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Convection-enhanced delivery of AAV2 in white matter—A novel method for gene delivery to cerebral cortex

N.U. Barua[a], M. Woolley[a,b], A.S. Bienemann[a], D. Johnson[a,b], M.J. Wyatt[a], C. Irving[a,b], O. Lewis[a,b], E. Castrique[a], S.S. Gill[a,*]

[a] Functional Neurosurgery Research Group, AMBI Laboratories, University of Bristol, BS10 5NB, UK
[b] Neurological Applications Division, Renishaw Plc., Gloucestershire, UK

HIGHLIGHTS

- High volume white matter infusions were well tolerated in a large animal model.
- CED of AAV2/5-GFP into white matter resulted in GFP expression in cerebral cortex.
- Gene therapies could be targeted to cerebral cortex using this novel method.

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ABSTRACT

Background: Convection-enhanced delivery (CED) is currently under investigation for delivering therapeutic agents to subcortical targets in the brain. Direct delivery of therapies to the cerebral cortex, however, remains a significant challenge.
New method: We describe a novel method of targeting adeno-associated viral vector (AAV) mediated gene therapies to specific cerebral cortical regions by performing high volume, high flow rate infusions into underlying white matter in a large animal (porcine) model.
Results: Infusion volumes of up to 700 μl at flow rates as high as 10 μl/min were successfully performed in white matter without adverse neurological sequelae. Co-infusion of AAV2/5-GFP with 0.2% Gadolinium in artificial CSF confirmed transgene expression in the deep layers of cerebral cortex overlying the infused areas of white matter.
Comparison with existing methods: AAV-mediated gene therapies have been previously targeted to the cerebral cortex by performing intrathalamic CED and exploiting axonal transport. The novel method described in this study facilitates delivery of gene therapies to specific regions of the cerebral cortex without targeting deep brain structures.
Conclusions: AAV-mediated gene therapies can be targeted to specific cortical regions by performing CED into underlying white matter. This technique could be applied to the treatment of neurological disorders characterised by cerebral cortical degeneration.

1. Introduction

Convection-enhanced delivery (CED) describes a method of direct drug delivery to the brain though intraparenchymal microcatheters along an infusion-mediated pressure gradient (Bobo et al., 1994; Lieberman et al., 1995). The technique allows drugs which do not cross the blood–brain barrier to be delivered in therapeutic concentrations throughout large volumes of brain tissue, whilst minimising systemic exposure.

Since Bobo et al.'s original description of pressure-mediated infusion of macromolecules in white matter (Bobo et al., 1994), CED of therapeutic agents has been applied to a wide range of neurological disorders in both experimental studies and clinical trials. The majority of clinical studies have focused on disorders in which the pathology is localised to either white matter, or to deep grey matter structures such as malignant glioma and Parkinson's Disease (Bruce et al., 2011; Gill et al., 2003; Kunwar et al., 2010; Lang et al., 2006). Direct delivery of therapeutics to the cerebral cortex, however, remains challenging.

Targeting therapeutic agents to the cerebral cortex could be beneficial in a number of neurologicial diseases, most notably Alzheimer's disease and other forms of dementia, amyotrophic lateral sclerosis, multiple sclerosis and stroke. Convection-enhanced

* Corresponding author. Tel.: +44 01173403957.
E-mail addresses: steven.gill@nbt.nhs.uk, annub@bristol.ac.uk (S.S. Gill).

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delivery of adeno-associated virus (AAV)-based vectors to the thalamus has previously been shown to result in transgene expression in widespread cortical areas (Kells et al., 2009) as a consequence of both anterograde and retrograde transport of AAV vectors. However, selective targeting of agents to limited areas of cortex would require convection-enhanced delivery to specific thalamic nuclei which are not easily identified with conventional imaging methods.

The aim of this study was to determine the feasibility of performing high volume, high flow rate infusions into white matter in a large animal (NIH miniature pig) model, and to determine the potential of using this method for AAV-vector based delivery of transgenes to adjacent cortical regions.

2. Materials and methods

All work was conducted in accordance with the Animals (Scientific Procedures) Act (1986) and with the authority of appropriate UK Home Office project and personal licences. Study protocols were pre-approved by the University of Bristol Ethical Review Board.

2.1. NIH minipig anaesthesia

A total of 6 NIH miniature pigs (aged 6 months, 40–45 kg) were used in this study. Pre-anaesthetic medication comprising azaparone (2 mg/kg, Janssen Ltd., Bucks, UK) and ketamine (10 mg/kg, Vetoquinol Ltd., Buckingham, UK) were administered by deep intramuscular injection into the dorso-lateral neck muscles. Propofol (Abbot Laboratories, Kent, UK) was used for induction of anaesthesia, and anaesthesia maintained with isofluorane (Isoflo, Abbot Laboratories). Morphine 0.1 mg/kg (Morphine sulphate, Martindale Pharmaceuticals Ltd., Essex, UK) and Meloxicam 0.4 mg/kg slow IV, (Metacam 20 mg/ml solution for injection, Boehringer Ingelheim Vet Medica GmbH., Ingelheim, Germany) were administered for analgesia by intravenous injection.

2.2. Head immobilisation and brain Imaging

Head immobilisation and brain imaging were achieved as we have previously described (Bienemann et al., 2012; White et al., 2011) with minor modifications. An MRI-compatible polyurethane head frame was attached to the animal using 2 zygomatic screws, a mouldable palate tray and Velco snout strap. A fiducial arc was then placed onto the frame to facilitate pre-operative surgical planning. The animal was transferred to the MRI scanner, where Flex-L coils were attached to the fiducial arc and pre-operative scanning performed.

A Pathfinder robotic arm (Prosurgics, High Wycombe, UK) and surgical planning software (Mayfield ACCISS-II) were used for all catheter implantation procedures. Catheter entry points, targets and safe trajectories were planned in ACCISS-II. Following identification of the site(s) for catheter entry into the brain, a U-shaped scalp flap was raised and the skull surface exposed. Robot-guided hand-drills were used for drilling of precise burr-holes into which the catheter hub would push fit. The burr hole tooling comprised of a 1.2 mm drill followed by a 5 mm drill.

2.3. Catheter design

The implantable catheter system previously used for chronic and intermittent infusions into the porcine putamen (Bienemann et al., 2012) was used for white matter infusions with minor modifications. CED catheters were supplied by Renishaw (Renishaw Plc., Wotton-under-Edge, UK). The catheter system was composed of two components:

1. A carbothane guide-tube inserted on a tungsten carbide guide rod (outer diameter of 0.7 mm and inner diameter of 0.6 mm).
2. A central catheter composed of a carbothane tube (outer diameter of 0.6 mm/inner diameter 0.4 mm) protruding a variable distance from the distal end of the guide tube.

2.4. Catheter insertion procedure

The 1.2 mm drill was used to penetrate the full skull thickness at the chosen catheter entry location, and also to perforate the dura. The 5 mm hand-drill was then used to form a burr hole to accommodate the guide-tube hub. The catheters were then implanted. In all pre-operative surgical plans, we aimed to place the guide-tube tip at the junction of the cortical grey matter and underlying white matter of the frontal corona radiata. The catheter tip extended between 5 and 7.5 mm from the guide-tube tip in all procedures.

The proximal end of the catheter was attached to a commercially available septum seal (SoloPort^{TM} – Instech Laboratories, USA). The septum seal was implanted subcutaneously and allowed infusions to be performed percutaneously by insertion of a needle attached to an infusion line. The wound was closed with continuous 3/0 Vicryl sutures.

On completion of CED infusions, the animal was then taken out of the head fixation device and the zygomatic wounds closed with interrupted 3/0 Vicryl sutures. The animal was then woken from general anaesthesia. When the animal was able to protect its own airway, it was extubated and allowed to recover in isolation for 24 h. Animals were inspected twice daily during the recovery period for signs of wound infection or breakdown, neurological deficits or abnormal behaviour.

2.5. Contrast infusions in white matter

Infusions of 0.2% Gadolinium-DTPA (Magnevist, Bayer Healthcare, Germany) mixed in sterile artificial cerebrospinal fluid (aCSF, Torbay Pharmaceutical Manufacturing Unit, Paignton, UK) were undertaken immediately following catheter implantation using the following ramping regime – 0.5 μl/min for 5 min, 1.0 μl/min for 5 min, 2.5 μl/min for 5 min, 5 μl/min for 10 min ± 10 μl/min until completion.

The total volume of infusion ranged from 182.5 μl to 700 μl per catheter. A total of 6 white matter infusions performed in 6 animals were used for volume of distribution analysis. In the sixth animal two catheters were also implanted into the anterior and posterior frontal white matter of the same hemisphere in order to determine whether high volume infusions might facilitate distribution of contrast throughout the whole frontal corona radiata.

2.6. Co-infusion of AAV2/5-GFP

In 3 infusions, adeno-associated virus of serotype 2/5 expressing green fluorescent protein (AAV2/5-GFP, Vector Biolabs, Philadelphia, USA) was co-infused with 0.2% Gadolinium-DTPA in aCSF. The constructs comprised an AAV5 capsid, an AAV2 Inverted Terminal Repeat sequence and CMV promoter. Infusions were performed at a concentration of 10^{10} Vg/ml in aCSF.

2.7. MRI acquisition

Imaging was undertaken using an MRI scanner with field strength of 1.5 T (Intera, Philips, UK). Pre-operative MR imaging comprised contiguous T1-weighted coronal slices (0.8 mm slice thickness) of a volume which included fiducials and brain. For subsequent T1-weighted imaging (repeated at 15 min intervals) the parameters were – FOV: AP 200 mm: RL 159 mm: FH72 mm; voxel size: AP 0.575 mm: FH 0.8 mm; matrix size: $M \times P$ 378 × 277. The