ABSTRACT

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

Candidate: Mgr. Marie Halašková
Supervisor: RNDr. Miloslav Macháček, Ph.D.
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Photodynamic therapy (PDT) is a clinically approved treatment modality that experienced great progress over the last two decades and that has been used in the treatment of several disorders, including age-related macular degeneration, psoriasis and certain cancers. PDT is based on the combination of three main components: light-sensitive drug (known as a photosensitizer, PS), light of an appropriate wavelength, and oxygen. The light-activated PS reacts with molecular oxygen to produce reactive oxygen species, which can interact with cellular components in a biological environment and trigger a cascade of reactions causing destruction of tumor cells, tumor vasculature and induction of a local inflammatory reaction. The result is a disruption of cell homeostasis leading to the cell death.

Like all the novel treatment modalities, further research is still needed including the development and optimization of PS synthesis in order to increase the efficiency of therapy and minimalize side effects. In this project, we decided to carry out an extensive study of the properties of several different PSs from the group of phthalocyanines (Pc) and their aza-analogues (AzaPc). Pcs are characterized by exceptionally good photophysical properties, especially strong absorption in 650-750 nm area and strong singlet oxygen production. These compounds represent a very promising group of PSs suitable for the use in PDT, however their potential application in PDT is often limited by their low solubility in water and strong tendency to aggregate.

In our work, we focused on the evaluation the photodynamic activity and properties of Pcs bearing different charges on a peripheral substituents. The photophysical properties were characterized on a series of anionic and cationic Pcs (hydrophilic and amphiphilic) and they were studied in terms of photodynamic activity under *in vitro* conditions on malignant and nonmalignant cell lines. All studied derivatives showed minimal toxicity without irradiation

and high toxicity upon irradiation (in the order of tens of nM). Solubility and aggregation in the water, effect of pH, binding to serum proteins, interaction with biomembranes and liposomes, subcellular localization and subsequent re-localization after irradiation, as well as morphological changes and the type of cell death were determined for all Pcs.

The subcellular localization of all Pcs was the endolysosomal compartment, which greatly influenced the activity of anionic derivatives. All anionic derivatives were aggregated at pH 4.9 (intralysosomal pH) while all cationic derivatives were not affected by this pH change. The activity of anionic compounds was also strongly suppressed by their binding to serum proteins (predominantly albumin) leading to excited states quenching and decrease of singlet oxygen production. After irradiation, cationic derivatives were rapidly released from lysosomes due to membrane rupture and subsequently relocated to the cytoplasm. The effect seemed to be stronger for hydrophilic compounds that were diffused in cytoplasm after the release from lysosomes, while amphiphilic compounds were localized primarily in the residues of membranous structures. The massive oxidative stress after the activation of Pcs led to severe morphological changes (*e.g.,* condensation of nuclear chromatin, shortening and rounding of mitochondria, reorganization of the cytoskeleton) and to the induction of necrotic or apoptotic cell death with secondary necrosis.

Based on all obtained results, a lower pH in the lysosomal compartment and binding to albumin were responsible for a substantially lower photodynamic activity of anionic Pcs compared to the cationic ones. Furthermore, this work demonstrated that an efficient bulky and rigid arrangement of peripheral cationic substituents efficiently inhibits aggregation, and that increased bulkiness of non-polar substituents on lipophilic part of the molecule of amphiphilic (Aza)Pcs does not improve anchoring into lipid bilayer compared to derivatives with non-bulky substituents that enter membranes almost immediately. Therefore, these PSs have a significant potential in light-triggered drug release from liposomes.