Light-induced photodynamic effects in cancer cell lines

Hana Kolarova [a], Katerina Tomankova [a], Robert Bajgar [a], Jakub Malohlava [a], Vera Cenklova [a], Adela Hanakova [a], Klara Pizova [a], Barbora Manisova[a], Lukas Malina [a], Jana Horakova [a], Kristina Kejlova [b], Dagmar Jirova [b]

[a] Department of Medical Biophysics, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University in Olomouc, Hnevotinska 3, 775 15 Olomouc, Czech Republic, [b] Centre of Toxicology and Health Safety, National Institute of Public Health, Srobarova 48, 100 42 Prague, Czech Republic
e-mail: hana.kolarova@upol.cz

Keywords: (light-emitting diodes, cancer cell lines, sensitizer)

Photodynamic therapy (PDT) is alternative treatment method for certain malign and nonmalign diseases [1]. The cytotoxicity results from a combined effect of sensitizer, oxygen and light. During this process reactive oxygen species (ROS) are produced that are involved among major agents triggering cell death by apoptosis or necrosis mechanism [2], [3]. In this study, we have focused on an effect of PDT on cancer cell lines using chloroaluminum phthalocyanine disulfonate ($ClAlPcS_2$) as the sensitizer. $ClAlPcS_2$ was obtained from Dr. Jan Rakusan (Research Institute for Organic Syntheses, CR). Irradiation was achieved with light-emitting diodes (670 nm, 10 $mW.cm^{-2}$) at the total dose range from 0 up to 50 $J.cm^{-2}$. Our study was performed on three cell lines (MCF7 human breast adenocarcinoma, G361 human melanoma, HeLa cervix carcinoma) as *in vitro* tumour models. The cells were grown in the presence of $ClAlPcS_2$ in the concentration range from 0 up to 10 $\mu g \cdot ml^{-1}$ at 37 °C, 5% CO_2 for 24 h. ROS measurement was performed using 10 μM 5-(6-)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (CM-H_2DCFDA) producing fluorescence dye CM-DCF in the presence of ROS. Fluorescence of CM-DCF was calibrated according to the fluorescence response of the probe to the additions of external H_2O_2. For identification of apoptosis the Vybrant apoptosis Assay Kit (annexin V conjugated to fluorescein and propidium iodide) was used. Microtubules in paraformaldehyde fixed adherent cell culture were detected by indirect immunofluorescence using mouse monoclonal anti-α-tubulin antibody, clone DM1A (Sigma-Aldrich) and AlexaFluor®488 dye conjugated to anti mouse secondary antibody (green fluorescent compound), and chromatin was stained by DAPI (blue fluorescent compound). The production of ROS, changes of microtubules, mode of cell death and distribution of $ClAlPcS_2$ in cells were visualized with an inverted fluorescence microscope. For atomic force microscopy (AFM) the cells were placed onto inverted optical microscope Olympus IX81 and imaged with Bioscope Catalyst AFM system in PeakForce QNM mode at scan rate of 0.2 Hz. DNP-10 silicon nitride tip with resonant frequency of 16 – 28 kHz and nominal spring constant 0.12 N/m was used. All images were processed in Gwyddion 2.34. We have found that $ClAlPcS_2$ at lower concentrations and lower doses of radiation induced lower ROS production. Cancer cells produced the highest amount of ROS in the presence of 10 $\mu g \cdot ml^{-1}$ $ClAlPcS_2$ after irradiation treatment by 50 $J.cm^{-2}$. We observed ROS production (CM-DCF fluorescence) diffusely localized in whole cells corresponding with the homogenous distribution of sensitizer within cells. The results from AFM showed that cells before treatment have a symmetrical shape and soft structure along the whole cell surface in comparison with PDT-exposed cells. In this study, the antitumour effect of $ClAlPcS_2$ induced by PDT was evaluated on cancer cells by fluorescence microscopy, atomic force microscopy (AFM) and quantitative fluorescence measurement of ROS production. Apoptosis was the predominant mode of cell death at lower PDT doses. This effect is probably associated with lysosomal and mitochondrial photodamage and DNA fragmentation. High PDT doses were associated with membrane photodamage and thus increased permeability to propidium iodide. Thus, the shift from apoptotic to necrotic cell death was a function of the phthalocyanine concentration and irradiation dose. The ability of PDT to produce a rapid apoptotic response may be an important element of successful photodynamic therapy. Our results showed that photodamage of MCF7, G361 and HeLa cells was dependent on the sensitizer concentration and irradiation dose. This work was supported by the grant project of Ministry of Health of Czech Republic IGA MZCR NT 14060-3/2013

[1] S. Yano , S. Hirohara , M. Obata , Y. Hagiya , S. Ogura , A. Ikeda , H. Kataoka , M. Tanaka , T. Joh, Journal of Photochemistry & Photobiology, C: Photochemistry Reviews, 12, (2011), 46-67.
[2] C.A. Robertson , D.H. Evans , H. Abrahamse , J Photochem Photobiol B. 17;96(1): (2009), 1-8.
[3] I. Baldea , A.G. Filip , J Physiol Pharmacol. 63(2),(2012) 109-18.

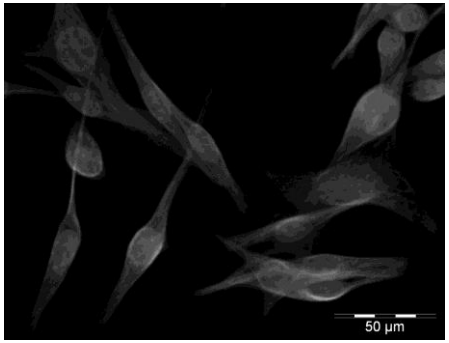

Figure 1. G361 melanoma cells – control without PDT.

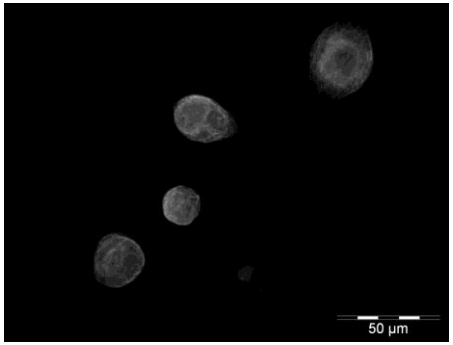

Figure 2. G361 melanoma cells – after PDT ($ClAlPcS_2$ concentration 8$\mu g \cdot ml^{-1}$)

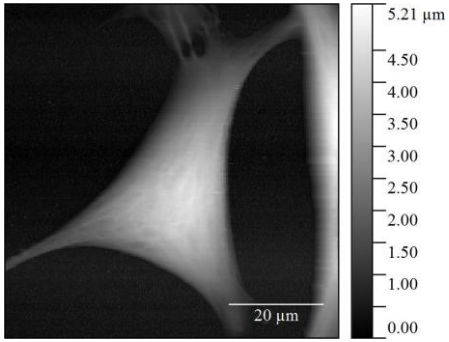

Figure 3. AFM study: HeLa cervix carcinoma cells – control without PDT.

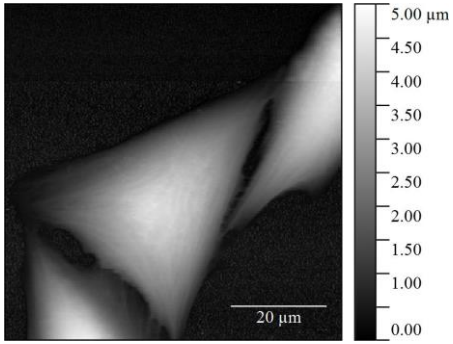

Figure 4. AFM study: HeLa cervix carcinoma cells – after PDT.