CONDITIONS OF PHOTODYNAMIC THERAPY OF TUMOR CELLS EXAMINED BY CARBONIZED COAL AND EPR SPECTROSCOPY

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Abstract

Coal samples carbonized at 600°C and TEMPO probes we applied in oximetry. EPR spectra of medium-ranked coal carbonized at 400, 500, and 700 °C were not sensitive to oxygen. Oxygen effects during photodynamic therapy of tumor cells were tested. Oximetric probes were used to examination of singlet oxygen O_2 formation in melanotic CRL-1424 tumor cells irradiated by laser (500 mW, λ=662 nm) at the presence of photolon as the photosensitizer. Tumor cells were irradiated during 7, 15, and 30 minutes. Changes in EPR spectra of coal probe and TEMPO after excitation of oxygen O_2 from triplet ground state (S=1) to diamagnetic singlet (S=0) state were analysed. Measurements of EPR spectra of coal carbonized at 600°C and TEMPO in: control cell culture, irradiated cells, and cells irradiated at the presence of photolon, were done. After PDT intensity of EPR lines of the used oximetric probes considerably increases. It was proved that the strongest formation of singlet oxygen in the studied cells appears after 15 minutes of laser irradiation.

Keywords: *paramagnetic centers, PDT, oximetric probes, EPR*

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Introduction

Free radicals and diamagnetic oxygen molecules O_2 destroy tumor cells during photodynamic therapy (PDT) [1]. It is known three different types of free radicals mechanisms in irradiated cells [1-3]. Free radicals are formed: 1) by laser irradiation, 2) after cells interactions with excited photosensitizer (for low oxygen concentration in cells), and, 3) in reactions of excited by photosensitizer diamagnetic oxygen O_2 molecules with cells (for high oxygen concentration in cells). Intensive production of both free radicals and reactive singlet oxygen is expected in optimal PDT.

In this work we searched high amount of singlet oxygen O_2 in melanotic tumor cells irradiated by laser at the presence of the photosensitizer. Paramagnetic probes we used in oximetry of irradiated cells. It is known that coal samples strongly interact with paramagnetic oxygen O_2 [4-6], and they are ambient for cell culture [7]. Coal samples and TEMPO we proposed as oximetric probes.

Materials and methods

Sample characterization

Photodynamic therapy of CRL-1424 tumor cells obtained from American Type Culture Collection (ATCC) was studied. The cells were grown in monolayer in Eagle's minimal Essential medium with Earle's BSS and 2mM L-glutamine (EMEM). The medium was supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin. The cell culture was incubated at 37°C and in humid atmosphere of 5% CO_2. Photolon (100 μg/mL) was used as photosensitizer.

The cells were irradiated by laser with wave 662 nm and power 500 mW. The number of cells in the cultures was determined by contrast phase OLYMPUS IX50 optical microscope. We used different times of laser irradiation: 7, 15, and 30 minutes.

EPR measurements

The measurements were performed by the use of an X-band (9.3 GHz) electron paramagnetic resonance spectrometer with magnetic modulation of 100 kHz. Microwave frequency was measured with MCM 101 recorder. In order to avoid microwave saturation EPR spectra of the oximetric probes were detected at low microwave power equal 0.7 mW.

Oxygen effects were studied by the use of oximetric probes: coal samples and TEMPO. Coal with a carbon content 64.8 wt% was heated at 400, 500, 600, 700 and 800°C. As oximetric probe was selected coal carbonized at 600°C with a carbon content 85.1 wt%, because of strong changes of its EPR lines in oxygen atmosphere.

Changes in EPR spectra of coal probe (600°C) and TEMPO caused by laser excitation of oxygen O_2 molecules to diamagnetic (S=0) state were analysed. The spectra of coal carbonized at 600°C and TEMPO located in control cell culture, irradiated cells, and cells irradiated at the presence of photolon, were compared.

Results and discussions

CRL-1424 cells are destroyed after laser irradiation and their number in cultures decreases. Death of tumor cells after laser irradiation is clearly visible from microscopic observations presented in FIGURE 1. Lower amount of tumor cells exist in irradiated culture (FIG.1b) than in control culture (FIG.1a).

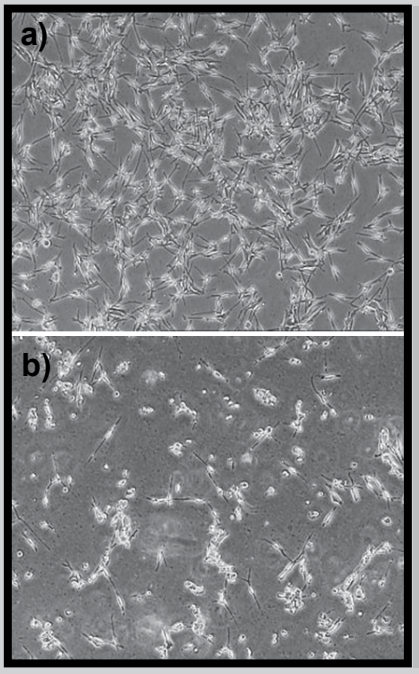

FIG.1. CRL-1424 cell cultures before (a) and after (b) laser irradiation at the presence of photolon as photosensitizer.

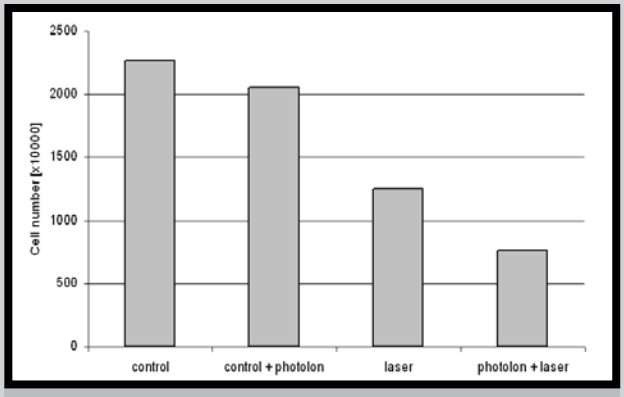

FIG.2. Cell number in control cell culture, control cell culture with photolon, and in irradiated cell culture without and with photolon.

Decrease of cell number in culture after laser irradiation is compared in FIGURE 2. The lower number of cells we obtained after laser irradiation of cells at the presence of photosensitizer. The number of cells in culture decreases with increasing of irradiation time.

EPR spectra of coal carbonized at 400, 500, 600, 700 and 800°C for samples in air were compared in FIGURE 3.

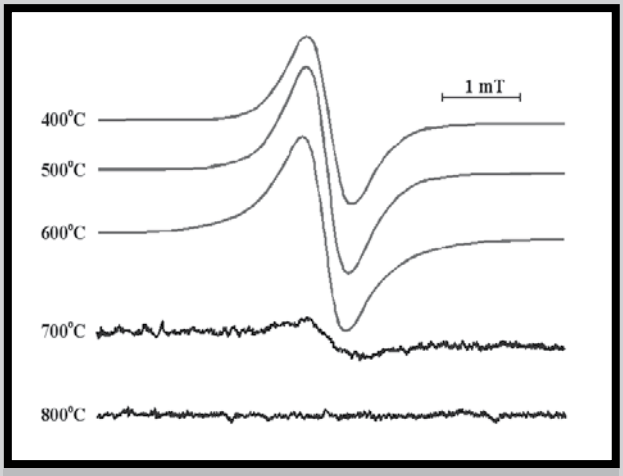

FIG.3. EPR spectra of coal carbonized at 400, 500, 600, 700 and 800°C for dry powdered samples in air.

Strong EPR lines were observed for coal heated at 400, 500, and 600°C. Only a weak EPR signal was measured for coal carbonized at 700°C. EPR lines were not measured for sample carbonized at 800°C. Concentrations of paramagnetic centers N in coal samples and parameters of their lines in air and in nitrogen N_2 environment are compared in TABLE 1. Higher concentrations were obtained for coal samples in nitrogen atmosphere.

	AIR			NITROGEN		
T [°C]	$Nx10^{18}$ [spin/g]	ΔB_{pp} [mT]	g [±0.0002]	$Nx10^{18}$ [spin/g]	ΔB_{pp} [mT]	g [±0.0002]
400	7.7	0.68	2.0034	7.9	0.67	2.0034
500	5.8	0.61	2.0033	6.2	0.58	2.0033
600	3.7	0.58	2.0033	4.8	0.52	2.0031
700	0.005	0.63	2.0029	0.006	0.58	2.0028

TABLE 1. Paramagnetic centers concentrations (N), g-factors and linewidths (ΔB_{pp}) of EPR spectra of coal samples carbonized at different temperatures. Data for samples in air and nitrogen atmosphere are compared.

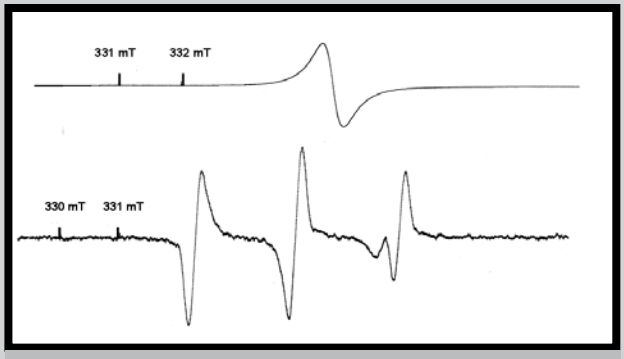

FIG.4. EPR spectra of coal oximetric probe and TEMPO located in the cells irradiated during 15 minutes by laser at the presence of photolon.

EPR lines of coal carbonized at 600°C saturate at the relatively higher microwave power. Such correlation is characteristic for multi-ring aromatic units [4-5]. Unpaired electrons of such molecular structures strongly interact with paramagnetic O_2 molecules [4-5]. The correlation indicates our proposal that sample carbonized at 600°C is valuable oximetric probe.

So as oximetric probe we selected coal carbonized at 600°C and additionally TEMPO. Exemplary EPR spectra of this coal probe and TEMPO in cell culture are shown in FIGURE 4. Parameters of EPR lines of coal oximetric probe (600°C) and TEMPO located in control cells, cells with photolon, cells irradiated by laser without and at the presence of photolon, are presented in TABLE 2 and 3. Intensities of their EPR lines increase after laser irradiation at the presence of photolon. Prolongation of irradiation time from 15 to 30 minutes gives decrease of EPR signals of both coal and TEMPO probes.

Similar effects appear during photodynamic therapy of SK melanotic tumor cells with coal and TEMPO probes [3]. Laser irradiation causes excitation of paramagnetic oxygen molecules (S=1) from ground triplet state to diamagnetic singlet state (S=0). Decrease of concentration of paramagnetic oxygen molecules in the environment of cells.

In this work we confirmed usefulness of coal probes to examination of oxygen effects in laser irradiated tumor cells. After laser irradiation of tumor cells concentration of paramagnetic oxygen O_2 molecules (S=1) decreases and amount of diamagnetic (S=0) excited oxygen molecules increases. Excitation of oxygen molecules in irradiated cell culture is accompanied by rise of intensity of EPR line of coal probe (TABLE 2). Creation of quasi-chemical bonds between paramagnetic oxygen O_2 and coal quenches its EPR signal [4].

Oximetric probe located in:	t [min]	I [a.u.]	ΔB_{pp} [mT]	g [±0.0002]
Control cell culture	0	2.45	0.36	2.0031
Cell culture with photolon	0	3.64	0.35	2.0031
Irradiated cells without photosensitize	7	1.78	0.35	2.0033
Irradiated cells with photolon	7	1.32	0.36	2.0032
Irradiated cells without photosensitize	15	3.34	0.37	2.0031
Irradiated cells with photolon	15	2.38	0.38	2.0031
Irradiated cells without photosensitize	30	3.18	0.38	2.0031
Irradiated cells with photolon	30	3.02	0.39	2.0033

TABLE 2. Integral intensities (I), linewidths (ΔB_{pp}) and g-factors of EPR spectra of the tested oximetric probes in cell culture during different conditions of photodynamic therapy. Cells were irradiated by laser during times (t): 7, 15, and 30 minutes. Data for coal probe carbonized at 600°C are shown.

Oximetric probe located in:	t [min]	I (-1) [a.u.]	$\Delta B_{pp}^{(-1)}$ [mT]	I (0) [a.u.]	$\Delta B_{pp}^{(0)}$ [mT]	I (+1) [a.u.]	$\Delta B_{pp}^{(+1)}$ [mT]	τ_c x10^{-9} [s]
Control cell culture	0	1.16	0.23	1.55	0.23	0.59	0.18	1.2
Cell culture with photolon	0	3.55	0.23	3.94	0.23	2.10	0.21	0.6
Irradiated cells without photosensitizer	7	0.87	0.27	0.51	0.21	0.40	0.19	0.1
Irradiated cells with photolon	7	11.02	0.21	13.71	0.23	9.02	0.21	0.5
Irradiated cells without photosensitizer	15	0.43	0.22	0.74	0.24	0.35	0.24	1.1
Irradiated cells with photolon	15	17.09	0.24	22.29	0.25	10.78	0.22	0.9
Irradiated cells without photosensitizer	30	0.53	0.23	0.57	0.23	0.14	0.14	1.6
Irradiated cells with photolon	30	7.45	0.23	8.13	0.23	3.46	0.18	0.8

TABLE 3. Integral intensities (I) and linewidths (ΔB_{pp}) of EPR lines of TEMPO probe in cell culture during different conditions of photodynamic therapy. Time (t) of laser irradiation was: 7, 15, and 30 minutes.

Diamagnetic oxygen molecules do not form such bonds and as the result increase of EPR signal appear.

In this work we proposed coal sample as oximetric probe in biological systems, because of its strong EPR line and high susceptibility to oxygen. Additionally this sample does not effect on cells in cultures. We observed similar growing of cell culture without and at the presence of coal probe. Such properties may possible observations of influence of photodynamic therapy on tumor cells and the optimal therapeutic conditions may be found.

As the second oximetric probe we tested TEMPO. Our experiment proved usefulness of TEMPO probe in oximetry and tumor cells studies. Similarly to coal probe intensity of EPR line of TEMPO increase after excitation of oxygen molecule from triplet to singlet oxygen.

Paramagnetic oximetric probes are known from a lot of work [7-14], but probes examined in our studies may be applied to cells, tissues and others biological species. The high sensitivity of their EPR spectra to oxygen O_2 magnetic state and absence of toxic reactions in cell medium are mainly important.

Our work indicates that electron paramagnetic spectroscopy and oximetric probes may be practically used in clinical applications. We use physical methods to determine of optimal conditions of photodynamic therapy of CRL-1424 tumor cells. Optimal conditions of PDT we found for laser irradiation of cells at the presence of photolon during 15 minutes. The lower amount of singlet oxygen is formed in tumor cells after laser irradiation during 30 minutes (TABLES 2,3).

Conclusions

On the basis of EPR examination of tumor cells irradiated by laser the following conclusion may be drawn:

1. Coal samples carbonized at 600°C and TEMPO may be used as oximetric probes for photodynamic therapy of CRL-1424 cells. Paramagnetic oxygen O_2 molecules strongly interact with these samples and decrease their EPR amplitude.

2. Singlet oxygen formation is more effective during laser irradiation of the studied cells at the presence of photolon.

3. The best conditions of PDT of CRL-1424 tumor cells appear when 15 minutes time of laser irradiation was applied.

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