Cationic versus neutral microbubbles for ultrasound-mediated gene delivery in cancer

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PURPOSE
To test whether plasmid-binding cationic microbubbles (MBs) enhance ultrasound-mediated gene delivery efficiency relative to control neutral MBs in cell culture and in vivo tumors in mice.

MATERIALS AND METHODS
Animal studies were approved by the institutional animal care committee. Cationic and neutral MBs were characterized in terms of size, charge, circulation time, and DNA binding. Click beetle luciferase (CBLuc) reporter plasmids were mixed with cationic or neutral MBs. The ability of cationic MBs to protect bound plasmids from nuclease degradation was tested by means of a deoxyribonuclease (DNase) protection assay. Relative efficiencies of ultrasound-mediated transfection (ultrasound parameters: 1 MHz, 1 W/cm(2), 20% duty cycle, 1 minute) of CBLuc to endothelial cells by using cationic, neutral, or no MBs were compared in cell culture. Ultrasound-mediated gene delivery to mouse hind limb tumors was performed in vivo (n = 24) with insonation (1 MHz, 2 W/cm(2), 50% duty cycle, 5 minutes) after intravenous administration of CBLuc with cationic, neutral, or no MBs. Tumor luciferase activity was assessed by means of serial in vivo bioluminescence imaging and ex vivo analysis. Results were compared by using analysis of variance.

RESULTS
Cationic MBs (+15.8 mV; DNA binding capacity, 0.03 pg per MB) partially protected bound DNA from DNase degradation. Mean CBLuc expression of treated endothelial cells in culture was 20-fold higher with cationic than with neutral MBs (219.0 relative light units [RLUs]/µg protein ± 92.5 [standard deviation] vs 10.9 RLUs/µg protein ± 2.7, P = .001) and was significantly higher (P < .001) than that in the no MB and no ultrasound control groups. Serial in vivo bioluminescence of mouse tumors was significantly higher with cationic than with neutral MBs ([5.9 ± 2.2] to [9.3 ± 5.2] vs [2.4 ± 0.8] to [2.9 ± 1.1] × 10(4) photons/sec/cm(2)/steradian, P < .0001) and versus no MB and no ultrasound controls (P < .0001). Results of ex vivo analysis confirmed these results (ρ = 0.88, P < .0001).
CONCLUSION
Plasmid-binding cationic MBs enhance ultrasound-mediated gene delivery efficiency relative to neutral MBs in both cell culture and mouse hind limb tumors.

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