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Differences in sensitivity to mTHPC-mediated photodynamic therapy of neurons, glial cells and MCF7 cells in a 3-dimensional cell culture model

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The effect of photodynamic therapy (PDT) on the cells of the nervous system is an important consideration in the treatment of tumours that are located within or adjacent to the brain, spinal cord and peripheral nerves. Previous studies have reported the sparing of nerves during PDT using meta-tetrahydroxyphenylchlorin (mTHPC, Foscan®) in patients and in animal models. The aim of this study was to investigate the effects of mTHPC on key nervous system cells using a 3-dimensional cell culture system for the accurate detection of differences in sensitivity.

Cultures of primary sensory neurons, satellite glia and astrocytes were obtained from rat tissue and the human adenocarcinoma cell line MCF7 was used as a comparator.

Uptake and localisation of mTHPC was studied using fluorescence microscopy which confirmed that the drug was incorporated into all of the cell-types studied. The sensitivity of the cells to PDT was determined by culturing them in a type-I collagen gel then exposing them to various concentrations of mTHPC (4 h) and light (10 min) from a transilluminator (0.5 mW/cm² at 633 nm). Cell death was detected using propidium iodide, nuclei were detected using Hoechst 33258, cell types were distinguished using immunocytochemistry. The cell types showed different sensitivities to mTHPC, with MCF7s, astrocytes and satellite glia being significantly more sensitive than neurons. This effect was particularly marked at 4 µg/ml mTHPC which caused no significant neuron death compared to untreated controls but was sufficient to elicit substantial levels of cell death in the other cell types. This apparent survival of neurons was studied further by exploring the effect of PDT using 4 µg/ml mTHPC on neurite growth. Initially this treatment was sufficient to reduce neurite length significantly compared to untreated controls. However, following treatment the neurons were able to extend neurites once again, confirming the viability of the surviving neurons.

In conclusion, this study demonstrates that neurons in culture can survive mTHPC-mediated PDT conditions that are sufficient to kill tumour cells and other nervous system cell types. Further work will be required to see whether this phenomenon occurs *in vivo* and whether it may help to explain the nerve sparing observed in other studies. Understanding the effects of PDT on the cells of the nervous system is a critical step towards the refinement and optimisation of PDT for treatment of tumours whose location makes nervous system damage a potential side effect.