Gene Activation and Gene Delivery with Ultrasound

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Abstract: Relatively low levels of ultrasound enhance gene expression following transfection. Possible mechanisms for enhancement of gene expression include increased cell permeability, but probably more important, gene activation. Acoustically active fluorocarbon gene delivery vehicles have also been developed to enable ultrasound-directed gene delivery.

INTRODUCTION

Gene therapy is being developed to treat a variety of different human diseases including autoimmune and vascular diseases as well as cancer. High levels of gene expression, as well as temporal control and regulation of gene expression are needed for effective clinical gene therapy. Better delivery vectors, which enable site-specific gene delivery, are also needed.

MATERIALS AND METHODS

In vitro experiments were performed in five different mammalian cell culture lines: HeLa, NIH/3T3, COS-1, C-127i, and N-MuLi. A 1.0-MHz, continuous-wave Rich-Mar Model No 25 therapeutic ultrasound transducer (Rich-Mar, Inola, OK) was used to insonate cells and tissues for in vivo experiments. Cell survival was assessed in cell culture by MTT assay.2 Transfection was performed using various commercially available cationic liposomes using plasmid genes p-chloramphenicol acetyl transferase (p-CAT), beta galactosidase (B-gal), and green fluorescent protein (GFP). Acoustically active cationic liposomes were prepared by formulating the cationic lipids with perfluorohexane as well as other fluorocarbon materials. Transfection experiments were performed in cell culture with these acoustically active liposomes and in vivo, in fish and mice, following intramuscular and intravenous injection. To assess effects of cell permeability caused by ultrasound, cells were exposed to FITC-labeled dextran after ultrasound. Reverse transcriptase-polymerase chain reaction (RT-PCR) was used to assess m-RNA levels of a variety of cell repair genes before and after ultrasound exposure in NIH/3T3 cells.

RESULTS

Ultrasound enhanced gene expression from transfection in all five cell lines; the increase was most marked in the NIH/3T3 cells, which only had very low levels of expression before ultrasound (Figure 1). The level of ultrasound energy required to enhance gene expression was insufficient to cause an appreciable rise in temperature or a decrease in cell survival. Ultrasound increased cell permeability to macromolecules and increased expression of cell repair genes. The acoustically active fluorocarbon gene carriers were more effective than the conventional cationic liposomes in increasing gene expression following transfection with or without ultrasound. Ultrasound enhanced gene expression in fish and mice following intramuscular and intravenous injection with both conventional and acoustically active gene carriers. Following IV gene administration, preferential gene expression was seen in the tissue of insonation.

CONCLUSION

Ultrasound enhances gene expression from transfection. The mechanisms underlying this phenomenon likely include increased cell permeability facilitating better delivery, but even more important, gene activation. Ultrasound-enhanced gene activation may involve increased translation and gene integration. Ultrasound may be used as an ‘on’ switch to control gene expression following transfection. Efficient new gene delivery vectors have been developed that provide site-specific gene delivery within the field of ultrasound insonation (Figure 2). Ultrasound holds great promise for improving efficacy and control of delivery in gene therapy.
REFERENCES


Effect of SonoPoration™ on Transfection Rates

![Graph showing CAT protein concentration (ng/ml) versus ultrasound parameters: 1.5 MHz, 0.5 W/cm², 100% DC.](image)

FIGURE 1

![Diagram illustrating the process of DNA entering a cell with a cationic microbubble and ultrasound](image)

FIGURE 2