Efficient Novel Unsymmetrical Lipopolyamine Formulations for Gene Delivery

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Introduction
Our aims are to design and develop efficient, non-toxic, non-viral vectors for in vitro and possible in vivo applications. These could be achieved using our novel spermine conjugates, based on changes to the type, length, position, and number of the hydrophobic anchors (Ahmed et al., 2005, 2006). We have designed a series of novel unsymmetrical lipopolyamine formulations (Table 1) for non-viral gene delivery. These cationic lipids are based upon consideration of the diglyceryl esters found in mammalian diglycerides where the two long lipid chains are not the same. Binding such lipids to the tetra-amine spermine, a natural DNA condensing agent, affords novel unsymmetrical lipospermines. These are not liposomal formulations, rather they form lipoplexes that will transfect target cells, in part because their particle size is in the nanoparticle range (100-180 nm, Fig. 1). These are vectors designed to have simplicity of use based upon DNA condensation by titration (Fig. 2).

Table 1. Lipospermines structures

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>R1</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>N^4,N^9-Didecanoyl spermine</td>
<td>CO(CH2)7CH2</td>
<td>CO(CH2)7CH2</td>
</tr>
<tr>
<td>N^8-Decanoyl-N^9-stearoyl spermine</td>
<td>CO(CH2)7CH2</td>
<td>CO(CH2)7CH2</td>
</tr>
<tr>
<td>N^8-Decanoyl-N^9-oleoyl spermine</td>
<td>CO(CH2)7CH2</td>
<td>CO(CH2)7CH2</td>
</tr>
<tr>
<td>N^8-Oleoyl-N^9-stearoyl spermine</td>
<td>CO(CH2)7CH2</td>
<td>CO(CH2)7CH2</td>
</tr>
<tr>
<td>N^8-Dioleoyl spermine (Lipogen*)</td>
<td>CO(CH2)7CH2</td>
<td>CO(CH2)7CH2</td>
</tr>
</tbody>
</table>

Fig. 1. Particle size of the pEGFP DNA complexed with different lipospermines

Fig. 2. DNA condensation (Agarose gel)

Fig. 3. DNase protection assay for DNA

Results and Conclusions
Unsymmetrical lipopolyamines (N^4-oleoyl, N^9-stearoyl), (N^8-decanoyl, N^9-oleoyl), (N^8-decanoyl, N^9-stearoyl), and (N^8-myristoyl, N^9-myristoyl spermine) show promising transfection results in tissue cultured skin fibroblast primary cells (FEK4) and also in the HeLa derived cancer cell line, with excellent cell survival ratios. There was no loss of potency on transfection in the presence of serum in either cell line. Therefore, the pEFGP DNA is effectively protected from DNase (Fig. 3 and Fig. 4). The results for both the transfection and cytotoxicity obtained with unsymmetrical C10,C18, C10,18 unsymmetrical and saturated (N^8-decanoyl, N^9-stearoyl spermine) analogue show that while transfection efficiencies of the HtTA cell line (~40%), and the primary skin cell line FEK4 (~60%) are both lower than a symmetrical diC10,18 (N^8,9-didecanoyl spermine (62% and 80% respectively), the formulations became significantly less toxic (cell viability up from ~10% to ~75%) (Fig. 5). Also, unsymmetrical diC18 (N^8-oleoyl, N^9-stearoyl spermine) displayed much higher transfection efficiency (~75%) and cell viability (~75%) (Fig. 5) than other unsymmetrical lipopolyamine formulations and was comparable to Lipogen.

Acknowledgements
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References