Gene Therapy for Neurologic Manifestations of Mucopolysaccharidoses

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Abstract

Introduction—Mucopolysaccharidoses are a family of lysosomal disorders caused by mutations in genes that encode enzymes involved in the catabolism of glycoaminoglycans. These mutations affect multiple organ systems and can be particularly deleterious to the nervous system. At the present time, enzyme replacement therapy and hematopoietic stem-cell therapy are used to treat patients with different forms of these disorders. However, to a great extent the nervous system is not adequately responsive to current therapeutic approaches.

Areas Covered—Recent advances in gene therapy show great promise for treating mucopolysaccharidoses. This article reviews the current state of the art for routes of delivery in developing genetic therapies for treating the neurologic manifestations of mucopolysaccharidoses.

Expert Opinion—Gene therapy for treating neurological manifestations of mucopolysaccharidoses can be achieved by intraventricular, intrathecal, intranasal, and systemic administration. The intraventricular route of administration appears to provide the most wide-spread distribution of gene therapy vectors to the brain. The intrathecal route of delivery results in predominant distribution to the caudal areas of the brain while the intranasal route of delivery results in good distribution to the rostral areas of brain. The systemic route of delivery via intravenous delivery can also achieve wide spread delivery to the CNS, however, the distribution to the brain is greatly dependent on the vector system. Intravenous delivery using lentiviral vectors appear to be less effective than adeno-associated viral (AAV) vectors. Moreover, some subtypes of AAV vectors are more effective than others in crossing the blood-brain-barrier. In summary, the recent advances in gene vector technology and routes of delivery to the CNS will facilitate the clinical translation of gene therapy for the treatment of the neurological manifestations of mucopolysaccharidoses.

Keywords

Enzyme replacement therapy; Gene therapy; Hunter syndrome; Hurler syndrome; Sanfilippo syndrome

1.0 INTRODUCTION

1.1. Mucopolysaccharidoses

The mucopolysaccharidoses (MPS) are a group of recessively inherited disorders included in the larger family of more than 40 identified lysosomal storage diseases (LSD). LSD comprise approximately 14% of all inherited metabolic diseases and affect nearly 1:7,700 births, of which ∼30% are MPS (1,2). MPS are caused by mutations in genes that encode lysosomal hydrolases, that are responsible for the degradation of glycosaminoglycans (GAG), primarily found in the extracellular matrix.. Deficiency for any of the enzymes involved in this process results in lysosomal accumulation of GAG storage material and resultant progressive, multisystemic disease (3). MPS diseases are typically manifest as symptoms including growth delay, organomegaly, cardiopulmonary disease, skeletal dysplasias, and obstructive airway disease. Additionally, patients with severe forms of MPS I, II, III, and VII exhibit severe neurocognitive decline. Symptoms develop within the first two years of life and, without treatment, severe MPS patients typically succumb to disease complications within the first decade of life. Current available therapies used to treat the MPS include allogeneic hematopoietic stem cell transplantation (HSCT) and recombinant enzyme replacement therapy (ERT) for MPS II, VI, and I. Although somewhat efficacious in treating manifestations of disease in peripheral organs, the benefits of ERT in the central nervous system (CNS) are limited due to the inefficiency of lysosomal enzymes to cross the blood brain barrier (BBB) (4). Thus, the focus of this article is to review gene therapy and routes of delivery as an alternative form of treatment that could be used as a supplement to HSCT and ERT in order to more effectively deliver lysosomal enzymes to the CNS.

1.2. Current Therapies for MPS Diseases

The molecular basis for developing treatments for MPS was first discovered in the laboratory of Elizabeth Neufeld in 1968. Cultured fibroblasts from a MPS I patient and fibroblasts from a MPS II patient demonstrated progressive accumulation of GAG over time. However, when cells from both genotypes were mixed together, they were able to complement each other and degrade GAG in normal fashion (5). This suggested that lysosomal enzymes made in one cell could be taken up by another cell to achieve "cross-correction" of the respective enzymatic deficiencies. Additional experiments revealed that lysosomal enzymes are translated in the rough endoplasmic reticulum (RER) before being transported to the lumen of the organelle through interaction between a hydrophobic amino terminal signal peptide on the nascent protein and an 11S signal recognition ribonucleoprotein (6,7). In the lumen of the RER and Golgi, the lysosomal proteins undergo posttranslational modification by the addition of oligosaccharide moeities (mannose and/or mannose 6-phosphate) to Asn residues (8). These modified proteins are then sorted in the Golgi by binding to mannose 6-phosphate receptors (MPRs) (9,10). The ligand-receptor

complexes are then coalesced into coated vesicles, which travel to lysosomes where the ligand-receptor complexes are dissolved by the acidic pH of the lysosome, thereby delivering the lysosomal proteins to their destination (11).

Although most of the lysosomal enzymes are effectively delivered to the lysosome, between 5–20% of the enzymes are released to the extracellular space without being trafficked to the lysosome (12). These extracellular enzymes can interact with MPRs on the surface of neighboring cells and become internalized before being shuttled to the lysosome. This "cross-correction" mechanism by which lysosomal enzymes produced in one cell are able to travel to and degrade GAG storage material in the lysosomes of neighboring cells forms the molecular basis for treating MPS. Current therapies based on this molecular principle include hematopoietic stem cell transplantation and recombinant enzyme replacement therapy.

1.2.1. Hematopoietic Stem Cell Transplantation (HSCT)—Treatment of MPS by transplantation of hematopoietic stem cells (HSC) is based on the rationale that donor-derived HSC are able to engraft in the recipient and differentiate into many cell types, including Kupffer cells in the liver (13), tissue macrophages in the spleen and lungs, and microglial cells in the brain (14). These and other donor-derived cells then provide enzyme to deficient cells via metabolic cross-correction, thereby clearing GAG storage material from host tissues. The first bone marrow transplant (BMT) for the treatment of MPS was performed in 1980 on a child with Hurler syndrome (MPS I H) (15). Since then, there have been approximately 500 children who have received BMT for MPS I, II, III, IV, VI, and VII. Most of these, to date, have been MPS I H patients (16). These patients have received cells from a variety of donor sources including bone marrow or peripheral blood stem cells (PBSCs) from either related or unrelated heterozygous or unaffected individuals as well as HSCs from unrelated umbilical cord blood (UBC) units. Since it is only possible to identify non-carrier human leukocyte antigen (HLA) matched bone marrow donors for about 1 in every 16 patients, many patients have recently been treated with unrelated HLA-matched cord blood as an alternate source of donor cells (17).

Prior to infusion of donor cells, patients receive a preconditioning regimen to create space for engraftment of donor cells by treatment with cyclophosphamide (Cy) and either busulphan (Bu) or total body irradiation (TBI) (17–19). In MPS I H, successful donor cell engraftment seems to be dependent on cell dose (greater success in patients receiving more than 3.5×10^8 cells/kg). 15–37% of patients failed to engraft and displayed autologous recovery after receiving more than 3.5×10^8 cells/kg. However, patients who received fewer than 3.5×10^8 cells/kg bone marrow cells failed to engraft at a rate of 72% (19–22). Of patients that did engraft, 45–86% engrafted to levels of 95% donor chimerism. Patients receiving unrelated cord blood transplants have overall fared better, with a graft rejection rate of 8% and with 97% of engrafted patients exhibiting 90% donor chimerism (23).

Following successful engraftment of donor HSC, patients rapidly display phenotypic correction. Within a few months of receiving a transplant, the lungs, spleen, liver, and upper respiratory symptoms show evidence of improvement. Most patients experience resolution of hepatosplenomegaly within a year after transplant. There is a decrease in cardiac

hypertrophy, although cardiac valve deformities still persist (23). Growth and development of the musculoskeletal system improves following transplant and many patients report an increase in joint mobility. Typically, corneal clouding partially resolves and many patients demonstrate improved hearing. Most importantly, overall survival of Hurler patients following transplant is between 50–85% with some of the first transplant patients living into the third decade of life.

With respect to neurological outcomes following HSCT, the age and severity of disease of the patient at the time of transplant is paramount to the overall benefit achieved (18–23, 24). The earlier the transplant is performed, the better chance of preventing lysosomal accumulation of GAG and associated secondary storage materials GM2 and GM3 gangliosides within the brain (25). There exists a wide variability in overall success of neurological outcomes of Hurler patients following HSCT. However, it has been reported that hydrocephalus improves and neurocognitive decline stabilizes in many patients following transplantation. Nonetheless, recipients continue to exhibit below-normal IQ and impaired neurocognitive capability (26,27). Furthermore, although some improvements in white matter and cribiform lesions have been detected using MRI brain-imaging techniques in patients with MPS II and VI after BMT, these improvements are incomplete, variable, and do not occur in all patients (28).

1.2.2. Enzyme Replacement Therapy—As recently as 2003, ERT was approved by the U.S. Food and Drug Administration for the treatment of MPS I. Today, ERT is available for the treatment of MPS I, II, and IVA, and VI in the forms of aldurazyme®, elaprase®, vimizim®, and naglazyme® respectively. The resultant drugs are administered by weekly intravenous infusion and most patients begin ERT immediately upon diagnosis. In the case of MPS I, mildly affected patients with Scheie syndrome (MPS I S) typically receive ERT while more severely affected Hurler-Scheie (MPS I H/S) and Hurler patients receive ERT followed by HSCT once an appropriate cell donor source has been identified.

Similar to the experience with HSCT, the response to ERT seems related to the severity of disease at the onset of treatment. Generally, following treatment with ERT, patients display a decrease in urine GAG excretion, resolution of hepatoslenomegaly, and improvement in cardiopulmonary function as measured by a 6-minute walk test within the first six months of treatment (29). Although greater than half of the patients treated with ERT develop antibodies to the recombinant proteins, they are mostly IgG antibodies and no connection has been made between development of these antibodies and decrease in efficacy of the drugs (30). Although the results of ERT are promising, intravenous infusion of ERT fails to provide enzyme to the CNS due to inability of the enzymes to efficiently cross the blood-brain barrier (31–36). This limitation has led to recent, ongoing clinical trials whereby these drugs are being infused intrathecally into the leptomeningeal space in order to directly access the CNS (37–38).

The goal of intrathecal ERT is to treat spinal cord compression and to provide enzyme to the brain through circulation within the cerebrospinal fluid (CSF). Following intrathecal infusion of laronidase into MPS I dogs, high levels of IDUA activity have been detected in the spinal, cervical, and lumbar meninges, associated with a 57–70% reduction in GAG

storage material in these tissues. Furthermore, in MPS I dogs, infusion of laronidase into the cisterna magna resulted in clearance of storage material in glia, perivascular cells, and neurons as well as normalization of quantified GAG (32,39). Currently, several clinical trials are underway to assess the safety and efficacy of intrathecal laronidase in MPS I patients. with results expected to be published shortly.

HSCT and ERT have both proved to be efficacious for the treatment of MPS. However, many challenges remain, including providing enzyme that is able to penetrate the cardiac valves, deep skeletal tissue, and the CNS in sufficient levels to prevent/reverse clinical outcomes. In the case of HSCT, identifying a proper source of donor HSC is not trivial and takes precious time during which patients continue to deteriorate. Additionally there is significant morbidity and mortality related to the transplantation procedure itself. With ERT, major limitations include the frequency of intravenous infusions (weekly) as well as the estimated costs of treatment that are among the highest for all diseases, between $250,000-$1,000,000 annually per patient (40)(http://www.medicalbillingandcoding.org/blog/the-11-most-expensive-medicines-in-america/). Moreover, questions remain regarding the overall efficacy of HSCT in preventing and/or reversing neurocognitive symptoms. Intrathecal ERT is a promising approach for delivering enzyme to the CNS, although effective infusion regimens need to be validated. At this time, more effective and affordable strategies to deliver lysosomal enzymes to the CNS, such as by gene transfer, must be developed as viable alternatives and/or adjunctive approaches to treat patients with MPS disorders.

2.0. GENE DELIVERY SYSTEMS

Many preclinical studies have been conducted during the course of the last few decades investigating the use of gene therapy vectors to deliver lysosomal enzymes to the CNS in animal models of MPS. Viral vectors generally have been used in these experiments including retroviruses, lentiviruses, adenoviruses, and adeno-associated viruses (AAV). However, non-viral approaches using *Sleeping Beauty* transposons have also been tested. Different routes of administration have been investigated including *ex vivo* transduction of HSC and *in vivo* infusions through intravenous, intrathecal, intracisternal, intraparenchymal, and hydrodynamic injections. The advantages and disadvantages of each system are discussed below.

2.1. Retroviral and Lentiviral Vectors

Retroviruses are a group of spherical 80–100nm diameter viruses that utilize two identical strands of RNA as genetic material. The basic retroviral genome contains three genes, *gag*, *pol*, and *env*, which encode proteins necessary for viral infection and reproduction. These sequences are flanked by long-terminal repeat (LTR) sequences required in *cis* for reverse transcription and integration. The reverse transcriptase enzyme, encoded by *pol*, produces a double-stranded DNA copy of the RNA viral genome (41) upon entry into a host cell. Once converted into double-stranded DNA and transported to the nucleus, the viral DNA is able to integrate into the host genome through interaction between the virally encoded integrase and the host genome, thereby creating a provirus. Activation of transcription and translation of the integrated viral genes by the host-cell machinery then results in production of viral genomes and proteins necessary to produce additional virions and complete the lytic life

cycle. Replication of viral vectors is generally unwanted. Hence, replication-incompetent vectors have been engineered whereby the viral genes and their promoter have been replaced by an alternative expression cassette comprised of a cellular promoter juxtaposed to the gene encoding the desired therapeutic protein, in this case a specific lysosomal hydrolase that is deficient in MPS patients. These vectors can be used to mediate integration of the therapeutic expression cassette into the host genome. Thus, long-term expression of the desired gene product can be achieved in target cells. We did the first clinical trial of gene therapy for a MPS disease, employing a gammaretroviral vector (42, 43). However, the use of gamma retroviral vectors as gene transfer vehicles is hindered by their inability to transduce non-dividing cells, which is necessary for transducing most cells in the brain.

HIV-1-based lentiviral vectors have been engineered to deliver therapeutic genes of interest into target cells (44). The advantage of lentiviral vectors compared to gamma retroviral vectors is that they are able to transduce non-dividing cells, including terminally differentiated cells such as neurons (45). However, since retroviruses and lentiviruses integrate selectively into transcriptional regulatory regions of the host genome (46), concerns remain about the risk of insertional mutagenesis when using these types of vectors. During clinical trials for X-linked Severe Combined Immunodeficiency (SCID) in France and the United Kingdom, several patients developed leukemia following treatment with a gamma retroviral vector due to insertional mutagenesis (47,48). However, further studies of integrating vectors has revealed that in most cases, adverse events have arisen from combinations of the vector, the transgene, and the corrected cells (49).

2.2. Sleeping Beauty (SB) Transposon System

The *Sleeping Beauty* (SB) transposon system is a nonviral, plasmid-based gene-transfer system that was constructed by reverse engineering of extinct DNA sequences found in salmonid fish (50–52). The system consists of two components - the transposon that is defined by inverted repeat (IR) sequences that flank a desired genetic cargo (an expression cassette encoding a therapeutic protein), and the SB transposase enzyme. The gene encoding SB transposase can be included on the same plasmid as the transposon (*cis* configuration) or on a separate plasmid (*trans* configuration). Following delivery into a target cell, two SB transposase molecules interact with each IR to excise the transposon from the plasmid delivery vehicle and simultaneously integrate the transposon into any of the approximately 2×10^8 TA dinucleotide sequences contained in mammalian genomes (53). These integration events allow for stable, long-term expression of genes contained in the genetic cargo of an engineered SB gene-therapy vector. The advantages of using the SB transposon system rather than viral vectors as gene therapy vehicles include that they are easier and less expensive to manufacture, and that the plasmids themselves have low immunogenicity in comparison to viruses (53). However, like retroviruses, the SB transposon system does have the potential risk of insertional mutagenesis. It should be noted that the SB transposon system is currently being used in clinical trials to treat B-cell lymphoid malignancies (54).

2.3. Adeno-associated Viral Vectors (AAV)

AAV is a small (26nm in diameter) single-stranded DNA parvovirus that causes no known pathogenic disease in infected humans. The wild type AAV genome is 4.7 kilobases in

length and contains two open reading frames, *rep* and *cap*, flanked by inverted terminal repeat sequences (ITRs). *Rep* encodes four overlapping genes encoding Rep proteins required for the viral life cycle. *Cap* includes three overlapping sequences encoding the capsid proteins VP1, VP2, and VP3, which assemble in a 1:1:20 ratio to form the icosohedral viral capsid (55–57). AAV requires co-infection by adenovirus or herpes simplex virus to complete its viral life cycle (58–60). To generate therapeutic AAV vectors, the *rep* and *cap* sequences are removed from the viral genome and replaced by an expression cassette including a promoter regulating expression of a therapeutic gene of interest. Three plasmids (one containing the ITRs flanking the therapeutic gene, one containing *cap* and *rep*, and one containing helper proteins) are co-transfected into producer HEK293 cells. The viral genome is packaged into virions produced within the triply transfected cells. Recombinant AAV then can be purified from cell lysates using density gradients, affinity columns, or ion exchange purification methods (61–62).

Recombinant AAVs (rAAV) have become effective tools for use as gene therapy vectors for several reasons. rAAVs are non-pathogenic and are capable of transducing a variety of tissues and cell types including brain, liver, heart, and muscle (63–66). Despite persisting in the form of extrachromosomal DNA once inside a host cell, rAAVs are able to maintain stable expression of transgenes for periods greater than 1.5 years in various animal models including mice, dogs, and hamsters. rAAV vectors have led to transgene expression for up to eight years for hemophilia (67). Although wild-type AAVs are capable of integrating into the AAVS1 site on chromosome 19 through interaction between the ITRs and the chromosome with the help of Rep proteins, rAAVs integrate into the host genome at very low frequency when the viral Rep gene has been removed (63,68). The low frequency of viral integration reduces the likelihood of insertional mutagenesis. In addition to these advantages of using rAAV vectors, several different serotypes have been identified and different capsid mutants have been either isolated from animal tissues or synthetically engineered. AAV serotype 2 (AAV2) was the first serotype discovered and cloned into bacterial plasmids and used to created rAAV vectors (59,69). Consequently, AAV2 is the most widely studied AAV serotype and, as of 2012, had been used to date in 75 clinical trials worldwide. Included in these clinical trials are 14 trials to treat neurological disease including Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, and epilepsy. Most of the preclinical studies involving the treatment of animal models of MPS disease have used AAV2 vectors, although as described below, several other serotypes such as AAV9 and rhAAV10 have advantages with respect to breaching the blood-brain barrier to treat neurological manifestations of disease.

3.0. ROUTES OF GENE THERAPY DELIVERY TO TREAT NEUROPATHOLOGICAL ASPECTS OF MPS

Gene therapy vectors have been administered in animal models of MPS disease with the intention of achieving gene transfer in the CNS to treat neurological manifestations of disease. Vectors have been infused into these animal models by the intravenous route as well as by direct injection into the CNS. Early studies demonstrated the limitations of the intravenous route to mediate gene transfer in the CNS due to the challenge of crossing the

blood-brain barrier. This has led to many studies that have explored different strategies for direct infusion of gene therapy vectors to the brain. The results of these experiments are outlined below.

3.1. Systemic Delivery

3.1.1. Intravenous Infusion—Extensive studies of intravenous vector administration have been conducted in animal models of MPS disease. In a seminal study, Daly *et al*. initially reported intravenous infusion of an AAV2 vector expressing murine β-glucuronidase (GUSB) into newborn MPS VII (GUSB-deficient) mice that resulted in GUSB expression in the brain; neurons, microglia, and meninges were cleared of pathological storage material (70). Similarly, intravenous infusion of an AAV2 vector expressing α-L-iduronidase (IDUA) into 1 day-old MPS I mice resulted in detectable expression of IDUA in the brain, clearance of pathological GAG, and improved neurobehavior in an open field test (71). Whether or not this high-level expression following vascular delivery in neonatal animals reflects non-physiological injury to the blood-brain barrier has been debated. Ponder and Haskins have conducted extensive studies of intravenous retroviral vector administration in rodent and larger animal models of MPS VII and MPS I (72). For example, intravenous infusion of a retroviral vector into newborn MPS VI and MPS I animals reduced accumulation of storage material in the brain, with the effect related to dosage (73). Intravenous infusion of a lentiviral vector at birth resulted in transduction of neurons in the brain, consistent with detectable IDUA expression and reduction of GAG storage material in the CNS that also was dose dependent (74) . MPS I dogs have been infused at birth with a gamma retroviral vector expressing canine IDUA, demonstrating that this route of administration can be used to deliver IDUA expression to the brain and clear GAG storage material from the CNS (72,75). Long-term studies of MPS VII dogs infused with retroviral vector encoding β-glucuronidase have demonstrated substantial correction of cardiac (76) and skeletal (77) manifestations of metabolic disease, with the potential for substantial neurological effectiveness as well (78).

Intravenous infusions into adult mice have also resulted in mixed outcomes. Tail-vein injection of a lentiviral vector encoding IDUA into 8 to 10-week old MPS I mice failed to mediate detectable IDUA expression or to decrease levels of GAG in the CNS of treated mice as assessed one month post-treatment (79). Lack of detectable IDUA expression was likely due to an immune response against human IDUA transgene product as enzyme-specific antibodies were detected, and a cytotoxic T-lymphocyte response resulting in clearance of IDUA-expressing transduced cells (80). However, immunomodulation prior to infusion with a gamma-retroviral vector resulted in a small amount of detectable IDUA activity (< 3% of normal) in the brains of treated MPS I mice with some evidence of reduction in pathological GAG storage (80). The immune response reported in these studies could be avoided by the use of a liver-specific promoter. Intravenous infusion into MPS II mice using an AAV2/8 vector expressing iduronate 2-sulfatase under transcriptional regulation of a liver-specific promoter resulted in detectable levels of IDS activity in brain tissue , reduction of GAG storage accumulation, and partial correction of neurobehavior as determined using an open field test (81). Use of a liver-specific promoter allowed for creation of a factory organ, from which the enzyme was produced , released into the

bloodstream, and then taken up by non-transduced organs via its receptor. Liver directed expression of β-glucuronidase and IDUA has been demonstrated after hydrodynamic delivery of *Sleeping Beauty* transposons encoding these proteins (82–84). Long-term, therapeutic levels of enzyme were achieved, however, only in animals that were immunodeficient or in animals that were immunosuppressed, again emphasizing the importance of dealing with the immune response.

Recent advances in delivery of therapeutic enzyme to the brain have been reported by systemic administration of AAV vectors packaged using alternate capsid types and by expression of enzymes that have been engineered to facilitate transit across the blood-brain barrier. Chen *et al*. used phage biopanning to identify peptides binding to brain endothelium of MPS VII mice, then demonstrated expanded biodistribution of AAV vector to the brain in MPS VII mice when the vector had been packaged using capsid engineering to display this peptide (85). Effective delivery to the CNS has been demonstrated after systemic administration of AAV serotype 9 in murine models of MPS IIIA (86) and MPS IIIB (87), resulting in substantial correction of CNS storage disease. Interestingly, AAV9-mediated delivery to the CNS is reportedly inhibited by increased levels of sialic acid in the CNS of MPS VII mice (88). For increased delivery of therapeutic enzyme to the brain, fusion of IDUA protein to antibodies targeting molecules expressed in the brain endothelium has been explored (89,90). Liver-directed expression of a minicircle DNA-based system provided continuous expression that resulted in substantial delivery of gene product (91). Results from these studies foster confidence that neurologic manifestations of MPS disease will eventually be addressed either by systemic administration of vector that achieves transit across the blood-brain barrier and transduction of target cells within the CNS, or by peripheral expression of a gene product that subsequently crosses the blood-brain barrier to the CNS for correction of storage disease.

3.1.2. Ex Vivo Transduction of HSC—The strategy of introducing autologous hematopoietic stem cells that have been genetically modified *ex vivo* builds upon the extensive experience accumulated in treatment of MPS disease by allogeneic HSC transplantation, particularly MPS I (see above, 1.2.1). Anticipated effectiveness for CNS manifestations is based on the engraftment of microglial cells in the brain derived from myelomonocytic differentiation of transduced donor HSC. The concept of *ex vivo* gene transfer for MPS disease was first demonstrated by Wolfe *et al*. (92), in which HSC from MPS VII mice were transduced with a retroviral vector encoding β-glucuronidase and engrafted in recipient MPS VII mice, indicating that low levels of transgene product were sufficient to reduce storage material, not only in a hematopoietic organ (the spleen), but in liver as well. Early studies of *ex vivo* IDUA gene transfer into a canine model of MPS I were confounded by immune response against the transgene product (93,94)). In another approach, stromal cells from the bone marrow were harvested and transduced *ex vivo* with a retroviral vector prior to being infused directly into the lateral ventricles of the brains of MPS VII mice (95). Two weeks after infusion, GUSB-positive cells were detected in the olfactory bulb, striatum, and cerebral cortex. GAG levels were reduced to normal, and the animals displayed neurocognitive improvement in a Morris water maze test. However, after 8 weeks, GUSB expression disappeared, likely due to an elicited immune response.

The MPS I mouse has proven to be a useful model for testing *ex vivo* gene therapy. In MPS I mice, transplantation of unmodified wild-type bone marrow cells into 6 to 8-week old animals did not result in detectable IDUA levels or reduced storage material in the brain (96). However, *ex vivo* transduction of bone marrow cells from MPS I mice with an IDUA-expressing retrovirus prior to transplantation into $IDUA^{-/-}$ recipients resulted in expression of IDUA as well as in partial correction of pathology, and detection of normal donor-derived microglia in the brain. *Ex vivo* transduction using a lentiviral vector encoding IDUA proved more successful as reported by Visigalli *et al*. (97), perhaps due to an overwhelming level of IDUA expression, leading to immune tolerance as well as correction of peripheral and neurologic manifestations of disease. Effective *in vivo* expression of IDUA can also be restricted to specific hematopoietic lineages after transduction of hematopoietic stem cells, as recently demonstrated for erythroid cells (98) and platelets (99).

For MPS diseases that are not known to be responsive to allogeneic HSC transplant, *ex vivo* gene transfer into HSC presents the opportunity for improved effectiveness through overexpression of the transgene product and a greater degree of metabolic cross-correction in the periphery as well as in the CNS. *Ex vivo* transduction of the sulfaminidase-encoding gene resulted in substantial correction of neurologic disease in MPS IIIA mice when using lentiviral transduced wt HSC (100), but effectiveness of transduced HSC from MPS IIIA mice required use of a myeloid-specific promoter to regulate sulfaminidase expression (101). *Ex vivo* iduronate sulfatase gene transfer into HSC from MPS II mice also resulted in substantial correction of metabolic disease and prevention of neurocognitive deficit in a murine model of Hunter syndrome (102). *Ex vivo* IDS gene transfer into human T cells has also been tested as an approach to provide IDS activity for mild Hunter syndrome, a trial that was conducted prior to the availability of ERT for this disease (42,103). Engraftment of genetically engineered hematopoietic cells remains a potentially feasible approach for gene therapy of MPS disease.

3.2. Direct Delivery to the CNS

3.2.1. Intracranial Infusion Into the Brain Parenchyma—Lysosomal enzymes have been expressed in the brain, mediated by direct intrastriatial infusion of therapeutic vectors. Following delivery of AAV2 and AAV5 vectors to the striatum by direct injection into the putamen, animals exhibited high levels of NAGLU expression in the brain, especially in the injected hemisphere of adult MPS IIIB mice (104) . Expression of NAGLU within neurons contributed to improved lysosomal function, reduced levels of GM2 and GM3 gangliosides, and improved neurobehavior in an open field test, elevated plus maze, and in a home-cage analysis. However, only partial neurological improvements were observed in many mice. AAV2 and AAV5 vectors encoding GUSB have been injected intrastriatally into MPS VII mice. Unilateral infusion of an AAV2 vector into adult MPS VII mice resulted in GUSB levels equivalent to that of unaffected heterozygote animals and cleared lysosomal storage lesions in the injected hemisphere of the brain (105). Bilateral intrastriatal infusions of a similar vector packaged into AAV5 capsids had a more successful outcome as GUSB was expressed at levels that cleared lysosomal storage material in both hemispheres. Since components of the striatal area of the brain are known to play a role in cognitive function these animals displayed improvement in a learning and spatial memory deficit using a

repeated acquisition and performance chamber (RAPC) assay (106). Furthermore, AAV2 and AAV5 vectors have been shown to mediate expression of IDUA throughout the brain of adult MPS I mice following a single unilateral intrastriatal infusion (107). This treatment prevented accumulation of GM2 and GM3 gangliosides and reversed lysosomal distension with the treatment being more effective in mice that received AAV5 vector compared to mice that received AAV2 vector. AAV5 vector has subsequently been injected into the striatum and cerebellum of adult MPS I dogs (108, 109) . Dogs that were fully immunosuppressed using a combination of mycophenolate mofetil and cyclosporine during the treatment exhibited widespread IDUA expression as well as reduced neuropathology, GAG, and secondary ganglioside accumulation. However, dogs receiving only partial immunosuppression with cyclosporine alone presented with subacute encephalitis, produced antibodies recognizing IDUA, and eliminated genetically modified cells (108).

Besides intrastriatal infusion, other direct intracranial approaches to delivery have been investigated. In contrast to a single unilateral intrastriatal infusion, multiple injections into both cerebral hemispheres combined with injection into the cerebellum of an HIV-1-based lentiviral vector expressing GUSB resulted in widespread expression of GUSB and reduction in pathology (110) . Another approach involved injections of an AAV2 serotype vector into the anterior cortex and hippocampus of newborn MPS VII mice. GUSB activity reached wild-type levels in the brain 18 weeks post-infusion, although much of the GUSB activity was concentrated near the injection sites. Lysosomal distention was prevented as was the CNS accumulation of GM2 and GM3 gangliosides. This resulted in improved performance of treated animals in a Morris water maze test of spatial navigation (111). Direct hippocampal injection of HIV-based lentiviral vector has been tested in a mouse model of MPS IIIB (112). Correction of CNS lesions has also been observed after intraparenchymal administration of AAV vector in MPS IIIA (113) and MPS IIIB (114) mice. Finally, AAV serotype rh10 was tested for intracranial delivery of the sulfaminidase gene in a gene therapy clinical trial for MPS IIIA (115). Early results from this trial show stabilization in 3 patients and possible improvement in one patient, although further follow up investigation is necessary.

3.2.2. Intracerebroventricular Infusion—An alternative strategy to direct infusion of therapeutics into the parenchyma of the brain is to take advantage of the ventricular system. There are four cerebral ventricles located within the mammalian brain, which are remnants of the neural tube formed during early development. Two paired, lateral ventricles are situated within the cerebrum and run parasagittally along much of the dorsal basal ganglia. The lateral ventricles communicate with the third ventricle, located within the center of the diencephalon, by way of the intraventricular foramina. The third ventricle is further connected to the fourth ventricle, located within the hindbrain, by the cerebral aqueduct, which runs through the midbrain. Three foramina further adjoin the fourth ventricle to the subarachnoid space and the central canal allows access between the fourth ventricle and the spinal cord. All of these ventricular spaces are filled with cerebrospinal fluid (CSF), which bathes the brain and spinal cord and protects these structures from injury.

Fraldi *et al*. exploited the ventricular system as a route of access to multiple brain structures using an AAV5 vector that co-expressed sulfaminidase (SGSH) and sulfatase-modifying

factor-1 (SUMF) for treatment of a mouse model of MPS IIIA (113). The investigators infused the vector bilaterally into newborn pups and detected SGSH activity throughout the brain while demonstrating that co-expression of SUMF and SGSH resulted in increased SGSH activity in comparison to animals infused with SGSH vector alone. With a similar AAV5 vector expressing green fluorescent protein (GFP), many transduced cells were visualized in the olfactory bulb, fewer cells in the choroid plexus and cerebral cortex, and very few cells in the striatum, hippocampus, thalamus, and cerebellum. Animals treated with the SUMF /SGSH bicistronic vector displayed a reduction in neuropathological storage material and GM2 ganglioside, decreased microglial activation, and prevention of astrogliosis compared to AAV5-GFP treated control animals. The functional results of the analysis demonstrated that the animals displayed improvement in gait as well as in both neurobehavioral and neurocognitive tests as measured using an open-field test and Morris water maze test, respectively.

Ventricular GUSB gene delivery using lentivirus vector was compared with intravenous delivery and found to provide superior transduction and GUSB expression in the CNS and improved neurobehavioral outcomes in MPS VII mice (116). Intracerebral ventricular (ICV) infusion of AAV vector has also been tested in MPS VII mice, demonstrating the effectiveness of GUS gene delivery to the fetal brain (117), and reversal of established neurocognitive deficit (118). ICV infusion of AAV8 vector into neonatal MPS I mice resulted in high level IDUA expression in all areas of the brain with correction of storage materials and prevention of neurocognitive deficit in the Morris water maze (119). Direct delivery to the CNS thus provides a strategy to achieve high levels of therapeutic enzymes in the brain to address neurologic manifestations of MPS disease.

3.2.3. Intracisternal Infusion—An alternative route of administration to deliver gene therapy vectors into the CSF is direct infusion into the subarachnoid cisterns. The cisterns are openings in the subarachnoid space located between the pia matter and arachnoid layers of the meninges, which surround the brain. The cerebellomedullary cistern (cisterna magna) is the largest of the subarachnoid cisterns and is connected by the flow of CSF from the fourth ventricle by the lateral and median apertures. Following intravenous administration of mannitol, an osmotic agent, used to increase extracellular space between epithelial cells in the CNS, infusion of an AAV2 vector expressing NAGLU into the cisterna magna resulted in increased longevity of MPS IIIB mice treated at 4–6 weeks of age (120). The overall effectiveness of the infusions was dose dependent. Animals treated with a high dose (5×10^{10} vector genomes) of the vector fared better than animals receiving a low dose of vector (1×10^{10} vector genomes). Long-term activity of NAGLU was detected in the brains of treated animals, and the highest levels occurred in the hind portion of the brains of animals treated with the high vector dose (about 4-fold higher than wild-type). IHC analysis revealed NAGLU expression in Purkinje cells of the cerebellum as well as in neurons of the brainstem and hypothalamus. GAG content in the brain was partially reduced and animals treated with a high dose of the AAV vector displayed improvement in a Morris water maze task, although there was no improvement in a rotarod test of motor function. Intracisternal infusion of AAV vector has recently been tested in the canine model of MPS VII, demonstrating considerable distribution and correction of lysosomal pathology in the brain

(121). In spite of efficacy of preclinical models using the intracisternal approach, clinical translational via the intracisternal route is more problematic since areas of the human brain involved in cardiac and pulmonary function would be susceptible to potential mechanical injury.

3.2.4. Intrathecal Infusion—Yet another strategy for delivering gene therapy vectors into the CSF is intrathecal injection into the leptomeningeal space of the spinal cord via lumbar puncture. Intrathecal delivery of an AAV2 vector expressing GUSB resulted in restoration of between 3% and 30% of wild-type GUSB activity levels in the brains of MPS VII mice treated as neonates and 8% of wild-type levels in animals treated as adults (122). This treatment resulted in a reduction of lysosomal storage vacuoles present in the cerebral cortex. Similarly, intrathecal infusion of an AAV2 vector expressing IDUA into adult MPS I mice resulted in enzyme activity levels in the hindbrain (123). IDUA-producing neurons were detected in the brains of treated animals by immunostaining of histologic sections, with the highest density of reactive cells detected in the olfactory bulb and cerebellum. In general, IDUA levels correlated with AAV vector dose, and the brains of animals treated with a high dose of vector (4×10^{10} particles) appeared to be entirely cleared of storage vacuoles. More recently, high levels of IDUA activity have been achieved throughout the brain following intrathecal infusion of AAV9-IDUA vector (124). Results from these studies support the concept of direct gene transfer to the CNS for MPS disease employing a less invasive route of administration into the CSF.

3.3. Comparison of Routes of Gene Delivery

A comparison of intra-carotid arterial (IA) and intracerebral ventricular (ICV) delivery of rAAV5 in MPS I mice was conducted by Janson *et al*. (125). In both approaches mannitol was also administered for disruption of the blood-brain barrier and the ependymal-brain interface. Both routes of delivery resulted in significant increases in enzyme activity in the brain, and corresponding decreases in GAG levels. However, rAAV5-IDUA delivery via the ICV route of administration allowed superior vector distribution within the brain whereas the IA route of administration was limited to the ipsilateral hemisphere.

A study of intraventricular vs. intravenous gene delivery for MPS IIIA was conducted by McIntyre *et al*. (126) using a lentiviral vector. Sulphamidase enzyme activity was detectable in various regions of the brain throughout the rostro-caudal axis in MPS IIIA mice after intraventricular administration. Enzyme activity was undetectable in the brains of mice after intravenous administration and in untreated controls.

Bielicki *et al*. (116) compared intraventricular vs. intravenous lentiviral mediated gene therapy for MPS VII. Intraventricular delivery resulted in β-glucuronidase activity thoughout the mid-rostro to caudal regions of the brain. Intravenous delivery resulted in no detectable enzyme levels in corresponding areas of brain. In addition, β-hexosaminidase activity, elevated in MPS VII mice, was reduced to wild-type levels in the brain after intraventricular treatment, while there was no decrease in activity observed in the brain following intravenous delivery of GUS vector. Histological analysis of the brain revealed

decreases in lysosomal storage in the frontal cortex, cerebellum, and other regions of the brain after intraventricular administration, but not after intravenous administration.

4.0. CONCLUSION

Gene therapy can improve neuropathological and functional deficits associated with neuropathic mucopolysaccharidoses. Preclinical studies demonstrate that a variety of vector systems can be used to deliver corrective genes to the brain. Intraparenchymal gene delivery usually results in very high level, but more focal distribution in the brain, whereas intracerebral, intraventricular or intracisternal delivery typically results in lower level, but a more global expression. Intravenous delivery of vector or vector-transduced hematopoietic stem cells can also provide widespread distribution of vector, but intraventricular delivery appears to provide the greatest distribution pattern within the brain for effective global therapy, at least in the preclinical setting.

5.0. EXPERT OPINION

Achieving limited therapeutic benefit in the central nervous system for MPS disorders has been achieved through HSCT in the case of Hurler syndrome, demonstrating proof-of – principle for the potential development of effective clinical treatments for CNS manifestations of MPS. However, other MPS disorders have proved remarkably unresponsive to this approach. Gene therapy is developing rapidly as a viable treatment option as many preclinical studies have been conducted with success and several clinical trials have begun to yield promising results in patients.

POTENTIAL

Gene therapy represents a viable method for widespread delivery of missing enzymes to multiple tissues including the CNS. However, the effectiveness of CNS-directed gene therapy for MPS disorders remains to be clearly demonstrated with respect to clear neurologic functional response in the clinic.

GOAL

Affordable treatments that restore full function to somatic tissues as well as neurological tissues. The biggest challenge in achieving this goal is the longevity of expression in the brain with restoration of cognitive function, or prevention of cognitive deficits when gene therapy is administered early.

CHALLENGES

In moving the field of gene therapy for neuropathic mucopolysacarridoses forward advances will be needed in understanding and blunting potential immune responses. Expansion of scale-up processes will also be needed to meet the demands of producing vector systems. Additionally, more detailed characterizations of the neurological benefits achieved are needed in both preclinical models and in the clinical setting. This could include both the standardization of neurobehavioral tests that are used in order to more easily compare the results obtained from different studies and also to develop clinically translatable biomarkers

of neurologic disease using brain imaging modalities such as MRI and PET and to incorporate these tests into future clinical trials.

FUTURE PROSPECTS

A Phase I clinical trial of gene therapy using direct surgical injection of rhAAV10 into the brain parenchyma of patients with Batten disease, and AAV9 into the brain of patients with MPS IIIA are being followed for evaluation of efficacy, and appear to relatively safe thus far. Similar studies in MPS IIIB are in progress. This approach is promising, and could yield an immediate treatment for those disorders of the brain. Safer, less invasive approaches to the CNS are being developed in animal models, and hold promise for even more practical therapies.

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LIST OF ABBREVIATIONS

AAV	adeno-associated vector
BBB	blood-brain-barrier
BMT	bone marrow transplantation
CNS	central nervous system
CSF	cerebral spinal fluid
ERT	enzyme replacement therapy
GAG	glycosaminoglycan
GFP	green fluorescent protein
GUSB	glucuronidase beta
HLA	human leukocyte antigen
HSC	hematopoietic stem cell
HSCT	hematopoietic stem cell transplantation
ICV	intracerebroventricular
IDUA	iduronidase
LSD	lysosomal storage disease
LTR	long terminal repeat
MPR	mannose-6-phosphate receptor
MPS	mucopolysaccharidoses
MPS 1H	mucopolysacarridosis type 1 Hurler
NAGLU	N-acetylglucosaminidase
PBSC	peripheral blood stem cell
SB	Sleeping Beauty
SCID	severe combined immunodeficiency
TBI	total body irradiation
UBC	umbilical cord blood

HIGHLIGHTS

- Gene therapy targeting the nervous system in mucopolysaccharidoses can complement systemic treatments such as enzyme replacement therapy and hematopoietic stem cell therapy.

- Adeno-associated viral vectors exhibit trophism for nervous system cells.

- Adeno-associated viral vector gene therapy can ameliorate cognitive deficits in preclinical animal models of mucopolysaccharidoses.

- Multiple routes of delivery have been evaluated for focal and global nervous system gene therapy.

- Intraventricular gene delivery achieves the greatest penetration of the brain with the broadest distribution.