Focused Ultrasound in Drug Delivery and Nanomedicine

Brian O’Neill, PhD

AAPM Annual Meeting
July 2014

Thanks to:
Nathan MacDannold, BWH
Kathy Ferrara, UCD
Natalya Rapoport, U. Utah
Motivation

• Delivering drugs exclusively to localized areas of disease should increase effectiveness and reduce side effects
• Nanoparticle drug carriers hold promise, but relying on natural targeting and drug release has failed to produce the expected results
• Focused ultrasound has advantages for “remote control” in tissue: deep penetration, accuracy on the mm scale, non-ionizing, feedback
Course Outline

• Mechanisms of Ultrasound-Material Interaction
• Ultrasound Alteration of Tissue Properties
  • Enhanced Delivery via Hyperthermia
  • BBB Disruption via Stable Cavitation
• Ultrasound Release from Drug Carriers
  • Induced Release from micelles and liposomes
  • Induced Release from microbubbles
  • Release from phase-change nanodroplets
Focused Ultrasound
(HIFU or FUS)

- Diagnostic ultrasound: 1-2 cycle pulses (time resolution), 1-15 MHz (spatial resolution)
- Therapeutic ultrasound: $10^3$-$10^4$ cycle pulses, 0.2-3 MHz
- Focused ultrasound: beam is directed to diffraction limited spot – ie. width ~ wavelength by geometry (single element), or electronic shift of phase (multiple element array)
- With sound speed ~ 1.5 mm/us, 1MHz ultrasound has wavelength 1.5 mm, so this is beam waist
- length depends on transducer diameter, for f=D, length~7 mm
- Intensity at focus is:

$$I_f = \frac{TAP}{area} = I_{xducer} \times \frac{D^2}{d^2}$$

- For InSightec D = 15 cm, so focusing factor is $10^4$
- $I_{xducer} = 3$ W/cm$^2$, so $I_f < 3 \times 10^4$ W/cm$^2$
Effects of Focused Ultrasound

Thermal effects

Hyperthermia (40-45 °C) -> altered blood flow, gene upregulation, inflammation, apoptosis

Thermal Ablation (50+ °C ) -> cell death through necrosis

Thermal Dose:

\[ TD_{43} = \int R^{T(t)-43} \, dt ; R \approx 4 (T < 43)|2(T > 43) \]

Thermal Conduction:

\[ \rho c \dot{T}(x, t) = \dot{Q}(x, t) + k \nabla^2 T - w_b c_b \Delta T_b \]
Effects of Focused Ultrasound

Mechanical effects

Cavitation (combination with bubbles)

\[ MI \propto \frac{p_r}{f^{0.5}} \]

\[ \omega_0 = \frac{1}{R_0} \left( \frac{3yP_0}{\rho_0} (1 + \sigma ...) \right)^{0.5} \]

Sonoporation and sonolysis -> cell membrane damage

Sonochemistry -> ROS production

Radiation force/shear -> mechanotransduction, bioeffects

\[ F = \alpha \frac{l(r)}{c_v} \]

Other??
Therapeutic Effects

control

pulsed-HIFU
HIFU-Enhanced Transport

• Working with ultrasound only – very attractive because clinical translation of device is much easier
• Idea is that tissue transport properties (diffusion, permeability) are altered by HIFU
• 5+ years of work on mouse and rabbit models to understand mechanism
Pulsed-HIFU treatment

O’Neill, et al., JMRI 2013
Treatment Results
Transport at 24 hours
(conclusion: thermal effect)
Ultrasound-mediated targeted drug delivery in the brain†

Nathan McDannold

Dept. Radiology, Brigham & Women’s Hospital, Harvard Medical School, Boston, MA

†used with permission
>98% of small molecule drugs do not cross the BBB

~100% of large molecule drugs do not cross the BBB

<1% of drug companies have a BBB drug targeting program

<1% of academic neuroscience programs emphasize BBB transport biology
BBB disruption with focused ultrasound

- Low-power, pulsed exposures
- Combined with ultrasound contrast agent (Optison, Definity)
- Temporary (~hours), localized, non-invasive
BBB disruption with Focused Ultrasound

- Occurs due to mechanically-induced changes and/or stimulation to vasculature
- Caused by microbubble/US interaction
- Not due to heating
- Exact mechanism(s) not known

Radiation Force  Bubble Oscillation  Acoustic Streaming
Electron microscopy study: tight junctions

Results: # TJ proteins reduced after BBBD; restored at 4h

BBB disruption with focused ultrasound

Mechanical interaction between US, microbubbles, and vessel walls results in:

- Transient disassembly of tight junction proteins
- Stimulation of active transport

At higher exposure levels, inertial cavitation occurs, leading to vessel damage

Hynynen et al., Neuroimage 2004
Electron microscopy study: active transport

BBB disruption with focused ultrasound

Small animal studies:
- Reliably induce BBB disruption without tissue damage
- Deliver a range of molecules to the brain, including therapeutics
- Improve outcomes in animal disease models
  
  *Glioma, Alzheimer’s*

Alzheimer’s model mouse
Endogenous IgG (green)
+Trypan blue bound to Amyloid plaque (red)

Raymond et al., PLoS One 2008
Characterizing BBBD with dynamic contrast enhanced MRI

Summary of therapeutic agents delivered via FUS-BBBD

• Chemotherapy
  BCNU, methotrexate, doxorubicin, liposomal doxorubicin

• Antibodies
  Herceptin, BAM10 (Alzheimer’s)

• Nanoparticles
  Magnetic nanoparticles
  Gold nanoparticles

• Neuroprotective agent
  BDNF, GDNF (Parkinson’s, stroke, traumatic brain injury)

• Viruses
  siRNA for Htt (Huntington’s disease)

• Cells
  Neural precursor cells (stem cells)
  Natural killer cells

• Nothing!
  BBBD alone might help Alzheimer’s disease, induce neurogenesis
FUS Induced Release from Nanoparticles

Two general approaches: Thermal and Mechanical

**Thermal:** Competes with many other modalities: RF, laser, AMF
Relies of heat sensitive liposomes, heat sensitive polymers – maybe reversible

**Mechanical:** based on cavitation
Microbubbles, nanodroplets – not reversible
Thermally sensitive liposomes

Liposomes are spherical lipid bilayers that can be used for carrying hydrophilic drugs. Problem: either too leaky or too stable. Sol’n: Lipid bilayers undergo gel to liquid phase transition with temperature dependent on composition. Leaky during transition due to phase mismatch. LTSL: developed at Duke, now used many places.

Mills & Needham, BBA Biomembranes, 2005; 1716(2):77–96
Thermal sensitive polymeric NPs

Many kinds of nanoparticles built of polymers as drug carriers – generally slow diffusion

Some polymers undergo conformational phase change that alters solubility in water (expansion, collapse, micelle formation, disassociation...)

Huge potential, barely scratching surface
Ablation + long circulating LTSL†

16 element annular array (IMASONIC)
3 MHz center frequency
14 MPa PPP, -7.7 MPa PNP
7 s CW, single spot >65 °C

†used with permission
$^{64}\text{Cu}-\text{LCL} - \text{no US}$
MRgFUS + $^{64}$Cu-LCL

6 hours 20 hours 48 hours
Complexation of Cu(II) and Dox within liposomes

**Problem:** Even liposomal doxorubicin has substantial cardiac toxicity and dose cannot exceed 500 mg/m² in lifetime.

**Solution:** Create a doxorubicin salt that is very stable in circulation

![Graph showing the relative fluorescence (Fl) of doxorubicin (Dox) at different pH levels for both ammonium sulfate and copper gluconate/TEA methods.](image)

*Kheirolomoom et al Molecular Pharmaceutics*
Complex of Cu(II) & Dox with liposomes

CuDox-lipo

Cu-lipo

Doxil

Kheirolomoom et al. Molecular Pharmaceutics 2010

100 nm

Tumor growth

- Control
- Control+US
- CuDox-LTSLs
- CuDox-LTSLs+US

Treat 2x/week, 4 weeks, 6 mg/kg

* p<0.05 compared to control
*** P<0.001 compared to control

Kheirolomoom et al, JCR 2013
Toxicity is low
Why do we favor thermally-sensitive nanoparticles?

%ID delivered depends on volume insonified, time of insonation

Assumes 5% blood volume in tumor
10 sec tumor blood refresh
5 L blood volume
Non-Thermal Release

Release driven via pressure changes, cavitation – rapid release, no change in T

Types include drug loaded microbubbles, gas containing liposomes, liposomes attached to microbubbles, phase shifting ‘nanodroplets’

The latter are PFC with bulk liquid-gas transitions around body temperature that are held together by Laplace pressure: $\Delta P = \frac{2\sigma}{R}$
Liposomes or oil carriers on bubbles

Microbubble

Nanoparticle

PEG

Fluid Concentration (Bubble-bead aggregates/mL)

UMB 2006, JCR, 2006 and 2007
Nanodroplets† (Courtesy of N. Rapoport, U. of Utah)
Versatile structures with properties that depend on the core and shell compositions

Core:
PFP, $T_b = 29 \, ^\circ C$
PFCE, $T_b = 140 \, ^\circ C$

Shell:
PEG-PDLA
PEG-PLLA
PEG-PCL
Pluronic

†used with permission
Ultrasound effect on the nanodroplet

Scheme of the ultrasound-induced drug release
Ovarian Carcinoma Model

- Chemotherapy by PTX/PFP/PEG-PLLA nanodroplets and ultrasound

MRgFUS

- Small Animal LabFUS System (Image Guided Therapy, Inc.)
- 16-element annular transducer, \( f = 3 \text{ MHz} \), \( r_c = 3.5 \text{ cm} \)

Rapoport, N. et al., J Control Release 2011; 153(1): 4-15
Treatment monitoring: MR Thermometry

**Ultrasound Parameters**
- 3-MHz
- $P = 3.4$ MPa
- 1 x 3 mm focal spot
- Grid trajectory, 4 x 5 mm
- 5 minute sonication time
MR thermometry response

Maximum temperature projection in time

MR Parameters
- SegEPI sequence, EPI=3
- 2x2x3 mm (ZFI to 1x1x3mm)
- 1.3 seconds
- TR/TE = 60/10 ms
- Flip angle = 15°
- 752 Hz/pixel
- Referenceless reconstruction

Temperature rise (°C) over time (sec)
Tumor Resolution

Tumor cells were transfected with RFP; only viable cells generated fluorescence.
Growth Curves
Pancreatic Cancer
**Lifespan results**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Average Life Span, weeks (mean ± std)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (N=7)</td>
<td>3.5 ± 0.5</td>
</tr>
<tr>
<td>No injection, MRgFUS (N=6)**</td>
<td>4.8 ± 2.3</td>
</tr>
<tr>
<td>Empty droplets, MRgFUS (N=6)**</td>
<td>3.5 ± 2.1</td>
</tr>
<tr>
<td>PTX droplets, no MRgFUS (N=7)</td>
<td>7.0 ± 0.8</td>
</tr>
<tr>
<td>PTX droplets, MRgFUS (CW, injection-MRgFUS time=8 hrs, N=8)*****</td>
<td>10.3 ± 1.6</td>
</tr>
</tbody>
</table>

**Mice that died within several days after treatment (P>4.2 MPa) were excluded**

***Survivors (N=2 for the grid trajectory) were excluded***
Results  Courtesy of N. Rapoport, U. of Utah

- PTX-loaded nanodroplets + MRgFUS dramatically decrease pancreatic tumor growth
- MR guidance improves treatment outcome
  - Detailed anatomic visualization
  - Tumor targeting and treatment planning
  - Real-time MR temperature imaging
- Treatment success is a function of ultrasound parameters
- In the absence of drug, hyperthermic conditions could increase perfusion and inflammation thus accelerating tumor growth.
Study Participants

Natalya Rapoport
Allison Payne
Christopher Dillon
Jill Shea
Roohi Gupta

University of Utah, Salt Lake City, Utah, USA
Understanding Ultrasound/Drug Synergy

- Going beyond the anecdotal evidence
- Look at thermal, mechanical interactions independently
- Understand biological mechanism
- Clues to what drugs might work best
HIFU Treatment with Drugs (Sonodynamic Therapy?)

Viability on day 3 (%)

- Control
- HIFU duty cycle = 0%
- HIFU duty cycle = 30%
- HIFU duty cycle = 50%

[Doxorubicin], ug/mL, N=8
‘Sonodynamic Therapy’

Four Hours after Treatment, N=3
Conclusions

Use of ultrasound to alter tissue properties or drive release from nanocarriers is a very promising approach to targeted drug delivery.

Challenges: need to visualize the target before you can hit it (metastatic disease problem)
- regular ultrasound limitations: no penetration in air, little through bone
Potential Areas for Application

Cancer – large or infiltrative tumor
Cardiac – plaques or thrombii
Neuro – target drugs to specific sites of the brain, spine
Orthopedic – joints, near surface bone lesions
Ophthalmology – drugs to the retina, through cornea
Others?