Brain Penetrating Nanoparticles for Brain Gene Therapy

Panagiotis Mastorakos1, Sneha Berry2, Clark Zhang3, Anthony J. Kim4, Graeme Woodworth4, Jung Soo Suk1, Justin Hanes1

1Department of Ophthalmology, Johns Hopkins University School of Medicine, Baltimore, MD 21231
2Department of Biotechnology, Krieger School of Arts and Sciences, Johns Hopkins University, Baltimore, MD 21231
3Department of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21231
4Department of Neurological Surgery, University of Maryland School of Medicine, Baltimore, MD 21201

pmastor1@jhmi.edu

ABSTRACT SUMMARY

We developed a strategy to formulate highly compacted, colloidal stable gene vector platforms that possess dense surface coatings of neutrally charged polyethylene glycol (PEG). The newly formulated gene vectors, termed brain penetrating nanoparticles, do not adhere to the brain extracellular matrix, rapidly penetrate and distribute throughout the brain tissue, and reach cells over a larger brain area following convection enhanced delivery (CED).

INTRODUCTION

Gene therapy constitutes an attractive approach for curing a variety of diseases in the central nervous system due to the numerous possible genetic targets that can alter the natural history of those diseases. Viral gene delivery, though relatively efficient, has been limited by immunogenicity, low packaging capacity and difficulties in manufacture and scale-up. Non-viral gene vectors offer an alternate strategy but face a number of obstacles to reach the target cells. While acknowledging the well-characterized blood brain barrier as a primary limitation, we focus our studies on surpassing the anisotropic and electrostatically charged extracellular space (ECS) found between brain cells. This ‘brain tissue barrier’, regardless of administration method, hampers widespread distribution of gene vectors in the brain, thereby limiting gene transfer to target cells. Previous studies have shown that exceptionally well coated sub-100 nm nanoparticles can rapidly diffuse in the brain parenchyma allowing the widespread distribution of therapeutics [1]. We aimed to achieve similar dense PEG coating of cationic polymer based gene vector system in order to formulate small and stable nanoparticles with a sufficient PEG coating to allow their penetration through brain parenchyma.

EXPERIMENTAL METHODS

Five kDa methoxy-PEG-N-hydroxysuccinimide was conjugated to 25 kDa branched polyethylenimine (PEI) to yield PEG5k-PEI copolymer with different PEG to PEI ratios. Poly-L-lysine (PLL) peptides were PEGylated with linear 5kDa PEG or ~15kDa (5kDa x 3 chains) branched PEG (BrPEG) using click chemistry. Conjugates were dialyzed, lyophilized, characterized via 1H-NMR and stored at -20°C. Cy5 and Cy3 fluorescently labeled plasmids and eGFP encoding plasmids were complexed into nanoparticles by the drop-wise addition of 10 volumes of plasmid DNA to 1 volume of a swirling PEI/PEG5k-PEI or PLL/PLL-BrPEG solution as previously described [2]. We thoroughly characterized physical characteristics of gene vectors using dynamic light scattering and laser Doppler anemometry, and microscopically visualized them using transmission electron microscopy.

Multiple particle tracking (MPT) was used to estimate the mean square displacement (MSD) of fluorescent gene vectors in ex vivo rodent brain slices as previously published [1]. Intracranial surgeries were performed on female Fischer F344 rats weighing 100-120g. A burr hole was made 3mm lateral to the sagittal suture and on the coronal suture. Cy5 and Cy3 fluorescently labeled plasmids in nanoparticle form were co-administered to the striatum using CED to assess in vivo distribution. Brains were harvested at 5 hours, fixed overnight, cryosectioned, stained with DAPI and imaged using Zeiss LSM 710 confocal microscope. The nanoparticle volume of distribution was then quantified by using a custom MATLAB script that thresholded the fluorescent intensities at 10% of the maximum intensity. A similar process was followed to study the distribution of transfected cells 48hours following administration of eGFP encoding nanoparticles. Student’s t-test and paired student’s t-test was performed for statistical analysis.

RESULTS AND DISCUSSION

We engineered and characterized densely PEGylated brain penetrating DNA nanoparticles (BPN) with PEI (PEI-BPN) and PLL (PLL-BPN). We compared them to conventionally PEGylated (PEI-CPN) [3] un-PEGylated (PEI-UPN) PEI nanoparticles and conventionally PEGylated PLL nanoparticles based on the technology
developed and clinically tested by Copernicus Therapeutics (PLL-CPN) [4] (table 1).

Table 1: Biophysical characterization of nanoparticles

<table>
<thead>
<tr>
<th></th>
<th>Size (nm)a</th>
<th>ζ-potential (mV)b</th>
<th>PDI</th>
<th>MSD@1sec (um²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEI-UPN</td>
<td>47±2</td>
<td>26±1.2</td>
<td>0.15</td>
<td>0.005</td>
</tr>
<tr>
<td>PEI-CPN</td>
<td>49±1</td>
<td>9.2±0.5</td>
<td>0.17</td>
<td>0.031</td>
</tr>
<tr>
<td>PEI-BPN</td>
<td>43±5</td>
<td>2.9±0.3</td>
<td>0.19</td>
<td>0.12</td>
</tr>
<tr>
<td>PLL-CPN</td>
<td>159±19.5</td>
<td>14.5±10.0</td>
<td>0.4±0.7</td>
<td>0.37</td>
</tr>
<tr>
<td>PLL-BPN</td>
<td>76.8±4.1</td>
<td>31.6±4.2</td>
<td>1.4±0.4</td>
<td>0.24</td>
</tr>
</tbody>
</table>

a For PEI nanoparticle size was measured by dynamic light scattering and are presented as average of 3 measurements ± Standard error (SEM). For PLL nanoparticles major and minor diameter were measured from transmission electron microscopy images using ImageJ software. Data are presented as average of 300 nanoparticles ± SEM.

b ζ-potential and polydispersity (PDI) were measured by dynamic light scattering and are presented as average of at least 3 measurements ± SEM.

c MSD at 1 sec was measured using MPT of fluorescently labeled gene vectors in rodent brain slices (Nance et al. 2012).

Based on our MPT analysis, we found that PEI-BPN and PLL-BPN formulations rapidly penetrated the brain parenchyma, whereas the PEI-UPN, PEI-CPN, and PLL-CPN were unable to do so (Figure 1). The ensemble-averaged mean-squared displacement, <MSD>, of PEI-BPN at timescale τ=1 sec was 20- and 4-fold higher than the PEI-UPN and PEI-CPN, respectively. Similarly, the <MSD> of PLL-BPN at timescale τ=1 sec was 6-fold higher than that of PLL-CPN (table 1).

In order to assess the translational importance of our findings, we co-delivered Cy5 labeled PEI-BPN and Cy3 labeled PEI-CPN using CED. In accordance with our ex vivo studies PEI-UPNs demonstrated 3-fold higher volume of distribution within the caudate putamen (Figure 2). Likewise, delivery of eGFP encoding PEI-BPN nanoparticles resulted in 3 fold higher volume in which cells were reached and transfected compared to PEI-CPN carrying the same plasmids.

CONCLUSION

Our results indicate that a dense PEG coating can allow an efficient penetration and distribution of gene vectors in the brain parenchyma thus providing a widespread gene transfer to the brain tissues. This novel strategy was applied in two different gene vector systems and we believe that it can be adapted to a variety of cationic polymers that have been designed to overcome other barriers, further enhancing gene transfer to the brain.

REFERENCES


ACKNOWLEDGMENTS

Financial support provided by the NIH R01 CA164789. Authors would also like to thank Ben Schuster for insightful discussions regarding particle tracking.