ABSTRACT

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Title of Doctoral Thesis **Study of novel phthalocyanine and azaphthalocyanine photosensitizers for the photodynamic therapy of cancer.**

Photodynamic therapy (PDT) of cancer is non-invasive treatment modality for solid tumour treatment using three basic components – molecular oxygen, light and photosensitizer (PS). These elements are essentially non-toxic on their own, but in the combination they induce reactive oxygen species (ROS; singlet oxygen mainly) production, causing damage to cellular components and subsequent cell death. Type of cell demise is dependent mainly on the type of PS, length of irradiation and subcellular localization of the drug. Singlet oxygen is highly reactive and is capable of limited diffusion in biological environment. Apart from direct cytotoxic effect, vascular shutdown (oxygen and nutrition deprivation) and activation of immune system are involved in tumour eradication. Combination of effective compound with delivery system, conjugation with targeting substances or synthesis of highly effective non-aggregating water soluble compounds are the main pathways in design of modern PSs. In our project we focused mainly on hydrophilic phthalocyanines (Pc) and azaphthalocyanines (AzaPc) with minimal dark toxicity (for our derivatives typically 10^2 – 10^3 μM) and high photodynamic activity (up to 10^-3 μM). Series of compounds were synthetized, photophysically and in vitro characterized on human cervical carcinoma cell line (HeLa) and for most promising compounds also on several other malignant and non malignant cell lines. Considering the importance of subcellular localization in the context of PS activity, we also focused on evaluation of primary target for our original compounds. In all cases, we observed endo lysosomal compartment out as the place of PS localization. After irradiation, the PSs damage membrane of those organelles and redistribute into cytoplasm, where they cause damage to other cellular components (still in the time-course of irradiation). Cationic derivatives subsequently redistribute also to the nucleus as the consequence of compromised nuclear membranes; their cationic nature allow them to bind negatively charged DNA. Redistribution to nucleus proceeds after irradiation and so no mutagenic effect of these novel PS is anticipated, which is general property of this type of drugs in the contrast to conventional chemotherapeutics. Extensive ROS production during photodynamic treatment leads, in the case of our lysosomal localized derivatives, to necrotic type of cell death accompanied with incomplete cell retraction, formation of large membrane blebs and blisters, shortening and rounding of mitochondria and reorganisation of actin and tubulin cytoskeleton. In the case of anionic AzaPc we were also able to describe the mechanism causing lower activity of negatively charged PS even in the absence of aggregation. Anionic PSs are bound to serum proteins (predominantly albumin) and triplet state quenching is taking place, which leads to the considerable decrease of singlet oxygen production. On the other hand, binding to the proteins also partly protects PS against negative influence of acidic lysosomal pH. In this environment the carboxylate groups lose charges and PS aggregate. Results of our research demonstrate that lysosomally localized (Aza)Pcs are potent PSs able to induce fast and effective oxidative damage leading to the cell death.