Porphyrin loaded nanoparticles enhance anticancer efficacy of sonodynamic therapy

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Sonodynamic therapy (SDT) is an innovative anticancer approach where ultrasounds are used to trigger the cytotoxicity of particular chemical compounds, known as sonosensitizers, which are per se harmless and ineffective alone. SDT takes advantage of the non-thermal effect of the acoustic cavitation generated by selected pulsed or continuous ultrasounds. The activated sonosensitizer then generates reactive oxygen species (ROS) leading to cancer cell death. Due to its non-invasiveness, lack of systemic toxicity, the possibility of it being focused on target tissues and its ability to penetrate deeply within the body, SDT has the potential to enhance cancer treatment, particularly for those solid tumours where surgical removal is difficult even in the early stages. As the physical-chemical structure of the sonosensitizer is essential for the success of SDT, the different aspects related to nanotechnology in medicine might well be able to enhance the triggering effect ultrasounds have on sonosensitizing agents. This prompted us to investigate into the potential poly-methyl methacrylate core-shell nanoparticles (NPs) loaded with meso-tetrakis (4-sulfonatophenyl) porphyrin (TPPS) have to function as an innovative sonosensitizing system i.e. TPPS-NPs.
The anticancer efficacy of sonodynamic treatment with shock waves (SW), generated by a piezoelectric device and TPPS, as a free or loaded nanoparticle formulation, was investigated on an in vitro and in vivo syngeneic cancer model. Moreover, SW treatment, TPPS and NPs alone, or in combination was carried out as a control.
The *in vitro* effects of sonodynamic treatment were evaluated on cancer cell growth (monolayer cell culture and multicellular tumour spheroids) and detailed investigation was also carried out on the mechanism of the sonodynamically-induced cytotoxicity, studying the cell death mechanism by imaging, flow cytometry analyses, mRNA and protein expression profiles.
The *in vivo* tumour volume changes after the sonodynamic treatment were assessed by magnetic resonance imaging (MRI) and histopathologic analyses.
The *in vitro* porphyrin incorporation into living cells was significantly higher with TPPS-NPs than with free porphyrin. A significant decrease in cancer cell growth was observed in two ($p<0.001$) and three dimensional ($p<0.001$) cell culture after 72 hours from the sonodynamic treatment with SW at an energy flux density of 0.43 mJ/mm^2 for 500 impulses (4 impulses/sec) and TPPS-NPs (100 μg/ml), compared to control. The sonodynamic treatment with SW and TPPS-NPs determined a significant increase ($p<0.01$) in the cell generation of ROS compared to cells exposed to the sonodynamic treatment with SW and free TPPS. The uncontrolled increase in the generation of ROS after the sonodynamic treatment with SW and TPPS-NPs led to cell damage i.e. the sonodynamic treatment was able to significantly increase apoptotic cell death.
There was a statistically significant reduction in the volume of the tumor mass i.e. up to 50% for sonodynamic treatment with SW and porphyrin loaded NPs, in a Mat B-III rat syngeneic breast cancer model when pre- and post-treatment volumes were compared to sonodynamic treatment with SW and free porphyrin, at 72 hours. In conclusion, the porphyrin properties were significantly enhanced once loaded onto NPs, due to an enhancement of the *in vitro* and *in vivo* anticancer efficacy of the sonodynamic treatment.