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Guided delivery of adeno-associated viral vectors into the primate brain

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Abstract

In this review, we discuss recent developments in the delivery of adeno-associated virus-based vectors (AAV), particularly with respect to the role of axonal transport in vector distribution in the brain. The use of MRI-guidance and new stereotactic aiming devices have now established a strong foundation for neurological gene therapy to become an accepted procedure in interventional neurology.

Keywords

gene delivery; AAV; non-human primate; MRI-guided delivery; thalamus; basal ganglia

1. Introduction

Disorders of the central nervous system (CNS) are some of the most difficult to treat and bestow an immense social and financial burden upon society. Traditional pharmacologic therapy often provides symptomatic relief but is plagued by both central and peripheral side effects, including serious drug-drug interactions due to the unrestricted access of many drugs to many tissues in the body. In addition, the problem of even getting drugs across the blood-brain barrier (BBB) is a major obstacle. That is perhaps one reason why more attention is being given to local, parenchymal drug delivery since the BBB can be by-passed, peripheral metabolic problems and side effect avoided, and CNS regional specificity maintained. However, as with any type of CNS delivery, a degree of complexity is expected and in our experience some of these include: i) delivery method, ii) target accuracy, iii) distribution of therapeutic agent and iv) number of injections required. These factors are further complicated when delivery of adeno-associated viral (AAV) vectors is considered. In this review, as part of the special issue discussing “*Delivery of therapeutics to the CNS*”, we discuss magnetic resonance image (MRI)-guided delivery of AAV vectors that employs a new hardware/software package and describe how this technological improvement is helping advance delivery of AAV in the CNS.

In order to achieve effective distribution of AAV in the human brain, it has been necessary to invoke methods of delivery beyond simple injection. Oldfield and colleagues first

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described convection-enhanced delivery (CED) 16 years ago [1]. CED involves the use of a pressure gradient from a cannula tip positioned within the target structure to generate 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 cannula by a mechanism clearly not based upon simple diffusion kinetics. The ultimate pattern and volume of distribution is dependent on the structural architecture of the target region and the physical properties of the agent being infused, including size, charge, viscosity and receptor binding. We have studied the basic mechanism of CED and have concluded that CED requires a pressure-driven engagement of the perivascular space to propel infusate over significant distances [2]. Improvements in the accuracy and reliability of AAV delivery have been greatly enhanced by our inclusion of MRI tracer (Gadoteridol) in vector suspensions (Fig. 1). This permitted vector infusions to be conducted in a magnetic resonance scanner where we could monitor distribution of AAV2 in real time by using the Gadoteridol signal as a surrogate marker [3]. This, together with advanced cannulae and frameless stereotactic aiming devices promises to transform neurological gene therapy into a standardized interventional procedure.

2. Characterization of MRI-Guided CED

With the above system in hand, we have been able to undertake a number of studies to optimize various parameters previously opaque to us. Issues such as optimal cannula placement within target structures have been addressed and have yielded some surprising and important answers. Related problems like reflux of infusate up the outside of the cannula and leakage into ventricles can also now be addressed intra-operatively, thereby improving safety and predictability.

2.1 Real-time MR imaging of AAV delivery

To validate real-time imaging of AAV infusions, we have co-infused Gadoteridol with AAV2 and found an excellent correlation between intra-operative MRI signal distribution and subsequent transgene expression within the non-human primate (NHP) brain [4]. Clinical use of MRI tracers for real-time CED has been previously performed in two patients treated at NIH [5] and shown to be safe within the human brain parenchyma. This innovation allowed us to use Gadoteridol CED as a surrogate for AAV2 distribution and has facilitated our study of CED parameters as described below. Thus, conduct of AAV infusions by interventional MRI (iMRI) is a clinically relevant method for providing guidance during cannula placement and feedback during infusions [6].

In addition to the advantages of co-infusing imaging tracers with therapeutic agents, this mixture allows us to confirm cannula placement within the NHP brain and in our experience, Gadoteridol encapsulated into liposomes [7–13] or free Gadoteridol [4, 14–16] has no discernible adverse effects. However, T2-weighted MR images can also be used to monitor infusate distribution without the need for a surrogate tracer [16, 17], although the inclusion of tracer makes detection much easier. In order to translate these direct brain delivery techniques into a reliable clinical procedure, we have, in collaboration with MRI Interventions Inc (formerly known as Surgivision Inc; Irvine, CA) and BrainLab (Heimstetten, Germany), developed an MRI-compatible delivery platform recently submitted to FDA for approval (Fig. 2). This technology platform includes a skull-mounted aiming device (SmartFrame®), MRI-integrated software package (Clearpoint®) and reflux-resistant CED cannula. The aiming precision of this delivery platform and the infusion capabilities of the CED cannula have been validated recently in NHP for infusion into the caudate, putamen, thalamus, substantia nigra, subthalamic nucleus, and hippocampus [15]. This device results in cannula tip placement that is within <1 mm of the visually identified target site, greatly increasing our ability to safely and reliably deliver gene therapy vectors.

It is important to note that this frame and associated software has now been approved by the FDA (510k number: **K102101**) for ventricular delivery of Cytarabine or aspiration of cerebro-spinal fluid (CSF) from the ventricles during intracranial procedures. Although the FDA views parenchymal delivery differently from ventricular delivery, this approval should make the regulatory path for MRI-guided AAV gene therapy considerably more straightforward.

2.2 Infusate Reflux

CED is a pressurized infusion method. As such, it is possible that the pressure generated at the cannula tip can exceed the shear modulus of the tissue-cannula contact surface. Under such conditions, infusate passes up the outside of the cannula into the lymphatic drainage system and further penetration of infusate into parenchyma ceases [13]. In order to prevent this adverse event, a reflux-resistant cannula was invented in our laboratory [7, 13]. In early clinical studies, the possibility of reflux was minimized by maintaining a flow rate less than 1 $\mu\text{l}/\text{min}$. However, the period of time required for significant volumes to be infused in patients can become problematic. The first CED-based gene therapy trial limited infusion to this rate because the infusion volumes were relatively small (50 μl) and we had no visual feedback during the progress of the infusion [18, 19]. Subsequently, we found that the reflux-resistant cannula effectively tolerated infusion rates up to 5 $\mu\text{l}/\text{min}$. However, at 8 $\mu\text{l}/\text{min}$ significant reflux was evident [20]. In addition, we observed that reflux was also associated with cessation of parenchymal distribution, suggesting that failure of the tissue-cannula wall seal created a low-resistance channel for the escape of fluid and validates the concept that the infusion pressure must exceed the hydrostatic pressure of the surrounding tissue in order to achieve CED-mediated distribution.

2.3 Cannula Placement

Optimal cannula placement is crucial for effective distribution of infusate within targeted CNS structures. Having accumulated a considerable number of independent infusions of MRI tracer into putamen, thalamus and brainstem of NHP, we first conducted a retrospective study of containment within the target structure versus cannula placement [21, 22]. In the putamen, particularly, we identified specific zones in which infusate was poorly contained within the structure, and this was driven by two kinds of untoward events: leakage into internal and external capsules as well as leakage into corpus callosum [21]. This “red” zone was correlated with proximity the cannula step of 3 mm or less to these structures. Somewhat further away, a “blue” zone provided much better containment and an optimal “green” zone was always associated with complete containment within putamen. We have also identified optimal zones for cannulae placements within the NHP thalamus and brainstem [22].

3. Thalamic axonal transport of AAV2

The thalamus is the largest relay center for both sensory and motor modalities. It is comprised of 50–60 nuclei, defined by their spatial location within the thalamus and their projection sites within the brain [23, 24]. Tracer studies have identified multiple synaptic inputs with reciprocal connections to and from the cerebral cortex {Briggs, 2008 #7456; Herkenham, 1980 #7457; Berendse, 1991 #7458; Cappe, 2009 #7460; Cappe, 2007 #7459; Jones, 1998 #7461}. The role of the thalamus is complex. It acts both as a modulator in the basal ganglia-thalamo-cortico circuitry [31, 32] and also mediates processing of cortico-cortical communication [33].

We have found that infusion of AAV2 (both GDNF and GFP transgenes) into this subcortical region resulted in robust transgene expression and distribution to motor and

sensory cortical regions distal from the site of infusion [34]. Figure 3 illustrates the overall result after target selection, trajectory planning, cannula insertion and infusate monitoring. In the example shown here, the NHP thalamus was infused with 300 μ L of AAV2-hGDNF/tracer into the left and right thalamus with no signs of leakage/reflux or untoward behavioral effects in NHP [35]. Volumetric reconstructions (iPlan Cranial®, BrainLab, Germany) of infusate distribution within both thalami revealed extensive infusate distribution (Fig. 4) leading consequently to robust cortical transgene expression (blocks 1–3) that could obviously not be detected by MRI (block 4; Fig. 3). This finding is consistent with the neuroanatomical, reciprocal connections between the thalamus and the cortex documented in several animal species [36–38] and particularly in reference to sensory modalities and motor attributes [28]. We believe that these observations may offer a means to treat certain neurological diseases with a cortical involvement.

4. Axonal transport of AAV2 in basal ganglia

We are developing AAV2-GDNF-based gene transfer for treatment of Parkinson's disease (PD). Selection of the correct anatomical targets for our approach is critically important. Our pre-clinical NHP studies were designed to address the influence of putaminal AAV2-GDNF therapy on the degenerated nigro-striatal pathways {Eberling, 2009 #6546; Kells, 2010 #6733}. Therefore, we wanted to understand how AAV2-GDNF (including expressed GDNF protein) is transported through the parkinsonian basal ganglia. In the unlesioned rat nigro-striatum, the infusion of vector into either striatum (ST) or substantia nigra (SN) resulted in GDNF expression in both locations, as well as in parts of the indirect pathway (e.g. globus pallidus, entopeduncular nucleus). In contrast, injection of vector into the parkinsonian nigro-striatum resulted in a much more restricted pattern of transduction. Striatal infusion of vector gave expression only in the substantia nigra pars reticulata (SNr) and parts of the indirect pathway {Ciesielska, 2010 #7346}. We interpreted the SNr data to be indicative of direct anterograde transport of vector via GABA-ergic projections. Injection of AAV2-GDNF into the lesioned SN did not result in transduction of ST even though the SNr was still intact, demonstrating the almost complete lack of retrograde transport of AAV2-GDNF and/or GDNF protein in the basal ganglia.

These data are consistent with our previous studies of intracranial delivery of AAV2 vector in NHP [39–45] and a phase 1 clinical study in PD patients [19]. For example, AAV2-hAADC and AAV2-TK delivery into the putamen of NHP resulted in detection of transgene in the GP and STN, but not cortex, strongly indicating anterograde transport only [46–48]. Similarly, anterograde axonal transport is indicated in animals that received AAV2-GDNF [43–45] or AAV2-hASM into the thalamus [49], where transgene was detected mainly in the cortex [49, 50]. In PD patients treated with putaminal AAV2-AADC, FMT PET detected robust AADC expression in the putamen, but failed to detect any AADC in the cortex, suggesting lack of retrograde axonal transport in humans [18], consistent with results obtained in MPTP-lesioned NHP [43].

Our finding that transport of AAV2-GDNF is almost exclusively mediated by anterograde axonal transport has important therapeutic indications. First, it suggests that AAV2-GDNF delivery into the putamen of PD patients with degenerated nigro-striatum should not inhibit GDNF trafficking to the SN, which offers the possibility of regeneration of remaining dopaminergic neurons in SNc (pars compacta). Second, anterograde axonal trafficking of GDNF via direct and indirect nigro-striatal pathways should deliver GDNF into other parts of the basal ganglia circuitry involved in PD, offering therapeutic potential beyond just putamen and SNc [51]. Third, there appears to be little rationale for exposing PD patients to unnecessary risks associated with AAV2-GDNF administration into the midbrain region that may lead to severe adverse effects such as bleeding or weight loss [52, 53]. Finally, since

AAV2 is transported anterogradely but not retrogradely, AAV2-GDNF delivery into the SN in PD patients is not expected to reach striatal regions, thus not permitting GDNF delivery to dopaminergic terminals in the striatum. In summary, the present study suggests that our current understanding of AAV2-GDNF transport within the parkinsonian basal ganglia supports a striatal but not nigral delivery in the clinical development of AAV2-GDNF for the treatment of PD.

5. Integrated Delivery Platform

Another constraint associated with stereotactic drug delivery into the brain, is the accurate determination of infusate volume. Humans and NHP have somewhat different brain anatomies in terms of size and location of various structures. Understanding these variables is essential to the translation of preclinical NHP studies into clinical trials in humans. We have made a number of quantitative comparisons between humans and NHP in order to clarify the spatial and anatomical adjustments required in this process, including consideration of relative AC-PC distances [54] and volumetric differences in striatum [55]. Together with optimal cannula placement determinations in NHP and mapping onto human brain, along with the development of new delivery hardware and software, we now have in hand an integrated AAV-based gene delivery system ready for clinical development. Doubtless, as we learn from clinical experience, further refinements to the system may be expected. In addition, a better understanding of how various AAV serotypes are trafficked within the brain, together with the development of exogenously regulated gene expression [56, 57], will offer yet more control and precision to what is rapidly becoming a viable medical procedure.

6. Conclusion

Neurological gene therapy may be one of most complex medical technology development programs ever undertaken, primarily because it requires highly invasive stereotactic surgery. Optimal surgical technique depends on immediate feedback to the surgeon of potential problems. We have documented a series of potential problems with parenchymal infusions, such as leakage of infusate into ventricles that prevents effective parenchymal distribution, reflux up the outside of the cannula that leads to exposure of vector to the peripheral immune system {Cunningham, 2008 #6240}, and incorrect cannula placement in the target structure that leads to untoward distribution of transgene. All these variables represent a real risk to patients. The development of gene therapy from a scientific program into a medical procedure relies very much on enhancement of safety. Visually monitored infusions will be an essential part of this development.

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Figure 1. Co-Infusion of AAV2 and MRI tracer monitored by MR Imaging

Real-time visualization of delivery of therapeutic agents into the NHP brain by co-infusion with surrogate MR tracer (Gadoteridol, 1 mM, ProHance®) provides immediate feedback regarding cannula placement and drug confinement within the targeted structure. Shown are putaminal (A, total volume infused 62 μ L), bilateral thalamic (B, 125 μ L per thalami) and brainstem infusions (C, 100 μ L). L/R = left/right side.

Figure 2. Demonstration of SmartFrame® Hardware

Main components of SmartFrame® (profile view) include the (A) attachment base that screws onto the array secured on the animal's skull and provides support to the cannula held within the fluid-filled stem. (B) Top view of the frame indicates location of color-coded adjustment knobs used for aligning cannula trajectory to targeted structure prior to parenchymal penetration ([1] yellow knob = medio-lateral, [2] green = anterior-posterior, [3] light blue = pitch, [4] orange = roll). (C) Demonstration of SmartFrame® mounted on the NHP skull. Based on the size of this frame and the small dimensions of the NHP skull, bilateral hemispheric targeting cannot be conducted simultaneously. Scale bar: 3 cm.

Figure 3. AAV2-hGDNF CED Infusion into Rat and NHP Thalamus

A) Naïve Sprague-Dawley rat infused via CED with AAV2-hGDNF (2.0×10^{13} vector genomes [vg]/mL) unilaterally into the left thalamus ($15 \mu\text{L}$ at $0.5 \mu\text{L}/\text{min}$; 6 week survival; stereotactic coordinates AP: -2.8 , ML: -1.6 , DV: -5.5 mm) resulted in robust expression of the GDNF transgene throughout the rat brain. **B)** Delivery of AAV2-hGDNF (2.3×10^{12} vg/mL) co-infused with MRI tracer (1 mM, ProHance®) into the NHP thalamus at approximately -11.70 mm on the AP axis (detailed methods described in [35]). We used the equipment shown in Fig. 2 to accurately target this subcortical region one side at a time (white and black arrows). Infusion parameters started at $1 \mu\text{L}/\text{min}$ (5 min) and escalated by $1 \mu\text{L}/\text{min}$ every 5 min until $5 \mu\text{L}/\text{min}$ where infusion continued for 50 min (total length of procedure 70 min/site; total volume $300 \mu\text{L}/\text{side}$; 5 week survival). Widespread distribution of GDNF transgene was found along the cortical surface starting at the site of infusion (AP -11.70 mm; Block 4) and reaching pre-frontal regions (AP $+5.85$ mm; Block 1). No leakage of MRI tracer was observed along the cortical surface where transgene was detected. Note that infusion into the right thalamus was completed 2 h prior to the contralateral (left side) evident in the reduction of tracer visualization. L/R = left/right side,

Th = thalamus, AP = anterior-posterior, ML = midline, DV = dorso-ventral. Scale bars for histology-processed sections A, B: 2 cm.

Figure 4. Three-Dimensional Reconstruction of a Bilateral Infusion in the NHP Thalamus

A surgical planning software called iPlan Cranial® (BrainLab, Germany) was used to reconstruct a 3D spatial map of the infusion described in Fig. 3B. A) MRI-guided infusion into the right thalamus was evidenced by the spread of tracer co-infused with AAV2-hGDNF. B–D) Reconstruction of the infusion represented as a red 3D object, together with cannula trajectory, positioning and extent of infusate coverage within the NHP thalamus demonstrated in three different planes (frontal, cranial and sagittal). E) Demonstration of accurate bilateral infusion into the thalamus after 3D reconstruction of MR images (F; total volume infused: 300 μ L/side). Intensity of MRI contrast signal between right and left thalamus is due to differences in infusion times.