DOCTORAL THESIS

Development of red light-activated porphycene-based photosensitizers for hypoxic anti-tumor photodynamic therapy

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ABSTRACT

This work focuses on the development of red light-activated porphycene-based photosensitizers for anti-tumor photodynamic therapy (PDT) under both normoxic and hypoxic conditions. A total of seven water-soluble porphycenes have been designed, synthesized and evaluated as potential PDT agents in terms of their photophysical and photobiological properties using principally the human nasopharyngeal carcinoma (HK-1) cells. Among the porphycenes synthesized, two were neutral amphiphilic aryl porphycenes, TDEGPPo and Zn(II) TDEGPPo, with relatively weak photo-cytotoxic activities even under normoxic condition. Two cationic porphycenes, TPyBPo and TriPyPPo, exhibited strong photo-cytotoxic activities, with LD_{50} of 0.3 μM at a light dose of 3 J/cm^2, under normoxic condition. However, much lower photo-cytotoxicity was observed under hypoxic condition for TPyBPo and TriPyPPo, with LD_{50} of 3 μM and 3.5 μM, respectively, obtained at high light doses (>10 J/cm^2). Two alkyl porphycenes with one and two sulfonoamide diglycol functionalities, TBPoS-OH and TBPoS-2OH, were synthesized and shown to exhibit very potent photo-cytotoxic activities, with respective LD_{50} of 53 nM and 20 nM (light dose 8 J/cm^2) under normoxic conditions. Most importantly, comparably potent photo-cytotoxicity was also observed for these porphycenes under hypoxic conditions, with respective LD_{50} of 65 nM and 50 nM (light dose 8 J/cm^2). In addition, these porphycenes were taken up by the HK-1 cells very rapidly, with >90% accumulated inside the cells after only 1 h of incubation. Confocal microscopy revealed that these porphycenes were localized at the lysosomes, mitochondria as well as endoplasmic reticulum. Furthermore, the predominant mode of cell death caused

by the PDT action of these porphycenes was shown to be apoptosis. In an attempt to effect mitochondria localization to enhance apoptotic cell death for these porphycenes, TBPoS-OH was conjugated with rhodamine B to produce the TBPoS-Rh B conjugate. This porphycene-Rh B conjugate also displayed very potent photo-cytotoxicity under both normoxic and hypoxic conditions, with LD_{50} of 52 nM and 85 nM, respectively, at a light dose of 8 J/cm^2. However, confocal microscopy revealed its principal subcellular localization was at the lysosomes, not the mitochondria. The PDT activities of these porphycenes were compared to a well-known patented PDT agent, EtNBS, which is active under both normoxic and hypoxic conditions, with LD_{50} of 58 nM and large than 1000 nM, respectively, towards the HK-1 cells. This comparison clearly shows that our sulfonoamido-porphycenes, TBPoS-OH, TBPoS-2OH and TBPoS-Rh B conjugate, display a 15- to 25-fold stronger hypoxic PDT activity relative to EtNBS, thus making these porphycenes excellent candidates for hypoxic anti-tumor photodynamic therapy.

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