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Photodynamic therapy with zinc-tetra(*p*-sulfophenyl)porphyrin bound to cyclodextrin induces single strand breaks of cellular DNA in G361 melanoma cells

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Abstract

The basis of photodynamic therapy (PDT) is the phototoxicity resulting from co-action of light, sensitizer and oxygen. In this study we demonstrate in vitro phototoxicity measurement on G361 cell lines using $ZnTPPS_4$ sensitizer bound to cyclodextrin hpβCD. We have proved its photodamage effect on cancer cell lines in the visible region of spectrum. We used the halogen lamp (24 V/250 W) as a source of radiation. After 24 h incubation of cell cultures with 10 μM $ZnTPPS_4$ and 1 mM cyclodextrine hpβCD, the cells were irradiated for 7.5 min at the total irradiation dose of 12.5 $J\,cm^{-2}$. Analysis of DNA damage in the cell line after PDT was proved by comet assay and using inversion fluorescent microscope with image analysis. This treatment method gave rise to DNA damage. The used radiation dose of visible light in the absence of sensitizers does not induce DNA breaks in tumour cells. In conclusion, binding of $ZnTPPS_4$ sensitizer to cyclodextrin hpβCD may improve the efficacy of PDT for the treatment of malign melanoma.

Keywords: Sensitizers; Light; Phototoxicity; Comet assay

1. Introduction

Photodynamic therapy of cancer (PDT) uses the interaction of sensitizer and light to destroy cancerous cells and tumors (Kessel, 2004; Allison et al., 2004). The photochemical interaction in the presence of molecular oxygen produces cytotoxic singlet oxygen ($^1O_2$) and other forms of active oxygen, such as hydroxyl radical etc. The tumor is destroyed either by $^1O_2$ (generated via energy transfer from excited sensitizer to triplet oxygen, type II mechanism) or radical products (generated via electron transfer from excited sensitizer, type I mechanism). The selectivity of tumor damage depends on specific retention of a sensitizer in the tumor tissue after systemic administration, combined with directed illumination (Moor, 2000). The cellular effects of PDT include plasma membrane, lysosomes and mitochondria damage leading to tumor ablation (Dahle et al., 1999). The tumor destruction happens via both types of cell death apoptosis or necrosis (Dougherty, 1993; Kessel and

Abbreviations: PDT, photodynamic therapy; $ZnTPPS_4$, zinc complex of *meso*-tetrakis(4-sulphonatophenyl)porphyrin; hpβCD, 2-hydroxypropyl-β-cyclodextrin; CD, cyclodextrin.

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Luo, 1997; Oleinick and Evans, 1998). Sooner or later, as a result of the processes is observed DNA damage (Green et al., 1996). A potential benefit of PDT is that it causes minimal damage to healthy tissue, because of elimination of sensitizers from healthy cells in the time of irradiation (Sibata et al., 2001). Hereto, it was synthesized a great number of sensitizers for PDT. In general they are sorted into two generations. The first-generation represents hematoporphyrin and its derivatives. The second-generation sensitizers show better photophysical and pharmacological properties. To the group belong porphyrins, phthalocyanines, texaphyrins, chlorins or bacteriochlorins. It is difficult to decide which ones are suitable for which pathological conditions (Nyman and Hynninen, 2004). In this work we studied cellular uptake and the phototoxicity of metallocomplex *meso*-tetrakis(4-sulfonatophenyl)porphyrin $ZnTPPS_4$, which improve phototoxicity (Mosinger et al., 2000) in the presence or absence of 2-hydroxypropyl-cyclodextrins (hpCDs) as nontoxic carriers, using G361 human melanoma cells. These substances create complexes with good penetration through the cell membrane. After the uptake of the sensitizer the cells are excited by appropriate light. Finally, we investigate the efficiency of photodynamic therapy by means of comet assay.

2. Materials and methods

2.1. Uptake

The G361 cells (ATTC, USA) were seeded in the amount of 10^4 to each well (Dynatech plates 8×12, flat bottom) and precultivated in DMEM with 10% FCS. The sensitizer was added into the wells in concentrations of 0; 0.1; 0.3; 1; 3; 10; 30 and 100 μM in the absence or presence of hpCDs in a hundred-fold concentration excess compared to the sensitizer. The cells were incubated in a CO_2 incubator (37 °C, 5% CO_2). After 1; 3; 6; 10; 16; 24 and 48 h of incubation, the medium above the adhering cells was removed. Each emptied well was 2× washed with 120 μl of DMEM. After washing 100 μl of DMEM was added into each well and fluorescence ($ZnTPPS_4$ excitation at 413 nm, emission at 606 nm) in G361 cells was measured by Perkin–Elmer LS50B fluorimeter equipped with well plate reader accessory (Perkin–Elmer Corp., Norwalk, CT). The whole plate was read once with a read time of 0.2 s for each well. We found these settings optimal, increasing the read time per well and/or adjusting slit widths did not improve the signal to background ratio. Subsequently, from each of the wells 10 μl of medium was withdrawn and replaced by 20% solution of SDS The plates were mildly shaken and incubated for 5 min; then their fluorescence was measured again.

2.2. Comet assay

2×10^6 cells of human melanoma (cell line G 361) were cultivated in DMEM with 10 μM $ZnTPPS_4$ and 1 mM hpβCD on Petri dish for 48 h at 37 °C. Then the growth medium was replaced by medium without the photosensitizer and cells were irradiated by visible light at room temperature. The cells were subsequently irradiated by a halogen lamp (24 V/250 W) at a dose of 12.5 $J\,cm^{-2}$. The halogen lamp has continuous irradiance spectrum (from 360 to 2700 nm) with maximum in visible and infrared region. Irradiance was measured by Radiometer RK 2500 (Meopta Prerov, Czech Republic). After the irradiation cells were cultivated for 24 h at 37 °C again and after this period their damage was assessed by means of the comet essay and fluorescent microscope, CCD camera and Olympus Micro Image software. 85 μl of 1% standard agarose in PBS was applied on the microscope slides precoated with 1% standard agarose in H_2O and, while still liquid, covered with a cover slip. Slides were placed in a refrigerator for at least 5 min to let the agarose solidify. Cells were trypsinated, collected by centrifugation and dispersed in 2ml PBS by vortexing. 20 μl of this solution (2.10^4 cells) was added to 85 μl of 1% LMP agarose in PBS at 37 °C. Finally, 85 μl of the mixture was transferred on each slide and placed in the refrigerator for another 5 min. Microscope slides were immersed in lysis buffer (2.5 M NaCl, 100 mM EDTA, 10 mM Tris and 1% Triton X-100; pH = 10) at 4 °C for 1 h. Slides were gently placed on platform in electrophoretic tank and immersed in cool electrophoresis solution (300 mm NaOH, 1 mM EDTA) for 40 min. Electrophoresis was run 30 mins at 2.5 $V\,cm^{-1}$. After electrophoresis the slips were washed 3×5 min with buffer (0.4 M Tris; pH = 7.5) at 4 °C and stained by ethidium bromide (20 μg/ml) for the visualization of DNA comet.

3. Results

Uptake of a sensitizer into tumor cells may also highly depend on the metabolic state of individual cells. The highest uptake was found for sensitizer $ZnTPPS_4$ in combination with hpβCD after 48 h of incubation. Fig. 1 shows time dependent accumulation of $ZnTPPS_4$ sensitizer bound to cyclodextrin carrier hpβCD. The concentration of sensitizers is 3 μM.

Comets representing different levels of DNA damage were analyzed quantitatively by standard method (Piperakis et al., 1999) and image analysis software. According to standard method the human eye readily discriminates comets. It was developed a scheme for visual scoring based on 5 recognizable classes of comets, from class 0 (undamaged, no discernible tail) to class 4 (almost all DNA in tail, insignificant head). For the final