Anti-Parkinson Drug Delivery to Brain by Targeting Blood-Brain Barrier

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ABSTRACT SUMMARY

The aim of the study is to develop a liposomal levodopa delivery system targeted to brain. The liposomes were prepared at different temperatures with different lipid compositions. The liposomes were PEGylated in order to increase the bioavailability of the liposomes by reducing the reticuloendothelial uptake and macrophage encapture. The brain targeted liposomes were prepared with conjugation of the maltodextrin molecule to the long chain PEG molecule to enhance the brain entry of the liposomal formulation via receptor mediated endocytosis.

INTRODUCTION

Parkinson’s disease is a progressive and permanent brain disorder [1]. Levodopa is the most commonly used drug in Parkinson’s disease treatment [1]. There is no brain targeted drug formulation in the market and in the clinical trials [1]. Liposomes are biocompatible, biodegradable, non-immunogenic, and nontoxic drug carrying systems [2]. In previous studies liposomes were targeted to brain with certain targeting molecules such as glucose, amino acids, and transferrin [2]. In this study, maltodextrin (MD) was selected as targeting molecule because of brain’s high sugars requirement.

EXPERIMENTAL METHODS

The multilamellar vesicles (MLVs) were prepared by lipid film hydration method. Large unilamellar vesicles (LUVs) were prepared from the MLVs by extrusion method. Maltodextrin was conjugated to PEG lipid via carbodiimide chemistry. The amount of Levodopa was determined with the Fluorescence Spectrometry (Turner Biosystems Modulus Fluorometer, UV Kit, USA). The particle size distribution of the LUVs was obtained by Dynamic Light Scattering Method (Malvern Mastersizer). The surface charge of the LUVs was determined by Zeta Potential Method (Malvern Nano-ZS90). The morphological characterization of the liposomes was performed by High Contrast Transmission Electron Microscopy (TEM) (FEI Technai G2 Spirit BioTwin). The cytotoxicity of liposomes was tested on 3T3 and SH-SY5Y (neuroblastoma) cells by MTT assay. In vitro Blood-Brain Barrier (BBB) transport experiments were performed using BBB Parallel Artificial Membrane Permeability Assay (PAMPA) (Pion Inc., USA).

RESULTS AND DISCUSSION

In initial optimization studies, the LUVs were prepared with three different molar lipid compositions (DPPC:Cho; 8:2, 7:3, and 6:4) at four different temperatures (38, 40, 42 and 44°C). Among the LUVs, the liposome DPPC:Cho 7:3 prepared at 40°C had the slowest cumulative drug release (29.99 ± 0.81 % and 44.98 ± 1.63 % at 24h and 48h, respectively). Therefore, PEGylation of DPPC:Cho (7:3) liposomes done at 40°C by addition of 18:00 DSPE-mPEG(2000) into lipid film with two different ratios (2 and 4 mole percentage of DPPC). 4% PEGylated liposome (4%PEG/LUV) was found optimal with slowest drug release (24.80 ± 2.88% and 36.19 ± 1.99 % cumulative drug release at 24h and 48h, respectively) (Figure 1). The decrease in drug release rate might be caused by entanglement PEG chains covering the liposome surfaces. Targeted liposomes were prepared with two different mole percentage of maltodextrin conjugation to 4%PEG/LUV (i.e. 2%MD-4%PEG/LUV, 4%MD-4%PEG/LUV). Cumulative drug release from the targeted
liposomes was similar to untargeted ones (Figure 1).

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Comparison of *in vitro* cumulative Levodopa release (%) from liposomal formulations

The liposomes had spherical morphology and unilamellar structures with monodisperse distribution (Table 1).

**Table 1.** Size distribution and zeta potential results

<table>
<thead>
<tr>
<th>Liposome</th>
<th>Z-average diameter (nm)</th>
<th>Polulidispersity Index (PDI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPC: Cho 7:3</td>
<td>123.9</td>
<td>0.018</td>
</tr>
<tr>
<td>4% PEG/LUV</td>
<td>129.2</td>
<td>0.030</td>
</tr>
<tr>
<td>4% MD-4% PEG/LUV</td>
<td>124.8</td>
<td>0.049</td>
</tr>
</tbody>
</table>

In-vitro liposome cytotoxicity experiments revealed that percent viabilities of the both cell lines were quite high for all liposomal groups; after 48h incubation, viability of 3T3 and SH-SY5Y cells were higher than 87 % and 81 %, respectively (Figure 2).

**Table 2.** Comparison of drug encapsulation efficiency, lipid recovery, and drug loading results of liposomes

<table>
<thead>
<tr>
<th>Liposome</th>
<th>% L-dopa EE</th>
<th>% Lipid Recovery</th>
<th>% Drug Loading</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPC: Cho 7:3</td>
<td>79.83 ±.95</td>
<td>43.54±.15</td>
<td>45.16±.70</td>
</tr>
<tr>
<td>4% PEG/LUV</td>
<td>74.57 ±.04</td>
<td>49.05±.18</td>
<td>38.01±.63</td>
</tr>
<tr>
<td>4% MD-4% PEG/LUV</td>
<td>92.47± 2.70</td>
<td>52.24±.19</td>
<td>44.25± .33</td>
</tr>
</tbody>
</table>

**CONCLUSION**

Among the liposomal formulations, targeted liposomes i.e., 4% MD-4%PEG/LUV, had highest potential in treatment of Parkinson’s Disease with controlled and sustained drug release property and low cellular cytotoxicity on 3T3 and SH-SY5Y cells. Blood Brain barrier experiments are continuing on different models for efficient targeting of the barrier cells and for successive transport efficiency.

**REFERENCES**


**ACKNOWLEDGMENTS**

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