ABSTRACT

Charles University in Prague
Faculty of Pharmacy in Hradec Králové
Department of Biochemical Sciences

Candidate: Petra Brázdová
Supervisor: Mgr. Miloslav Macháček
Title of diploma thesis: Study of the novel phthalocyanines for the vascular-targeted photodynamic therapy of tumors

Cancer is one of the leading causes of death in developed countries, therefore a big effort is devoted on research of novel anticancer drugs. Photodynamic therapy (PDT) is currently a well-established method in this area, but targeting its effect on tumor vasculature has not been considered until recently. In comparison with PDT, vascular-targeted photodynamic therapy (VTP) takes the advantage of a much shorter interval between administration of the photosensitizer (PS) and irradiation (drug-light interval) and thus turning the effect of cytotoxic agents to tumor vasculature. It is intended to disrupt the oxygen and nutrients supply to malignant cells and thus cause their damage and death.

Aim of this work is to evaluate the efficacy of newly synthesized PSs from the group of phthalocyanines and azaphthalocyanines using the VTP protocol under *in vitro* conditions. The PSs used in this study have been investigated recently with classical PDT protocol, in which they have demonstrated very promising activity against tumor cells and exhibited low inherent toxicity.

The main objective of this study was to evaluate the ability of the cytotoxic effect of the PSs activated by an irradiation with red light immediately after their application. Evaluation of cell viability was carried out in 96-well plates using a neutral red uptake assay. Experiments were performed on two types of cell lines – human endothelial cell line EA.hy926 and malignant human cervical cell line HeLa.

Fluorescence microscopy and microscopy in differential interference contrast were employed for evaluation of morphological changes of the cells after activation of the PS. For detection of selected subcellular changes, the fluorescent probe JC-1 was used for the determination of mitochondrial damage and propidium iodide (PI) in combination with

Hoechst 33342 to distinguish changes in the nucleus during cell death. The latter was further confirmed by flow cytometry with cells fluorescently labeled with PI and FITC-Annexin V. The possible involvement of autophagy in the process of cell death was assessed by fluorescent labeling with monodansylcadaverin.

The results of the experiments confirmed the high activity of these compounds against selected cell lines and in the VTP protocol. The overall best PS was proved to be peripherally substituted phthalocyanine derivative having high activity after irradiation (EC_{50} = 0,57 μM for EA.hy926 or 0,24 μM for HeLa cells) and especially very low inherent toxicity (TC_{50} > 1500 μM).