*International Journal of Applied Science & Environmental Engineering – IJASET 2018*

*Volume 1 : Issue 1 Publication Date : 28 December, 2018*

# *Low-level laser radiation at 1265 nm triggers cellular effects through mitochondrial damage*

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***Abstract*—The mechanism responsible for the oxidative stress induction due to laser irradiation at 1265-1270 nm is still unclear. Thermal effects caused by irradiation are the main factors to be eliminated. In this study, low-level laser radiation (LLLI) has been used at 1265 nm to avoid side effects associated with the thermal denaturation of biomolecules and provide conditions that at least theoretically exclude singlet oxygen generation by direct 3O2 → 1O2 transition. Here, we report on the experimental results highlighting mitochondrial role in the oxidative stress provoked by LLLI within the wavelength range 1260-1275 nm. We study the dynamics of oxidative stress, mitochondrial potential, cardiolipin oxidation, cell death, mitochondrial and nuclear DNA damage in the HCT-116 cell line exposed to low-level laser irradiation at 1265 nm. We demonstrate that the laser radiation at 1265 nm can induce the oxidative stress and disturb mitochondrial functioning at the energy density as low as 3.15 J/ cm2 and 9.45 J/cm2, respectively. Noteworthy, LLLI at 1265 nm damages mitochondrial DNA but does not affect the nuclear DNA. The performed experiments brought us to the conclusion that the laser irradiation at 1265 nm can affect intracellular processes through mitochondrial damage.**

***Keywords*—low-level laser irradiation, infrared lasers, 1265 nm laser, DNA damage, mitochondrial function, oxidative stress.**

## *1. Introduction*

Nowadays, low-level laser irradiation (LLLI) is widespread for medical purposes. Lasers operating within the wavelength range 600-1070 nm at the power below 5 W/cm2 are of specific scientific interest [1,2,3,4]. It has been shown that most of intracellular endogenous sensitizers are localized in mitochondria making them main acceptor of laser radiation [5,6,7]. Interacting with photoactive molecules in mitochondria, in particular with cytochrome aa3, LLLI can increase O2*-production in both cytoplasm and mitochondria leading to intracellular oxidative stress.

Comprehensive studies have demonstrated that LLLI at the wavelengths in the range 600-1070 nm can affect the redox state of mitochondria and modulate intracellular oxidative stress [8,9]. Apart from this wavelength range, there are ranges, at which laser irradiation is able to induce intracellular oxidative stress. IR range of 1265-1270 nm is the most studied among them [10,11,12,13,14].

Some authors report singlet oxygen generation in cell cultures under laser exposure at the wavelength of 1265-1270 nm. In these studies, high-power lasers with high energy density from 60 J/cm2 up to 400 J/cm2 have been employed [10,11,12]. Lasers with such energy may cause side effects associated with inadequate functioning of intracellular mechanisms resulted from thermal denaturation of protein molecules, which, in turn, may lead to oxidative stress having no direct connection with singlet oxygen generation. In these experiments, temperature control is actually monitoring of the culture medium temperature. Light energy absorption by cells is neglected in the studies [10,11,12]. Moreover, it was considered that a direct 3O2→1O2 transition is prohibited according to spin-orbital selection rules and orbital symmetry [16].

## *2. Materials and methods*

A semi-conductor laser (Yenista Optics, OSICS T100 Tunable Laser Module T100 1310) with a tuning range from 1260-1360 has been used as the source of irradiation. The average output power is 2 mW with the linewidth less than 1 nm. The surface dose (energy density) of laser radiation absorbed by a biological tissue (E, J/cm2) is calculated as a ratio:

$$E=Pt/S,$$

where P is the average output power (W); t is the exposure time (sec), S is the laser spot area on biological tissue (cm2).

Experiments have been performed with colorectal cancer HCT-116 cells (ACCT ® CCL-247TM) taken from American Type collection, maintained maintained in DMEM/F12 medium with 10% fetal bovine serum and 50 µg/ml gentamycin at at 37°C, 95% and 5% CO2.

Irradiation is conducted in 8-well slide chamber (SPL LifeSciences) at the energy density between 0.3 and 9.45 J/cm2 24 hours after a passage.

Fluorescent microscopy is an assay of choice for cell viability evaluation [15], intracellular ROS concentration [16], mitochondrial potential and cardiolipin oxidation level [17] and it is performed 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 [18].

Mitochondrial and nuclear DNA damage assay is set up according to [19,20] with some modifications due to optimize assay conditions.

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 $<0.05$.

## *3. Results*

To evaluate mechanisms of ROS generation under irradiation at the wavelength of 1265 nm a series of experiments has been performed. Fig. 1A shows the results

on ROS generation in the HCT-116 cells under laser irradiation at the energy density of 9.45 J/cm2 within the wavelength range of 1260-1275 nm. The diagram shows the maximal intensity of intracellular ROS generation registered at the wavelengths of 1265 and1266 nm. Fig.1B shows the dependence of intracellular ROS generation on the laser energy density. ROS concentration has been determined 5 minutes after irradiation. As the diagram shows, this dependence is linear.

Fig. 2 shows the amount of cells with apoptotic and necrotic signs one hour and 24 hours after irradiation. We have found that 24 hours after laser irradiation level of both apoptotis and necrotis increases.

Fig. 3 shows changes of mitochondrial potential in HCT-116 cells exposed to 1265 nm laser at the energy density of 9.45 J/cm2, as well as the decreasing of the unoxidized cardilipin level.

Fig 4 shows the calculations of the relative amount of lesions for 10 kb DNA. No significant differences have been observed between the damage levels of DNA localized in nucleus and mitochondria for the cells in the control group. However, in the group subjected only to the 1265 nm laser irradiation at the energy density of 9.45 J/cm2 a significant increase of mitochondrial DNA damage has been recorded compared with the nuclear DNA damage. After irradiation, the relative amount of mitochondrial DNA lesion increases in 6.5 times compared with the corresponding control values.

## *4. Discussion*

In the present study, the 1265 nm LLLI has been used to explore its effect at the cell level. Previously, it has been reported for 1262-1270 nm laser irradiation at higher power within the energy density range of 40 - 400 J/cm2. It has been concluded that the main damaging effect from the lasers operating at the wavelength range 1262-1270 nm is due to singlet oxygen generated from a direct $3O2 \rightarrow 1O2$ transition. In the isolated molecule of this kind the transition is forbidden according to the selection rules of spin and orbital symmetry [21] highlighting small chance of singlet oxygen generation without photosensitizers.

In our experiments, we analyze the dependence of oxidative stress value on the radiation wavelength in the range 1260-1275 nm (Fig. 1A). The maximal oxidative stress value has been observed for the 1265-1266 nm range. CW irradiation at such intensities causes a thermal damage of organic materials. It has been previously reported that the effects observed in cells under the 1270 nm laser radiation at power of 350 mW/cm2 can be attributed to the thermal effects [22].

Noteworthy, in our case, the oxidative stress has been registered under irradiation at the wavelength of 1265 nm at the intensities of about 10 mW/cm2, i.e. at the intensities employed for photodynamic therapy (Fig. 1B). Moreover, our experiments show that the oxidative stress has been observed at the intensity as low as 2mW/cm2 (the energy density is 3.15 J/cm2 (Fig.1B)). All this indirectly indicates the presence of endogenous photosensitizer capable of generating free radical molecules.

We analyze the dependence of oxidative stress value on the radiation wavelength in the range 1260-1275 nm (Fig. 1A). The maximal oxidative stress value has been observed for the 1265-1266 nm range. However, at the wavelength of 1270 nm, a resonant frequency of $3O2 \rightarrow 1O2$ transition, no oxidative stress has been registered in HCT-116 cells (Fig. 1A). Thus, the experimental results are in agreement with the estimations given above. It allows assuming the presence of endogenous cell photosensitizers having the resonance absorption frequency at the wavelengths of 1265-1266 nm.

In our experiment, laser irradiation at 1265 nm causes a decrease of mitochondrial potential (Fig. 2A). As the experiments show, mitochondrial destruction due to MPTP can be responsible for a mitochondrial mass decrease. Also, the mitochondrial mass decreases due to cardiolipin oxidation resulted by ROS generation of a mitochondrial respiratory chain. It has been shown that the cardiolipin is bound with 10-N-Nonyl acridine orange (NAO) in unoxidizedintact state [23]. Thus, the laser effect at 1265 nm on mitochondrial potential is associated with MPTP formation from oxidation of sulfhydryl groups.

To identify the source of biomolecule damage we estimate the level of nuclear and mitochondrial DNA lesions after laser irradiation. In our case, mitochondrial DNA damage is observed only. All this supports our assumption that the laser light at 1265 nm interacts with endogenous photosensitizers included into mitochondria.

## *5. Conclusion*

In our study, we have demonstrated that the laser irradiation at 1265 nm can induce the oxidative stress and disturbance of mitochondrial functioning at the energy density as low as 3.15 J/cm2 and 9.45 J/cm2, respectively. LLLI at 1265 nm damages the mitochondrial DNA only without producing any effects on the nuclear DNA. The experimental results have brought us to conclusion that the 1265 nm laser irradiation affects intracellular processes through interaction with mitochondrial photoactive molecules.

### *Acknowledgment*

The work is supported by the Ministry of Education and Science of the Russian Federation: State Task, Pr. No.14.Z50.31.0015.

### *Conflict of interest*

None of the authors have any conflicts of interest.

### *Figures*

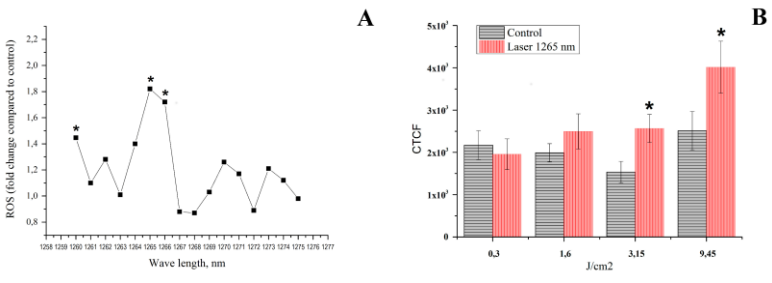

Fig.1. Mechanisms of ROS generation under LLLI. Fig.1A. The dynamics of intracellular ROS concentration in HCT-116 cells 5 minutes after exposure to the laser irradiation at the wavelengths 1260-1275 nm. The energy density is 9.45 J/cm2. Data are given as the ratio between ROS concentration in the experimental and control group. Fig.1B. The dependence of intracellular ROS concentration on the energy density of the 1265 nm laser. All results are given as mean values ± S.D. * - statistically significant difference between control and other groups. ($p<0.05$).

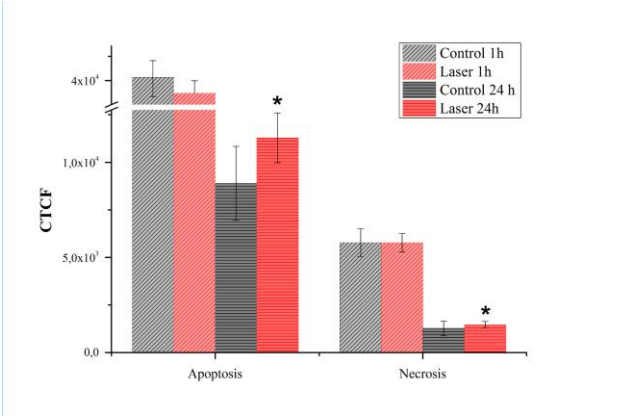


Fig.2. Amount of cells with apoptotic and necrotic signs 1 hour and 24 hours after irradiation. Energy density is 9.45 J/cm2. Apoptosis is expressed as YO-PRO-1 corrected total cell fluorescence (CTCF). Necrosis is expressed as propidium iodide corrected total cell fluorescence (CTCF). * - statistically significant differences between control and irradiated cells.

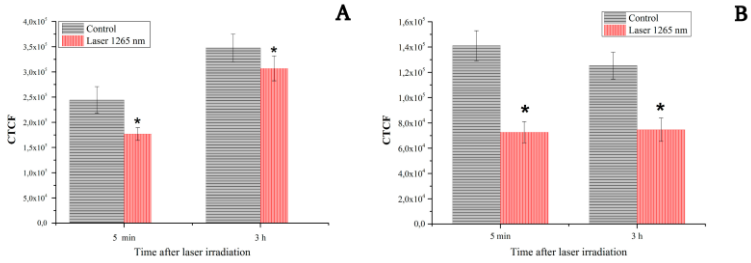


Fig. 3. The laser effect at 1265 nm on the total mitochondrial potential and mitochondrial mass. Fig. 3A. Changes of mitochondrial potential in HCT-116 cells exposed to the 1265 nm laser irradiation. Energy density is 9.45 J/cm2. Mitochondrial potential is expressed as TMRE corrected total cell fluorescence (CTCF). Fig. 3B. Changes of mitochondrial mass in HCT-116 cells exposed to the 1265 nm laser. Mitochondrial mass is expressed as nonyl-acridine orange corrected total cell fluorescence (CTCF). All results are given as mean values ± S.D. * - statistically significant difference between control and other groups ($p<0.05$).

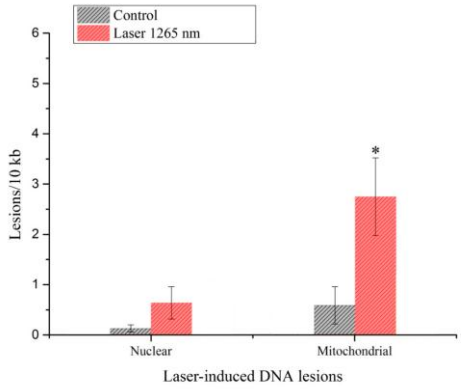


Fig. 4. 10 kb DNA lesions in HCT-116 cell line after laser irradiation at the wavelength sof 1265 nm. * - statistically significant difference between the control group cells and irradiated cells ($p<0.05$).

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