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Analysis of the efficiency of using 1265-nm cw laser radiation for initiating oxidative stress in the tissue of a solid malignant tumour

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Abstract. **The possibility of laser initiation of oxidative stress was studied by the example of the tumour tissue of cervix. The laser facility with the operating wavelength 1265 nm that falls within the region of resonance absorption of molecular oxygen was used for initiation. The source of radiation in the experiments was a fibre SRS laser with the repeated cascade conversion of radiation of a 1125-nm ytterbium laser.**

Keywords: *fibre SRS laser, oxidative stress, cervical carcinoma.*

9. Introduction

At present the problem of malignant tumour growth is one of the most urgent problems in biology and medicine[1]. The authors of [2,3] demonstrated the role of active forms of oxygen and lipid peroxidation (LP) induced by them in the mechanisms of carcinogenesis. An important role in the protection of cells from damage by active forms of oxygen is played by the system of antioxidant protection [4]. The condition of LP activation against the background of depression or deficiency of natural antioxidant systems is referred to as oxidative stress [5].

It is generally accepted that the most efficient agent, damaging cancer cells, is singlet oxygen, which is explained by its high chemical activity [6], since it can participate in chain free-radical reactions, oxidize amino acids in proteins, guanine in DNA, and initiate LP [7]. The result of such disturbances in the case of exceeding the reparative abilities of the cell is its destruction. The light–oxygen effect consists in the activation of damage of a biological system by optical radiation (depending on the light dose) via direct photo excitation of diluted molecular oxygen into the singlet state.

One of the photo excitation bands (the most active one) of the singlet oxygen is localised in the region 1.26–1.27 μm [8,9]. The absorption line of O_2 in this region of the spectrum is most active because of practically full absence of absorption by competing chromophores. The role of a high-power radiation source, able to operate efficiently in this region, can be played by the SRS laser pumped by an ytterbium fibre laser [10]. In papers [11,12], by the example of organic liquids and cell cultures, using a tunable SRS laser it is shown that the maximal generation of singlet oxygen is achieved by irradiation at the wavelength 1264–1270 nm. In this case, the FWHM of the spectrum amounts to 15–20 nm. Yusupov et al. presented [13] the results of clinical application of a 1262-nm SRS laser, which demonstrated exclusive potential capabilities of using such light sources in treatment of oncological diseases. In the present paper we estimate the possibility of initiating oxidative stress in a tumour tissue of cervical carcinoma using cw laser radiation with the wavelength 1265 nm.

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10. Materials and methods of study

To obtain a model of cervical carcinoma (CC-5) in white outbred mice we used the tumour strain, produced at the Research Institute of Experimental Diagnostics and Therapy of Tumours, N.N. Blokhin Cancer Research Centre, Russian Academy of Medical Sciences (Moscow).

As a source of radiation we used the SRS laser with the repeated Raman conversion of radiation from an ytterbium fibre laser ($\lambda = 1125$ nm) [14]. The active medium of the Raman converter was a telecommunication fibre. The maximal output power of the SRS laser was about 4 W at the wavelength 1265 nm, the spectrum of its radiation is presented in Fig. 1.

The dose F of radiation, absorbed by the biotissue, is determined by the ratio $F = W/S$, where W is the energy of laser radiation, absorbed by the tissue (in J); S is the area of the laser spot on the irradiated biotissue (in cm^2). In 10 min after irradiation the dose of high-intensity radiation will amount to 106.2 J cm^{-2}, and for two-minute exposure it will be 5 times

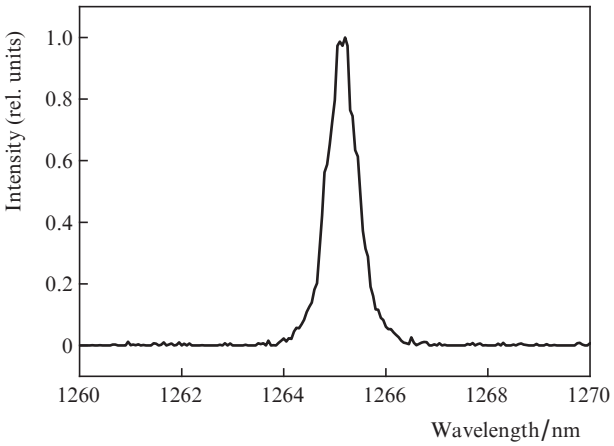


Figure 1. Spectrum of SRS laser radiation.

smaller. Thus, the total dose after 10 such sessions in the first case will amount to 1062 J cm^{-2}, and in the second case 212 J cm^{-2}.

The intensity of LP processes was estimated by measuring the content of the secondary product, the malonic dialdehyde (MDA), in the tumour tissue of animals with CC-5 during the test with thiobarbituric acid, in correspondence with the modified method by Andreeva [15]. When studying the enzymatic stage of the antioxidant protection, the activity of superoxide dismutase (SOD) was determined using the method by Dubinina and Nishikimi [16, 17], and that of catalase (CT), glutathione reductase (GR) and glutathione transferase (GT) was found using the method by Karpishchenko [18]. For protein assessment the method by Bradford [19] was used.

Morphological investigation was performed using the computer video test system with the densitophotometry program Mecos C1. In analysing histological sections of the tumour, the specific volumes of tumour parenchyma, tumour stroma and necrotic areas were calculated, as well as the mitotic and apoptotic indices.

The statistical significance of the obtained results was estimated by means of the nonparametric Mann–Whitney criterion. The differences between the groups were considered as significant provided the statistical significance parameter is $p \leqslant 0.05$.

11. Results of the study and their discussion

The studies of the influence of 1265-nm cw laser radiation (the wavelength of resonance absorption of the molecular oxygen O_2) on neoplasm showed that the irradiation by the dose 212 J cm^{-2} significantly reduces the level of MDA on the 30th day after the tumour transplantation (Fig. 2). The dynamics of activity of enzymes is multidirectional: the negative dynamics is observed in the activity of GP, while that of GT is positive, and the indicators of catalase and SOD level do not significantly change (Table 1).

The exposure dose of 1062 J cm^{-2} at the same duration of the tumour growth gives rise to opposite changes in the LP–antioxidant system: an increase of the MDA level in the neoplasm (Fig. 2) is observed together with statistically significant reduction of the activity of SOD and GT, the level of GR remaining significantly unchanged (Table 1). Such dynamics may indicate the development of an oxidative stress in the tumour tissue.

Table 1. Indicators of antioxidant protection system in neoplasm on the 30th day of the CC5 tissue growth after irradiation by SRS laser.

Indicator	Number of measurements and dose of radiation		
	$n = 16$ ($F = 0$)	$n = 12$ ($F =$ 212 J cm^{-2})	$n = 12$ ($F =$ 1062 J cm^{-2})
GR/mmol min^{-1} mg^{-1}	0.212±0.016	0.418±0.013*	0.238±0.023
GT/mmol min^{-1} mg^{-1}	1.502±0.292	0.509±0.051*	0.602±0.078*
SOD/arb. unit mg^{-1}	26.57±6.02	29.01±1.25	8.48±0.24*
CT/mmol s^{-1} mg^{-1}	0.537±0.100	0.442±0.083	0.667±0.287

* data differing form those without irradiation with statistical significance, $p \leqslant 0.05$.

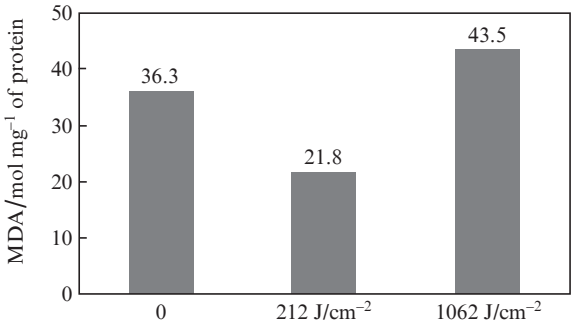


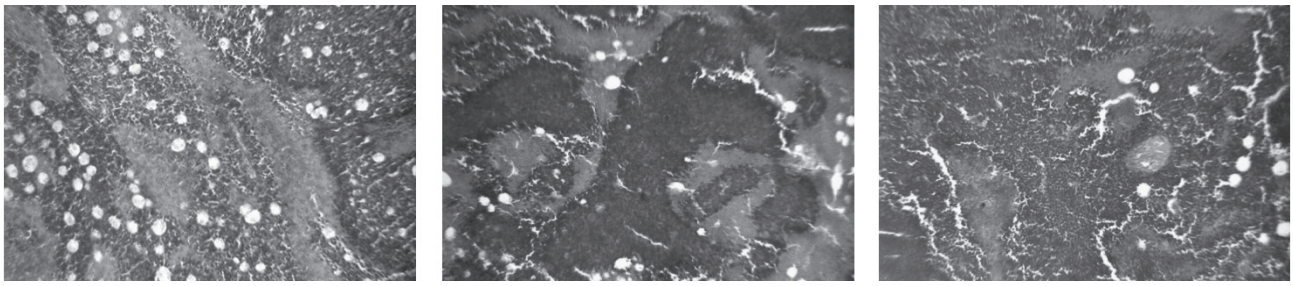
Figure 2. MDA level in neoplasm on the 30th day of the CC-5 tissue growth after exposure to SRS laser at doses $F = 0$, 212, and 1062 J cm^{-2}.

The morphological assessment of the tumour tissue SS-5 after irradiation with a SRS laser showed that the laser impact facilitates a significant reduction of the apoptotic index (Table 2). At the same time the mitotic index remains significantly unchanged (Table 2), and the volumes of necrotic (Fig. 3b) and tumour stroma (Fig. 3c) regions increase.

Table 2. Morphometric indicators of tumour tissue after irradiation with SRS laser.

Radiation dose/ J cm^{-2}	Specific volume (%)			Mitotic index (‰)	Apoptotic index (‰)
	tumour parenchyma	tumour stroma	regions of necrosis		
0	65.34±2.78	0.20±0.04	34.64±2.76	18.00±3.18	67.00±4.25
212	58.24±3.99*	0.32±0.04*	41.45±3.96	16.88±3.13	31.25±5.00*
1062	56.79±18.80	0.36±0.11	42.86±18.73	16.25±1.25	41.88±1.88*

* data differing form those without irradiation with statistical significance, $p \leqslant 0.05$.



a b c

Figure 3. Microphotographs (×400) of the CC-5 tumour biotissues on the 30th day: without irradiation (a) and after irradiation with the dose 212 (b) and 1062 J cm^{-2} (c). Staining by haematoxylin eosin.

12. Conclusions

In the present paper it is shown that:

(i) Laser irradiation at the wavelength 1265 nm causes dose-dependent changes in the indicators of the LP–antioxidants system in the solid CC-5 tumour, which can be an evidence of the possibility of oxidative stress development.

(ii) The cw laser irradiation of the experimental CC-5 tumour by the light with the wavelength 1265 nm, the total dose being 1062 J cm^{-2}, leads to the enlargement of the necrosis regions and stroma volume.

(iii) Laser radiation with the wavelength 1265 nm (in the region of resonance absorption of molecular oxygen) may be efficiently used for destruction of solid tissues of malignant tumours, e.g., cervical carcinoma.

The obtained results demonstrate the promising potentialities of using the considered SRS lasers for photodynamic therapy, based on the light–oxygen effect and not implying the use of sensitizers [6]. Thus, this technique may advantageously differ from the conventional methods (radiotherapy and surgery) of treatment of oncological diseases, including the cervical carcinoma, by the absence of topical, organ and systemic complications, the possibility of multiple repetition of the treatment procedure, relative cheapness and practical absence of contraindications.

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