Ultrasonic Enhancement of Gene Transfection in Murine Melanoma Tumors

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(*Received* 2 *March* 1999; *in final form* 13 *July* 1999)

The enhancement of gene transfection by ultrasound (US) was evaluated *in vitro* using the B16 mouse melanoma model. Cultured cells were either exposed in suspensions *in vitro* or implanted subcutaneously in female C57BL/6 mice for 10-14 days and, subsequently exposed, *in vivo*. For comparison to results with a luciferase plasmid, a reporter plasmid for green fluorescent protein (GFP) was used to evaluate transfection efficiency. US was supplied by a system, similar to a Dornier HM-3 lithotripter, that produced shock waves (SW) of 24.4 MPa peak positive and 5.2 MPa peak negative pressure amplitudes at the focus. The plasmids were mixed with the suspension to achieve 20 uL mL^{-1}, or were injected intratumorally to provide 0.2 mg DNA per mL of tumor. Acoustic cavitation was promoted by retaining 0.2 mL of air in the 1.2-mL exposure chambers *in vitro* and by injecting air at 10 % of tumor volume *in vitro*. In vitro, cell counts declined to 5.3% of shams after 800 SW exposure, with 1.4% of the cells expressing GFP after 2 days of culture. *In vivo,* 2 days after 400 SW exposure, viable-cell recovery form excised tumors was reduced to 4.2% of shams and cell transfection was enhanced by a factor of about 8, reaching 2.5% of cell counts ($p<0.005$ in *t*-test). These results show that strong tumor ablation induced by US shock wave treatment can be coupled with simultaneous enhancement of gene transfection.

USTUR-0142-99