Multifunctional Anionic Nanoparticles for the Targeted Delivery of Therapeutic Agents to the Brain for the Treatment of Dementias

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Introduction

Genetic therapies offer great promise for enhancement, replacement, modification and regulation of gene expression. No other area of medical research has the potential to offer such a wide range of possibilities for the development of new therapeutics in the central nervous system (CNS). One of the major obstacles in the treatment of CNS disorders is the inability to effectively bypass the blood brain barrier (BBB) and deliver the therapeutic nucleic acids to the affected region.[1] Therefore, the development of novel strategies to increase the delivery of genetic therapies to the brain is required to progress treatments in the clinic. Nanoparticles are extensively used as delivery vectors for gene delivery due to their biocompatibility and the protection they afford the gene when delivered *in vivo*.[2] Convection enhanced delivery (CED) is a method widely used to circumvent the BBB and allow focused delivery of therapies to a particular region.[3] However, there is an urgent need to be quantitatively measure the distribution of therapeutic delivery *in vivo*. The strategy we have implemented is the use of anionic nanoparticles that contain gadolinium for MR imaging and labels for fluorescence microscopy/histology. This nanoparticle imaging strategy not only allows increased distribution and therapeutic delivery of nucleic acids to the target cell type, but also allows monitoring of distribution throughout the brain using MRI.

Methods

Nanoparticle formulation: Liposomes were formulated using the film hydration method consisting of DOPG:DOPE:DOPE-Rhodamine and the gadolinium containing lipid[4] $GdDOTA(GAC12)_2$ at a molar ratio of 35:49:1:15 mol% respectively, followed by sonication. Nanoparticles were prepared at a charge ratio of 3 (liposome): 2 (peptide): 1 (DNA) for anionics and 0.5:5:1 for cationic formulations respectively, by first mixing eGFP DNA with a dual-functionality peptide (containing a K16 sequence to condense DNA and a 12-amino acid targeting sequence based on neurotensin) and then the liposome. The mixture was incubated at room temperature for 30 min to allow complex formation. Size and charge of the nanoparticles were analysed using a Malvern Nano ZS (Malvern, UK). ***In Vivo Brain Delivery:*** Male Wistar rats (B&K Universal, Hull, UK) were anaesthetised and placed in a stereotactic frame, burr holes were drilled to allow cannula implantation to corpus callosum on the left and striatum on the right hand side of the brain. Administration of the nanoparticles (n=6 per formulation) was via a 220 µm outer diameter fused silica cannula at a rate of 0.5 µl/min at each site (2.5 µl for corpus collosum and 5 µl for striatum) using an infusion pump (World Precision Instruments, Inc, Sarasota, FL, USA). Following infusion, the cannula was left in situ for 5 min and withdrawn at a rate of 1 mm/min. Animals were killed by transcardial perfusion fixation using 4% paraformaldeyhde (pH 7.4) under terminal anaesthesia for MRI and fluorescence histology analysis at 4 and 48 h after treatment. ***MRI:*** MRI measurements were performed on a 9.4T VNMRS horizontal bore scanner (Agilent, Palo Alto, CA) using a 59/26 Rapid quadrature volume coil. Fixed rat brains were imaged using a T1-weighted gradient echo 3D sequence (TR=17 ms, TE=4 ms, FA=52°, 40 µm isotropic resolution, Ave=6). Distribution volumes were measured by segmenting the hyperintensities caused by the gadolinium containing nanoparticles using Amira (Visage Imaging Inc, San Diego, CA, USA). ***Histological Assessment:*** Brains were sectioned and images obtained using a Leica DM5500 microscope and digital camera (MBF, Germany), using rhodamine from the nanoparticles to corroborate the MRI and also GFP to determine gene expression mediated by the nanoparticles.

Results

The formulations created produced stable nanoparticles at both anionic and cationic ratios of 174 ± 6 nm and 216 ± 7 nm respectively, ideal for *in vivo* delivery. The distribution volumes of the nanoparticles, as seen in Figure 1 A+B vs D+E, was determined to be significantly larger for the anionic nanoparticles when compared to cationic ($p < 0.01$, Figure 2 A). Administration of the nanoparticles to the corpus callosum led to a significantly increased distribution for cationic and anionic nanoparticles when compared to the striatum ($p < 0.01$, Figure 2 B). Injection of anionic nanoparticles into the striatum had a significantly improved distribution when compared to cationic nanoparticles ($p < 0.01$). However, delivery to the corpus callosum gave a further improvement in distribution ($p < 0.001$, Figure 2 B). MRI results were confirmed by fluorescence microscopy using the rhodamine lipid demonstrating the increased distribution of the anionic nanoparticles (Figure 1 B+E). Functional delivery mediated by the nanoparticles was confirmed by GFP expression for both anionic and cationic nanoparticles (Figure 1 C+F).

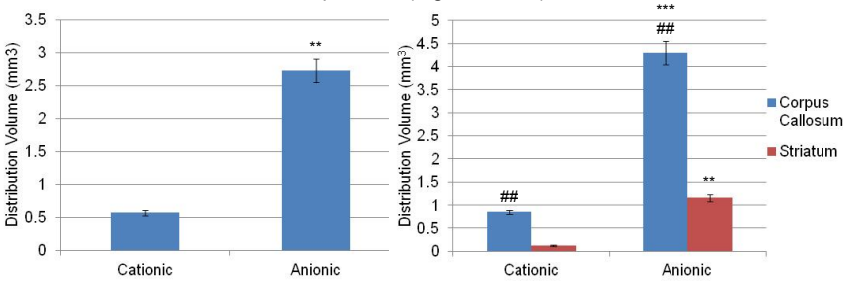

Figure 2. A. Anionic and cationic distribution volumes (** = $p < 0.01$). **B.** Distribution volumes for striatum and corpus callosum (** = $p < 0.01$, *** = $p < 0.001$ for cationic vs anionic and ## = $p < 0.01$ for corpus callosum vs striatum).

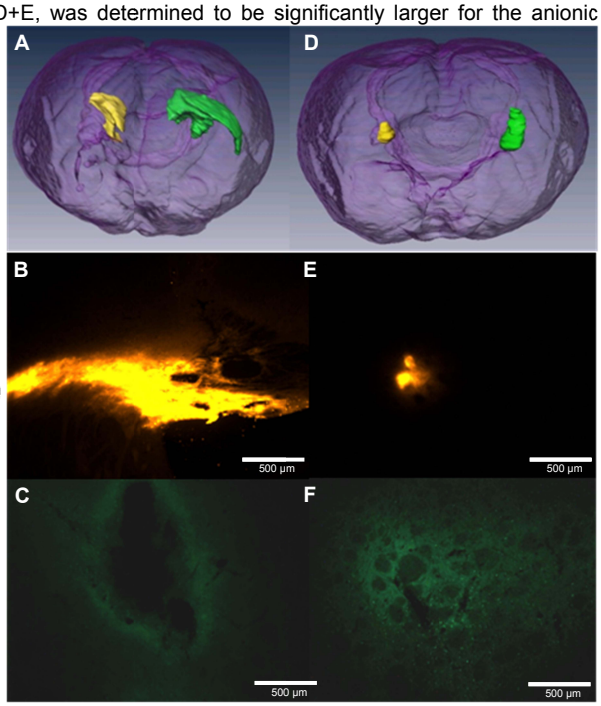

Figure 1. Anionic (A, B and C) and cationic (D, E and F) nanoparticles delivered via CED to the brain. Nanoparticle distribution volumes from 3D MRI images (A and D), corroborated by fluorescence miscroscopy (B and E) and functional gene delivery visualised by GFP expression (C+F).

Discussion

Our nanoparticles are the first that not only allow for increased brain distribution from a single injection, reducing invasiveness, but also mediate functional delivery of DNA. In addition the incorporation of MRI and fluorescence labels allows the simultaneous monitoring of delivery. These results clearly show that our anionic nanoparticles have real potential for the future treatment of a wide range of neurodegenerative diseases and permit real time monitoring of therapeutic distribution noninvasively by MRI.

References

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