The role of ultrasound in image-guided drug delivery

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Outline

Use thermal or mechanical effects of ultrasound, in combination with drug or immunotherapy to cure cancers

- Temperature sensitive liposomes + hyperthermia
- Long circulating liposomes + hyperthermia
- Long circulating liposomes + ablation
MRfUS
Collaboration with Image-Guided Therapy (IGT)

- 16 element annular array
- Diameter: 45mm
- Radius of curvature: 35mm
- Operating frequency: 3MHz
- Acoustic efficiency > 65%
- Power rating: 150Wacoustic
- Bandwidth: 300KHz.
- Focal spot size (approx.): 1.5mm x 1.5mm x 2mm

Fite, PLoS One, 2012
Mild hyperthermia with ultrasound reduces CD4+
Stabilized temperature sensitive liposomes
Treat 2x/week, 4 weeks, 6 mg/kg
Enhanced delivery to tumors- highly stably and thermally sensitive particles

- Direct and local release can increase concentration and decrease systemic toxicity
- Small molecule can penetrate tissue
Complexation of Cu(II) and Dox within liposomes

Problem: Doxorubicin has substantial cardiac toxicity, and dose cannot exceed 500 mg/m² in lifetime.

Solution: Create a doxorubicin salt that is non-toxic at neutral pH, toxic at low pH of lysosomes or tumor.

Dox fluorescence validates delivery

Tumor growth

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

Survival, %

Day post treatment

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

† p<0.001
One Way ANOVA/Tukey

NDL Tumor growth, %

-500 0 500 1000 1500 2000 2500 3000 3500 4000 4500 5000 5500

Days post treatments

CuDox-LCLs
insonified 40 min

CuDox-LTSLs
insonified 40 min

Control-saline

40 min-US

20 min-US
Long Circulating Liposomes + Hyperthermia
Optimize ultrasound-enhanced permeability and retention effect (U-EPR)

(Red arrow shows insonified tumor)

PET (shell)  %ID/cc

Optical (drug)  Optical Efficiency ($10^{-5}$)

Watson, Cancer Research, in review
Cumulative Equivalent Minutes at 43˚C Comparison

%Injected dose/gram

<table>
<thead>
<tr>
<th>CEM43</th>
<th>&lt;1</th>
<th>~1.5</th>
<th>4.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial CNT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insonified</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelial</td>
<td>*</td>
<td>**</td>
<td>***</td>
</tr>
</tbody>
</table>

PNP (MPa)

| 1.1 | 1.1 | 2.4 | 2.4 |

*p<0.01, **p<0.005, ***p<0.001
Combination of thermal and mechanical effects

W/O Heat, NO effect

- Control
- Mechanical

With Heat + ↑ PNP, Enhanced Accumulation

- CNT
- 1.1
- 2.4

<table>
<thead>
<tr>
<th>PNP (MPa)</th>
<th>CEM43</th>
<th>Time (min)</th>
<th>Est. temp. incr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>&lt;1</td>
<td>35</td>
<td>+0.5°C (37.5°C)</td>
</tr>
<tr>
<td>2.4</td>
<td>~1.5</td>
<td>7</td>
<td>+5°C (42°C)</td>
</tr>
</tbody>
</table>

*
Combination therapy regressed epithelial tumors

N = 32 mice

Dox: 33 mg/m², twice a week (intravenous injection, iv)

Rapa: 0.9 mg/kg, 3 times a week (intraperitoneal injection, ip)

US: 1.5 MHz, 42°C for 2 min, MI 1.9

*p < 0.05
Histological & immunohistochemical evaluation

Control      Rapa      CuDox-lipo      CuDox-lipo/US      CuDox-lipo/Rapa      CuDox-lipo/Rapa/US

3 mm

↑ Mammary lymph node

↑ Viable tumor

3 mm

**p < 0.01, ***p < 0.001

WBC K/μL

<table>
<thead>
<tr>
<th>Treatment</th>
<th>WBC K/μL</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.78</td>
</tr>
<tr>
<td>Doxil</td>
<td>6.76</td>
</tr>
<tr>
<td>Normal</td>
<td>1.904</td>
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</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total nuclei x 10^5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
</tr>
<tr>
<td>Rapa</td>
<td>5</td>
</tr>
<tr>
<td>CuDox-lipo</td>
<td>1</td>
</tr>
<tr>
<td>CuDox-lipo/US</td>
<td>0</td>
</tr>
<tr>
<td>CuDox-lipo/Rapa</td>
<td>0</td>
</tr>
<tr>
<td>CuDox-lipo/Rapa/US</td>
<td>0</td>
</tr>
</tbody>
</table>

Normal 5-14.7
Long Circulating Liposomes + Ablation
Ultrasound ablation (CEM43>200 for 2-3 seconds)

- 6 hours
- 18 hours
- 24 hours

Max Accumulation (%ID/g)

Time (hours)

Control
Ablation Untx
Ablation
Blood

Blood (%ID/g)
LCL + Ablation Unilateral Tumors
(ablate 1x1x2 mm), 4x ablation, 8x drug
### LCL + Ablation Histology

<table>
<thead>
<tr>
<th>No Treatment</th>
<th>Dox Only</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>2 mm</td>
<td></td>
</tr>
</tbody>
</table>

- **No Treatment**
  - Image 1: Tissue sample without treatment, showing normal histological structure.

- **Dox Only**
  - Image 2: Tissue sample treated with Dox, showing no significant changes.

- **US Only**
  - Image 3: Tissue sample treated with ultrasound, showing minimal changes.

- **US + Dox**
  - Image 4: Tissue sample treated with ultrasound and Dox, showing significant changes.
Conclusion

Use thermal or mechanical effects of ultrasound, in combination with drug or immunotherapy to cure cancers

- Temperature sensitive liposomes + hyperthermia
- Long circulating liposomes + hyperthermia
- Long circulating liposomes + ablation
NCI and NIBIB

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CMGI (Dave Kukis)
Pfizer Oncology
Targeson (Jack Rychak)
Image-Guided Therapy (Erik Dumont)
Siemens Medical Solutions
Single ablation + long circulating nanodrug

% Change in volume

- Control
- Drug
- Drug + ablation contralateral
- Drug + ablation

Time (days)
CuDox-LTSLs
40 min US

Dox spectrum

Dox fluorescence validates delivery

CuDox-LTSLs
20 min US

Heart

US
40 min

Heart

Tumor

US
40 min

Heart

US
20 min

Heart

US
20 min

Heart

US
20 min

Heart

US
20 min

Heart

Tumor+US

Contralateral tumor

Dox FI
($\times 10^6$ photons/cm$^2$/s)

US+20 min

US+40 min

***
Thermal effects
MRI maps temperature and drug release
Mechanical disruption of RBC phantom (>12 MPa)