Photodynamic therapy (PDT) is a treatment for neoplastic disease that involves the selective destruction of tumors using light-activated sensitizer compounds that preferentially accumulate in target tissue areas (1,4,8). The photochemical interactions of the sensitizer, light, and molecular oxygen produce cytotoxic singlet oxygen and other forms of active oxygen, such as peroxide, hydroxyl radical and superoxide ion resulting in damage of organelles within malignant cells and leads to tumor ablation (2,9). The major sites of PDT damage are membranous organelles, such as mitochondria, lysosomes and plasma membrane. The efficiency of sensitizers in situ is most likely to be dependent on their local accumulation and specific cellular uptake in the tumor site, stimulating research toward the development of water soluble and efficient in vivo sensitizer – delivery system with a high potential to target specific organs. In the present study we used meso-tetrakis(4-sulphonatophenyl)porphine (TPPS4) and paladium complex of TPPS4 (PdTPPS4) as model sensitizers (6,7). We report here the uptake of a sensitizer into G361 human melanoma cells in the presence or absence of 2-hydroxypropyl-cyclodextrins (hpCDs).

**Material and methods**

**Cellular uptake:** The G361 cells (ATCC, USA) were divided in the amount of $10^4$ to each well (Dynatech plates 8 x 12, flat bottom) and filled in DMEM with 10% FCS. The sensitizer was added into the holes in concentrations of 0; 0.1; 0.3; 1; 3; 10; 30 and 100 mM in the absence or presence of hpCDs in a 100-fold concentration excess compared to the sensitizer. The cells were incubated in a thermobox (37°C, 5 %CO₂). After 1; 3; 6; 10; 16; 24 and 48 hours of incubation, the medium above the adhering cells was removed. Each emptied hole was 2x washed with 120 µl of DMEM. After washing 100 µl of DMEM was added into each hole and self-fluorescence (TPPS4 excitation at 415nm, emission at 645nm, PdTPPS4 excitation at 423nm, emission at 645nm) in G361 cells were measured by Perkin-Elmer LS50B luminometer equipped with well plate reader accessory. Morphological changes in cells have been evaluated using inversion fluorescent microscope Olympus IX 70 and image analysis. The uptake of the sensitizer PdTPPS4 at the given time interval from 1 to 48 hours is markedly higher than the uptake of TPPS4. The highest uptake was found for sensitizer PdTPPS4 in combination with hpβCD. TPPS4 and PdTPPS4 especially in the supramolecular complex with nontoxic cyclodextrin carriers represent efficient sensitizers for photodynamic therapy in vitro on G361 cells.

**Key words:** Sensitizers; Uptake; G361 human melanoma cells
the same one of 20% SDS. The holes were mildly shaken and incubated for 5 minutes; then their fluorescence was measured again.

**Self-fluorescence of sensitizers in G361 cells:** Twice washed trypsinated G361 cells were divided in the amount of 10⁴ to each well and filled in DMEM with 10% FCS in a total volume of 80 µL. After 24 hours of cultivation at 37°C in 5% CO₂ the 20 µL of sensitizer was added. Cells were cultivated with sensitizers at concentrations ranging from 0.1 to 125 µg/ml. The total volume of 100 µl (cells with additives) were cultivated for 24 hours. Cell uptake and morphological changes in cells have been evaluated using inversion fluorescent microscope Olympus IX 70 and image analysis.

**Results**

Fig. 1 shows that the uptake of the sensitizer PdTPPS₄ at the given time interval is markedly higher than the uptake of TPPS₄. The presence of the hpCD carrier did not affect the accumulation of TPPS₄, but significantly affect uptake of PdTPPS₄. The highest uptake was found for sensitizer PdTPPS₄ in combination with hpβCD.

The presence of the hpCD significantly increases the level of an accumulation of PdTPPS₄ in cells after a long-time period of incubation and gives no saturation character even after 48 hours of incubation. This is in contrary of free PdTPPS₄ that reaches saturation after 24 hours (Fig. 2).

Self fluorescence of sensitizers in cells was evaluated by inversion fluorescent microscope Olympus IX 70 and image analysis. The major sites of cell uptake are plasma membrane, mitochondria and lysosomes (Fig. 3).

**Discussion**

Efficiency of PDT is affected by various factors including photophysical properties of a sensitizer, wavelength of the activation light, depth of the light penetration in the biological tissue, tissue response on singlet oxygen, etc. (3,5,10). The wavelength of used light that activates the sensitizer dictates the proper absorption spectrum of a sensitizer as well as the depth of the treatment effect. Uptake of a sensitizer into tumor cells may vary depending on the metabolic state of individual cells. The measurement of the uptake of sensitizers into the G361 cells shows the difference between the free sensitizers and bound to hpCD carriers. The kinetic of PdTPPS₄ uptake is higher than for TPPS₄. While the presence of hpCDs does not notably affect the uptake of TPPS₄, in the case of PdTPPS₄ cyclodextrin carriers hpβCD cause a significant magnification in accumulation of the sensitizer. The most effect on the level of distribution of the sensitizers in G361 cells was found for PdTPPS₄ in combination with hpβCD. G361 cells are sensitive to photodynamic damage by sensitizers in the absence or presence of hpCDs (3). In conclusion, PdTPPS₄ and TPPS₄ especially in the supramolecular complexes with hpCDs carriers represent efficient sensitizers.

![Fig. 1](image1.png)

**Fig. 1:** The uptake of TPPS₄ (3 µM) and PdTPPS₄ (3 µM) sensitizers in the absence or presence of 100 fold molar excess of hpCDs into G361 cells (10⁴) after 24 hours of incubation in DMEM with 10% FCS.

![Fig. 2](image2.png)

**Fig. 2:** Time dependent uptake of TPPS₄ and PdTPPS₄ sensitizers (3 µM sensitizer, 10⁴G361 cells in DMEM with 10% FCS) bound to hpβCD.

![Fig. 3](image3.png)

**Fig. 3:** Self fluorescence of PdTPPS₄ sensitizer (12 µg/ml) bound to hpβCD after 24 hours of cultivation in DMEM with 10% FCS in G361 cells. Excitation wavelength - 420 nm. (3a: transmitted light, 3b: accumulation of sensitizer on plasma membrane and mitochondria, 3c: accumulation of sensitizer on lysosomes.)

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