Ultrasound-Mediated Drug Delivery using Echogenic Liposomes

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Seminar Overview

- Definition of the clinical problem
- Targeted nanoparticle development
  - Echogenic liposomes (ELIP)
- Ultrasound-enhanced drug delivery (1 MHz)
  - Ex vivo arterial flow model
- Thrombolytic efficacy of tPA-loaded ELIP (120 kHz)
  - Time-lapse thrombolysis videos in vitro
- Thrombolytic efficacy of tPA and Definity® contrast agent (120 kHz)
  - Ex vivo arterial flow model
- Sustaining stable cavitation with contrast agents

Cardiovascular Disease

- #1 Cause of Death and Disability Worldwide (WHO)
- Accounts for 34.3% of all deaths in the US (Lloyd-Jones D, et al. Circulation, 2009)

Development of US techniques

- Evaluation of the progression of atherosclerosis
- Identification of the morphology of atheroma and thrombus
  - Landini et al., UMB 1986
  - Barzilai et al., Circ Res 1987
  - Picano et al., Circulation 1988
  - Jones et al., Ultrasonic Imaging 1987 & 1989
  - Ng et al., Circulation 1993

Functional anatomy of vasa vasorum


Development of Ultrasound Contrast Agents Targeted to Atheroma or Thrombus

- Diagnosis - Identification of plaque vulnerable to rupture
- Therapeutics - thrombolytic delivery
  - Ischemic Stroke
  - MI
  - DVT
  - AV Fistula Maintenance for Dialysis Access
**Molecular “Velcro” Targeting Strategies**
- Linear hexapeptide coupled to lipid (Aerosomes, InaRx, Tucson, AZ, USA)
- Antibodies targeting Glycoprotein IIb-IIIa receptor (Bracco Research SA, Geneva, Switzerland)
- ICAM-1 to target atheroma (Mastrobattista et al. *Biochim Biophys Acta*, 1999)
- Inactivated rt-PA in ELIP to target fibrin (Klegerman et al., *J Liposome Res*, 2008)

**Targeted Immunoliposomes**
Klegerman ME et al.
*J. Controlled Rel.* (2010)

**ELIP Size Distribution**

**Size Distribution of rt-PA-ELIP**
Ultrasound Controlled Drug Delivery Paradigm:

- Rapid Fragmentation – shell fragments thereby liberating gas

- Acoustically Driven Diffusion – forced diffusion of gas into the medium

Smith DAB et al., UMB, 2007

Interaction of UCA with US

- Primary Radiation Force - net displacement of UCA in the direction of wave propagation
Drug Delivery with 1-MHz CW US Using ICAM-1-targeted ELIP

- Artery permeability after ultrasound + bubbles
  - Red blood cells and dyes observed leaking
- Could work for therapeutics as well
- Hypothesize a stable cavitation mechanism
- Objective: To investigate potential for ultrasound-induced stable cavitation to enhance delivery to the artery wall


Materials and Methods: Flow System

- Mouse aortas kept viable in flow system
- Artery chamber for flow and ultrasound coupling

Materials and Methods: Passive Cavitation Detection

- Passive Cavitation Detection
- Materials and Methods
- Protocol approved by IACUC of U. of Cincinnati
- Removed aorta of 16 mice at 17 – 24 weeks
- Flow of 0.5% bovine serum albumin, 3.4 mL/min
- 30-second bolus of ELIP at 1.8 mg lipid/mL
  - Fluorescently labeled with Rhodamine dye
  - Targeted with antibody to Intercellular Adhesion Molecule-1 (ICAM-1)
- Allowed albumin flow to continue for 3 minutes
- Collected perivascular fluid to monitor for ELIP

Materials and Methods

- Half of arteries insonated at 1 MHz, continuous wave, 0.49 MPa
  - Stable cavitation regime
- Numbers of arteries exposed/Number of arteries excluded due to loss of endothelium during handling

<table>
<thead>
<tr>
<th></th>
<th>1 MHz Ultrasound</th>
<th>No Ultrasound</th>
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<tbody>
<tr>
<td>ELIP</td>
<td>8 / -3</td>
<td>8 / -3</td>
</tr>
<tr>
<td>No ELIP (negative control)</td>
<td>3 / -1</td>
<td>4 / -2</td>
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</table>

- Additional 6 positive controls with ELIP injected into perivascular fluid

Results: Stable Cavitation

- Determined cavitation thresholds using PCD in 0.5% bovine serum albumin with:
  - ELIP
  - Flow
  - 1 MHz continuous wave
- Stable cavitation: above 0.43 ± 0.02 MPa
- Inertial cavitation: above 0.51 ± 0.01 MPa
- Confirmed stable cavitation throughout ultrasound exposure of arteries
Results: Factor VIII Stain

No ultrasound

With ultrasound at 1 MHz, 0.49 MPa

Results: Red fluorescence due to Rhodamine-labeled ELIP

No ultrasound

With ultrasound at 1 MHz, 0.49 MPa

Results: Fluorescence Under Blue + Red Filters

No ultrasound

With ultrasound at 1 MHz, 0.49 MPa

Results – ELIP targeting to arteries

• 2 of 16 ELIP-exposed arteries excluded due to poor histological outcome
• Enhanced fluorescence on endothelium of 10 of 14 ELIP-exposed arteries
• ELIP penetration beyond endothelium in
  – 5 of 5 ultrasound-treated arteries
  – 0 of 5 non ultrasound-treated arteries
  – p-value of 0.008 with Fisher’s Exact Test
• No full-thickness penetration

Results – Analysis of perivascular fluid

• Spectrofluorometry of perivascular fluid showed high levels of Rhodamine-labeled ELIP

Mass of Rh-ELIP in Perivascular Fluid (mg)

Results

Quantification of Rhodamine Penetration

Red Area / Endothelial length [µm]

0.4

0.3

0.2

0.1

0.0

Ultrasound Treated

(n=5)

non-Ultrasound Treated

(n=5)

Results: Analysis of perivascular fluid

• Spectrofluorometry of perivascular fluid showed high levels of Rhodamine-labeled ELIP
Discussion

- *Ex vivo* approach allows use of living tissues combined with control of variables
- Leakage from side branches limits use of small arteries for full penetration studies
- Endothelial damage to arteries observed likely due to handling
- Ultrasound-enhanced penetration into arterial wall demonstrated in this proof-of-concept study

Conclusions

- This model allows passive detection of cavitation
- Stable cavitation can be sustained in an artery with ELIP at low acoustic pressures
- Anti-ICAM-1 targets liposomes to endothelium
- 1-MHz Ultrasound at 0.49 MPa\textsubscript{p-p} enhanced liposome penetration of arterial wall
- Careful selection of ultrasound variables may prevent undesirable vascular effects

Thrombolytic Efficacy of rt-PA loaded ELIP

1. To what degree is the lytic efficacy of rt-PA changed when the drug is encapsulated?
2. Can 120-kHz pulsed ultrasound be used concurrently with rt-PA-loaded ELIP to enhance thrombolysis?


Methods

- Human whole blood clots
  - Venipuncture of 22 volunteers
  - 7-0 Silk sutures in 20 µl glass tubes
  - 6-8 µl clots
  - Clot Width (W\textsubscript{c}): 238.5 ± 34.6 µm (215 clots)

Experimental Set-up

- Water tank at 37°C
- Human fresh frozen plasma (hFFP)
- Inverted microscope and CCD camera
- Field of view: 260 µm x 340 µm
- Entire width of clot in field of view

Methods

- T-ELIP Formulation
  - Reconstituted using supersaturated de-ionized water
- rt-PA Concentration:
  - Free rt-PA added to hFFP: \([rt-PA]_{\text{free}} = 3.15 \, \mu g/ml\)
  - rt-PA-loaded ELIP (t-ELIP), added to hFFP: \([rt-PA]_{t-ELIP} = 3.15 \, \mu g/ml\)
Methods

- Ultrasound Calibration
  - 120 kHz, 0.35 MPa\text{p-p}
  - PRF = 1667 Hz
  - DC = 50% (36 cycles)
- Protocol: 30 min exposure to:
  1) Free rt-PA alone
  2) t-ELIP alone
  3) Free rt-PA + US
  4) t-ELIP + US

Time-lapse Thrombolysis

- t-ELIP Alone
  - 28 min
  - 1 frame of movie ≡ 10 seconds of data
  - 1 second of movie ≡ 1 minute of data
- US & t-ELIP
  - 30 min

Clot Width vs. Time

- The clot width ($W_C$) is corrected for suture width (SW) and normalized ($W_{CN}$)

\[
W_{CN}(t) = \frac{W_{Corrected}(t)}{W_{Corrected}(0)} = \frac{W_C(t) - SW}{W_C(0) - SW}
\]

Average SW: $95 \pm 15$ mm
Clot Width vs. Time

Clot Width vs. Time

Clot Width vs. Time

Potential Mechanisms for Thrombolytic Enhancement with t-ELIP

- Better targeting than free rt-PA
  - Fibrin binding for t-ELIP is twice that of free rt-PA (Tiukinhoy-Laing et al., *J Drug Target*, 15:109-114, 2007)
- Less scavenging of rt-PA
- Nucleation of stable cavitation with encapsulated bubbles

Ultrasound-assisted thrombolysis for stroke therapy

Better Thrombus Break-up with Bubbles

- Ultrasound (US) enhances rt-PA thrombolysis\(^1\) via stable cavitation\(^2\)
- Ultrasound-driven bubble activity
- One clinical study demonstrated increased intracerebral hemorrhage \(^3\)
- Later modeling showed peak negative pressures > 1.0 MPa, standing waves \(^4\)

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Stroke, In Press 2010
Ultrasound-enhanced clot lysis *ex vivo*

- **Research Question:** If stable cavitation is maximized, is thrombolytic enhancement also improved?
- Whole-blood clots injected into excised living porcine carotid arteries
- Protocol approved by IACUC at U. of Cincinnati
- Mounted in flow system and perfused with porcine plasma
  - Physiologic pH, pressure, temperature
  - Oxygen \(23.3 \pm 1.5\) mg/L
  - Flow \(2.7 \pm 1.8\) mL/min to represent ischemia
- Arteries and clots examined histologically after treatment

Hitchcock et al., In press 2010

Results: US-enhanced clot lysis *ex vivo*

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<tr>
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<td>6</td>
<td>5</td>
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- 1.25 cm of 1.9 ± 0.2 cm clot inside US beam (66%)

Cavitation Detection

- Cavitation is the formation and collapse of gaseous and vapor bubbles in a liquid due to acoustic pressure field
- Two types of cavitation:
  - Stable
  - Inertial

Flow system

- Arteries kept viable in flow system
- Mounted in flow system and perfused with porcine plasma
- Physiologic pH, pressure, temperature
- Oxygen \(23.3 \pm 1.5\) mg/L
- Flow \(2.7 \pm 1.8\) mL/min to represent ischemia
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US Parameters chosen to maximize stable cavitation
- Intermittent US, 0.44 MPa peak-to-peak amplitude
- Delivered in intervals of 8.5 s on 19.5 s off
- Quiescent intervals permit influx of fresh Definity®

Number of clots in each treatment group:

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Can stable cavitation be sustained with a clinical ultrasound scanner using an ELIP infusion scheme?

Experimental details...
- ELIP 0.02 mg/ml lipid in 0.5% BSA
- 5 ml/min pulsatile flow
- 6-MHz Color Doppler pulse from L12-5 (Philips HDI 5000)
- PRF = 700 Hz
- FR = 12 Hz
- 10-MHz PCD confocally aligned with first line of Color Doppler pulses

PCD signal processing
- PCD signals recorded and processed in MATLAB
- Computed power spectrum averaged over 20 s
- Subtracted averaged electronic noise in high frequency band beyond PCD and L12-5 -6dB bandwidth

Can stable cavitation be sustained with a clinical ultrasound scanner using an ELIP infusion scheme?

YES!
Acknowledgements

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