Drug Delivery

Goals:
- focal delivery instead of systemic delivery (brain and body)
- increase the drug delivery
Outline

• Thermal effects:
  • Heating induced effects on the parenchyma to enhance drug delivery
  • Liposomes – Thermally gated drug uncaging

• Mechanical effects:
  • Microbubbles – Cavitation-induced opening of blood-tissue barriers and sonoporation
  • Microbubbles – Cavitation-induced opening of blood-tissue barriers
  • Nanodroplets – Drug release with acoustic droplet vaporization
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Thermal Bioeffects for Drug Delivery

• Heating $\rightarrow$ vasodilatation $\rightarrow$
• Increased blood volume $\rightarrow$
• Increased intravascular pressure $\rightarrow$
• Increased vascular and lymphatic drainage $\rightarrow$ decreased interstitial pressure

• Also, increased capillary leakiness $\rightarrow$ increased drug extravasation

• Also, direct cytotoxic effects with excess heating noted in thermal therapy lecture
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Thermosensitive liposomes

- Liposomes:
  - Take liposome packed with drug
  - Incorporate thermosensitive polymer or lipid with differential phase change temperature between more solid and liquid regimes
  - Under mild hyperthermia, small rafts of thermosensitive polymer/lipid act as pores for drug release

- Can combine with prior noted bioeffects to get up to 25x drug concentration in tumor compared with analogous non-thermosensitive liposomal preparations (e.g. ThermoDox vs Doxil)

- Celsion marketing ThermoDox (thermosensitive liposome packed with doxorubicin, an otherwise approved chemotherapeutic)
  - Still awaiting FDA approval AFAIK
  - Clinical trials ongoing for liver and breast cancers
Thermosensitive liposomes

• Challenges:
  • Hard to maintain higher temperature stably over extended period of time in large area
    • FUS treatment focus size goes down with frequency; heating efficiency goes up with frequency
  • For intracranial applications, given cranial geometry and reflections from skull base, etc, getting reliable tissue temperature rise is that much harder, and getting tissue temperature rise only in near tumor but not in brain is even harder – especially at brain periphery and skull base
  • Tumor vascular response and EPR effects not necessarily the same as what is seen in models, and patient-to-patient
  • If can heat the tissue, why not ablate?
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Mechanisms of vascular permeability/sonoporation

- a – Push, expansion
- b – pull, suction
- c – jetting
- d – streaming, shear
- e – translocation

Reality is probably a mix of these
Microbubble expansion - simulation

• 3 µm microbubble can expand to >8 µm under sonication

Figure 5. A snapshot of the predicted pressure field induced by a microbubble with an initial diameter of 3 µm as it oscillates in a microvessel with an inner diameter of 8 µm in an ultrasound field with a PRF of 0.5 MPa and center frequency of 1 MHz.

Ferrara et al
Microbubble effects - visualization

- Can visualize vessel deformations with microbubble oscillations on the order of µs
Sonoporation

- Sonoporated cells will die through osmotic effects and/or apoptosis
- Sonoporation most effective within 2-3x microbubble diameter
Microbubble-assisted therapy

• Deliver functionalized microbubble targeted to tissue to ablate

• Visualize targeting with very low intensity US

• After binding, ramp up intensity to MI>0.5 to induce inertial cavitation and ablate tissue

Kaneko and Willmann, 2012
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Blood Vessels

In the Body

- Endothelial cells
- Pores

In the Brain

- Endothelial cells
- Pericytes
- Astrocytes
- Tight Junctions
- CSF

This barrier protects the brain from toxins, pathogens
Blood Brain Barrier Opening

- Gad is 500-600 Daltons
- BBB stops > 100 Daltons
- stays open 4-24 hours

- inject microbubbles and sonicate
  - doxorubicin, cisplatin, BCNU
  - antibodies, herceptin, BAM
  - targeted genes
  - neural stem cells
  - immunotherapy agents

Hynynen, Radiology (2001)
Microbubble-assisted drug delivery

Ultrasound-mediated blood–brain barrier disruption for targeted drug delivery in the central nervous system

Muna Aryal\textsuperscript{a,b}, Costas D. Arvanitis\textsuperscript{b}, Phillip M. Alexander\textsuperscript{b,c}, Nathan McDannold\textsuperscript{b,*}

Sample therapeutic agents that have been delivered across the BBB or BTB.

<table>
<thead>
<tr>
<th>Therapeutic agent</th>
<th>Size (Da)</th>
<th>Use</th>
<th>Delivered to</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carmustine</td>
<td>194</td>
<td>Chemotherapy</td>
<td>Gioma model (9L)\textsuperscript{a} [141]</td>
</tr>
<tr>
<td>1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU)\textsuperscript{b}</td>
<td>214</td>
<td>Chemotherapy</td>
<td>Gioma model (C6)\textsuperscript{a} [138,152]</td>
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<tr>
<td>Cytarabine</td>
<td>243</td>
<td>Chemotherapy</td>
<td>Normal brain [140]</td>
</tr>
<tr>
<td>Boronophenylalanine</td>
<td>330</td>
<td>Agent for boron neutron capture therapy</td>
<td>Gioma models (G8M 8401 [149]; 9L [150])</td>
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<tr>
<td>Doxorubicin</td>
<td>540</td>
<td>Chemotherapy</td>
<td>Normal brain [96]</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>545</td>
<td>Chemotherapy</td>
<td>Normal brain [139]</td>
</tr>
<tr>
<td>siRNA</td>
<td>-13 kDa</td>
<td>Huntington’s disease therapy</td>
<td>Normal brain [135]</td>
</tr>
<tr>
<td>Glial cell line-derived neurotropic factor (GDNF)\textsuperscript{b}</td>
<td>24 kDa</td>
<td>Neuroprotective agent</td>
<td>Normal brain [153]</td>
</tr>
<tr>
<td>Brain-derived neurotropic factor (BDNF)</td>
<td>27 kDa</td>
<td>Neuroprotective agent</td>
<td>Normal brain [136]</td>
</tr>
<tr>
<td>Herceptin (trastuzumab)</td>
<td>148 kDa</td>
<td>Anti-cancer antibody</td>
<td>Normal brain [147]; breast cancer brain met. model (BT-474)\textsuperscript{b} [148]</td>
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<tr>
<td>BAM-10 Aβ targeted antibodies</td>
<td>-150 kDa</td>
<td>Therapeutic antibody for Alzheimer’s disease</td>
<td>TgCcrND8 Alzheimer’s model mice [138]</td>
</tr>
<tr>
<td>BCNU-VEGF\textsuperscript{b}</td>
<td>-150 kDa</td>
<td>Angiogenic-targeted chemotherapy</td>
<td>Gioma model (C6)\textsuperscript{a} [155]</td>
</tr>
<tr>
<td>Plasmid DNA (pBDNF-EGFP)\textsuperscript{b}</td>
<td>-3600 kDa\textsuperscript{a}</td>
<td>Gene therapy</td>
<td>Normal brain [154]</td>
</tr>
<tr>
<td>Epinucin in magnetic nanoparticles \textsuperscript{b}</td>
<td>-12 nm</td>
<td>Magnetic targeted chemotherapy</td>
<td>Gioma model (C6)\textsuperscript{a} [144]</td>
</tr>
<tr>
<td>Doxorubicin in magnetic nanoparticles\textsuperscript{b}</td>
<td>-6–10 nm</td>
<td>Magnetic targeted chemotherapy</td>
<td>Gioma model (C6) [146]</td>
</tr>
<tr>
<td>BCU in magnetic nanoparticles\textsuperscript{b}</td>
<td>-10–20 nm</td>
<td>Magnetic targeted chemotherapy</td>
<td>Gioma model (C6) [145]</td>
</tr>
<tr>
<td>Adeno-associated virus (AAV)</td>
<td>-25 nm</td>
<td>Gene therapy vector</td>
<td>Normal brain [160–162]</td>
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<tr>
<td>Liposomal doxorubicin (Lipo-Dox) \textsuperscript{b}</td>
<td>90 nm</td>
<td>Chemotherapy</td>
<td>Normal brain [83]; Gioma model (9 L)\textsuperscript{a} [142,163]</td>
</tr>
<tr>
<td>Adenovirus receptor targeted Lipo-Dox</td>
<td>100–</td>
<td>Chemotherapy</td>
<td>Gioma model (8401) [143]</td>
</tr>
<tr>
<td>Interleukin-4 receptor targeted Lipo-Dox</td>
<td>120 nm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neural progenitor cells</td>
<td>7–10 μm</td>
<td>Stem cell</td>
<td>Normal brain [159]</td>
</tr>
<tr>
<td>Natural killer cells (NK-92)</td>
<td>-10 μm</td>
<td>Cell therapy for brain tumor</td>
<td>Breast cancer brain met. model (MDA-MB-231-HER2) [151]</td>
</tr>
</tbody>
</table>
Extravasation homogeneous for small molecules

Punctate regions of enhancement for large molecules

What about antibodies (150kDa)? Ongoing work…
BBB Disruption for Brain Tumors

D270 tumor
Human GBM

7day
Post FUS 11

KTrans

FUS-
FUS+

Normalized
BBB Disruption for Brain Tumors

D270 tumor
Human GBM

7day
Post FUS 11

KTrans

FUS-
FUS+

Tumor already leaky
BBB Disruption for Brain Tumors

In Tumors

- Tumor already leaky
- FUS increases extravasation into the tumor

D270 tumor
Human GBM

7day Post FUS 11

K Trans

FUS-
FUS+

Normal

Pores
Endothelial cells

High Pressure decreases extravasation?
BBBo changes Microenvironment

- BBB opening affects the microenvironment, consistent with SIR
- Need to investigate how this helps/hurts immune response to tumors
- Also saw activation of microglia and astrocytes.
- Was this due to hemorrhage (presence of albumen)?

Z Kovacs, Kim S, Jikaria N, Qureshi F, Milo B, Lewis BK, Bresler M, Burks SR, Frank JA. Proc Natl Acad Sci U S A. 2017 Jan 3;114(1):E75-E84
What about activation of microglia and astrocytes?

- good or bad
- potentially necessary/helpful/correlated to mounting immune response
What about activation of microglia and astrocytes?

- good or bad
- potentially necessary/helpful/correlated to mounting immune response

BBB opening with FUS

Control

blue: DAPI (nuclei)
red: NeuN (neurons) / Trypan blue
green: Iba1 (activated microglia)
Results,

Hemorrhage not necessary for activated microglia and astrocytes

- Useful pressure range is narrow
- Why the variability?
Cavitation

- **Stable Cavitation** is gentle oscillation of microbubbles

  Depends on ultrasound
  - pressure (or intensity)
  - frequency
  - bubble size

- **Inertial Cavitation** includes violent collapse of microbubbles

Can we Monitor Cavitation?

In our hands, cavitation signal only when there is hemorrhage
How do we control cavitation?

- Modeling
- Need to know acoustic pressure (or intensity)

Clinical translation of microbubble-assisted BBB opening

• First in human demonstration recently completed at Sunnybrook Hospital/U. Toronto
  • 220 kHz transducer
  • Used adaptive design to find pressure to go with; increased pressure with successive bubble injections to look for second harmonic
  • Done as a Phase I study in patient otherwise having that region resected (unfortunately the histological sample too degraded to quantify drug penetration)

• For clinical translation:
  • Need better way to calibrate sonication field to target a particular in situ pressure
  • Need to find US pressure that induces stable cavitation, detection with second harmonic or subharmonics
  • Need to decide how many BBB opening spots to make and where to put them w.r.t. tumor to maximize drug penetration while not doing it ‘too much’
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Practical synthesis and stability
Drug release kinetics independent of sonication site/vessel

- Drug release profile similar regardless of drug loaded
- Kinetics similar to that known for each drug
- Release profile similar whether sonicating aorta (femoral sampling) or brain capillaries (jugular sampling)

Ultrasonic drug uncaging is safe and non-toxic

✓ No evidence of toxicity or damage by:
  ✓ Clinical measures
  ✓ MRI
  ✓ Histology

✓ Over 200 rats have undergone ultrasonic drug uncaging without evidence of toxicity
  ✓ Some completed up to 9x uncaging experiments over several weeks, without issue

Assessing spatial resolution with PET

✓ Measured $^{18}$FDG uptake with PET with sonication (650 kHz; 50 ms pulses, 1 Hz PRF for 4 min) after IV bolus of 1 mg/kg encapsulated propofol vs blank particles

Wang, Aryal et al. Neuron. 2018