria), respectively. Other reagents and salts have been purchased from Sigma-Aldrich with purity grade >99 % or higher unless otherwise stated.

Laser irradiation of cells

Irradiation of cells has been performed in the middle of the exponential growing phase using an incubator microscope (UNO, OkoLab) prepared for culture maintenance during 5–30 minutes. The laser light sources are fixed at the bottom side of the plate at a distance of 0.5 cm from the slide chamber. While one cell is irradiated, another (control) is shielded by a steel foil. Laser energy densities at 9.45 J/cm2 for the narrowband laser and 66.6-400 J/cm2 for the wideband laser are set depending on the exposure time. Each experiment includes at least three irradiation sessions.

Fluorescent microscopy

Cell viability is evaluated 24 h after irradiation (9.45 J/cm2; 66.6-400 J/cm2). The cells are stained by a fluorescent dye propidium iodide and kept in a thermostat for 20 minutes. Analysis is performed with the fluorescence microscope as described in [35].

Intracellular ROS concentration is determined using DCFH-DA 30 minutes and 3 hours after 1265 nm laser irradiation at 9.45 and 400 J/cm2. Assay is performed as described in [17].

To assay the mitochondrial potential, cells are incubated in 50 nM TMRE solution in culture medium after irradiation at 9.45 and 400 J/cm2 as described [17]. The analysis is performed 30 minutes and 3 hours after laser exposure.

To assay the reduced glutathione, cells are exposed to 1265 nm laser at 9.45 and 400 J/cm2 and incubated with 5 μM monochlorobimane [17] 30 minutes and 3 hours after irradiation.

In each experiment using the microscope, fluorescence of more than 300 cells has been analyzed with each technique. All images are captured using an optical system comprising Nikon Ti-S microscope, DS-Qi1MC camera, Nikon S Plan Fluor ELWD 20 × 0.45 lens and appropriate filter and PC with NIS-elements 4.0 package.

Quantitative image analysis is performed using Image J software. The corrected total cell fluorescence (CTCF) = Integrated Density – (Area of selected cell x Mean fluorescence of background readings) [36]. All means are presented as the CTCF fold change compared to unirradiated control cells.

DNA damage assay

To compare laser irradiation dose effects, we use further methods for nuclear DNA damage analysis.

We use a qPCR-based assay for DNA damage described by Santos et al. [37] with some modifications [14, 38] and Comet assay in alkaline solution as described [17, 39]. Both assays are performed immediately after irradiation sessions.

Statistical Analysis

Each test has been performed in triplicate and results have been expressed as mean ± SD. Differences between irradiated and control cells are regarded as statistically significant when P calculated by the two-sided Student t-test is <0.05.

RESULTS

A. Effect of 1265 nm laser irradiation on cancer cell viability

We have compared different doses of 1265 nm laser irradiation on cell viability 24 hours after exposure. The results of the analysis are performed on Figure 1. Doses employed are 9.45, 66.6, 200 and 400 J/cm2. Figure shows that 24 hours after irradiation, the amount of dead cells increases with increasing energy density of the laser radiation. In this case, a statistically significant effect is observed even at the lowest dose.

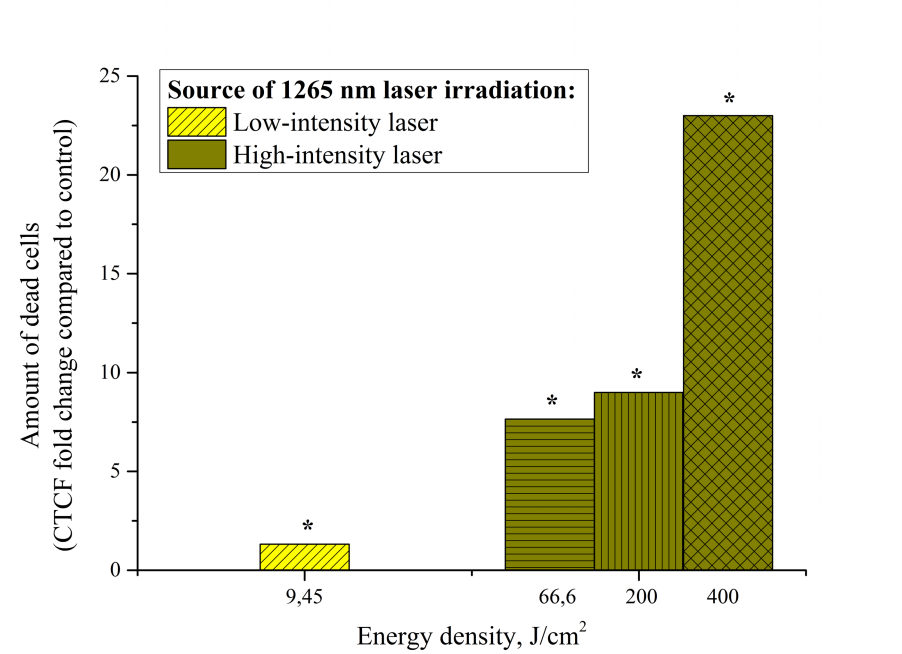

Figure 1. Effects of the narrowband and wideband laser irradiation at the wavelength of 1265 nm on the HCT116 cell viability 24 hours after irradiation. * - statistically significant difference between control and experimental groups, $p<0.05$.

B. Oxidative stress after 1265 nm laser irradiation

To estimate the dynamics of the laser radiation effect on generation of oxidative stress at a wavelength of 1265 nm, the intracellular concentration of reactive oxygen species (ROS) was determined 30 minutes and 3 hours after irradiation. For analysis, the minimal and maximal doses of 9.45 and 400 J/cm2, respectively, were applied. The results are shown in Fig. 2. 30 minutes after irradiation, in both cases, a statistically significant increase of ROS concentration was observed. Three hours after exposure, the effect of laser irradiation at 400 J/cm2 increases almost in 2.5 times (CTCF ratio of 1.45 and 3.5 for low and high doses, respectively), while at 9.45 J/cm2 the irradiation effect on HCT116 cells in the experimental group decreases to values comparable with in the control group.

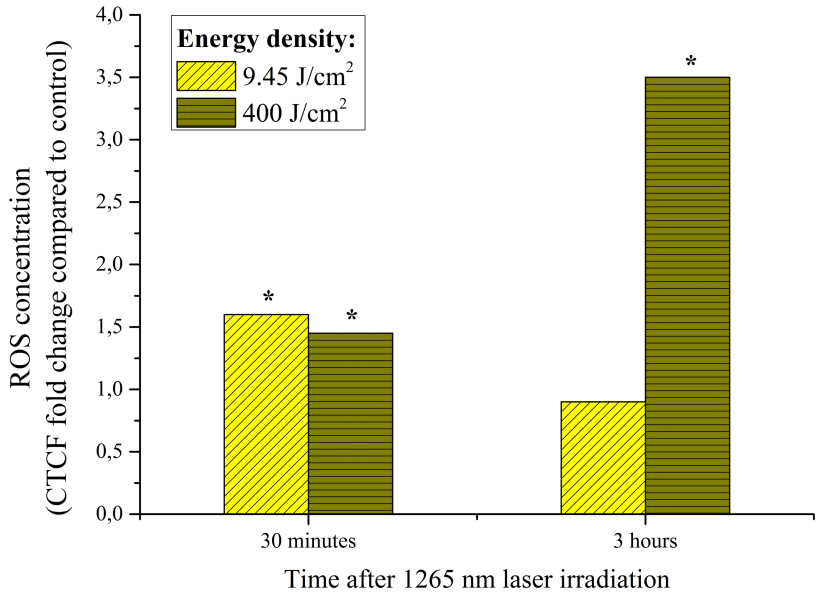

Figure 2. Levels of intracellular ROS in HCT116 cells exposed to the 1265 nm low-power and relatively high-power laser sources 30 minutes and 3 hours after irradiation. * - statistically significant difference between control and experimental groups, $p<0.05$.

C. Changes in mitochondrial potential after 1265 nm laser irradiation

The mitochondrial membrane potential was determined 30 minutes and 3 hours after laser exposure at 1265 nm and energy densities of 9.45 and 400 J/cm2. The results are shown in Fig. 3. In the experimental group, 30 minutes after irradiation at the minimal dose a decrease in the mitochondrial potential was observed. Three hours after, this value increases but remains significantly lower than in the unirradiated control group. The mitochondrial potential of HCT116 cells irradiated at the wavelength of 1265 nm at the maximal dose of 400 J/cm2 did not change with respect to the unirradiated control group 30 minutes and 3 hours after exposure.

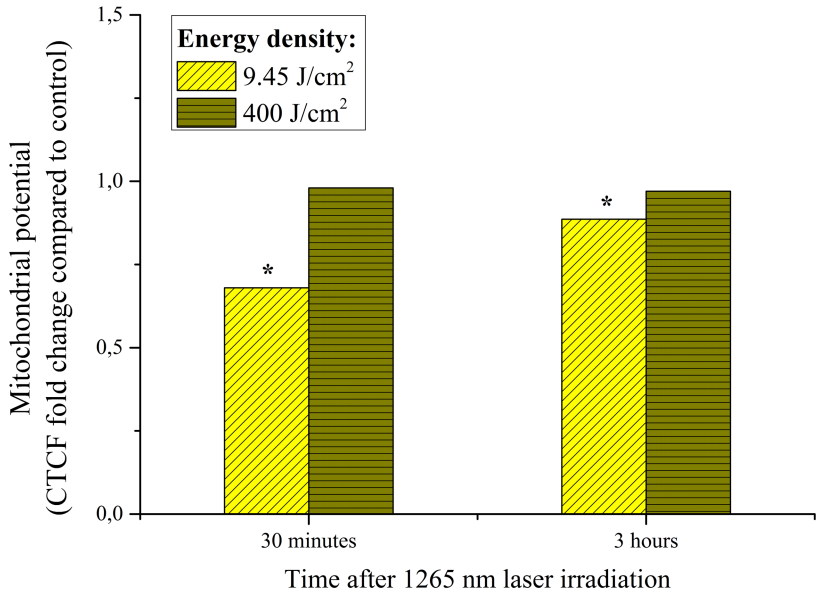

Figure 3. Changes of mitochondrial potential in HCT116 cells exposed to the 1265 nm low-power and relatively high-power laser sources 30 minutes and 3 hours after irradiation. * - statistically significant difference between control and experimental groups, $p<0.05$.

D. 1265 nm laser irradiation effects on reduced glutathione level

The level of reduced glutathione (GSH) was evaluated 30 minutes and 3 hours after exposure to the laser irradiation at the wavelength of 1265 nm and energy densities of 9.45 and 400 J/cm2. The results are shown in Fig. 4. 30 minutes after irradiation at the dose of 9.45 J/cm2, no change in the GSH level was registered in HCT116 cells compared to the control group. But 3 hours after exposure, an increase of 38% was observed. 30 minutes and 3 hours after irradiation of HCT116 cells at the dose of 400 J/cm2, the opposite effects were observed: the GSH level decreased significantly relative to the control value. Also, a slight increase in the GSH level was demonstrated 3 hours after exposure. However, this value was twice as low as in the GSH level in the control.

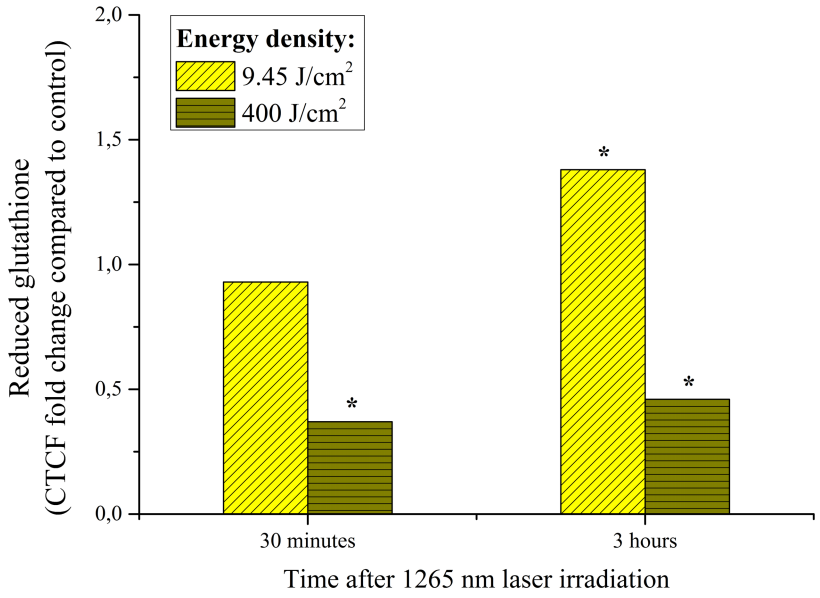

Figure 4. Changes of intracellular reduced glutathione level in HCT116 cells exposed to the 1265 nm low-power and relatively high-power laser sources 30 minutes and 3 hours after irradiation. * - statistically significant difference between control and experimental groups, $p<0.05$.

E. DNA damage after 1265 nm laser irradiation at low and relatively high doses

To estimate the effect of laser radiation at the wavelength of 1265 nm and dose of 9.45 J/cm2 on the nuclear DNA damage, DNA strand breaks were examined by a quantitative PCR. Also, the total number of DNA strand breaks was studied by the Comet assay under the laser irradiation at the maximal dose of 400 J/cm2. The analysis was performed immediately after irradiation to determine the direct effect of laser radiation on DNA damage and to minimize damage from intracellular reactions.

In the first case, the analysis showed that laser radiation at 1265 nm at a dose of 9.45 J/cm2 did not produce damaging effect on nuclear DNA. When analyzing the effect of laser radiation at 1265 nm and dose of 400 J/cm2, a statistically significant increase in the number of DNA breaks by 2.5 times was observed.

Table 1. Relative amount of DNA lesions in HCT116 cancer cells immediately after 1265 nm laser radiation exposure.

Energy density, J/cm2	Relative amount of DNA lesions (fold change compared to control)
9.45	1,25
400	2,5 *

* - statistically significant difference between control and experimental groups, $p<0.05$.

DISCUSSION

We have studied vital parameters of the HCT116 cancer cells exposed to 1265 nm laser irradiation of two continuous wave laser sources, providing energy densities at 9.45 and 66.6-400 J/cm2, respectively.

To determinate the dependence of cell death on the absorbed dose, the cells have been irradiated by the laser at the wavelength of 1265 nm and energy densities of 9.45 J/cm2, 66.6 J/cm2, 200 J/cm2, 400 J/cm2. 24 hours after exposure, a dose-dependent increase in the relative number of dead cells is observed (Fig. 1) highlighting this process is not quite linear. The result could be explained by the different mechanisms underlying the effect of a low-intensity narrow-band (highly coherent) laser and highly intensive Raman laser and by a specific response of the cancer cells to the doses used. Wu et al. have demonstrated the dependence of the programmed cell death activation mechanism on the energy density: at a higher dose absorbed by the studied cell cultures activation of apoptosis directly by active forms of oxygen has been observed, whereas at a lower dose, the caspase cascade is activated [40]. Kong et al. have revealed different types and

mechanisms of DNA damage in the cell induced by laser systems with different parameters [41]. Also, it has been demonstrated that the total energy required for the simulation of the same process (H2AX phosphorylation) by different systems could be different.

The study of the dynamics of oxidative stress shows that 3 hours after 1265 nm low-intensity narrowband laser irradiation at energy density of 9.45 J/cm2, the HCT116 cell level the concentration of ROS to the control values (Fig 2). Noteworthy, despite the absence of significant differences in this case, some processes are triggered in cells in response to the oxidative stress. This is evidenced by the presence of negative intracellular effects: a significant cell death 24 hours after irradiation, a decrease in mitochondrial potential almost immediately after exposure, persisting 3 hours after (Fig. 3). Also, our recent study has showed that 1265 nm narrowband laser can lead to mitochondrial induced oxidative stress at doses three times lower, and cause oxidative damage of biological molecules such as cardiolipin and mtDNA [14]. At the same time, the fact that the level of reduced glutathione, an indirect indicator of increased oxidative reactions in mitochondria, is not changed relative to the unirradiated control, and 3 hours after irradiation significantly exceeds the control values by 38%, is not explained at the moment. (Fig. 4)

30 minutes after exposure, the wideband high-power laser irradiation at energy density of 400 J/cm2 does not show any differences from the level of ROS in the HCT116 cells for PBMT at 9.45 J/cm2, but 3 hours after, this value significantly increases. One of the probable mechanisms to increase oxidative stress is effect of "ROS-induced ROS release", which has been well described for mitochondria as the main suppliers of ROS inside the cell [42]. Laser radiation at 400 J/cm2 causes nuclear DNA damages, including in dynamics [17], which is not been observed at lower doses, indicating the role of this mechanism in later oxidative stress. Also, a significantly low level of reduced glutathione, which does not recover 3 hours after exposure, is indirectly points to mitochondrial disturbance (Fig. 4) [43].

In addition, the absence of any effects of 1265 nm wideband relatively high-power laser irradiation at 400 J/cm2 on cancer cell mitochondrial potential is registered. (Fig. 3). At the same time, we have showed this type of laser radiation can increase mitochondrial potential in the non-cancer CHO-K cells in our work [17]. It may be associated with the inhibited ATP synthesis in cancer cells due to activation of glycolysis [44].

Linewidths of low-power and relatively high-power lasers are $\Delta\lambda_l \sim 10^{-3}\ nm$ and $\Delta\lambda_h \sim 2\ nm$, respectively. Thus, for the present research, power spectral density of a low-power laser at the maximum is higher than the corresponding value of the relative high-power laser. All this indicates the possibility of the narrow-band resonance formation, presumably on the surface of the mitochondrial membranes. This assumption requires further review and verification, but could shed light on some of the laser radiation effects, not explained to date, e.g., the so-called "light-oxygen effect" [45].

CONCLUSION

Our study has demonstrated comparative effects of a narrowband (highly coherent) low-intensity semi-conductor laser and wideband relatively high-intensity fiber laser at energy densities of 9.45 and 66.6-400 J/cm2, respectively. The experimental results have brought us to conclusion that the PBMT source as well as the high-intensity laser induce the oxidative stress, leading to cancer cell death and can disturb mitochondrial functioning at each energy density employed. In our case, however, the sources of laser radiation have had specific physical properties, which, as it turned out, can be essential for the interpretation of some results and further research.

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