In vitro study of sonodynamic and photodynamic treatment on human cancer cell lines

F. Foglietta[1], R. Canaparo[1], A. Francovich [2], P. Civera [2], C. Ferretti [1], G.P. Zara [1], L. Serpe [1]

[1]Dipartimento di Scienza e Tecnologia del Farmaco, University di Torino,
[2]Dipartimento di Elettronica, Politecnico di Torino, Torino, Italy

Photodynamic therapy (PDT) is an anticancer treatment that uses light to activate cytotoxic compounds killing cancer cells. Sonodynamic therapy (SDT) is a new anticancer treatment where ultrasound is used to trigger the cytotoxic effect of chemical compounds known as sonosensitizers. SDT is able to focus the ultrasound energy, generated by selected continuous or pulsed ultrasound such as shock waves (SW), onto malignant sites situated deeply inside tissues overcoming the main drawback linked to the use of PDT: the poor penetration of light in biological tissues. Even if the SDT mechanism is still under debate, some researchers suggest a common basic principle for PDT and SDT. Therefore, *in vitro* comparison of PDT and SDT effects can provide insight into SDT mechanism of action. We have investigated PDT and SDT effects on human melanoma and fibrosarcoma cell lines, previously exposed to 5 aminolevulinic acid (Ala), through cell proliferation, cell death and gene expression analysis.

The human melanoma, A2058, and fibrosarcoma, HT-1080, cell lines were previously exposed to Ala (0.45 mM) for 12 and 4 h, respectively. SW generated by a piezoelectric device (Piezoson 100, Wolf) were used for SDT. In particular, A2058 were treated with an energy flux density (EFD) of 0,32 mJ/mm^2 for 1000 shots (4 shots/sec) while HT-1080 were treated with an EFD of 0,43 mJ/mm^2 for 500 shots (4 shots/sec). A LED lamp at 405 nm was used for PDT and both cell lines were treated for 5 min at 15 mW. Cell growth was evaluated by WST-1 assay, cell death by flow cytometric analysis with SYTOX Green and APC-Annexin V and mRNA expression by real time RT-PCR.

In A2058 both treatments determined a significant cell growth reduction even if SDT produced a progressive cell growth decrease compared to PDT reaching the greatest decrease at 72 h ($p<0.01$). Moreover, cell death evaluation highlighted a 25 % increase of apoptotic cells at 48 h from SDT. Both PDT and SDT determined a significant overexpression of the pro-apoptotic gene *BAX* and of the genes involved in the oxidative stress, *NQO1* and *SOD2*. In HT-1080 SDT was more effective than PDT with a more significant increase of apoptotic cells compared to PDT ($p<0.01$). After both treatments a significant over-expression of the pro-apoptotic gene *APAF1* was observed.

After PDT and SDT a similar gene expression profile was observed in both cell lines, even though SDT seems more effective on fibrosarcoma cells and PDT on melanoma cells.