HIGH ENERGY SHOCK WAVES INCREASE PACLITAXEL EFFICACY IN A SYNGENIC MODEL OF BREAST CANCER

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The combined effect of High Energy Shock Wave (HESW), generated by a piezoelectric device, and paclitaxel was examined on Mat B-III rat breast cancer cells *in vitro* and *in vivo* animal model.
In vitro cytotoxicity were investigated by cell growth curves. Mat-B-III cells were exposed to paclitaxel (0.1-100 nM) and HESW treatment (0.22 mJ/mm^2, 1000 shots), and viable cells growth was determined by ELISA assay at 48 and 72 h after treatment.
In vitro cell death were investigated by flow cytometry analysis. Mat B-III cells (1x10^6 cells/ml) were exposed to paclitaxel (1 and 10 nM) and HESW (0.22 mJ/mm^2, 1000 shots), and stained with annexin-V-fluorescin (A-V-FITC)/propidium iodide (PI) at 24 h after treatment. Viable cells were defined to be A-V-FITC and PI negative.
For *in vivo* animal study, inbred female Fisher 344 rats were handled according to European guidelines (Directive CEE 86/609) and the experimental protocol was reviewed and approved by the Local Animal Committee.
Control animals were treated with one single i.v. injection into the tail vein of physiological solution at day 7 and 11, and experimental animals with a single i.v. injection of paclitaxel (2.5 mg/kg) at day 7 and 11, HESW (0.50 mJ/mm^2, 500 shots) at day 11 alone or in combination with paclitaxel at day 7 and 11.
Control and experimental animals were sacrificed at the end of the study (at day 12) and primary tumour tissues were removed for histological and gene expression studies.
For apoptosis detection, terminal deoxynucleotidyl transferase (TdT) mediated deoxyuridine triphosphate (dUTP) nick end labelling (TUNEL) in situ cell death detection assay was carried out and pro-apoptotic genes *Bad* and *Casp3* mRNA expression was evaluated by quantitative SYBR Green real time RT-PCR.
In *vitro* results show that combined exposure to paclitaxel (0.1-100 nM) and HESW (0.50 mJ/mm^2, 500 shots) resulted in a significant reduction of Mat B-III cell proliferation at 48 and 72 h after treatment in respect with cells treated with paclitaxel alone. Moreover, an earlier induction as well as an enhancement of apoptosis was observed in cells subjected to combined treatment with HESW and paclitaxel (1 and 10 nM).
In vivo findings the apoptotic index, as detected by the TUNEL method, and *Bad* and *Casp3* mRNA expression confirm a significant enhancement of apoptosis in tumour tissues subject to the combined treatment with HESW and paclitaxel.
In conclusion, HESW can enhance paclitaxel cytotoxicity in Mat B-III rat breast cancer cells through apoptotic pathway *in vitro* and *in vivo* animal model.