Trans-Nasal Mucosal Delivery of Brain-Derived Neurotrophic Factor in Rat Brain using Liposomes-in-Gel Formulation

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1. OPPORTUNITY

Abstract
Purpose: Delivering drugs in the brain for treating neurological disorders is limited by the presence of the blood brain barrier (BBB). Brain-derived neurotrophic factor (BDNF) is a high molecular weight protein, responsible for dopaminergic neuron survival. Trans-nasal mucosal route utilizing a thermosensitive liposome-in-gel (LiG) formulation offers an effective strategy to deliver proteins like BDNF to the brain.

Methods: Cy5-labeled ovalbumin (ova cy5) or BDNF was formulated using either cationic or anionic liposomes. The liposomes suspended in 20% (w/w) Pluronic F127 were used for trans-nasal mucosal delivery in Sprague Dawley rats by creating a surgical window in the BBB, which is repaired using nasal mucosal grafts from a donor rat. The graft was positioned on the craniotomy creating an internal reservoir directly above the graft. Upon installation of the formulations, uptake and distribution studies in the brain were carried out by fluorescence microscopy for Cy5-labeled ovalbumin and quantitatively by ELISA for BDNF. The level of BDNF in the different regions of the brain was compared between the LiG formulation and controls.

Results: The uptake studies with Cy5-labeled ovalbumin showed that cationic LiG formulations had better encapsulation and uptake in rat brain. Additionally, distribution studies with BDNF showed that cationic BDNF LiG formulation resulted in the protein distribution throughout the rat brain as compared to BDNF in solution.

Conclusions: The preliminary results show that the novel trans-nasal mucosal delivery can help in transport of large molecular weight disease modifying therapies, such as BDNF, in the treatment of chronic neurodegenerative diseases.

2. APPROACH

2.1 Formulation and characterization of ovalbumin cy5 and BDNF liposomes and liposomes-in-gel formulations.
- Cationic lipids (DOTAP) and anionic lipids (DPPC) along with cholesterol (stabilizer) and DSPE-PEG 2000 were used in different ratios to optimize the liposomes to get desires size, charge and maximum encapsulation efficiency for optimum delivery of the protein to the brain.

2.2 Heterotopic mucosal engraving procedure for trans nasal delivery to rat brain

Donor mucosal graft isolated and 3 mm craniotomy outlined using a drill to create hole
The dura was removed and donor graft was placed on craniotomy
Skin flaps sutured back to engrat for 3 days
Propylene reservoir placed on craniotomy and secured using dental cement

2.3 Quantitative uptake of cationic cy5-labeled ovalbumin liposome-in-gel in rat brain, and a comparison between the uptake of cationic and anionic liposomes-in-gel in rat brain.

Rats were dosed with ova cy5 in gel, ova cy5 anionic liposomes, ova cy5 anionic and cationic liposomes-in-gel formulations with saline controls
Rats were sacrificed after 72 hours
Quantitative uptake using ICYTE
Qualitative uptake using confocal microscopy and IVIS

2.4 In vivo quantitative evaluations and pharmacokinetics (PK) of the delivery of BDNF liposome-in-gel in rats.

Rats were dosed with only saline, BDNF in saline and BDNF cationic liposomes-in-gel formulations
Rats were sacrificed after 48 and 72 hours
Quantitative uptake and distribution using BDNF ELISA

3. RESULTS

3.1 Characterization data for liposomes

<table>
<thead>
<tr>
<th>Liposomes</th>
<th>Average size (nm)</th>
<th>Average PDI</th>
<th>Charge (± SD)</th>
<th>Encapsulation efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cationic</td>
<td>279.6 ± 49.6</td>
<td>0.302 ± 0.032</td>
<td>2.45 ± 0.15</td>
<td>97.02 ± 0.75</td>
</tr>
<tr>
<td>Anionic</td>
<td>148.85 ± 1.35</td>
<td>0.2075 ± 0.007</td>
<td>-1.685 ± 0.015</td>
<td>73.73 ± 13.73</td>
</tr>
<tr>
<td>Anionic ovum</td>
<td>148.85 ± 1.35</td>
<td>0.2075 ± 0.007</td>
<td>-1.685 ± 0.015</td>
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<td>-1.685 ± 0.015</td>
<td>73.73 ± 13.73</td>
</tr>
<tr>
<td>Cationic ovum</td>
<td>202.15 ± 2.05</td>
<td>0.279 ± 0.006</td>
<td>12.55 ± 1.55</td>
<td>98.3 ± 0.1</td>
</tr>
</tbody>
</table>

3.2 Heterotopic mucosal engraving histology study

Day 3
Day 7

3.3 In vivo uptake of ova cy5 in rat brain

3.3 In vivo uptake of ova cy5 in rat brain using IVIS

3.4 In vivo uptake of BDNF in rat brain

4. IMPACT

- The unique feature about my innovation/research is: Trans nasal drug delivery to deliver proteins to the brain
- This addresses the problem of: delivering high molecular weight protein therapeutics to the brain