Characteristics of photosensitizers from cyanoarylporphyrazines group: from viscosity sensitivity to immunogenic properties in photodynamic therapy of cancer

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Abstract

Photodynamic therapy (PDT) is a minimally invasive procedure that widely used in the therapy of different types of cancer. However, there is a still need to review and revise the current PDT approaches, in particular by creating of new photodynamic agents with improved photophysical and photobiological properties for boosting the PDT efficacy and maintaining the quality of patients' lives.

This study was focused on a detailed characterization of photophysical properties of four tetracyanotetra(aryl)porphyrazine dyes (**pz I-IV**) and analysis of their biological effects on cancer cells during PDT. The experiments were performed using the following constant cell lines: murine glioma GL261, murine fibrosarcoma MCA205, human epidermoid carcinoma A431, and human keratinocytes HaCaT cells. The cells were cultured according to standard protocols. For photodynamic activity estimation, the cells were exposed to light irradiation at the dose of 20 J/cm^2 using a LED light source (λex 615–635 nm, 20 mW/cm^2).

It was shown that all four **pz** have intensive light absorption, with a maximum in the red spectral region at 592–598 nm (Q-band) and a molar extinction coefficient exceeding10^4. **Pz I-IV** easily enter cancer cells and efficiently induce cell death under light irradiation in a dose-dependent manner with a lower cytotoxic effect on normal keratinocytes. Application of confocal imaging technique allow to establish the localization of **pz I-IV** in cancer cells predominantly in the Golgi apparatus and endoplasmic reticulum. Our studies revealed that **pz II** and **pz IV** have a unique photophysical properties as molecular rotors. Depending on viscosity of the surrounding medium, **pz II** and **pz IV** drastically change intramolecular twisting or rotation of side aryl groups, resulting in significant alterations of non-radiative relaxation and subsequent changes in quantum yield and fluorescence lifetime. The use of **pz II** and **pz IV** as viscosity sensors in combination with time-resolved microscopy allows to obtain viscosity maps of cancer cells and visualizing the features of cancer cells' reactions to PDT at the subcellular level. Interestingly, cancer cells undergone PDT with **pz I** and **pz III** exhibit pronounced ICD hallmarks. It was shown the release of crucial DAMPs (e.g., ATP and HMGB1) from PDT-induced fibrosarcoma MCA205 cells with subsequent activation of phagocytic activity and phenotypic maturation of bone marrow-derived dendritic cells *in vitro*. In the prophylactic tumor vaccination model *in vivo*, application of dying MCA205 cells contributed to the protection mice from tumor growth after challenge with the viable cancer cells that points to the activation of the adaptive immune system effectively combatting with tumor.

All together, the data suggest that **pz I-IV** combine the features of potent photodynamic agents with immunogenic properties and viscosity sensors that opens possibility for both efficient anti-

cancer treatment and PDT dosimetry providing a tailoring the PDT treatment regimen for each patient.

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