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Changes in Free Radicals System of *Imr-90* and *C-32* Cells During Photodynamic Therapy

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Abstract

Free radicals in *IMR-90* and *C-32* cells during photodynamic therapy (PDT) were studied by electron paramagnetic resonance spectroscopy at X-band (9.3 GHz) and optical microscopy. Tumor cells were irradiated by a laser (635 nm) with the power of 10 mW. ALA was used as a photosentsitizer. Free radical formation in the analysed cells was observed after laser irradiation and damage of cells. Irradiation of the cells at the presence of ALA is accompanied by formation of a high amount of free radicals and their recombination. It was proved that both *IMR-90* and *C-32* cells are susceptible to photodynamic therapy.

Keywords: tumor cells, ALA, photodynamic therapy, free radicals, electron paramagnetic resonance

Introduction

Laser radiation indicates changes in biological structures that result from free radicals' reactions [1]. These processes are accompanied by negative effects in cells and finally by apoptosis or necrosis. The induced biological changes depend on the type of cells and parameters of laser radiation. In this work two different kind of amelanotic tumor cells were studied: *IMR-90 human normal fibroblasts* and *C-32 human amelanotic melanoma*. Formation of paramagnetic centers during radiation in above mentioned cells is not known so far. Evolution of paramagnetic centers in analysed cells during photodynamic therapy with ALA as photosensitizer has been observed. The results were compared to our earlier project where *SK melanotic human melanoma* and *human colon adenocarcinoma* cells were used during PDT with ALA [2].

Experimental procedures

The *IMR-90* and *C-32* cells were obtained from American Type Culture Collection (ATCC). The analysed cells were grown in monolayer in Eagle's Minimal Essential medium with Earle's BSS and 2 mM L-glutamine (EMEM). The medium was supplemented with 10 % fetal bovine serum and 1 % penicillin and streptomycin. The cell culture was incubated at 37 ^{o}C and in a humid atmosphere of 5 % CO_2. ALA (100μg/ml) was used as a photosensitizer. The cells were irradiated with 635 nm light by laser Terapus R 635/650. The power of irradiation was 10 mW. Cells were irradiated during the time of 20 min. Free radical properties in original cells, cells cultured with photosensitizer – ALA and cells irradiated by laser with and without ALA were studied. Microscopic observations of all the tested samples were performed.

EPR spectra were measured by RADIOPAN (Poznań) spectrometer with modulation of magnetic field 100 kHz and Rapid Scan Unit produced by the JAGMAR firm from Cracow. Single EPR line was recorded at short time 10^{-4} s. EPR spectra were measured at low microwave power of 0.7 mW to avoid microwave saturation. The EPR curves were analysed using Elf program of JAGMAR firm. Integral intensities (I), linewidths (ΔB_{pp}) and g-factors were determined.

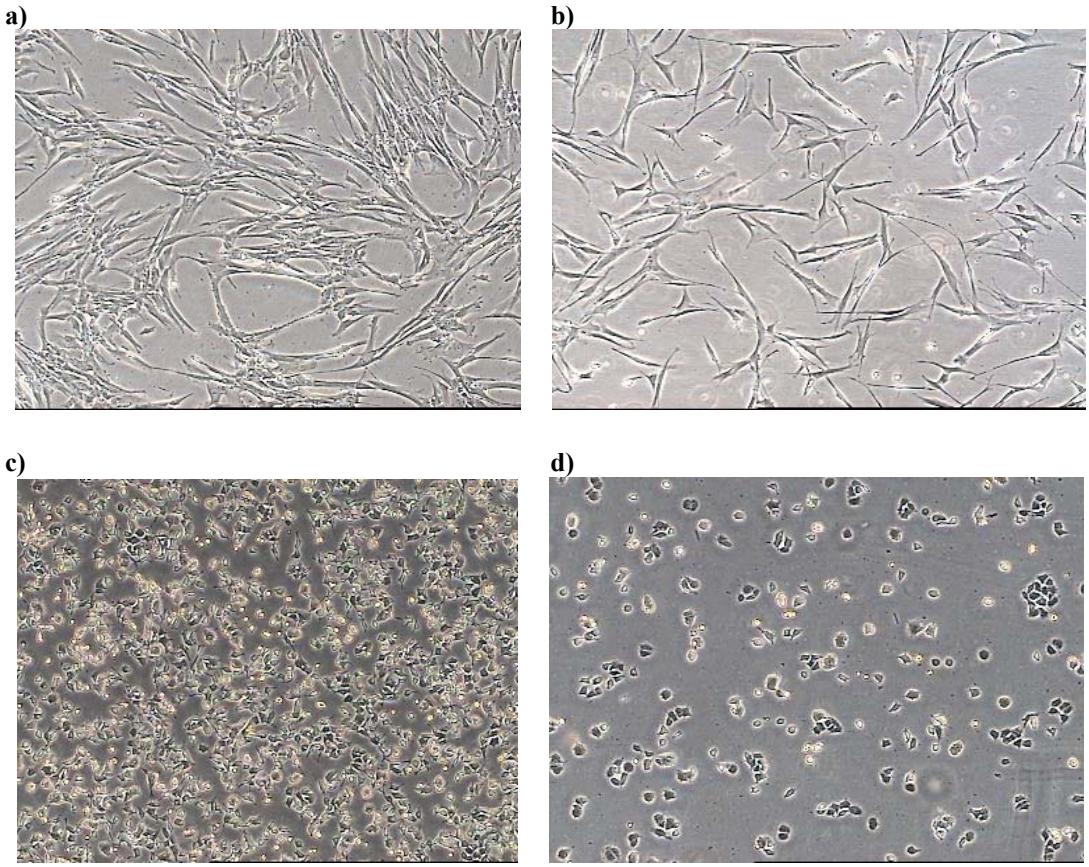

Fig. 1. Control *IMR-90* (a) and *C-32* (b) cells cultures, and *IMR-90* (c) and *C-32* (d) cells irradiated by laser

Table 1. Linewidths (ΔB_{pp}) and integral intensities (I) of EPR spectra of *IMR-90* and *C-32* cells. Data for control cells, cells cultured with photosensitizer ALA, and for laser irradiated control cells and laser irradiated cells with ALA.

sample	***IMR-90* cells**		***C-32* cells**	
	ΔB_{pp} [mT] ($\pm$0.02)	**I [a.u.] ($\pm$0.5)**	**ΔB_{pp} [mT] ($\pm$0.02)**	**I [a.u.] ($\pm$0.5)**
control cells	3.59	20.8	3.52	27.8
control cells + ALA	3.59	23.0	3.29	21.7
control cells + laser	3.54	29.4	3.23	30.4
control cells + ALA + laser	3.23	19.8	3.04	15.8

Results

Free radicals with g-factors near 2.0030 and single broad EPR lines were found in all the studied *IMR-90* (ΔB_{pp}: 3.23-3.59 mT) and *C-32* (ΔB_{pp}: 3.04-3.52 mT) cells. Parameters of their EPR spectra are shown in Table 1. The number of free radicals strongly changes after laser irradiation of the analysed two kinds of cells. After the irradiation with laser integral intensity of EPR lines of the tumor cells increases, since the chemical bonds are ruptured in cell units and free radicals are creating. Irradiation of *IMR-90* and *C-32* cells cultured with ALA led to decrease in free radicals in the examined samples. The higher amount of free radicals should appear in cells irradiated by laser at the presence of ALA than in cells irradiated without photosensitizer. The photosensitizer excited by laser play a role of an additional source of energy in process of rupturing chemical bonds. Probably recombination of large number of free radicals in cells irradiated by laser at the presence of ALA is responsible for the observed quenching of their EPR lines.

Microscopic observations of tumor cells brought to light damage of cells after laser irradiation. Formation of the free radicals detected by EPR method is responsible for this process. Control *IMR-90* and *C-32* cells are presented in Figure 1 a and Figure 1 b, respectively. *IMR-90* and *C-32* cell culture with ALA and irradiated by laser are shown in Figure 1 c and Figure 1 d, respectively.

Discussion of results

It was proved that after laser irradiation of *IMR-90* and *C-32* cells without photosensitizer free radicals were formed and the tumor cells died. Application of laser with the power of radiation equal to 10 mW indicates rupturing of chemical bonds in tumor cells and number of free radicals increases. ALA rises free radical formation in irradiated *IMR-90* and *C-32* cells, which is accompanied by their recombination and as the result of it the decrease in intensity of EPR lines was measured. Similar correlations were observed for *SK* cells during PTD with ALA used as a photosensitizer [1, 2].

References

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