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AAV viral vector delivery to the brain by shape-conforming MR-guided infusions

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ABSTRACT

Gene transfer technology offers great promise as a potential therapeutic approach to the brain but has to be viewed as a very complex technology. Success of ongoing clinical gene therapy trials depends on many factors such as selection of the correct genetic and anatomical target in the brain. In addition, selection of the viral vector capable of transfer of therapeutic gene into target cells, along with long-term expression that avoids immunotoxicity has to be established. As with any drug development strategy, delivery of gene therapy has to be consistent and predictable in each study subject. Failed drug and vector delivery will lead to failed clinical trials. In this article, we describe our experience with AAV viral vector delivery system, that allows us to optimize and monitor in real time viral vector administration into affected regions of the brain. In addition to discussing MRI-guided technology for administration of AAV vectors we have developed and now employ in current clinical trials, we also describe ways in which infusion cannula design and stereotactic trajectory may be used to maximize the anatomical coverage by using fluid backflow. This innovative approach enables more precise coverage by fitting the shape of the infusion to the shape of the anatomical target.

1. Introduction

Central nervous system (CNS) drug discovery along with drug development are seen as one of the most challenging areas of pharmaceutical development. Pharmaceuticals for neurological diseases are frequently plagued by poor efficacy and serious side effects. Accordingly, the introduction of first-in-class neurological medications has become infrequent. More than any other factor, the conflicting roles of a given drug target in one brain region versus another make finding a balance between efficacy and safety extremely difficult. Thinking of the brain as a single functional entity, therefore, may not be the best way to affect optimal therapeutic outcomes. We have focused for many years on advancing the technology of local delivery of genes and drugs to affected regions of the brain (and brain tumors) to treat disease. Particularly in the past 5 years, we have brought into the clinic an infusion technology in which intra-operative MRI is used to guide cannula placement and to monitor infusate distribution. The potential flexibility and power of this approach permit to achieve specificity through anatomical localization while minimizing off target effects.

Gene therapy is potentially an excellent way to deliver therapeutic agents directly to the human brain using the adeno-associated virus (AAV). However, there are various aspects of AAV-based gene therapy that present significant challenges as identified below.

AAV vectors are classified into serotypes based on their capsid sequence. Our studies have focused on AAV2 for clinical development because it is neuron-specific, offers essentially permanent expression [1], and has been used in more patients than any other vector [2,3]. AAV2 is subject to anterograde axonal transport in the brain. When infused into the thalamus, for example, neurons in brain regions that receive thalamic projections, like cortex, are abundantly transduced [4]. In contrast, AAV6 is transported in an entirely retrograde direction [5,6] and AAV9 appears to be bidirectional (in press). While it is clear that

Abbreviations: 16-G, 16-gauge; 3D, three-dimensional; 6-OHDA, 6-hydroxydopamine; AAV, adeno-associated virus; AD, Alzheimer's disease; CED, convection enhanced delivery; cGMP, current Good Manufacturing Practice; CN, caudate nucleus; CNS, central nervous system; CsCl, caesium chloride; DA, dopamine; FDA, Food and Drug Administration; FDG PET, fluorodeoxyglucose positron emission tomography; GABA, gamma-amino butyric acid; GAD, glutamic acid decarboxylase; GCH-I, cyclohydrolase I; GDNF, glial cell-derived neurotrophic factor; GFP, green fluorescent protein; hAADC, human aromatic L-amino acid decarboxylase; HD, Huntington's disease; min, minute; mm, millimeter; MP-RAGE, magnetization prepared rapid acquisition gradient recalled echo; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MR, magnetic resonance; MRI, magnetic resonance imaging; MSA, multiple system atrophy; NE, norepinephrine; NHP, non-human primate; NIH, National Institutes of Health; NTN, neurturin; OD, outer diameter; PD, Parkinson's disease; PDQ-39, 39-item Parkinson's disease questionnaire; PUT, putamen; SNpc, substantia nigra pars compacta; STN, sub thalamic nucleus; TH, tyrosine hydroxylase; UCSF, University of California San Francisco; UPDRS, Unified Parkinson's Disease Rating Scale; VTA, ventral tegmental area.

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AAV-based gene delivery for CNS disorders has tremendous therapeutic potential there are several issues related to its administration to achieve predictable, reliable and reproducible gene delivery that could be used clinically.

Three key technologies were harnessed to enable a dramatic improvement in the precision and safety of brain infusions. Pressurized infusions, also called convection-enhanced delivery (CED), permitted direct, efficient, and controlled distribution throughout target brain structures. This approach required development of an infusion cannula that allowed such pressure to be exerted without significant reflux [7]. The critical feature of this cannula that prevents infusate reflux is the inclusion of a shorter stepped design closer to the cannula tip. There have been subsequent refinements to the design as we have developed other components of the infusion technology. Other complementary innovation to the cannula design was the co-administration of an MRI contrast agent with the infusate. This contrast agent allowed monitoring all the infusions intra-operatively in real-time giving surgeon information regarding status of AAV delivery to the brain. This was a transformative innovation because, for the first time, it permitted quantification of CED dynamics [8–10] and the intra-operative detection of leakage and reflux [11]. It further allowed optimizing cannula placement and gains a level of precision previously unattainable [12–14]. Finally, innovations also have been applied to improve neuronavigation hardware (in collaboration with MRI Interventions Inc.) and software (in collaboration with BrainLab AG), creating a complete clinical platform for MRI-guided administration of therapeutics into the brain [15,16].

All these innovations enhanced the accuracy of the intracerebral drug delivery as well as the feasibility and reproducibility of the delivery technique. Despite significant progress in anatomical targeting of AAV, considerable work still needs to be done to optimize surgical trajectories for infusion cannulae. Specifically we strive to customize the shape of the infusions to conform closely the contours of target structures to ultimately provide a reliable brain delivery platform [15]. This development will improve infusate distribution and ultimately gene transfer of many brain structures such as, hippocampus, caudate and putamen among others. These elongated and curved structures resist filling with a single infusion, and multiple passes are required to maximize the coverage, therefore, trajectories for infusions in the long axis of the anatomical structure may ultimately allow for complete coverage with while minimizing number of cannula tracts.

In this article, we describe an effort aimed to modify intra-operatively the shape of the infusion while maximizing the coverage of such brain regions. Currently with the existing techniques, we can achieve about 50% coverage of the putamen in Parkinson's disease (PD) patients, but we believe that better approaches can improve the coverage while minimizing number of cannula tracts. Two clinical trials in PD are currently ongoing using MRI-guided CED technology (Clinicaltrials.gov: NCT01973543, NCT01621581) and we believe that the development of this technology has implications for a range of brain diseases beyond PD, such as Alzheimer's (AD) and Huntington's disease (HD), as well as different types of brain cancer like glioblastoma (Clinicaltrials.gov: NCT02022644).

2. CNS delivery platform. Real-time MRI-guided AAV delivery

Controlled distribution of the therapeutic agent seems to be a recurrent problem when trying to reproduce preclinical results in clinical trials, probably due to the use of simple injections for parenchymal delivery [17–19]. Convection-enhanced delivery (CED) seems to be a more efficient system to achieve complete coverage of target structures. CED, first described by Oldfield and colleagues [20], is a parenchymal infusion technique that, by means of a pressure gradient at a cannula tip positioned within the target structure, generates bulk flow of macromolecules within the interstitial fluid space. This method allows higher quantities of therapeutic agents to be distributed through large volumes of brain tissue from a single point source. Slow infusions through the stepped cannula direct pressure-driven engagement of the perivasculature to propel infusate over significant distances [21,22]. Since CED relies on fluid convection rather than passive diffusion to achieve its distribution and bypasses the blood brain barrier, which confounds vascular-based delivery approaches, it can deliver high concentrations of fairly large macromolecules over considerable volumes.

Our group has worked extensively in optimizing this method over the years in NHP [3,13,14,23,24]. Our current technique permits monitoring of parenchymal infusions by adding Gadoteridol, a tracer visible with magnetic resonance (MR), in the therapeutic agent preparation and performing intracranial dosing in an MRI scanner (Fig. 1).

This method greatly enhanced the accuracy and effectiveness of AAV delivery since it provides real-time visualization of the infusion [22]. In fact, MRI tracers for real-time CED have been already used in 12 patients treated at the NIH and UCSF and shown to be safe within the human brain parenchyma [25]. In our experience, regardless of the encoded transgene, co-infusion of AAV2 and Gadoteridol with MRI monitoring shows an excellent correlation with the transgene expression as assessed in NHP brain by immunohistochemistry (Fig. 2) [24,26]. Thus, performing infusions by interventional MRI represents a tremendous advantage in predicting the infusion outcome, achieving optimal cannula placement within target structures, and monitoring possible hemorrhages or infusate leakage outside the target structure [24].

Development of real-time CED in NHP studies led to the development of a clinical platform for MRI-monitored CED in the brain [13,14]. In collaboration with MRI Interventions Inc. and BrainLab AG, we have developed an MRI-compatible delivery platform that includes a skull-mounted aiming device (SmartFrame®), a reflux-resistant CED cannula (SmartFlow®) and an MRI-integrated software package (ClearPoint®) that communicates with both the console and the operating neurosurgeon in the MRI suite [14]. This device results in cannula tip placement that is within <1 mm of the visually identified target site [13], greatly increasing our ability to safely and reliably deliver gene therapy vectors as evaluated in the NHP model for infusion into different regions of the brain (Fig. 3) [13].

Since CED uses a pressurized infusion method, under certain conditions, e.g. high flow-rate, the pressure generated at the cannula tip can exceed the shear modulus of the tissue-cannula contact surface and result in reflux of the infusate through the outside of the cannula [7]. In order to avoid this phenomenon, we designed a ceramic, fused silica reflux-resistant cannula with a 3-mm stepped tip that is now the FDA-approved SmartFlow® cannula [7,23] and discovered that infusion rates up to 10 μL/min [7] or even higher (KSB, unpublished data), can be safely achieved, significantly reducing the procedure time. For this custom-designed cannula, we also identified specific cannula-placement zones within different target structures, or “green” zones that would ensure anatomically contained infusion of the therapeutic agent [27,28].

MRI enables characterization of the anatomical structures that impact CED and can be used to monitor the infusion process. Once the preferred infusion origin has been established, however, it was necessary to have a cannula implantation system that enabled the infusion plan. We addressed this by leveraging an intra-operative MR guidance method (ClearPoint®) that was initially developed for implanting deep brain stimulator electrodes [29,30]. This system was adapted to place an infusion cannula rather than an electrode, but otherwise corresponds closely to the existing platform. The trajectory guide-stem is mounted onto a surgically implanted plastic adapter plug and features adjustments with four degrees of freedom (pitch and roll around a pivot point and X-Y translation) that can be manually guided by geared controller knobs. The fluid-filled trajectory guide-stem has an MR-visible alignment cannula that reveals its present orientation. The guidance software allows the user to identify a target and provides feedback on how to manipulate the trajectory guide to achieve alignment with the target. Initially, pitch and roll adjustments provide coarse alignment. Subsequently, images are acquired along the orientation of the alignment cannula and