

# ABSTRACT

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Title of diploma thesis: *In vitro* evaluation of photodynamic activity of tetrapyrroldiporphyrin derivatives for treatment of solid tumors

Nowadays, cancerous diseases belong among the main causes of death, incidence continues to rise. Therefore research in the area of tumor treatment significantly focuses on the development of new potential anticancer drugs and therapeutic approaches. Photodynamic therapy (PDT) belongs to clinically approved therapeutic methods for the treatment of the malignant and nonmalignant diseases. PDT is composed of three main components – photosensitizer (PS), light and oxygen, all of them are non-toxic on their own. However, their interaction leads to a generation of highly reactive molecules. This method is based on absorption of light with appropriate wavelength by PS and subsequent energy transfer from photon to surrounding molecules, especially to oxygen. This transfer leads to a generation of cytotoxic reactive oxygen species (ROS) of which singlet oxygen plays a main role. ROS subsequently causes a damage of surrounding cell structures, which leads to irreversible damage of cell functions and subsequent cell death. Type of cell death is closely related to the localization of PS within the cell. Effect of the treatment is based on three mechanisms, which are interconnected – direct lethal cytotoxic effect of ROS, damage of cancer vasculature and induction of immune reaction in the organism.

Aim of this diploma thesis is the evaluation of photodynamic activity of newly synthesized PS from the group of tetrapyrroldiporphyrin derivatives *in vitro*. Experiments were performed on HeLa cell line, which is derived from human cervix

cancer cells. Cells were irradiated with red part of the visible spectrum using a Xe lamp ( $\lambda > 570$  nm, 12.4 mW/cm<sup>2</sup>, 11.2 J/cm<sup>2</sup>) after 12 hour incubation with PS. Dark toxicity of PSs (without the presence of activating light) was also performed. Evaluation of cell viability was carried out in 96-wells plates using neutral red uptake assay. Uptake profile of PS was also performed, amount of PS was measured in the cell lysate by fluorescence measurement.

Subcellular localization was detected by fluorescence microscopy. Cell organelles were labelled with specific fluorescent probes MitoTracker Green FM and LysoTracker Blue DND-22. Morphological changes of the cells induced by photodynamic effect of PS were studied by fluorescence labelling of cell nuclei (Hoechst 33342, propidium iodide), cell membrane (Cell Mask Green Plasma Membrane Stain), mitochondria (MitoTracker Red CMXRos) and F-actin (Alexa Fluor 488 conjugate of phalloidin) using fluorescence and confocal laser scanning microscopy. Type of cell death was evaluated by flow cytometry by staining cells with Annexin V-FITC and propidium iodide.

Cationic derivatives have shown to be effective PS with exceptionally high toxicity after activation ( $EC_{50} = 3,89 \pm 1,79$  nM), while they have shown a very low inherent toxicity of hundreds of  $\mu$ M without irradiation. Changes of cell morphology were already detected during the irradiation period. Photodynamic effect manifested in membrane blebbing, changes of mitochondria shape and later in nuclear changes (chromatin condensation and nucleus fragmentation) and reorganization of actin cytoskeleton. The results of flow cytometry together with induced morphological changes indicate rather necrotic cell death. Non-charged derivatives have not proven to be effective under our experimental conditions.