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TITLE
Magnetic nanoparticle thermal treatment potentiates PTX activity in breast cancer cells

HYPOTHESIS:
Nanoscale hyperthermia using superparamagnetic iron oxide nanoparticles (SPION) potentiate PTX and triggers mitotic exit of PTX-pretreated cells, reversing taxol resistance in breast cancer.

BACKGROUND/AIMS:
Taxanes such as paclitaxel (PTX), are among the most effective agents in breast cancer treatment, however intrinsic and acquired resistance to taxanes represent the most limiting factors for their use. Overcoming resistance to these drugs would represent a major advance in breast cancer treatment. PTX induces mitotic block; sensitive cells exit mitosis and die by mitotic catastrophe. Resistant cells remain in block and continue proliferation after drug decay, denoting one of the PTX resistance mechanisms. It has been demonstrated that mild hyperthermia (HT) triggers mitotic exit of PTX-pretreated cells, reversing PTX resistance in breast cancer cells. This suggests HT-forced mitotic exit as a promising strategy to elevate efficacy of PTX.

METHODS:
MCF-7 parental breast adenocarcinoma cells and a PTX-resistant subline were seeded in an 8-well strip plate. Seven conditions were established for the experiments: control, PTX, SPION at 37 °C, external HT, SPION HT, PTX→external HT, and PTX→SPION HT. PTX solution in media was added at a final concentration of 1, 2.5 and 25 nM and incubated for a total of 18 hours. After 16 hours of incubation with PTX, SPIONs were added to the cells at a concentration of 1.5 mgFe/mL in media. The cells were exposed to a magnetic field to apply HT at 42 °C for 2 hours using the induction heater. For external HT, the cells were exposed for 2 hours at 42 °C in a humidified incubator. The cells were washed and incubated at 37 °C four days post treatment. Cell survival was analyzed by image analysis using Hoechst 33342 to stain the nucleus. Fluorescent images were taken and the total number of cells were counted using ImageJ. The number of cells per well was normalized relative to control cell. Additionally, cells were classified based on nuclear morphology (interphase, mitotic, micronuclei, apoptotic) in selected conditions.

RESULTS & CONCLUSIONS
The efficacy of treatments was evaluated at increasing concentration of PTX, resulting in cell survival of < 60% for WT and negligible effect on TR, while the PTX+SPION combined treatment resulted in < 10% and < 35% of cell survival in WT and TR cells, respectively. Our results indicate that SPION HT is more effective than external HT at potentiating response to PTX in sensitive and resistant cell lines. More importantly, combination of SPION HT with PTX in the resistant cell line led to significant reduction in cell survival at a PTX concentration that otherwise had no effect. The combined treatment resulted in an increase in the percentage of micronucleated cells in both cell types, an indication of forced mitotic exit. In addition, we observed an increase in apoptotic cells for the resistant cell subline. SPION HT by itself did not cause an increase in micronuclei formation, but it did increase the percentage of apoptotic cells for the PTX resistant cell line. These results suggest that SPION HT potentiates the PTX effect by significantly reducing cell survival, and, in contrast to the external HT, triggers programmed cell death.