ULTRASOUND-MEDIATED ACTIVITY BY VARIOUS METAL-PORPHYRIN COMPLEXES IN HUMAN COLON CANCER AND FIBROBLAST CELLS

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Introduction: Porphyrins are well-known photosensitisers as their tetrapyrrole ring structure can be activated by light to generate reactive oxygen species (ROS) which cause damage to cell structures and cell death. In recent years, increasing evidence suggests their ability to also behave as sonosensitisers in sonodynamic treatment (SDT) of cancer. SDT is an innovative approach developed from photodynamic therapy in which a physical stimulus, e.g. ultrasound (US) instead of light, is used to trigger the cytotoxic nature of sonosensitisers, which are ineffective on their own. Porphyrin's ability to generate ROS following electronic excitation renders porphyrins an excellent platform to explore the sonodynamic process. Moreover, some studies suggest that the synergistic cytotoxic effect of porphyrins and US might be selective between cancer and non-cancerous cells.

Materials and methods: The effects induced by US exposure of different metal complexes of 5,10,15,20-tetrakis (N-methylpyridinium-4-yl) porphyrin tetrachloride (TMPyP), i.e. TMPyP-Zn(II) (P-Zn) and TMPyP-Pd(II) (P-Pd) were investigated on cancer and non-cancerous cells, in order to evaluate possible differences in responsiveness to the treatment. Briefly, sonodynamic treatment was performed with low intensity US generated at 1.8MHz on human colon cancer (HT-29) and human primary dermal fibroblast (HDF 106-05) cells. Effects of light exposure of the same porphyrin concentrations were evaluated on both cell lines, as a reference. To highlight possible differences between HT-29and HDF 106-05cells, basal content of glutathione (GSH) was determined by a colorimetric assay and porphyrin cellular uptake by fluorescence measurements. Effects of the sonodynamic treatment with P-Zn or P-Pd were then evaluated on cell growth, ROS production and mitochondrial membrane potential. Due to US interaction with cell membrane for the energy transfer to intracellular sonosensitisers, differences on cell membrane response were also investigated during US exposure, evaluating the effects of US on cell membrane poration and calcium flux by flow cytometry assays, and cell membrane fluidity assessed using a polarized fluorescence spectrometer.

Results: HT-29and HDF 106-05cells showed similar intracellular GSH levels suggesting a similar role of GSH system in defence against ROS. The maximum P-Zn and P-Pd uptake was reached on both cell lines after 24hours. Effects of sonodynamic treatment with US and P-Zn or P-Pd used at non-cytotoxic concentration per se, resulted in significant ROS production and cytotoxicity only on HT-29cells. The results also showed a porphyrin dose-dependent sonodynamic effect, ROS-mediated cytotoxicity and mitochondrial function impairment only on HT-29cells. Conversely, P-Zn or P-Pd under light exposure showed similar cytotoxicity on both cell lines. It is worth mentioning that US was not able to induce membrane poration on both cell lines. However, HT-29and HDF 106-05cells showed different behaviour during US exposure in terms of membrane fluidity and calcium flux. Specifically, HT-29cells showed a significant increase in membrane fluidity along with an increase of calcium flux, whereas HDF 106-05cells showed a significant decrease in cell membrane fluidity and an increase of calcium flux followed by a rapid decrease.

Discussion and conclusion: The efficacy of sonodynamic treatment with US and metal-porphyrin complexes in killing cancer cells is dependent on ROS generation triggered by US-mediated sensitiser activation. The US-mediated sensitiser activation seems to be selective according to cell type, being effective against cancer cells but not to non-cancerous cells, despite what has been observed for photodynamic treatment. Experimental evidence suggests that outcome and selectivity of the sonodynamic treatment, supported by intramembrane cavitation hypothesis of sonosensitiser activation, could be strongly regulated by different ROS production and cell membrane properties in response to US exposure.