

# 57. High Energy Shock Waves for Sonodynamic Therapy: Efficacy in a Syngeneic Model of Colon Cancer

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## Device and producing company:

Piezoson 100, Richard Wolf, Knittlingen, Germany

## Introduction:

The cytotoxic effect of the natural porphyrin precursor 5-aminolevulinic acid (ALA) exposed to high energy shock waves (HESW), generated by a piezoelectric device, was investigated in DHD/K12/TRb rat colon cancer cells in vitro and in vivo.

**Methods:** In vitro, cells were exposed to ALA (50 g/ml) for 24 h and then to HESW E1 (EFD = 0.22 mJ/mm<sup>2</sup>, 1000 shots) or E2 (EFD = 0.88 mJ/mm<sup>2</sup>, 500 shots). Viable cell growth was determined by trypan blue assay at day 1, 3 and 7 whereas cell death were investigated by flow cytometry with annexin-V-fluorescein and propidium iodide staining at day 1. In vivo, eight weeks after cell implantation in the caecum, syngeneic adult male BD-IX were treated with one i.v. injection of saline/ALA (375 mg/kg) into the tail vein 3 h before HESW E2 treatment alone or in combination with ALA. After 24 h in primary tumour tissues TUNEL in situ cell death detection assay was carried out and pro-apoptotic genes Bad and Bcl-2 mRNA expression was evaluated by quantitative SYBR Green real time RT-PCR. Moreover, as marker of activation of caspases during apoptosis, the cleavage of poly-ADP ribose polymerase (PARP) was investigated by western blot assay.

## Results:

ALA exposed to HESW resulted in a significant reduction of in vitro cancer cell proliferation at day 3 with respect to cells exposed to ALA (p.1 and 6.4 fold increase vs. ALA) or E2 (3.4 and 5.3 fold increase vs. ALA). In vivo, we observed Bad enhanced mRNA expression, Bcl-2 decreased mRNA expression (p<0.05) and enhanced apoptotic index (2.0 fold increase) in respect with ALA exposure. Moreover, after combined treatment with ALA and HESW, PARP cleavage was observed.

## Discussion:

In vitro findings show how the two schedules of HESW treatment investigated perform the same cytotoxic effects. In vivo, the E2 schedule treatment shows a significant enhancement of apoptosis in tumour tissues subject to the combined treatment with ALA. Therefore, the piezoelectric device is able in vivo to activate protoporphyrin IX after exogenous administration of ALA, with specific target of tumor cells.

## Conclusion:

ALA and HESW determine cytotoxic effects in rat colon cancer cells either in vitro or in vivo.