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Targeted gene transfer to the brain via the delivery of brain-penetrating DNA nanoparticles with focused ultrasound

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ABSTRACT

Gene therapy holds promise for the treatment of many pathologies of the central nervous system (CNS), including brain tumors and neurodegenerative diseases. However, the delivery of systemically administered gene carriers to the CNS is hindered by both the blood–brain barrier (BBB) and the nanoporous and electrostatically charged brain extracelluar matrix (ECM), which acts as a steric and adhesive barrier. We have previously shown that these physiological barriers may be overcome by, respectively, opening the BBB with MR image-guided focused ultrasound (FUS) and microbubbles and using highly compact "brain penetrating" nanoparticles (BPN) coated with a dense polyethylene glycol corona that prevents adhesion to ECM components. Here, we tested whether this combined approach could be utilized to deliver systemically administered DNA-bearing BPN (DNA-BPN) across the BBB and mediate localized, robust, and sustained transgene expression in the rat brain. Systemically administered DNA-BPN delivered through the BBB with FUS led to dose-dependent transgene expression only in the FUS-treated region that was evident as early as 24 h post administration and lasted for at least 28 days. In the FUS-treated region ~42% of all cells, including neurons and astrocytes, were transfected, while less than 6% were transfected in the contralateral non-FUS treated hemisphere. Importantly, this was achieved without any sign of toxicity or astrocyte activation. We conclude that the image-guided delivery of DNA-BPN with FUS and microbubbles constitutes a safe and non-invasive strategy for targeted gene therapy to the brain.

1. Introduction

Gene therapy approaches have shown promise for the treatment of Parkinson's disease [1–6], Alzheimer's disease [7,8], lysosomal storage diseases [9,10] and brain tumors [11]. Viral gene vectors have been used in clinical trials for neurological disorders and shown to be therapeutically effective [12]. However, viral vectors, such as adenovirus, adeno-associated viruses and herpes simplex viruses have significant limitations, including safety concerns, limited packaging capacity, technical difficulties in scale up and high production costs [13]. Moreover, prior exposures and/or repeated administrations of these vectors lead to neutralizing immune responses that ultimately reduce the efficiency of transgene delivery [14,15]. DNA-bearing nanoparticles (DNA-NP) have emerged as a versatile and easily adaptable platform for gene therapy devoid of the aforementioned limitations.

Regardless of the type of gene vectors used, the blood–brain barrier (BBB) prohibits delivery of systemically administered vectors to the central nervous system (CNS), resulting in minimal transgene expression [16]. Even specific viral vectors or DNA-NP with BBB-targeting ligands achieve only minimal accumulation in the brain when administered at very high doses, which are associated with potential adverse effects in peripheral organs [17]. For this reason, the majority of preclinical and clinical studies have focused on direct intracranial administration of gene vectors. However, the invasive nature of this approach and the risks associated with surgery limit the applicability of this strategy and its potential use for repeated administrations. Various methods for circumventing the BBB, such as intra-arterial infusion of osmotic agents, have been proposed, but they are invasive and non-targeted [18,19], leading to transgene expression in an uncontrolled fashion.

Currently, focused ultrasound (FUS) is the only modality allowing repeated, non-invasive, and temporary BBB permeabilization, leading to localized therapeutic delivery to the brain [20,21]. Circulating ultrasound contrast agent microbubbles (MBs), when exposed to low intensity FUS, oscillate in volume with acoustic rarefaction and compression [22]. Ultimately, interactions between these activated MBs with the vascular wall lead to disruption of tight junctional complexes [23] and induction of active transport processes across the BBB [24]. Importantly,

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Table 1
Physiochemical properties of DNA-BPN.

	Hydrodynamic Diameter ± SEM (nm)[a]		ζ-potential ± SEM (mV)[b]	PDI[a]
	Number mean	z-average		
DNA-BPN	56 ± 2	106 ± 1	1.5 ± 0.3	0.18
DNA-BPN in plasma[c]	65 ± 7	130 ± 2	−1.8 ± 0.8	0.25

[a] Size and PDI were measured by DLS in 10 mM NaCl at pH 7.0 and data are presented as the average of at least 3 measurements ± standard error of the mean (SEM).
[b] ζ-potential was similarly measured by laser Doppler anemometry and data are presented as the average of at least 3 measurements ± SEM.
[c] Physicochemical characteristics were measured following 5 min incubation in PHP at 37 °C.

high capillary density in the brain permits many points of entry after FUS application, potentiating improved distribution compared to local injection. BBB opening is temporary, typically resolving within 4–6 h [20,25], and has shown safety in several experimental animal models, including rhesus macaques [26]. Furthermore, both preclinical and clinical studies have demonstrated the potential of FUS to deliver systemically administered payloads including imaging agents [27,28], ~100 nm liposomes [29,30], ~150 kDa antibodies [31,32], recombinant proteins [33], ~20 nm viruses [34,35] and ~10 μm neural stem cells [36] into the brain. Toward this end, the size of BBB opening is dependent on FUS acoustic pressures [37], suggesting the FUS parameters can be tuned to accommodate delivery of therapeutics of different sizes. FUS can be aimed with guidance from magnetic resonance imaging systems, allowing for accurate targeting of predefined brain structures; devices capable of targeting ultrasound through the human skull with sub-millimeter precision are currently in clinical trials [38,39].

Once beyond the BBB, the brain parenchyma provides an additional barrier to the diffusion of nanoparticles (NP). This brain-tissue barrier (BTB) consists of a nanoporous microstructure of negatively charged ECM macromolecules that hampers the distribution of NP [40,41] and viruses [42] via adhesive interactions and/or steric obstruction. It has recently been shown that sub-115 nm NP densely coated with neutrally charged and bio-inert polyethylene glycol (PEG) are able to overcome the BTB and rapidly diffuse within the brain tissue [40]. We have demonstrated that BBB opening with MR-guided FUS and MBs can facilitate the delivery of colloidally stable, densely PEGylated 60 nm fluorescent tracer brain-penetrating NP (BPN) across the BBB [21]. Once delivered across the BBB, BPN exhibited wide dispersion into the tissue away from the vessels of entry, allowing for homogeneous distribution in the FUS-treated tissue.

In this study, we used colloidally stable DNA-NP with a dense PEG coating (DNA-BPN) previously shown to achieve remarkable penetration through the BTB and high levels of transfection following direct intracranial administration [43]. By combining FUS-mediated BBB opening with systemically administered DNA-BPN, we formulated a non-invasive strategy to achieve safe, highly localized, robust, and sustained transgene expression in the CNS.

2. Results and discussion

We formulated highly PEGylated DNA-BPN based on a gold-standard cationic polymer, polyethylenimine (PEI), as previous described [43–45]. This technique allowed the formulation of highly compact and colloidally stable 56 ± 2 nm DNA-BPN with a PEG to PEI *w/w* ratio of 50 that is substantially higher than PEGylation ratios used traditionally [46–48]. Effective shielding of the NP positive surface charge was confirmed by the near-neutral ζ-potential (+1.5 ± 0.3 mV; Table 1). We further measured the stability of DNA-BPN in pooled human plasma (PHP; Innovative Research, Novi, MI); DNA-BPN retained their colloidal stability following incubation in PHP at 37 °C, as evidenced by the well-preserved hydrodynamic diameters (65 ± 7 nm), near-neutral surface charge (−1.8 ± 0.8 mV) and polydispersity index (PDI) of 0.25 (Table 1). Despite a minimal increase in size, DNA-BPN did not aggregate. DNA-BPN retained their sub-100 nm diameter and DNA compaction over at least 30 min of incubation in PHP at 37 °C, as demonstrated by the hydrodynamic diameter histograms and transmission electron micrographs (Fig. 1a, b). This may be attributed to the inclusion of free PEI in the formulation of BPN allowing the formation of strongly positive polymer core that leads to efficient DNA compaction in spite of the steric hindrance imposed by the use of dense amounts of PEG.

To measure the *in vivo* transfection efficiency of DNA-BPN, we formulated DNA-BPN with a plasmid containing a luciferase reporter gene driven by a long-acting β-actin promoter (pBAL). These DNA-BPN were intravenously co-injected at 3 different concentrations (50 μg, 100 μg and 200 μg) with MBs in Sprague–Dawley rats (n = 5 per dose) and FUS was applied to the striatum of the left hemisphere. Gene expression was measured using an *in vivo* Imaging System (IVIS100; Xenogen, Alameda, CA). FUS-mediated BBB permeabilization led to targeted DNA-BPN delivery to the brain and robust bioluminescence at the ultrasound focus (i.e. anatomical location where FUS was applied) (Fig. 2a). Bioluminescence was not detected in brain tissue outside of the FUS focal region. Furthermore, extending the IVIS scan to include the entire rat revealed that transgene expression was not detectable in any other off-target organs, including the liver (Fig. S1).

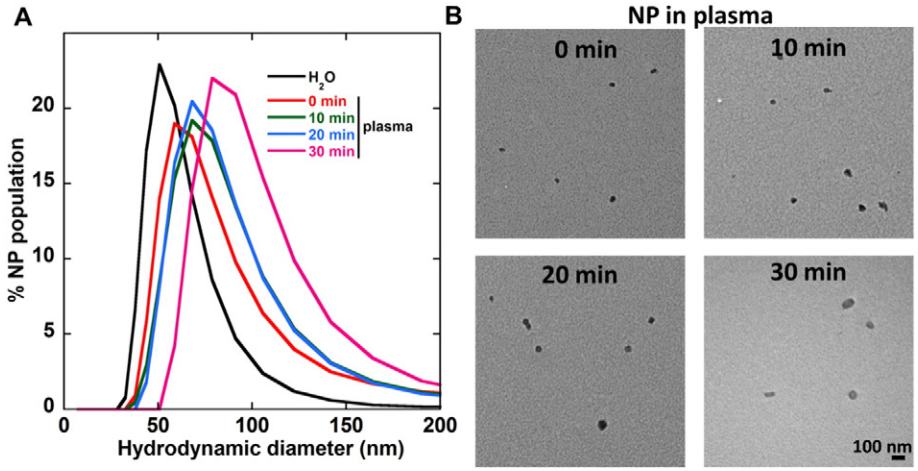

Fig. 1. DNA-BPN stability in PHP. (A) Gene vector hydrodynamic diameter (number mean) distribution following incubation in PHP at 37 °C for 0, 10, 20 and 30 min. Size was measured by DLS in 10 mM NaCl at pH 7.0. (B) Transmission electron microscopy images of gene vectors following incubation in PHP at 37 °C. Scale bar: 100 nm.