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Topic: Porphycenes in photodynamic therapy of cancer cells and photodynamic inactivation of bacteria

Abstract

Photodynamic therapy (PDT) is a method of treatment based on simultaneous introduction of two factors: light and the substance called photosensitizer to the targeted cell or tissue. Their combined application results in generation of reactive oxygen species (ROS) and the death of the targeted cells. PDT is an approved method for the treatment of several cancer and pre-cancer conditions. Recently it gathers also a substantial interest in the field of treatment of infections. Antimicrobial application of PDT is often called photodynamic inactivation (PDI).

In this PhD thesis the group of molecules from the family of porphycenes were tested for their photosensitizing properties and their applicability in PDT and PDI. The studies included several sections. Investigations started from the basic studies for evaluation of photophysical properties of differently substituted porphycene-core molecules. This helped to estimate the photosensitizing potential of the studied compounds, such as their ability to produce ROS, which is necessary for PDT and PDI applications. The second part was the selection of delivery system for introducing the studied photosensitizers to the biological environment for the studies on bacteria and cancer cells. This part was particularly important, since porphycenes are highly hydrophobic, water-insoluble compounds, which cannot be directly transferred to the water phase, due to high tendency to aggregation. Finally after selection of carrier medium the PDI studies were conducted, including evaluation of the efficiency of the compounds against three strains of Gram-positive bacteria: *E. faecalis, S. aureus* and *S. epidermidis*. The dependence between the substituents of porphycene and the PDI activity was also evaluated. The other thread of biological studies included *in vitro* PDT trials conducted on HeLa cells and microscopic imaging to establish the localization of the chromophores inside the cells.

The research presented in the thesis proves that porphycenes are promising candidates for photosensitizers in therapeutic applications, with high absorption coefficients and the quantum yields of singlet oxygen generation (ϕ_Δ) oscillating around 30%.

Pluronic F-127 micelles was selected as the best carrier medium for the biological applications. Bactericidal studies revealed very pronounced PDI effect of unsubstituted porphycene against Gram-positive bacteria. The total eradication and over 6 log decrease of the investigated species was observed after treatment with 7 μM of photosensitizer and red light irradiation with the dose of 6 – 30 J/cm^2. The structure-activity relationship studies provided an interesting discovery that the *tert*-butyl substituents lower the bactericidal activity of porphycenes and can eventually block it completely, whereas apparently similar methoxyethyl moieties retain the activity of the porphycene core. The steric hindrance affecting bacterial cell wall penetration is the postulated reason. The results obtained during PDT studies of HeLa cells were surprisingly matching very well the conclusions from the PDI experiments. Also in this case the photosensitizing effect recorded for most of the studied compounds was very pronounced, but introduction of the *tert*-butyl groups was blocking the activity. The reason was the location of *tert*-butyl substituted compound in the lysosomes, whereas the other compounds occupied cytoplasm and membrane of HeLa cells.