EPR examination of singlet oxygen formation in Caco-2 cells irradiated by laser at the presence of chlorin e6

Małgorzata Latocha¹, Barbara Pilawa², Dariusz Kuśmierz¹, Elektra Sliupkas-Dyrda¹

¹Department of Cell Biology, School of Pharmacy, Medical University of Silesia, Narcyzów I, 41-200 Sosnowiec, ²Department of Biophysics, School of Pharmacy, Medical University of Silesia, Ostrogóraska 30, 41-200 Sosnowiec

Electron paramagnetic resonance studies were performed for examination of excitation of oxygen molecules O₂ from paramagnetic triplet (S = 1) to diamagnetic singlet (S = 0) state in human adenocarcinoma Caco-2 cells during irradiation by laser. Laser with 662 nm and 500 mW was used. Different time of irradiation was applied: 7, 15, and 30 minutes. Laser irradiation was done for Caco-2 cell culture at the presence of photosensitizer chlorin e6.

Singlet oxygen existence in the cell environment was tested by the use of TEMPO spin probe as the oximetric sample. EPR spectra at X-band were measured by RADIOPAN spectrometer with magnetic modulation 100 kHz. Microwave frequency was measured by MCM102 recorder. Low microwave powers (0.7 mW) were used during experiments to avoid microwave saturation of EPR lines. TEMPO was added to cell culture, and cells with both photosensitizer and TEMPO were irradiated by laser.

The typical for TEMPO three lines were measured and their parameters were determined. Amplitudes of TEMPO spectrum strongly depend on triplet oxygen concentration in cells. Amplitudes of the three TEMPO lines (I, II, III) in arbitrary units for control cell culture, and for culture with chlorin e6 irradiated during different times are compared in Figure 1. It is shown that amplitudes of EPR spectrum of TEMPO strongly increase after laser irradiation of cells. This effect is higher for the longer times of irradiation. The observed increase of EPR amplitudes of TEMPO after laser irradiation is the result of formation of singlet oxygen from triplet oxygen in the cells. Paramagnetic oxygen O₂ (S = 1) quenches EPR signals. Excitation of oxygen molecules to singlet state is accompanied by decrease of paramagnetic triplet oxygen in the cells, so finally signals increase. The discussed correlations are presented in Figure 1.

Figure 1. Amplitudes of EPR lines of TEMPO in Caco-2 cells.
Microscopic studies of the irradiated Caco-2 cells indicate that singlet oxygen damages cells, and the number of cells in the culture decreases with increasing time of irradiation. This correlation is visible from Figure 2 a-d.

Figure 2. Caco-2 cell culture: a) control culture, b-d) cell culture irradiated by laser during 7, 15, and 30 minutes, respectively.

The performed spectroscopic studies indicate that TEMPO probe may be used as reference for singlet oxygen formation during laser irradiation of Caco-2 cells. EPR spectra of TEMPO are susceptible for oxygen content in cell culture. EPR spectroscopy is proposed as the very useful technique for determination of photodynamic therapy parameters of tumors.