Journal of Controlled Release 262 (2017) 37–46

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Biodegradable brain-penetrating DNA nanocomplexes and their use to treat malignant brain tumors

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ARTICLE INFO

Keywords:
Biodegradable polymer
DNA nanocomplex
Non-viral gene therapy
Tumor tissue barrier
Convection enhanced delivery

ABSTRACT

The discovery of powerful genetic targets has spurred clinical development of gene therapy approaches to treat patients with malignant brain tumors. However, lack of success in the clinic has been attributed to the inability of conventional gene vectors to achieve gene transfer throughout highly disseminated primary brain tumors. Here, we demonstrate *ex vivo* that small nanocomplexes composed of DNA condensed by a blend of biodegradable polymer, poly(β-amino ester) (PBAE), with PBAE conjugated with 5 kDa polyethylene glycol (PEG) molecules (PBAE-PEG) rapidly penetrate healthy brain parenchyma and orthotopic brain tumor tissues in rats. Rapid diffusion of these DNA-loaded nanocomplexes observed in fresh tissues *ex vivo* demonstrated that they avoided adhesive trapping in the brain owing to their dense PEG coating, which was critical to achieving widespread transgene expression throughout orthotopic rat brain tumors *in vivo* following administration by convection enhanced delivery. Transgene expression with the PBAE/PBAE-PEG blended nanocomplexes (DNA-loaded brain-penetrating nanocomplexes, or DNA-BPN) was uniform throughout the tumor core compared to nanocomplexes composed of DNA with PBAE only (DNA-loaded conventional nanocomplexes, or DNA-CN), and transgene expression reached beyond the tumor edge, where infiltrative cancer cells are found, only for the DNA-BPN formulation. Finally, DNA-BPN loaded with anti-cancer plasmid DNA provided significantly enhanced survival compared to the same plasmid DNA loaded in DNA-CN in two aggressive orthotopic brain tumor models in rats. These findings underscore the importance of achieving widespread delivery of therapeutic nucleic acids within brain tumors and provide a promising new delivery platform for localized gene therapy in the brain.

1. Introduction

Glioblastoma (GBM) is the most common and aggressive form of primary brain tumor [1]. GBM is characterized by a heterogeneous tumor microenvironment and diffuse tumor cell infiltration throughout the brain [2]. The standard of care is surgical resection followed by radiation and intensive chemotherapy, but this aggressive multimodal therapeutic regimen only marginally improves median survival time [3]. Further, the standard regimen is associated with poor patient life quality due to its toxic nature. Development of a well-tolerated and effective therapeutic option that may replace or complement the current frontline therapy is sorely needed.

Recent genetic association studies have unraveled linkages between specific genetic mutations and glioma sub-phenotypes, leading to the identification of common molecular pathways that are critical for the maintenance, progression and/or recurrence of brain tumors [4,5]. In

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http://dx.doi.org/10.1016/j.jconrel.2017.07.009
Received 23 February 2017; Received in revised form 21 June 2017; Accepted 7 July 2017
Available online 08 July 2017
0168-3659/

addition, innovations in plasmid engineering and gene editing techniques [6] make it possible to have long-term and/or tumor-specific transgene expression. To this end, gene therapy has emerged as a potent therapeutic opportunity to potentially arrest or even reverse the progression of this devastating disease [7,8]. Encouragingly, intracranial administration of viral and non-viral gene vectors has yielded promising outcomes in several preclinical studies and has been well tolerated in Phase I clinical trials [9]. However, subsequent clinical trials have failed to demonstrate significant therapeutic benefits in humans. Inadequate efficacy has been primarily attributed to poor distribution of therapeutic transgene expression achieved with the gene delivery systems tested to date [7,10–13]. Of note, invasive cancer cells that migrate away from the bulk of the tumor mass into the healthy brain parenchyma are difficult to access, and thus, are primarily responsible for tumor relapse [14].

Convection-enhanced delivery (CED) provides a continuous pressure-driven flow of fluid into brain tissue from an implanted catheter. Thus, CED has been widely explored in an attempt to increase the penetration of therapeutics within the brain parenchyma [15]. CED of a retroviral replicating vector carrying a suicide transgene enhanced survival in an orthotopic rodent U87MG glioma model compared to an intratumoral bolus injection of the same vector [16]. However, most preclinical and clinical studies have shown that CED exerted limited effect on the distribution of common vectors [17–20] and, thus, provided marginal improvement at best in anti-tumor efficacy. These failures are likely due to the limited ability of conventional gene vectors to avoid steric and/or adhesive trapping [21,22] at the point of administration, which prevents them from spreading with the CED infusate through the brain extracellular space.

We have previously demonstrated that synthetic DNA nanocomplexes possessing small particle diameters (~50 nm) and non-adhesive surface coatings (requiring an exceptionally dense polyethylene glycol (PEG) corona) are capable of efficiently penetrating healthy rodent brain parenchyma *ex vivo* and *in vivo*[23,24]. However, whether these DNA-loaded brain penetrating nanocomplexes (DNA-BPN) can also efficiently spread within brain tumor tissue has yet to be determined. Compared to healthy brain parenchyma, brain tumor tissues possess a more heterogeneous microenvironment with varying cellular density and collagen content [25,26], as well as necrotic areas [27]. We formulated DNA-BPN composed of a blend of a promising biodegradable polymer, poly(β-amino ester) (PBAE) [23], with PEG conjugated to two terminal ends of PBAE (PBAE-PEG), and evaluated vector distribution and transgene expression in two aggressive orthotopic brain tumor models.

2. Results and discussion

2.1. Formulation and characterization of DNA-loaded nanocomplexes

Plasmid DNA were condensed either with PBAE (Fig. 1A) alone or a blend of PBAE with PBAE-PEG (Fig. 1B) to yield DNA-loaded conventional nanocomplexes (PBAE-CN) or brain-penetrating nanocomplexes (PBAE-BPN), respectively [23]. The hydrodynamic diameters of PBAE-CN and PBAE-BPN were 94 ± 5.8 nm and 52 ± 2.6 nm, respectively (Fig. 1C, Table S1). PBAE-BPN had near neutral surface charge (ζ-potential = −3.6 ± 0.4 mV) whereas PBAE-CN possessed highly positive surface charge (ζ-potential = 26.9 ± 1.1), thus confirming the dense PEG corona on PBAE-BPN that masked the cationic (*i.e.* amine-rich) PBAE core (Fig. 1D, Table S1). The size and morphology of PBAE-CN and PBAE-BPN were confirmed by transmission electron microscopy (Fig. 1E). We previously demonstrated that nanoparticles as large as 70 nm in diameter efficiently penetrated orthotopically-established 9L gliosarcoma (GS) if the particle surface was passivated with a dense PEG corona [28]. Surface plasmon resonance analysis has also revealed that such nanoparticles resist adhesion of brain extracellular matrix (ECM) molecules on their surfaces [29].

Based on these findings, densely PEGylated PBAE-BPN were expected to diffuse within brain tumor tissue without being significantly hindered. However, physicochemical properties of nanoparticles are often altered in physiological environments [30]. We thus evaluated colloidal stability of PBAE-BPN and PBAE-CN in artificial cerebrospinal fluid (aCSF). While PBAE-CN instantaneously aggregated following the incubation in aCSF to 310 ± 35 nm, the size of PBAE-BPN remained unchanged in aCSF (53 ± 1.2 nm), suggesting that PBAE-BPN retained the physicochemical properties required for efficient brain penetration. This is in good agreement with our previous finding where a dense PEG coverage prevented polyethylenimine (PEI)- and poly-L-lysine (PLL)-based DNA-BPN from aggregating for up to 24 h in aCSF, whereas non-PEGylated and/or conventionally PEGylated (*i.e.* lower PEG density) counterparts lost colloidal stability shortly after the exposure to aCSF [24,31].

2.2. In vitro transfection efficiency of DNA-loaded nanocomplexes

We next assessed *in vitro* gene transfer efficacy of PBAE-CN and PBAE-BPN carrying plasmid DNA encoding luciferase protein. For comparison, transfection efficiencies of other conventional DNA-loaded nanocomplexes based on PEI (PEI-CN) and PLL (PLL-CN) were also evaluated. PEI is the most widely explored cationic polymer for gene delivery applications *in vitro* and in preclinical settings [32], including brain gene transfer studies [33,34], and has been tested in clinical trials for gene therapy of several cancers [35,36]. The PLL-CN used here is composed of DNA condensed into nanocomplexes using block copolymers of a 30-mer PLL connected to PEG *via* a cysteine residue; this DNA nanocomplex was well tolerated by cystic fibrosis patients [37] and demonstrated promising results in rodent brain [38,39].

Two different types of malignant rat gliomas, including F98 GBM and 9L GS cells, were selected to assess *in vitro* transfection efficiency and to establish orthotopic brain tumor models for subsequent *ex vivo* and *in vivo* studies. F98 GBM forms highly aggressive tumors that mimic many of the hallmarks of human GBM, including a highly invasive pattern of growth, overexpression of protein-based tumor markers and resistance to many chemotherapeutics and irradiation [40–42]. We also note that while being less invasive compared to F98-based models, orthotopic models established with 9L GS have been widely used in the preclinical setting to determine the potential of novel therapeutic approaches to be tested in clinical trials [43,44]. Indeed, clinical trials for Gliadel® [45–47] and OncoGel™ [48–50] were initiated based on preclinical evaluation in the 9L GS model.

We discovered that the *in vitro* transfection efficiencies of PBAE-BPN in F98 GBM and 9L GS cells, as well as differentiated mouse hippocampal neuronal cells (HT22), were significantly lower than those achieved using PBAE-CN ($p < 0.05$; Fig. S1). This result is in agreement with previous findings that PEGylation reduces *in vitro* transfection efficiency of non-viral gene delivery systems by reducing cellular uptake and/or intracellular processing [51,52]. However, despite the dense PEGylation, PBAE-BPN exhibited a similar or significantly higher *in vitro* transfection efficiency compared to non-PEGylated PEI-CN and conventionally PEGylated PLL-CN in rat brain tumors cells (Figs. S1A, B). This result is likely due to the biodegradable nature of PBAE that may facilitate intracellular release of plasmid DNA [53], unlike DNA-loaded nanocomplexes formulated with non-biodegradable polymers such as PEI. The transfection efficiency of PLL-CN was comparable to or only marginally improved from carrier-free plasmid DNA control (Fig. S1).

Importantly, *in vitro* transfection efficiency of PBAE-BPN was at least two orders of magnitude higher in rapidly dividing F98 GBM or 9L GS cells than in slowly dividing neuronal HT22 cells (Fig. S1). The differentiation state-dependent doubling rates may at least partially explain the differences in *in vitro* transfection efficiency. Mitosis transiently opens the nuclear membrane, a significant obstacle to intracellular gene delivery, thereby allowing nucleic acid payloads to