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Influence of dendrimers in the subcellular localization of protoporphyrin IX in carcinoma cells for Photodynamic Therapy

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Introduction: The greater efficacy of photodynamic therapy (PDT) depends on the selective accumulation of the photosensitizer in the cells mitochondria. Protoporphyrin IX (PpIX) is an efficient photosensitizer when administered in the form of its non-photosensitizer precursor, the 5-aminolevulinic acid. However, when administered directly, PpIX high hidrophobicity and diffuse distribution into the cells impaired PDT efficacy. Target PpIX to the cells mitochondria could improve PDT efficiency with this drug. This target delivery can be achieved with the use of drug delivery systems, such as the dendrimers. Polyamidoamine generation – 4 hydroxyl terminated dendrimer (PAMAM G4-OH) are hyberbranched polymers capable to form complexes with PpIX, increasing PpIX aqueous solubility (Patente BR 10 2012 002494 2 (2012)) and likely changing its inner cell distribution. **Objective:** The aim of this work was to study the influence of PpIX-PAMAM G4-OH complexes in PpIX carcinoma subcellular localization for further PpIX-PDT. **Methods:** PpIX-PAMAM complexes were prepared by dispersing an excess of PpIX in dimethylformamide (DMF) with 0.2% of PAMAM G4-OH. This mixture was stirred overnight before the organic solvent was evaporated at ambient temperature. The residue formed was dispersed in water, centrifuged, filtrated and the solution recovered was dried by freeze-drying. The amount of PpIX in the PpIX-PAMAM complex formed was determined by spectrofluorimetry at 400/635 nm (excitation/emission). Subcellular localization of PpIX administrated from dendrimer complexes was studied using squamous cells carcinoma (ATCC A431) and confocal laser scanning microscope (LSCM). The A431 cells were cultured ($1x10^5$) in lamina with 8 wells. After 24 h each well was incubated with PpIX free or complexed with PAMAM G4-OH at 20 ng/mL of PpIX along 4 h. For the last 45 min of incubation the cells were co-stained with 200 nM of Mito Tracker Green FM (MTG), a mitochondria marker, following the manufacturer's instructions and analyzed by LSCM. For PpIX observation, the cells were excited at 405 nm and PpIX fluorescence was monitored at 600 -700 nm; for MTG, excitation was at 488 nm and emission monitored at 515-555 nm. **Results**: The molar ratio of PpIX/PAMAM complexes was around 6:1. LSCM observation of the cells suggested that PpIX was able to penetrate through carcinoma cells membrane either when it was free or complexed with PAMAM. Nonetheless, administration of PpIX from the simple solution (free) showed a weak and diffuse fluorescence along all the cytoplasm. On the other hand, PpIX-PAMAM fluorescence seemed to be more intense and clearly localized in some parts of the inner cell. It is not possible yet to affirm that PpIX-PAMAM was localized in the mitochondria because in the condition that the experiment was performed, co-visualization of PpIX and the mitochondria probe, MTG, was not possible. Adjustments in MTG concentration will be done for further experiments. **Conclusion**: PpIX-PAMAM G4-OH complexes certainly changed PpIX distribution into carcinoma cells. The effect of this change in PpIX-PDT has still to be studied.

Keywords: protoporphyrin IX, PDT, dendrimer, subcellular localization, carcinoma cells.

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