

# **Viral Vector Gene Delivery to the Brain to Treat the Disseminated Lesions of Neurogenetic Diseases: Focus on Lysosomal Storage Diseases**

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## Introduction

Neurogenetic diseases have a widely distributed pathology in the brain because most defective genes are involved in metabolic pathways (the inborn errors of metabolism) and consequently affect cells throughout the CNS. Gene transfer can correct biochemical defects within a diseased cell, but delivering a gene to a whole brain is a significant challenge, especially in humans, whose brains are 2000–3000 times larger than a mouse brain.

Certain viral vectors can be transported along neural pathways to distal sites. Combining virus properties with selected neuronal pathways can thus form a strategy for delivering a therapeutic gene over a large volume of brain from localized injection sites. Several strategies have attempted to deliver genes across the blood–brain barrier, as the vascular bed is distributed uniformly throughout the brain. Some vectors have shown promise for intravascular gene delivery, which would obviate the need for surgery. In addition to vector properties, some proteins' properties allow for wider delivery of the therapy, e.g., the secretion uptake mechanism of many lysosomal enzymes, which are responsible for a large number of the neurogenetic disorders. Recent advances in animal experiments suggest it may be possible to treat a large portion of the human brain using a clinically feasible number and volume of injections.

## Brain Size and Structure

The human brain is ~800 g at 1 year of age (when many human genetic-disease patients are likely to be treated) and grows to ~1200 g in adults. In contrast, the mouse brain is only ~400 mg in the adult: 2000–3000 times smaller. Although rat brains are about 5 times larger (2 g), there are very few human neurogenetic disease models in the rat. The brains of domestic mammals are much larger than rodent brains, and a large number of animals with human genetic diseases have been identified, mainly in cats and dogs, many of which involve the CNS. Their brains are approximately 100–200 times larger than a mouse brain, and they have a gyrencephalic structure (cortical folds with gyri and sulci), compared to the lissencephalic rodent brain. The large animals model the obstacles to global gene and/or protein delivery in humans better than mice.

## Animal Models of Human Neurogenetic Diseases

Testing gene therapy methods in valid animal disease models is valuable for designing successful and predictable approaches for human gene therapy. With human patients, it is not feasible to study the large numbers of individuals, at multiple time points, needed to compare various parameters of vector design, control of gene expression, and the fate of transduced cells. For these purposes, a large number of mouse models of human neurogenetic diseases have been identified or created. Many are good phenocopies of the human disorder, but they vary in fidelity from one gene to another. A large database on the genetics, biochemistry, pathology, and comparison of various treatment modalities now exists for these models, providing the basis for comparing and improving new methods of therapy.

Testing experimental therapies under actual disease conditions provides information on the responses of the affected organs that would not be apparent in normal animals. Genetic diseases typically result in lesions distributed throughout the brain, requiring global distribution of the gene vector or the therapeutic protein. The large animal brains provide a more accurate model of the conditions that are present in human diseases. The large animals also provide better models for testing noninvasive imaging modalities, such as magnetic resonance imaging or spectroscopy of disease processes, and can be performed using human clinical magnets and apparatus.

Another advantage of large animal models is that they are treated and evaluated as individual patients, so the range of success and failure can be assessed. At the same time, large statistically significant cohorts of affected animals and normal controls can be produced and evaluated. Variations in age at treatment and analysis, class of gene therapy vector, route of administration, and other experimental variables can be studied. In human genetic disease populations, by comparison, it would be virtually impossible to assemble the statistically significant cohorts or to treat and evaluate normal controls. In addition, human genetic disease populations are small but often harbor a variety of mutations, whereas genetically diseased animal breeding colonies typically contain a single mutation. These animal colonies provide a uniform genetic mutation that causes the disease, while the genetic background of large animals within a colony is relatively outbred compared with inbred strains of mice. Another

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advantage for translation to clinical trials is that the large animal species live much longer than rodents. This longevity allows studies on long-term effects of treatments, both for efficacy and adverse results.

### **Lysosomal Storage Diseases: Biochemical and Cell Biological Basis for Correction**

The lysosomal storage diseases (LSDs) are caused by inherited deficiencies of lysosomal enzyme activity. More than 60 such human diseases have been identified. Although most are caused by mutations in the enzyme structural genes, some involve defects in modifying genes that are necessary for enzyme maturation or transport molecules. Most of the LSDs are characterized by severe, progressive degenerative syndromes with onset in early childhood that affect multiple organ systems.

Treatment strategies for these diseases are based on the observation that most lysosomal enzymes can be released from normal cells and taken up by mutant cells. The extracellular enzyme is endocytosed and is activated when the endosome fuses with the acidified lysosome. In the lysosome, it degrades the accumulated substrate to correct the metabolic deficiency (called cross-correction). Studies of corrected cell transplantation (*ex vivo* gene therapy) and direct viral gene transfer (*in vivo* gene therapy) in animal disease models have shown that sufficient levels of normal enzyme can be expressed in order to arrest or reverse some of the pathological effects of the diseases. Gene therapy for LSDs thus depends on correcting a portion of the deficient cells in the patient, which can then supply the missing enzyme to non-transduced cells. Although the effectiveness varies with the disease, treatment performed at a young age produces a better clinical outcome than in older animals. However, most patients with a LSD are not diagnosed until they begin to display significant pathology during early childhood, making it important to understand the effects of intervention after the disease is considerably advanced.

Treating the brain disease is critical in human patients with LSDs, most of which involve developmental disabilities in brain function. Although non-CNS pathology can be at least partially treated (using bone marrow transplants, enzyme replacement therapy, or gene therapy to provide soluble enzyme into the circulation), treating the CNS component is more difficult because the enzyme does not easily cross the blood–brain barrier.

### **Viral Vectors**

Many animal models of LSDs have been treated using viral vector-mediated gene transfer strategies to deliver the normal enzyme. Similar distribution of the gene can be expected for most other genes as well, but efficacy will depend on the biochemistry and cell biology of the gene product. The most commonly used vector systems for *in vivo* transduction have all been studied in the CNS in animal models of LSD. Variable amounts and patterns of transduction can be achieved in the brain with lentivirus (LV), herpes simplex virus (HSV), adenovirus (AdV), or adeno-associated virus (AAV) vectors (Cearley and Wolfe, 2009). When animals are treated as neonates or fetuses, the progression of disease can be arrested before significant pathology occurs. However, initiating treatment in adult animals even when the pathology is advanced has shown that significant reversal of established lesions can be achieved; also, improvements in behavioral tests and neurodegeneration parameters can occur. Furthermore, studies have demonstrated that in some neural pathways, the enzyme can be transported to distal neuronal cell bodies, which can amplify the sphere of correction far beyond the injection site.

### **Lentivirus**

Lentivirus (LV) vectors have been developed from human immunodeficiency viruses, simian immunodeficiency virus, feline immunodeficiency virus, and equine infectious anemia virus (Jakobsson and Lundberg, 2006). The major advantage of LVs for delivery to the CNS is that they can transduce post-mitotic cells such as neurons; thus, they can be injected directly into the brain. LV vectors become stably integrated into the host-cell genome and can mediate long-term expression of the transgene. To reduce the potential for activating a proto-oncogene, LV vectors are designed to be self-inactivating; in LVs, the long terminal repeat promoter and enhancer are deleted from the integrated provirus form. This design also eliminates competition with the internal promoter in the expression cassette.

LV vectors can transduce most cell types within the CNS, including neurons, astrocytes, oligodendrocytes, and stem cells. The host range can be expanded by pseudotyping LVs with surface glycoproteins from a number of other enveloped viruses, including vesicular stomatitis virus G, MuLV, Mokola, lymphocytic choriomeningitis virus, and rabies virus. Each pseudotyped vector transduces different subgroups of cells within regions of the CNS. The selectivity is mediated by variable expression

of the cell-surface receptors to which the viral envelope protein binds. Thus, targeted transduction within the CNS can potentially be achieved using a combination of cell-tropism and cell-type-specific promoters. Some evidence also shows that LV vectors undergo retrograde transport, but this does not occur in all nervous system pathways.

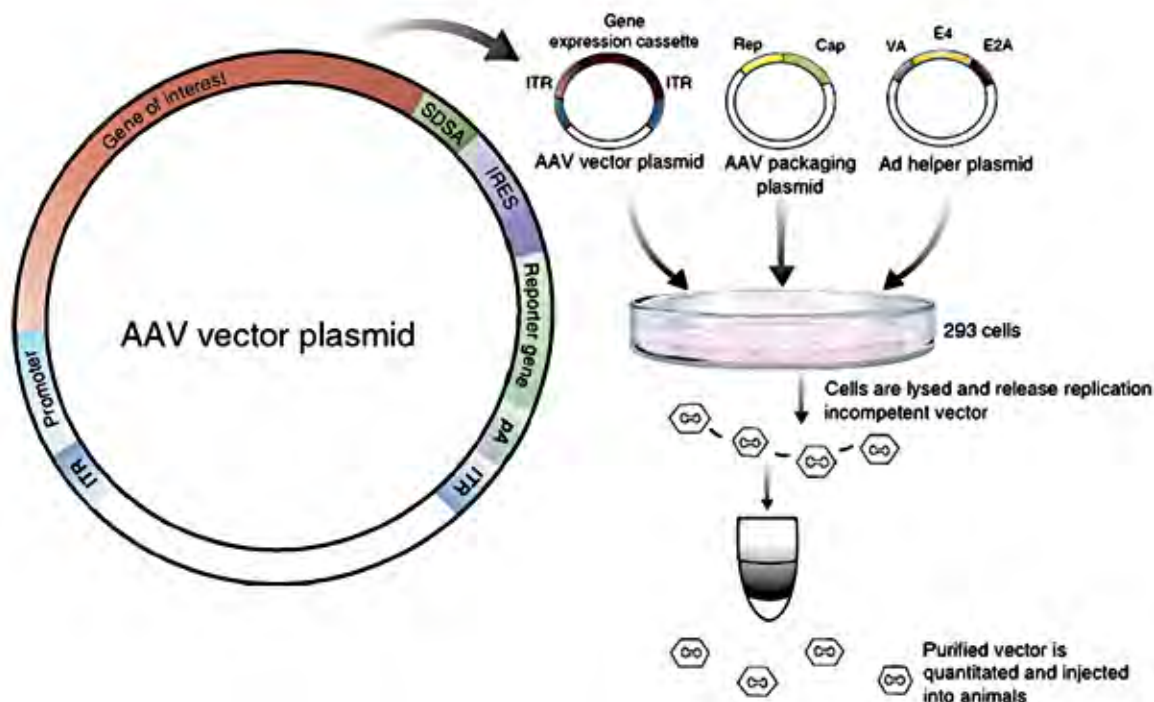
### Herpes simplex virus

HSV is the only naturally neurotropic virus used as a vector. HSV has a large double-stranded DNA genome with approximately 80 genes, but many of the genes are not required for replication (Mata et al., 2003; Berges et al., 2007). Thus, much of the genome can be deleted so that HSV vectors can potentially hold large inserts of foreign DNA. Human

HSV-1 is the most commonly used herpes virus vector system for experiments involving the CNS, although porcine pseudo-rabies virus has been used for short-term tract-tracing studies. HSV-1 vectors establish an episomal latent infection in neurons; thus, insertional mutagenesis is not thought to occur. There are two types of HSV-1 vectors: recombinant replication-competent vectors and non-replicating amplicons that must be made in packaging cells. Both types can mediate gene delivery to CNS cells.

### Recombinant HSV vectors

Recombinant HSV vectors consist of a wild-type HSV genome, selected for either loss of viral pathogenic effects or deletion of genes necessary for replication. Because HSV-1 is a naturally neurotropic



**Figure 1.** Example of viral vector design and production. To make an AAV vector, a 3-plasmid transfection system is used, consisting of the AAV vector plasmid, an AAV packaging plasmid, and an Ad helper plasmid. The AAV vector plasmid (enlarged view on the left) contains the gene of interest, flanked by the inverted terminal repeats (ITRs), which are the only elements from the wild-type AAV included in the plasmid. The AAV packaging plasmid contains the AAV genes necessary for genome replication (Rep) and capsid protein formation (Cap), but these are not flanked by ITRs and thus are not incorporated into the virion. AAV is a dependovirus, which requires functions supplied by a helper virus, e.g., AdV or HSV. For vector production, a helper plasmid encoding the minimum gene functions needed for replication is used. The 3 plasmids are transfected together into a packaging cell line, such as 293T cells, and normal cellular pathways are used to express the genes and proteins leading to the assembly of viral particles. The particles are released into the media after cell lysis or budding from the cell surface, depending on the type of virus. The vector particles are isolated from the lysate using cesium chloride gradient, pelleted by centrifugation, or column-purified. The vector viral particles are then quantified and can be used for experimental purposes. Production of most vector viruses is done using a similar strategy, with the functions supplied either in trans on plasmids, or by helper viruses, which vary depending on the virus type. IRES, internal ribosomal entry site; pA, poly-adenylation; SDSA, splice donor–splice acceptor.

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virus, only low titers are needed in order to spread the replicating virus in the CNS. Recombinant HSV vectors are thought to establish a persistent latent state as a nonintegrated nuclear element, similar to wild-type HSV. In natural infection, after latency is established, only one viral gene is expressed: the latency-associated transcript (LAT), which is expressed for the lifetime of animals. Thus, the promoter of the LAT sequence has been used to drive long-term expression of therapeutic genes in the CNS.

#### **Nonreplicating HSV amplicon vectors**

The HSV amplicon vector system is based on a eukaryotic expression plasmid that can be packaged (with the viral genes supplied in trans) or a helper virus-free method. HSV amplicons have a theoretical packaging capacity of up to 130 kb. Because the HSV amplicon vector can accommodate such a large insert, typical vectors are constructed with 13–15 kb foreign sequences, which are concatamerized in a single vector. This packaging results in an increased transgene dose per infected cell. A disadvantage of using the amplicon-based system is that these vectors result in relatively limited transgene expression, and expression in dividing cells is transient because vector DNA is lost during mitosis. For these reasons, hybrid vectors have been developed that combine components of the HSV-1 amplicon with genetic elements from other types of viral vectors. HSV–AAV hybrid vectors have been made that combine the benefits of both vector systems, resulting in a vector that can package very large inserts, target multiple cell types, and result in high expression levels for an extended period of time.

#### **Adenovirus**

Adenovirus (AdV) vectors are non-enveloped, with a double-stranded DNA genome. AdV vectors have both significant advantages and disadvantages for gene therapy experiments in the CNS (Davidson and Breakefield, 2003). Advantages include their simplicity to generate and ability to transduce a variety of cell types, both dividing and post-mitotic, including neurons, astrocytes, oligodendrocytes, and ependyma. Transduction using AdV vectors also results in high levels of transgene expression. Another potential advantage is that, in some cases, AdV vectors are transported along neuronal pathways.

The main disadvantage of using AdV vectors is that they are highly immunogenic, a quality that is the main contributor to loss of transgene expression after administration. AdV vectors target dendritic cells,

which facilitates immune stimulation. Most humans carry circulating antibodies against adenoviruses as a result of natural infection, which is a potential barrier to clinical use. One approach to reduce this effect is to alter the capsid protein to resemble less immunogenic forms. Another is to use a “gutless vector,” which has a genome deleted of all the Ad proteins, which are supplied in trans to form a virus. Gutless AdV vectors have mediated, stable, long-term expression of a transgene in the CNS and can accommodate an insert of approximately 30 kb, whereas replication-defective AdV vectors carry only approximately 8 kb.

#### **Adeno-associated virus**

AAV is a category of non-enveloped parvoviruses with a single-stranded DNA genome. AAVs are naturally replication-defective and can only replicate when co-injected with a helper virus (AdV or HSV). AAV vectors are made in a transfection cell system in which the replication and other functions are provided in trans (Mandel et al., 2006; Wu et al., 2006). This system results in the vector genome containing only about 300 nucleotides of viral sequence.

AAV vectors are one of the most widely used gene therapy vectors in CNS experiments. When directly injected into the brain, AAV vectors transduce mostly neurons, but some serotypes can also transduce other neural cell types. AAV vectors result in a delayed-onset but persistent transgene expression in the CNS. AAV vectors can be concentrated and purified to very high titers, resulting in widespread and stable transduction with low toxicity. The limitations of AAV vectors include their small cloning capacity (4.5 kb), which limits their use for large therapeutic genes or many genetic regulatory elements. AAV vectors are relatively inefficient for *in vitro* experiments compared with other vectors, but they can transduce a number of neural cell types. AAV vector production is also labor-intensive and time-consuming.

Numerous serotypes of AAV capsid genes are available (~100) and each is a sequence variant of the capsid protein (Cearley et al., 2008). Each serotype has been shown to have different transduction characteristics in the CNS. The AAV2 serotype vector has been widely applied in CNS applications, though it has relatively low transduction. The use of several other serotypes in the CNS is increasing, especially serotypes 1, 5, 9, and rh10, and many new variants have been tested. Different serotypes can produce different patterns of transduction when injected into

specific regions of the CNS, and these characteristics can be used to selectively target certain cells. There is evidence that the AAV vector serotypes 1, 5, 9, rh10, and others are transported within the CNS along various neuronal pathways.

### Combining Vector Properties with Routes of Delivery to Increase Enzyme Distribution

The properties of vectors and lysosomal enzymes have been combined in different ways to target larger volumes of brain tissue in mice. For example, AAV4 injected into the ventricles can transduce ependymal cells, and the secreted enzyme is distributed via the circulating CSF (Liu et al., 2005). When AAV1, 9, or rh10 is injected in a small volume into projection nuclei originating in the brainstem/pons region, it can be transported widely enough so the subsequent enzyme secretion from the dispersed gene expression sites is sufficient to treat the whole brain (Cearley and Wolfe, 2007). Other sites with widespread connections within the brain (including the hippocampus, striatum, and thalamus) have been used to disseminate the enzyme and/or gene (Cearley and Wolfe 2007; Baek et al., 2010). These studies have shown that when the correct vector and neuronal system are used, the volume of treated brain tissue can be significantly increased while reducing the number and volume of injections. Another promising approach is the recent finding that intravenous injection of AAV9 crosses the blood–brain barrier and can deliver therapeutic amounts of a lysosomal enzyme (Foust et al., 2009; Fu et al., 2011).

### Human Trials

Phase 1 clinical trials have been performed with AAV in two neurogenetic diseases: Canavan disease and late infantile Batten disease. These trials were designed to assess safety of the reagents and procedures. Although there is little evidence that the diseases were improved, that is probably the result of the limited number of injections allowed and use of AAV2, which is the least effective AAV. Thus, the total amount of normal protein delivered was probably below the threshold needed for efficacy. This conclusion is supported by experiments using multiple injection sites spaced throughout the brain, which have been shown to be medically beneficial in large animal disease models such as cats with alpha-mannosidosis (Vite et al., 2005). Using more efficient serotypes and targeting the injections to the neural systems most likely to disseminate the gene and/or protein may allow effective scale-up to

the human brain. Additional trials are planned in Batten's disease and other LSDs using AAV serotypes that have shown wider distribution in animal studies.

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