Microbubbles and ultrasound for gene therapy

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Microbubble ultrasound for gene therapy

- Basic principles
- What does the in vitro data tell us?
- Recent in vivo studies
- How near to clinical applications?
Clinical trials in gene therapy:

- Retrovirus: 34%
- Adenovirus: 27%
- Poxvirus: 6%
- Adeno-associated virus: 2%
- HSV: 1%
- Others: 2%
- RNA transfer: 1%
- Naked DNA: 11%
- Lipofection: 12%
- N/C: 4%
Safe efficient site specific delivery: Viral

• **Pro:**
  – Better transfer efficiency

• **Con:**
  – Immunogenicity (especially adenovirus)
  – Cytopathic effects (especially herpes)
  – Undesirable viral tropisms
  – Limitations on the length of DNA that can be carried
Safe efficient site specific delivery: Non-viral

• Pro:
  – Ease of preparation
  – Better safety
  – Less immunogenicity and inflammatory side effects
  – Can carry relatively large DNA sequences

• Con:
  – Efficiency and target precision poor even when complexed
  – Short duration of effect
Ultrasound potentiates transfection

Ultrasound

Can create pores in cell membranes
“Sonoporation”

Generally higher power than FDA limits for diagnostic use

Tachibana et al. Lancet 1999
Method

1. FITC-dextran 5mins
2. Wash

“Negative control”: No ultrasound
“Positive control”: Already in contact with FITC when US applied
4 kDa FITC-dextran

RMFI

Positive control
Negative (no US)
10 sec
30 sec
10 mins
30 mins
60 mins
180 mins
270 mins
4KDa FITC-dextran: early time points

RMFI

US

5 mins before 1 min before Just before Just after 2 seconds after 5 seconds after Control
Effects of US power

Overall transfection

4ug DNA 20S
Effects of exposure duration

1. Exposure time:
   - Control
   - 1s
   - 5s
   - 10s
   - 20s
   - 30s
   - 60s

2. Gene transfection intensity:
   - 2W/cm², 4ug DNA

3. Overall expression:
   - 2W/cm², 4ug DNA
Cell death increases with power
Cell death increases with exposure duration
QuickTime™ and a Cinepak decompressor are needed to see this picture.

Kathy Ferrara and colleagues, UC Davis
Microbubbles: efficient promoters of ultrasound bioeffects

Microbubbles could enhance extravascular delivery

Tissue specific microbubbles

Blomley MJ et al. Stimulated acoustic emission in the liver parenchyma with the ultrasound contrast agent Levovist. Lancet 1998; 351(9102): 568
Blomley MJK et al. Improved imaging of liver metastases in the late enhancement phase of the ultrasound contrast agent Levovist,. Radiology 1999: 210(2): 409-16
Albrecht T, Blomley M, Burns P et al. Improved detection of liver metastases during the liver phase of SHU508A. Radiology 2003; 227(2):361-70
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Targeted/preloaded microbubbles

After Unger
Targeted microbubbles

- Microbubbles which target P-selectin and $\alpha_v$-integrins (Klibanov, Linder and colleagues)
- Thrombus specific microbubbles which target GPIIb/IIIa (Unger, Schneider and colleagues)
No-invasive assessment of angiogenesis by US and microbubbles targeted to $\alpha_v$ integrins. Leong-Poi...Lindner. Circulation 2003; 107: 455-60

Figure 5. Confocal microscopy images of matrigel neovessels (left panel) and examples demonstrating retention of $\text{MB}_E$ (center panel) and $\text{MB}_\alpha$ (right panel) microbubbles (arrows) retained within neovessels.
Antibody-labelled microbubbles targeting to cells
Microbubbles as site-specific delivery vehicles

after Unger
• Plasmid only  Plasmid/Optison
Plasmid/Optison/US
- Plasmid DNA only
- Plasmid/US
- Plasmid/microbubble
- Plasmid/microbubbles/US
An Extra digit (#3) produced by Sonoporation
Gene Induction

12hrs after Sonoporation
Oligonucleotides

Evidence for:
- potentiation of transfer with US
- binding to some microbubbles

Oligonucleotides showing considerable promise especially in gene modulation / silencing strategies
- antisense
- decoy strategies
- RNA interference
Recent oligo studies

Erikson et al. Mol and Cellular Cardiol 2003
• Antisense to TNF-α in the heart with microbubble ultrasound: downregulated

• Decoy to NFκ-B: improved delivery with microbubble ultrasound and improved survival of rat allografts
How near are we to clinical use?
Ultrasound widely used and available and safe
Microbubbles are also available clinically in many countries
Definite promise as a non-viral delivery tool for genes and oligonucleotides
Bioeffects of microbubble ultrasound do need more evaluation
Substantial development work still needed for therapeutic applications