Alzheimer’s: Inhibiting Plaque Formation

Immunoliposome directed drug delivery
Alzheimer’s Impact

- Alzheimer’s lowers quality of life
- Importance of memory
- Longer life (Alzheimer’s is ultimately fatal)
- An estimated 26.6 million people 65 years and older had Alzheimer’s worldwide in 2006
Beta Amyloid Protein

- Strong correlation between Alzheimer’s and beta-amyloid plaques
- Causes neural degeneration
- Beta amyloid protein vs. beta pleated sheets
Purpose

- To target a specific component of Alzheimer’s
  - Beta-amyloid protein
- Reduce neural damage
  - By inhibiting the formation of plaques

Figure by MIT OpenCourseWare.
Epigallocatechin gallate (EGCG)

- A polyphenol with many antioxidant properties found in many plants

  - Binds to BA precursor protein, prevents it from taking final shape with its potent iron chelating abilities
General System Diagram

- Immunoliposomes
  - Goes to brain
- Attaches to BA precursor protein
- Releases EGCG
Immunoliposome Structure

- Liposome
- Antibody
- Bacteria (E. coli)
- EGCG
- Beta-Amyloid Precursor Protein
- Beta-Amyloid Binding Site
Modified antibodies detect beta-amyloid plaques

Feedback system: based on concentration of plaques

Immunoliposomes containing EGCG
Devices

Drug Delivery Device
- Immunoliposome

Beta-amyloid Detector
- Receptor sequence on antibody

Trigger
- Release of EGCG

Stop/Feedback Mechanism
- Large amount of EGCG inhibits production of EGCG
Drug Delivery Device (Immunoliposome)

Beta-amyloid Protein Detection

Releases EGCG

Immunoliposomes

EGCG inhibits production of more

Immunoliposomes go to brain

Reaches beta-amyloid protein

Releases EGCG

Device Diagram 2
Immuno-liposomes go to brain
Reaches beta-amyloid protein
Releases EGCG
Excess of EGCG

Timing Diagram

DDD  Drug Delivery Device
A  Immunoliposomes go to brain
B  Reaches beta-amyloid protein
C  Releases EGCG
D  Excess of EGCG
## Parts

<table>
<thead>
<tr>
<th>PARTS</th>
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<tbody>
<tr>
<td>Liposome</td>
<td>DSPE-PEG</td>
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<tr>
<td>Monoclonal Antibody</td>
<td>OX26</td>
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<tr>
<td>Radioligands</td>
<td></td>
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<tr>
<td>Beta-Amyloid Sensor</td>
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<tr>
<td>E. Coli Promoter</td>
<td>PEC3876</td>
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<tr>
<td>EGCG gene</td>
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<tr>
<td>EGCG Inhibitor Enzyme</td>
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<tr>
<td>Transcription Terminators</td>
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</table>
Parts (Sequence)

EGCG

EGCG inhibitor → PEC3876 → EGCG → TXN Term.

Liposome

PEC3876 → DSPE-PEG → Radio-ligand → TXN Term.

Antibody

PEC3876 → OX26 → BA precursor sensor → TXN Term.
In excess concentrations of EGCG, EGCG binds to inhibitor which binds to the promoter site to prevent the production of more EGCG.
Clinical trials with mice with Alzheimer’s

Need to determine:
1. Amount of EGCG needed
2. Side effects of excess EGCG

Test that:
1. Liposome binds to antibody
2. Insertion of bacteria
3. Immunoliposome attaches to BA proteins
4. EGCG is released
Debugging Steps

1.  
2.  
3.  
4.
Overall System

- Image brain for beta-amyloid plaques
  - If plaques have not formed
    - Overall system has the desired effect
  - If plaques have formed
    - Overall system is not working
    - Check individual parts of the system for problems
1. Formation of Immunoliposome

- Test in vitro
  - Chromatography
    - Separates liposomes, antibodies, and bonded antibody-liposomes
    - Bonded immunoliposomes heaviest
    - Select for attached antibody-liposomes
2. Insertion of Bacteria

- Test in vitro
  - Set-up: Known amount of bacteria into solution with immunoliposome
  - Chromatography:
    - Immunoliposomes with bacteria will be heavier and travel a shorter distance than immunoliposomes without bacteria
3. Beta–Amyloid Detection

- Isolate BA precursor protein from brain
- Introduce known amount of immunoliposomes in culture
- Determine change in immunoliposome levels

“Radioligands” – probe that can pass through blood-brain barrier and detect/label plaques, which can then be imaged
4. Drug Release

- Place immunoliposomes with EGCG-containing bacteria in solution with beta-amyloid precursor protein
- If all previous steps work
  - Concentration of EGCG in solution should increase when beta-amyloid protein introduced
  - If concentration of EGCG does not increase check trigger device
<table>
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<tr>
<th>Unknowns/ Possible Issues</th>
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<tbody>
<tr>
<td>Does B–A secrete anything?</td>
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<tr>
<td>Make sure fat degrades in brain and does not build up</td>
</tr>
<tr>
<td>How does binding to B–A trigger release of EGCG? (What pathway?)</td>
</tr>
<tr>
<td>How do we put the bacteria into the immunoliposome? Polycarbonate membrane extrusion</td>
</tr>
<tr>
<td>Can we replicate the parts of immunoliposomes with E.Coli?</td>
</tr>
<tr>
<td>Do the antibodies have to be specific to each person?</td>
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<tr>
<td>Can we have the bacteria degenerate with the immunoliposome?</td>
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Important Pros and Cons

**Pros**
- Immunoliposome gets past the blood-brain barrier
- Efficient/Concentrated Drug Delivery
- Beta-amyloid Specific Targeting
- Feedback based on concentration of EGCG

**Cons**
- Bacteria in the brain
- Does not address all causes of Alzheimer’s
- May need large quantities of EGCG to be effective
- Hard to test in vitro

**May cure disease but have side effects**
Costs

- DSPE-PEG 2000 in Chlorophyll Solution (C) 25mg => $195
- E. coli promoter (PEC3876) => $80
- Radioligands => $150
- Antibodies (OX26) => $3,900

- Estimated Cost: ~$5000
Main difficulty: moving substances into brain past blood-brain barrier (BBB)

Current methods:
- direct injection into brain
- surgical implantation/catheters
- All invasive, dangerous, and have limited effectiveness
No Go?

- Competition – several current techniques in development:
  - drugs to temporarily open BBB
  - attachment to nanoparticles
  - Ultrasound

- Unknowns:
  - Side effects of excess EGCG
  - The base cause of Alzheimer’s
References

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- http://www.pnas.org/content/97/13/7609.full?ck=nck