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Crossing the blood–brain-barrier with viral vectors

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The abundant vasculature of the CNS provides a compelling route of administration for the delivery of gene therapy vectors if the limitations imposed by the blood–brain-barrier (BBB) can be overcome. There are two general approaches to transporting viral vectors across the BBB: either by transient disruption of brain microvasculature endothelial tight junctions, or through the use of receptor-mediated transcytosis. Advances in BBB disruption have led to pre-clinical success for both global and localized gene delivery, while therapies based on receptor-mediated transcytosis have recently advanced to phase I clinical trials in humans. Both approaches show long term promise for treating a wide range of CNS diseases.

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Introduction

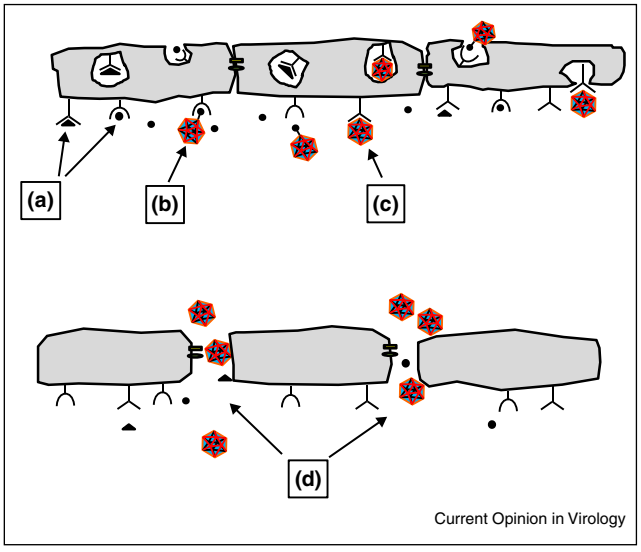
The central nervous system (CNS) is a critical target for gene therapy for the treatment of genetic, developmental, and acquired diseases. While some conditions, such as cancer, Parkinson's disease, stroke, or epileptic seizure may benefit from highly localized gene transfer, others, primarily genetic neurodegenerative diseases, require global gene delivery. New approaches are being developed to address both of these gene therapy modalities. The extraordinarily abundant vasculature of the brain lends itself to a systemic delivery approach to access the CNS, in that no neuron is more than 8 μm from a capillary. Standing in the way of this entry route is the blood–brain-barrier (BBB), an assembly of brain vascular endothelial cells, astrocytic endfeet, and pericytes, forming tight junctions that prevent most molecules greater than 400 Da from entering the CNS via the blood circulation [1]. While the BBB in mice is somewhat permeable in neonates, it is fully formed at birth in humans, and for clinical relevance, this review will therefore focus on gene delivery in adult animal models.

Paracellular transport by disruption of tight junctions

There are two very general approaches to transporting a viral vector across the BBB. The first is receptor-mediated transport across brain vascular endothelial cells by transcytosis; the second is transient disruption of the BBB, allowing the vector to enter by paracellular transport into CNS interstitial spaces (Figure 1). This has conventionally been accomplished by osmotically shrinking the cells that make up the BBB, using intravenous (IV) administration of highly concentrated mannitol (25%), a sugar alcohol that does not penetrate cells, and is rapidly excreted via the kidneys. This treatment has been used for decades to relieve intracranial pressure after traumatic brain injury or encephalitis, and has also been applied to increase the transport of chemotherapeutic drugs across the BBB to reach brain tumors [2,3]. While there are other methods available to increase the permeability of the BBB, including adenosine receptor activation and human serum albumin nanoparticles, they have not as yet been applied to viral vector delivery [4,5].

Mannitol-mediated osmotic disruption of the BBB has been used for CNS delivery of adenovirus (Ad), herpesvirus, adeno-associated virus (AAV), and SV40 [6–8]. This approach to treating neurodegenerative disease was demonstrated to be therapeutically beneficial in an adult mouse model of mucopolysaccharidosis type IIIB (MPS IIIB) [9•]. The disease, caused by mutations in N-acetylglucosaminidase (NAGLU), is characterized by profound CNS neurodegeneration due to accumulation of heparan sulfate and its derivatives in lysosomes. However, there are also significant somatic manifestations including hepatosplenomegaly, skeletal anomalies, and lysosomal storage in the peripheral nervous system, making MPS IIIB an ideal candidate for systemic gene delivery as long as sufficient vector can reach the CNS [10]. The deficient NAGLU gene was delivered IV using AAV serotype 2 (AAV2), which does not normally cross the BBB, in conjunction with mannitol pretreatment. While the number of cells transduced in the brain using this method was relatively low at approximately 1–2%, the uniform distribution within the brain parenchyma, combined with the cross-correction effects of the secreted NAGLU enzyme, provided global correction of CNS pathology. The timing of IV vector delivery relative to mannitol infusion was critical, with 10-fold greater transduction achieved at 8 min after

Figure 1



Brain microvascular endothelial cells (gray), with tight junctions between cells, form the primary barrier to CNS entry from the bloodstream. Receptor-mediated transcytosis **(a)** transports specific nutrients across the BBB by endocytosis from the apical side of the endothelium, and subsequent release on the basolateral side. Viral vectors can be fused or conjugated to the ligands for these receptors **(b)** allowing them to be transcytosed across the BBB. Alternatively, viral vectors can interact directly with the transcytosing receptors **(c)**, either naturally or by directed evolution. Finally, the tight junctions can be transiently disrupted by osmotic shock or other stresses, allowing paracellular transport of viral vectors **(d)**.

mannitol pre-treatment compared to 5 min or 10 min (Figure 1).

Localized disruption of the BBB

A more concentrated and localized vector transduction pattern can be achieved by transient disruption of the BBB using magnetic resonance-guided focused ultrasound and IV administered microbubbles, followed by IV administration of AAV vector [11•,12,13]. This method of BBB permeabilization has been developed for the treatment of a wide range of disorders, and relies on the mechanical action of the lipid/gas microbubbles, in response to ultrasonic pressure waves, to disrupt the brain endothelium for periods of approximately 6 h [14]. By IV infusion of AAV2/9 vector after local BBB disruption, foci of transduction could be directed to areas as small as 2 mm or as large as the right hemisphere of the mouse brain. Although the AAV9 capsid can naturally cross the BBB (see below) gene delivery to the CNS could be achieved at doses 10–50-fold lower than would typically be needed, and the transduced areas could be exquisitely controlled. This relatively non-invasive approach would be particularly well-suited to gene delivery strategies to mitigate brain injury or damage from stroke.

Localized gene delivery via a compromised BBB has also been studied in an epileptic seizure model wherein affected regions of the brain become accessible in response to the injury [15]. This offers the potential for delivery of therapeutics aimed directly at the seizure site. A directed evolution strategy was used to select AAV vectors from a library of eight DNA-shuffled serotypes after IV injection into rats with kainic acid-induced seizure [16]. Capsid coding sequences were recovered from the seizure-affected, neuron-rich piriform cortex and hippocampus at three days post-infusion, and the selection process was repeated twice more to enhance vector tropism for the seizure site. The selected vector clones were subsequently tested using a GFP reporter in the seizure model, and showed a striking specificity for the BBB-compromised brain regions. While each unique clone contained components of several AAV serotypes, they all contained the same region of AAV8 capsid. When compared to the parent AAV8 vector however, the selected clones showed higher levels of transduction at the seizure sites, and markedly lower transduction of other tissues, including liver, heart, and skeletal muscle. These highly specialized vectors may therefore offer a gene delivery treatment pathway in the immediate aftermath of an epileptic seizure.

Receptor-mediated transport via transcytosis

While paracellular transport through a compromised BBB has achieved some significant successes in animal models, the alternative strategy of exploiting receptor-mediated transcytosis to cross the BBB has shown a great deal more promise recently. These approaches have fallen into three general categories: using a viral vector that naturally crosses the BBB (archetypally, AAV9); modifying vectors to attach to a specific transcellular transport receptor; or, using directed evolution to create a vector that interacts with an effective receptor, without necessarily choosing what that would be. All of these take advantage of highly specialized pathways for moving large molecules across the BBB, mostly targeting well-studied transporters such as the transferrin receptor, LDL receptor-related proteins (LRP-1 and LRP-2), or insulin receptor [17].

Re-targeting adenovirus vectors

Adenovirus (Ad) vectors have been modified to bind specifically to a wide range of cellular receptors, either using conjugated adaptor molecules or peptide fusions into the fiber, penton, or hexon proteins [18]. One such modification, directed specifically at transport across the BBB, was targeted to the LRP-1 receptor. In this case, the extracellular domain of the coxackie-adneovirus receptor (CAR), which binds to the Ad fiber, was fused to melanotransferrin, which is transported by LRP-1 [19]. The fusion protein, sCAR-MTf, was pre-mixed with Ad vector and applied to the apical surface of polarized bovine brain microvasular endothelial cells grown in a transwell culture, modeling the BBB *in vitro*. Transcellular transport of