Alzheimer’s Disease: The Long Dark Tea-Time of the Brain

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Alzheimer’s Disease

- Most common form of dementia
- Current estimated prevalence of 5.2 million Americans
- Increasing yearly rate of incidence

Two major challenges:

1. To develop robust diagnostic methods widely applicable *in vivo*.
2. To develop treatments which are able to prevent the onset and progression of Alzheimer’s disease
Pathology of Alzheimer’s Disease

- Amyloidβ – Oligomers, Fibrils, Plaques
- Tauopathy-Neurofibrillary Tangles
- Neuroinflammation

All Images on this slide courtesy of the National Institute on Aging/National Institutes of Health
Alzheimer’s Disease Components

Amyloid\(\beta\) – Oligomers, Fibrils, Plaques
Antibodies against Aβ Reduce Plaques

Figure 1 | Amyloid plaque reduction with aducanumab: example amyloid PET images at baseline and week 54. Individuals were chosen based on visual impression and SUVR change relative to average one-year response for each treatment group (n = 40, 32, 30 and 32, respectively). Axial slice shows anatomical regions in posterior brain putatively related to AD pathology. SUVR, standard uptake value ratio.

Sevigny et al., Nature 537(2016)50
Aβ Plaque Reduction also Preserves Some Aspects of Cognition

Dementia

MMSE

Sevigny et al., Nature 537(2016)50
Plaque formation in the brain stimulates the activation of microglia macrophages.

Activated microglia propagate inflammation by producing cytokines which stimulate a secondary immune response.

Cytokine production within cells is regulated by the transcription factor NF-κB.

One can Image Aβ with PET

- But, Aβ are small ~ 50 µm.

- There is no method for imaging plaques within the living brain with this resolution. Even with radiation, the images have a resolution like trying to read the New York Times through cheesecloth.
MRI Can Image Individual Plaques

Resolution is sufficient (30 microns at 9.4 T) to see individual plaques in Tg mice. Contrast between Aβ plaques and surrounding tissue is due to the different susceptibility between brain water and amyloid protein in the plaque.

Has been done in Tg mice, but scan times are long. Needs Bo > 7 T.

However, current resolution at clinical field strengths (1.5 – 3 T) is too low, ~300-500 microns to achieve single plaque detection in humans.
Attempts to solve this include the use of contrast agents the bind specifically to the plaques. First results were with Gd3+ (1999) in mice. Now Gd is mainly a $T_1$ agent and so reports actual plaque sizes, but relaxivity of Gd is low ($r_1 \sim 5-10$ Hz/mM) so that doses up to 2 mM need to be administered.

Gd3+ binds to but does not aid the resolution of a plaque. Only animals have been scanned with this since 1999 so it does not look like it will make it into the clinic.
MRI Using Superparamagnetic Nanoparticles.

- SPIIONs made of Iron Oxide are non-toxic.
- Ferumoxytol is approved by the FDA for IV use in humans. The injected Fe in the SPIIONs is metabolized and enters the body’s Fe pool.
- SPIIONs are primarily T₂ (T₂*) agents (r₂ ~ 800 Hz/mM) so doses are μM.
- There are many MRI sequences that emphasize differences in T₂ (T₂*).
Nanoparticle bioconjugates

Iron Oxide Nanoparticle

5-100 nm

Therapeutic agent*

*cytotoxic drug
prodrug - ganciclovir
suicide gene (herpes simplex virus thymidine kinase gene)
Since SPIONs are particles one needs a mechanism for them to enter the brain through the BBB and this has been a challenge unmet by attempts to use SPIONs up until now.

Even though SPIONs appear attractive, progress has been limited and results even in animals have not been very useful.
SPIONs

One must therefore engineer the nanoparticles if success is to be forthcoming:

- **Zeta potential (must be positive) so that the surface charge is (-) for entry into the brain parenchyma. (Aβ is extracellular)**
- **The particles must be lipophilic. (polysorbate 80)**
- **Small size (<100 nm).**
- **Must carry a targeting ligand directed against a robust brain target (E.g., an anti-Amyloid antibody).**
- **Must carry a ligand that promotes brain uptake. (ApoE ε2).**
- **Must avoid the RES (polyethyleneglycol).**
MRI of Control Mouse Brain
MRI of APP/PS-1 Tg Mouse Brain
How do we tell if the SPIONs work?

**Z-score Measurement**

The Z-score for this plaque was 19.2.

**Diagram Description**

- **Average Background = 208**
- **Background Standard Deviation = 9.2**
- **Pixel Intensity**
- **Plaque Signal = 30.3**
- **Z-score = (208 - 30.3) / 9.2 = 19.2**
Effect of SPIONs on Z-scores in Tg Brains

![Graph showing the effect of SPIONs on Z-scores in Tg Brains]

The graphs illustrate the frequency distribution of Z-scores for Tg Control and Tg + SPIONs groups. The left graph shows the frequency distribution across different Z-scores, while the right graph displays the cumulative sum of N(Z) for each group.
Volumetric Distribution of Plaques

Tg Control

Tg + SPIONs
Optimization of MRI Detection

- Improve Nanoparticles (FePt)
- Develop a theory of MRI contrast
- Use this to optimize MRI sequences.
**Improve Nanoparticles: SIPP Micelle (SM) Synthesis**

**Thin Film Self Assembly**
1. Thin film: evaporate in chloroform
2. Resuspend in water/buffer
3. Extrude
4. Magnetic Purification

<table>
<thead>
<tr>
<th>Method</th>
<th>Size [nm]</th>
<th>Stdev [nm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLS</td>
<td>42.9</td>
<td>8.2</td>
</tr>
<tr>
<td>TEM</td>
<td>44.2</td>
<td>13.1</td>
</tr>
</tbody>
</table>
PS80 Promotes the Entry of the FePt micelles into the Brain
Mechanism of MRI Contrast is $T_2^*$

$T_2^* = 44.4$ ms

$T_2^* = 3.2$ ms

- Cortex
- Plaque core
- $T_2^*$ theory
- Expon. (Cortex)
The Z-score (contrast-to-noise ratio) for a Plaque

The Z-score (contrast-to-noise ratio) for each individual plaque centered at \((x,y,z)\) is defined as:

\[
Z(x,y,z) = \frac{(B - M(x,y,z))}{\sigma}
\]

Where \(B\) is the average pixel intensity of the tissue surrounding each plaque;

\(\sigma\) is the standard deviation of the MRI background noise,

And \(M(x,y,z)\) is the minimum intensity of the hypointense area at \((x,y,z)\):

\[
Z = \frac{(B - M)}{\sigma}
\]
Dependence of the Z-score on Echo Time

Model: \[ Z (B, t, T_{2b}, M, T_{2m}, \sigma) = \frac{(B e^{-t/T_{2b}} - M e^{-t/T_{2m}})}{\sigma} \]

Where \( t \) is the echo time,

\( T_{2b} \) is the effective transverse relaxation time (\( T_2^* \)) of the brain background,

And \( T_{2m} \) is the effective \( T_2^* \) of the water surrounding the SPION in the plaque core;

We can determine \( M \) from the fact that at \( t = 0 \),

\[ Z = 0 \]
\[ => B - M = 0, \]
\[ or \ B = M \]

\[ Z (B, t, T_{2b}, T_{2m}, \sigma) = \frac{B (e^{-t/T_{2b}} - e^{-t/T_{2m}})}{\sigma}, \]

and we can replace the term \( B/\sigma \) by \( S \), the signal to noise ratio in the image, giving:

\[ Z (S, t, T_{2b}, T_{2m}) = S (e^{-t/T_{2b}} - e^{-t/T_{2m}}). \]
Dependence of the Z-score on Echo Time
Increasing the Echo Time Results in the Detection of More Plaques

TE = 4 ms

TE = 20 ms
The optimum echo time at which the Z-Score was a maximum could be found by setting the time derivative of Eqn. [4] to zero and solving for \( t \).

\[
\frac{\partial Z}{\partial t} = -S \left[ \frac{e^{-\frac{t}{T_2b}}}{T_2b} + \frac{e^{-\frac{t}{T_2m}}}{T_2m} \right] = 0
\]

which gives (assuming \( S \neq 0 \)):

\[
t_{opt} = - \frac{T_{2b} T_{2m} \ln \left( \frac{T_{2m}}{T_{2b}} \right)}{T_{2b} - T_{2m}}
\]
Optimization of the Echo Time

With $T_2b = 51.2$ ms, and for $T_2m = 2.8$ ms, the optimum echo time is 8.6 ms.
Optimization Produces Higher Plaque Visibility

But, notice that the Plaques seem Larger
Longer Echo Times Produce Larger Plaques
Gradient Recalled Echo Images Grow with Echo Time

TE = 4 ms

TE = 26 ms

TE = 40 ms
NMR Signal Loss in the Gradient of SPIONs Attached to LNCaP Cells for a GRE Image at various TE Values.

NMR Signal Loss (%) vs Distance from Cell (microns)

- TE = 4 ms
- 12 ms
- 26 ms
- 80 ms
Gradient Recalled Echo Images Grow with Echo Time
Theory vs. Experiment

But, does this work for SPIONs too?
Gradient Recalled Echo Images of SPION-labeled Plaques Grow with Echo Time

![Probability vs Plaque Area Graph]

- TE = 4 ms
- TE = 20 ms

Probability (A)

Plaque Area (mm$^2$)
Resveratrol and Other *trans*-stilbenes as a Drug to Target the NF-κB Pathway

- Numerous reports have revealed that resveratrol is able to reduce inflammation
- Resveratrol is a naturally occurring polyphenol which possesses antioxidant properties
- Wang *et al.*, demonstrated that resveratrol is able to cross the blood brain barrier
- Heynekamp *et al.*, have established a role of resveratrol in the inhibition of NF-κB activity
- LD-55 was made to mimic resveratrol, but without its OH groups

Experimental Model

- Procured six-week old double transgenic mice
  - First transgene encoded for a β-amyloid precursor protein containing double Swedish mutations
  - Second transgene encoded for presenilin 1 (a subunit of γ-secretase) with a deletion of exon 9

Group Design

- Control group was fed a regular mouse diet
- Experimental group was supplied a diet supplemented with 100 ppm resveratrol

Mice were observed over a 14-month feeding period
Confocal Microscopy of Aβ Plaques and Activated Microglia

Untreated Tg

Tg, Resveratrol treated

Tg, LD55 treated
Effect of drugs on the plaque density in various brain regions

![Graph showing plaque density in different brain regions]
Using SPIONs to Monitor Treatment for Alzheimer’s

MRI Sequence Optimized Plaque Detection

Control, 3X Tg

Resveratrol
Effect of drugs on the microglial density in various brain regions

- Control
- Resveratrol
- LD55

Microglial density (mm²)
Using SM80s to Monitor Treatment for Neuroinflammation in Alzheimer’s

Control, 3X Tg

Resveratrol
Conclusions

- We have developed targeted superparamagnetic nanoparticles that penetrate the BBB and bind to Aβ and Microglia.

- We have optimized the MRI detection scheme for these particles.

- The resulting lesions have sizes (200-700 µm) that are now in the range needed for MRI at high field in patients.

- We can now monitor the treatment of AD mice, and have shown that trans-stilbene inhibitors of NF-κB lower both Aβ plaque density and neuroinflammation.
Future Research

Translation to patients.

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