Central nervous system therapy for lysosomal storage disorders

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Most lysosomal storage disorders are characterized by progressive central nervous system impairment, with or without systemic involvement. Affected individuals have an array of symptoms related to brain dysfunction, the most devastating of which is neurodegeneration following a period of normal development. The blood–brain barrier has represented a significant impediment to developing therapeutic approaches to treat brain disease, but novel approaches—including enzyme replacement, small-molecule, gene, and cell-based therapies—have given children afflicted by these conditions and those who care for them hope for the future. (DOI: 10.3171/FOC/2008/24/3-4/E11)

**KEY WORDS**
- bone marrow transplantation
- enzyme replacement therapy
- gene therapy
- lysosomal storage disorder
- stem cell transplantation
- substrate reduction therapy

Significant advances in approaches to the diagnosis and treatment of inborn errors of metabolism have occurred in recent years. Expanded newborn screening using tandem mass spectrometry has led to the ability to identify and treat neonates who have metabolic conditions before symptoms appear. Further developments in nutritional support and medical treatment, such as alternative pathway therapy for urea cycle disorders, biopterin for phenylketonuria, or small-molecule therapies for LSDs, have also led to better outcomes. Patients undergoing solid organ transplantation have benefited from innovative techniques such as the use of nonsteroidal immunosuppressive regimens, which has resulted in improved postoperative growth and decreased incidence of hypertension, obesity, and cosmetic side effects. Hematopoietic stem cell and umbilical cord blood transplantation have also improved outcome in some disorders. Despite these advances, significant hurdles must be overcome in order to treat the devastating brain disease associated with many of these conditions. The LSDs represent a particular challenge in this regard. The majority of these disorders are characterized by substantial brain involvement, with developmental regression, seizures, and mental retardation being relatively common features. This review focuses on LSDs associated with significant brain impairment, including the particular challenges associated with treatment of these conditions and the potential for novel therapies, especially cell-based techniques, to impact brain disease.

**Lysosomal Storage Disorders**

Most LSDs are caused by a specific inherited enzyme deficiency that leads to accumulation of complex macromolecules, such as sphingolipids, glycoproteins, mucopolysaccharides, and glycoproteins in lysosomes. Rare forms are associated with defective targeting of enzymes to the lysosome or abnormalities in lysosomal transporters. More than 40 LSDs have been identified and, although each individual disorder is rare, in aggregate the worldwide prevalence is approximately 1 case per 8000 live births. Lysosomes are membrane-bound cytoplasmic organelles that contain acid hydrolases, enzymes that degrade a variety of macromolecules as part of normal cellular function. Intracellular and secreted lysosomal enzymes are initially synthesized in

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**Abbreviations used in this paper:**
- AAV = adeno-associated virus
- ARSA = arylsulfatase A
- ASM = acid sphingomyelinase
- BBB = blood–brain barrier
- BMT = bone marrow transplantation
- CNS = central nervous system
- ERT = enzyme replacement therapy
- GAG = glycosaminoglycan
- Gd-DTPA = gadolinium diethylenetriamine pentaacetic acid
- HSCF = hematopoietic stem cell transplantation
- IDUA = α-L-iduronidase
- INCL = infantile neuronal ceroid lipofuscinosis
- LINCL = late-INCL
- LSD = lysosomal storage disorder
- MLD = metachromatic leukodystrophy
- MR = magnetic resonance
- MPS = mucopolysaccharidosis
- MSC = mesenchymal stem cell
- M6P = mannose-6-phosphate
- PPT1 = palmitoyl protein thioesterase–I
- rAAV = recombinant AAV
- TPP-I = tripeptidyl peptidase I.
the endoplasmic reticulum and targeted to lysosomes by an M6P-mediated pathway (Fig. 1). An M6P residue, applied in the cis-Golgi network, allows an enzyme to be targeted specifically to lysosomes, and, therefore, represents a distinct molecular recognition signal. Enzymes tagged by an M6P marker bind to receptors located in the membranes of the vesicles that bud from the trans-Golgi network. Subsequently, these vesicles fuse with lysosomes and release the “tagged” enzyme into the lysosomal matrix. There are also M6P receptors on the surface of cell membranes; a proportion of enzymes destined for lysosomes are extracellularly secreted and re-enter the cell via M6P receptors located on the outer cell membrane. This extrinsic pathway is the basis for exogenous enzyme replacement therapy.

Enzyme Replacement Therapy

Deduve first proposed that ERT could be used to treat LSDs in 1964. Thereafter, pioneering work by Neufeld and others led to the discovery of the M6P-receptor–mediated pathway. Further experiments showed that stored intralysosomal macromolecules could be cleared through the addition of lysosomal enzymes to the media of enzyme-deficient fibroblasts obtained from patients with LSDs. Significantly, only 1–5% of normal cellular enzymatic activity was needed to correct the abnormal accumulation of lysosomal substrate.

Starting in the 1970s, ERT clinical trials were performed by infusing normal human enzymes into individuals who had a variety of LSDs, including Sandhoff disease (β-hexosaminidase A derived from urine), Pompe disease (placental α-glucosidase), Fabry disease (placental, plasma, and splenic α-galactosidase A), and Gaucher disease Type 1 (placental acid β-glucosidase). These studies showed both evidence of substrate degradation and rapid clearance of the intravenously delivered enzymes, but no significant clinical benefits.

Gaucher disease Type 1 is characterized by hepatosplenomegaly, failure to thrive, bone disease, and hematological abnormalities. In 1990, a landmark study performed by Barton and coworkers demonstrated that ERT using human mannose-terminated acid β-glucosidase was safe, well tolerated, and effective in treating systemic manifestations of Gaucher disease Type 1 in a child. Gaucher disease Type 1 has since become the paradigmatic disorder for treatment by ERT, and acid β-glucosidase (manufactured by genetically engineered Chinese hamster ovary cells) was approved for clinical use in 1991.

Recombinant DNA techniques for manufacturing highly purified therapeutic enzymes have led to the practical application of ERT to other disorders that do not primarily affect the CNS. Fabry disease is an X-linked disorder that features progressive accumulation of globotriaosylceramide in microvasculature, leading to renal failure, painful
crises, strokes, cardiac disease, and early death. Enzyme replacement therapy using recombinant α-galactosidase A clears stored globotriaosylceramide from plasma and tissues, decreases pain, and stabilizes renal disease. Infantile Pompe disease is an autosomal recessive condition that causes severe cardiac disease (hypertrophic cardiomyopathy) and myopathy, with children typically dying from cardiorespiratory failure or respiratory infection before 2 years of age. An initial study in 3 infants < 4 months of age who received twice-weekly intravenous infusions of recombinant acid α-glucosidase showed that ERT was capable of improving cardiac and skeletal muscle function in infants with Pompe disease. A pivotal trial involving 18 infants showed that ERT using recombinant acid α-glucosidase was generally well tolerated and improvements were noted in cardiac function, growth, motor development, and overall survival (treatment reduced the risk of death by 99% during the study period compared to an untreated control group).

More recently, ERT has been developed for the MPSs, disorders that often feature both systemic and brain involvement. Common manifestations include coarse physical features, progressive neurological deterioration, corneal clouding, hearing loss, obstructive sleep apnea, mucoid rhinitis, cardiac disease, hepatosplenomegaly, contractures, and severe bone disease. Enzyme replacement therapy with recombinant human arylsulfatase B significantly improved endurance, as measured by a 12-minute walk test and 3-minute stair climb test, in patients with MPS VI (Maroteaux–Lamy syndrome), a disorder characterized by severe skeletal manifestations and normal intelligence. Although systemic manifestations improve with ERT, treatment has not had an effect on CNS disease. An open label trial in 10 patients 5–22 years of age with MPS I of various degrees of severity (ranging from the classic severe Hurler phenotype to the mild Scheie phenotype) showed decreased liver and spleen sizes, improved joint range of motion, and decreased excretion of a biochemical marker of disease (urinary GAGs) following infusions of recombinant α-L-iduronidase (laronidase). A larger placebo-controlled trial involving 45 patients with MPS I showed that patients had significantly improved respiratory function, physical capacity, and reduced GAG storage. Mental function was not assessed. Similarly, a multicenter Phase II/III clinical trial involving 96 patients with MPS II (Hunter syndrome), showed that recombinant iduronate-2-sulfatase (I2S) therapy resulted in improved forced vital capacity, physical endurance, joint range of motion, liver and spleen size, and decreased urine GAG excretion.

**Enzyme Delivery Across the Blood–Brain Barrier**

The brain microvasculature consists of a continuous layer of endothelial cells, joined by tight junctions, that restrict the exchange of serum proteins, ions, and water-soluble drugs between blood and brain (the BBB). Furthermore, astrocytic foot processes that ensheath brain capillaries form an additional barrier to polar molecules or any large proteins. Bulky recombinant enzymes used for treatment of LSDs are widely considered to be unable to penetrate across the BBB, limiting their utility for treating disorders characterized by brain pathology. Nevertheless, a variety of methods have been used in attempts to bypass this barrier. Reversible osmotic barrier opening in rats was accomplished by infusing a hyperosmotic solution of arabinose into the external carotid artery. Intravenously injected α-mannosidase, a lysosomal enzyme, was able to enter the brain immediately after barrier opening and was incorporated into neuronal lysosomes within the 1st day after infusion. Another approach exploits the BBB receptor–mediated transport system to deliver enzymes directly to the CNS. Following intravenous administration in adult mice, bacterial β-galactosidase conjugated to the rat transferrin receptor via a streptavidin-biotin linkage localized to brain parenchyma. Interestingly, studies using murine models have shown that high-dose intravenous administration of recombinant enzymes may result in uptake across the BBB in aspartylglycosaminuria, α-mannosidosis, MLD, and MPS VII (Sly syndrome). The M6P/insulin-like growth factor 2 receptor appears to be responsible for transcytosis of phosphorylated enzymes across the BBB.

Although the ability of enzymes to enter the brain in these animal models may be related to dosage, developmental stage may also be a significant factor, because M6P receptor–mediated transcytosis is progressively lost with maturation. In MPS VII mice, β-glucuronidase improves abnormal brain storage if treatment is begun before the age of 2 weeks. After the first few weeks, the M6P-receptor transport mechanism is lost, so that by 7 weeks of age mice have little or no transport capacity. Mucopepsacyrhidosis IIIA (Sanfilippo syndrome) is characterized by progressive, severe neurodegeneration and behavior abnormalities caused by a deficiency of sulfamidase. Sulfamidase can enter the brain in a murine model of MPS IIIA until the BBB completely closes at 10–14 days of age. Treatment of MPS IIIA mice from birth resulted in normal behavior and ability to learn. In contrast, untreated mice or mice treated after 6 weeks of age were significantly impaired. Increased lifespan and substantial improvement in gait in twitcher mice, the murine model of Krabbe disease, was apparent after intraperitoneal injection of recombinant galactocerebroside in animals 10–20 days of age. Although therapy for brain pathology appears to work best in neonatal animal models, it may be possible to enhance adult brain uptake of enzymes through pharmacological manipulation of the M6P pathway. Epinephrine was shown to restore M6P-receptor–mediated functional transport of phosphorylated β-glucuronidase in adult MPS VII mice to levels seen in neonates.

Convection-enhanced delivery (infusion of enzyme into intracerebral microvasculature under slight hydrostatic pressure) has also shown potential for the treatment of brain disease. Glucocerebrosidase, the enzyme deficient in Gaucher disease, localizes to cortical neurons following infusion into the striatal region of rats. Convection perfusion of glucocerebrosidase into primate frontal lobe or brainstem resulted in significantly increased levels in the cortex, white matter, and pons of treated animals. More recently, convection-enhanced delivery was used to treat a 13-month-old boy with the severe, neuropaithic form of Gaucher disease. Glucocerebrosidase was administered along with an imaging tracer (Gd-DTPA), so that the distribution of enzymes within the brain could be tracked by MR imaging. Enzyme perfusion with stereotactic cannula placement was first performed into the right frontal lobe and, 3 months later, into the pons. Real-time MR imaging
demonstrated progressive and complete filling of the targeted brain regions with enzymes and Gd-DTPA infusate. Follow-up 9 months later showed the patient to be at the preinfusion neurological baseline.80

Intrathecal and Intraventricular Enzyme Delivery

Direct infusion of enzymes via an intrathecal or intraventricular route is another potential method for bypassing the BBB. An early attempt at direct delivery of recombinant enzymes by intrathecal injection in a patient with Tay–Sachs disease failed, probably, at least in part, because of the limited amount of enzyme infused.102 Despite the lack of early success, the intrathecal route for enzyme administration has received more attention in recent years. Mice with MPS IIIA (Sanfilippo syndrome) received up to 7 monthly or bimonthly injections of recombinant human sulfamidase into the cisterna magna. A dose-dependent reduction on stored substrate was observed in various brain regions and the spinal cord. Mice receiving enzyme injections on a fortnightly basis showed improved open-field behavior when compared to animals receiving monthly treatment. Most mice developed systemic antibodies to recombinant human sulfamidase, but this was not accompanied by any adverse effect.50

In another attempt to bridge the BBB, intraventricular administration of iduronidase was studied in rats. Iduronidase was widely distributed throughout the brain following injection into the lateral ventricle. Enzymes also crossed the ependymal lining of the ventricle and entered neurons, diffusing into brain tissue.8 Intrathecal enzyme infusion in a canine model of MPS I (Hurler syndrome) also showed encouraging results. Mean total brain GAG content was reduced to normal levels and there was a 57% reduction in meningeal GAG levels, which was accompanied by histological improvement of lysosomal storage in all cell types.84 Similarly encouraging findings were apparent after intrathecal infusion of recombinant human iduronidase into the cisterna magna of 15 dogs with canine MPS I at monthly or quarterly intervals for a total of 4 or 3 injections, respectively. Brains of the canines were examined at the end of the treatment regimen. Iduronidase was distributed widely throughout the brain, spinal cord, and meninges, with accompanying significant reduction of GAG levels. Both regimens normalized total brain GAG levels and decreased spinal cord GAG storage by 58–70%. One dog that had neurological impairment before starting intrathecal therapy showed clinical improvement, while a second dog with neurological signs showed no improvement.85 (All other animals had no neurological signs before or during the study.)

A human trial using intrathecal iduronidase to treat CNS manifestations of MPS I has recently started to recruit participants (ClinicalTrials.gov identifier NCT00215527).

Small-Molecule Therapies—Substrate Reduction and Chaperones

Because of the inherent difficulties in delivering enzymes across the BBB, approaches utilizing small molecules that have the ability to cross into brain parenchyma are also being developed as possible therapies for lysosomal disorders. One method attempts to diminish substrate storage through partial inhibition of enzymes responsible for biosynthesis of complex macromolecules that are degraded in lysosomes. By decreasing substrate flux into lysosomes with impaired ability to degrade these compounds, storage is decreased. Substrate reduction therapy with N-butyldexoyojirimycin (miglustat) improves organomegaly and hematological abnormalities associated with the non-neuropathic forms of Gaucher disease.24 Miglustat may also benefit patients with Gaucher disease who have neurological involvement, because it has the ability to cross the BBB. An adult patient with Gaucher disease Type 3 had decreased seizure activity, improved speech, and overall improved neurological status after 2 years of treatment with a combination of miglustat and ERT.18 Miglustat therapy may also help neurological symptoms in Niemann–Pick disease Type C, a neurodegenerative disorder characterized by a defect in cellular lipid trafficking and secondary glycosphingolipid storage.101 The accumulation of GM1 ganglioside is prevented, and ganglioside storage markedly reduced, in Tay–Sachs mice and symptom onset was delayed in Sandhoff mice treated with miglustat.58,108 In 2 children with infantile Tay–Sachs disease, miglustat prevented the occurrence of macrocephaly, but did not arrest neurological deterioration.9

In contrast to substrate reduction therapy, chaperone therapy is designed to enhance innate enzyme activity by restoring the shape of misfolded lysosomal enzymes, so that the enzymes can subsequently leave the endoplasmic reticulum and traffic to the lysosomes, instead of undergoing endoplasmic reticulum–associated degradation. Once the appropriately folded enzymes reach the lysosomes, the chaperones dissociate, helped by the intralysosomal acidic pH, and can then degrade stored substrate. Some missense mutations and small in-frame deletions may lead to a misfolded protein without significantly affecting the active site; patients harboring such mutations, in theory, would be candidates for a chaperone approach. Chaperones are in the early stages of clinical development, but have shown promising results in cell culture and animal models for a number of lysosomal disorders, including Gaucher disease, Fabry disease, Pompe disease, MPS I, GM1-gangliosidosis, and Tay–Sachs disease.38,56,74,86,97,102,121,139,146

Bone Marrow, Hematopoietic Stem Cell, and Umbilical Cord Blood Transplantation

Allogeneic BMT, HSCT, and, more recently, umbilical cord blood transplantation have been mainstays of therapeutic intervention for many LSDs that have significant brain involvement, although therapeutic efficacy has been variable. After transplantation, donor cells with normal lysosomal enzyme function populate the bone marrow. Hematopoietic cells, including activated lymphocytes, monocytes, and precursors of microglia, have the potential to cross the BBB.3,105 Because a proportion of lysosomal enzymes synthesized intracellularly are secreted and then internalized and directed to lysosomes via the M6P pathway, cross-correction of neighboring cells lacking a normal lysosomal enzyme is possible. Release of enzymes into plasma, with subsequent uptake by enzyme-deficient cells throughout the body, may also occur following BMT.53 Animal studies have demonstrated the efficacy of BMT or HSCT in restoring normal enzyme activity in different tissues, such as circulating blood cells, liver, and the periphery.
eral nervous system.\textsuperscript{53,141} Importantly, in some instances BMT may have an effect on the CNS. Bone marrow–derived stem cells can differentiate into blood monocytes that may migrate to the brain and further differentiate into microglial cells.\textsuperscript{73} Microglia of donor origin have the potential to produce therapeutic amounts of the missing or defective enzyme, leading to clearance of stored substrate in the brain. Increased brain α-mannosidase activity was found in a feline model of α-mannosidosis after BMT; functional enzymes were detected in neurons, glial cells, and cells associated with vasculature.\textsuperscript{133} Similarly, BMT in a mouse model of Krabbe disease showed brain enzyme activity reaching 15–20% of normal.\textsuperscript{82}

Transplantation, however, is associated with significant risks, including the development of graft-versus-host disease, graft rejection, early death from infection, and regimen-associated toxicity.\textsuperscript{33} Nevertheless, transplantation may still be considered a reasonable option because of the devastating neurological involvement in many LSDs.\textsuperscript{70} Initial attempts to treat patients with LSDs by BMT started in the 1980s. Hobbs and coworkers\textsuperscript{52} reported reversal of clinical manifestations of MPS I (Hurler syndrome) in a 1-year-old boy following BMT, including resolution of hepatomegaly and clearing of corneal clouding. The long-term clinical outcome in patients with MPS I who have undergone BMT has been encouraging overall. In a study of 11 patients with MPS I, 9 survived the procedure, with all survivors showing at least partial donor engraftment. Leukocyte α-iduronidase activity was maintained at donor levels, urine GAG excretion declined to near normal within 5 months, brain imaging showed stabilization of volume loss, and all children with a developmental quotient above 80 before transplantation maintained IQ scores above this level (median follow-up 5.5 years).\textsuperscript{134} In a smaller series of cases, BMT slowed the clinical regression of the disease significantly, although IQ continued to decrease in 2 patients.\textsuperscript{54} More recently, HSCT using banked cord blood showed clear benefit in children with MPS I who had a median age at transplantation of 16 months. Serial neurodevelopmental assessments demonstrated stabilization or improvement in all surviving children. Three of the 4 oldest children had average-to-high IQs, although on the basis of their genotypes and results from protein analysis they would have been predicted to have severe disease associated with rapid neurocognitive decline.\textsuperscript{120} In children with MPS I, early transplantation, before the manifestation of neurological disease, has the greatest likelihood of success, because long-term intellectual benefit may be limited by damage sustained before BMT.\textsuperscript{120,134} Transplantation for globoid cell leukodystrophy (Krabbe disease) has also shown promising results, especially if performed before significant neurological impairment has occurred. Two children who underwent BMT at ages 7 and 12 years experienced improvements in neuropsychological function.\textsuperscript{100} Five children who underwent allogeneic HSCT demonstrated restoration of normal leukocyte galactocerebroside levels and improvement in brain MR imaging abnormalities. In the 4 patients with late-onset disease, CNS deterioration was reversed, and in 1 patient with the infantile form, no signs or symptoms of the disease had appeared at the time the report was written.\textsuperscript{72} A larger trial used umbilical cord blood transplantation to treat infantile Krabbe disease; the trial included 11 asymptomatic neonates (age range 12–44 days) and 14 symptomatic infants (age range 142–352 days). Children who underwent transplantation after the onset of symptoms demonstrated minimal neurological improvement. In contrast, all asymptomatic children showed progressive central myelination and continued gains in developmental skills. A few had mild-to-moderate delays in expressive language and mild-to-severe delays in gross motor function.\textsuperscript{77,88}

Although transplantation has been relatively successful in some LSDs, other disorders, including MPS II (Hunter syndrome), MPS III (Sanfilippo syndrome), and LINCL have not responded well to such treatment.\textsuperscript{51,75,90,114,128} Most patients with MPS II and MPS III who have undergone BMT have continued to show developmental regression and steady progression of physical handicap.\textsuperscript{89,128} Allogeneic HSCT has been attempted in LINCL without success in an animal model and affected children.\textsuperscript{30,75} Because relatively few patients with these disorders have undergone transplantation, it is difficult to draw specific conclusions about the utility of BMT in these conditions.

**Gene Therapy**

The brain has limited capacity for regeneration, so any technique employing direct intraparenchymal or intraventricular injection must minimize direct damage to brain tissue while delivering the therapeutic vector as widely as possible. Immune-mediated and inflammatory responses should also be kept at a minimum in order to maximize therapeutic benefit. Because only a fraction of normal enzyme activity would be expected to result in clinical benefit, a gene therapy approach is attractive for treating brain disease. In addition, because lysosomal enzymes are secreted and internalized by M6P-mediated endocytosis, any corrected cells should be able to have an effect on neighboring tissue. Recombinant adeno-associated viral vectors, especially new serotypes such as rAAV2/1 and rAAV2/5, and lentiviral vectors have shown the ability to provide sufficient enzyme levels to neural tissues and cells in contact with axonal projections from transduced cells.\textsuperscript{15,27,39,94,99,113} A comprehensive account of gene therapy approaches for the treatment of LSDs that affect the brain is beyond the scope of this review. There has, however, been progress in developing gene therapy methods for treating CNS disease, and a few recent studies are summarized below. Ex vivo gene therapy approaches (initial cellular transduction followed by cell transplantation) are discussed further in the following section on cell-based therapies.

Adeno-associated viral and retroviral vectors have been used extensively in various animal models of lysosomal disorders with brain involvement, including MPS I, MPS III, MPS VII, Krabbe disease, MLD, Niemann–Pick disease, and the infantile (INCL) and late-infantile (LINCL) forms of neuronal ceroid lipofuscinosis. A single injection of rAAV2 or rAAV5 vectors carrying recombinant L-iduronidase (IDUA) in the striatum of adult MPS I mice corrected the underlying neuropathological condition.\textsuperscript{31} Intracerebral injection of an AAV5 vector in an immunosuppressed canine MPS I model was associated with broad brain dispersion of vector genomes, prevention of GAG and ganglioside accumulation, and drastic reduction of neuropathology.\textsuperscript{22} Widespread correction may have been the result of additional transport of IDUA along neuronal
processes.20 It is possible that IDUA has the ability to diffuse throughout the brain more readily, because direct injection of vectors carrying genes for other enzymes has resulted in only localized expression.41,113 Interestingly, intravenous delivery of retroviral vectors carrying the IDUA transgene in adult murine and neonatal canine models not only improved the systemic manifestations of MPS I, but also decreased lysosomal storage in brain tissue, possibly by enzyme diffusion across the BBB.83,124 This theory is supported by the finding that neonatal MPS I mice receiving a high-dose intravenous injection of retroviral vector showed nearly complete correction of brain storage but brain disease was not improved with low-dose vector infusion.21 A combination of intravenous and intracisternal rAAV2 vector injections, following intravenous mannitol infusion, was used to treat a murine MPS IIIB model. Treatment resulted in variable correction of lysosomal storage in brain and systemic tissues, improved behavior, and significantly prolonged lifespan.40 Adeno-associated viral vector delivery to MPS VII mice by direct brain injection has resulted in long-term expression of β-glucuronidase. The brain distribution of the enzyme also increases over time and is associated with clearance of the stored lysosomal substrate.90,100,113 Treated MPS VII mice may display improved spatial learning as well.19

Intracranial administration of AAV vectors carrying the galactocerebrosidase transgene has resulted in improved histopathology and increased enzyme expression (even to supernormal levels), although no improvement in behavior or longevity was observed.76,106 Injection of a lentiviral vector carrying ARSA directly injected into the brains of ARSA-deficient mice (model for MLD) resulted in sustained enzyme expression and protected against hippocampus neuropathology–related learning impairment.23 Long-term, widely distributed ARSA expression associated with cross-correction of neurons and astrocytes may be achieved with the use of AAV vectors.112 A global reversal of pathology correlated with recovery of motor and cognitive functions was apparent in the Niemann–Pick mouse following a combination of brain and systemic injections of rAAV encoding human ASM.98 Newborn PPT1-deficient mice (a model of LINCL) had localized increases in PPT1 activity, decreased autofluorescent material, improved histological parameters, and increased brain mass following direct intracranial injection of an AAV vector (AAV2-PPT1) at multiple brain sites. However, seizure frequency and longevity were not improved in the INCL mice.45 AAV2 vectors have also been used for CLN2 gene transfer in rodents and nonhuman primates to explore the potential to correct the defect in LINCL. Direct stereotactic injection of AAV serotype 2 or 5 vectors carrying a human CLN2 transgene into a mouse model of LINCL resulted in a marked reduction of autofluorescent storage in injected brain regions and in adjacent regions, as well as a significant decrease in cellular curvilinear bodies.99 After AAV2 vector injection, long-term expression of TPP-I, the enzyme deficient in LINCL, was achieved in rats and African green monkeys, without significant safety concerns being identified.48,109 Because of the lack of efficacy of previous HSCT attempts and the limitations inherent in systemic delivery of recombinant enzyme, Crystal and coworkers developed a gene therapy approach using direct intracranial injection of a recombinant AAV vector carrying CLN2 complementary DNA for the treatment of LINCL.25,115 The rationale for this approach was based on the knowledge that 1) genotype–phenotype correlations suggest 5–10% of normal TPP-I activity is sufficient to prevent clinical manifestations of LINCL; 2) TPP-I is secreted, so transfection of only a fraction of cells should result in cross-correction of large numbers of neighboring cells; 3) AAV vectors have been demonstrated to transfer genes to neurons in vivo and have negligible toxicity; 4) clinical experience exists with AAV vectors in human trials, including trials with direct intracranial injection; and 5) preclinical data in rats and nonhuman primates has demonstrated the feasibility of delivering the CLN2 transgene.25 The clinical trial started in March 2004, and is ongoing (ClinicalTrials.gov identifier NCT 00151216).

Although craniotomy and direct local injection of viral vectors have been most commonly employed in attempts at brain gene therapy, a transvascular approach (across the BBB) has the potential to deliver a transgene globally by means of nonviral, noninvasive intravenous administration.114 For example, DNA encapsulated in a liposome that has been conjugated with polyethylene glycol strands forms, in essence, a stable nanocontainer for gene delivery. To enable delivery across the BBB, the tips of 1–2% of the polyethylene glycol strands must be conjugated to a targeting monoclonal antibody, to form a pegylated immunoliposome.111 Global brain expression of a plasmid encoding bacterial β-galactosidase was demonstrated in a Rhesus monkey brain following pegylated immunoliposome encapsulation and intravenous injection.114 Nonviral plasmid DNA delivery across the BBB using liposomes was also performed in a murine model of MPS VII, with a greater than 10-fold increase in activity in brain, liver, spleen, lung, and kidney.145

Cell-Based Therapies

For similar reasons as discussed when considering the potential therapeutic benefits of BMT and gene therapy, cell-based therapies are also attractive candidates for treating lysosomal disorders associated with significant brain disease. The possibility of cross-correction secondary to the ability of neighboring cells to internalize and traffic secreted enzymes to lysosomes via the M6P-receptor system, as well as the need to increase enzyme activity to only a fraction of normal to have a potential therapeutic benefit, are especially compelling reasons for pursuing a cell-based approach. Moreover, the brain is relatively immunologically privileged, with reduced innate and adaptive immunity compared to other tissues,41,158 which could lead to better engraftment and survival of transplanted cells. In addition, unique properties of stem cells, such as the capacity for regeneration and the ability to migrate and differentiate into various cell types, including neurons, astrocytes, and oligodendrocytes, make them particularly attractive for treating damaged neural tissue. In this regard, transplanted stem cells could serve not only as a reservoir for secreting lysosomal enzymes, but also as the means for replacing or repairing damaged brain parenchyma.

The discovery of neural stem cells in the early 1990s created the field of CNS transplantation biology and established a paradigm for treating diseases of the CNS. Reynolds and Weiss107 first reported that adult rat forebrain
Central nervous system therapy for lysosomal storage disorders

cells cultured in serum-free medium containing epidermal growth factor formed free-floating spheres of cells and labeled these entities "neurospheres." Neurospheres exhibit self-renewal and are multipotent, with the ability to differentiate into neurons, astrocytes, and oligodendrocytes, satisfying the definition of organ-specific stem cells. When transplanted into adult immunosuppressed rats, neurospheres respond to environmental cues and migrate along routes normally taken by the endogenous precursors.

Intravenous or intracranial injection of various cell types has been tested in LSD and leukodystrophy animal models. In some cases, a combined gene therapy and cell-based therapy approach was used; enzyme production was enhanced by prior transduction of cells with gene therapy vectors before transplantation into the host. Mesenchymal stem cells derived from bone marrow produce lysosomal enzymes and are able to fuse with Purkinje cells, creating binucleate heterokaryons that can then redifferentiate into a Purkinje cell lineage. Adult bone marrow-derived MSCs injected intravenously in a mouse model of Niemann–Pick disease Type C fused with Purkinje neurons, leading to a reduction of cholesterol and sphingomyelin storage. The pathology associated with the cerebellum in Niemann–Pick disease Type C was thought to augment the ability of MSCs to fuse. Direct intracranial injection of MSCs into the hippocampus and cerebellum of Niemann–Pick disease Type A mice significantly delayed Purkinje cell loss, reduced sphingomyelin storage, and prolonged survival. The MSCs in this case had been previously transduced with a retroviral vector so that ASM was overexpressed. Transplanted cells migrated away from the injection sites and survived for at least 6 months. Combined intravenous and intracranial injection of MSCs, previously transduced to overexpress ASM, into neonatal Niemann–Pick Type A mice led to near normal levels of ASM activity, dramatic improvements in brain and systemic organ histology and normalization of cerebellar function. However, the effects were reversed following the development of anti–human ASM antibodies by Week 24.

Following initial encouraging studies in animal models, allogenic bone marrow–derived MSCs were transplanted into 5 patients with MPS I (Hurler syndrome) and 6 with MLD; all patients had previously undergone successful BMT with bone marrow from an HLA-identical sibling. Cell infusions were tolerated well, but there was no apparent change in overall health or physical development, although bone mineral density was either maintained or slightly improved. The MSCs were completely of host origin at 60 days in most patients, although in 2 individuals 0.4 and 2% donor MSCs were present at this time point.

Transplantation of neural progenitor cells, derived from the external germinal layer of neonatal mouse cerebellum, into the cerebral ventricles of MPS VII mice led to donor cell engraftment and differentiation throughout the neuraxis. The expression of β-glucuronidase resulted in widespread correction of lysosomal storage in neurons and glia. Other investigators have transduced human neural stem cells or murine bone marrow stromal cells with viral vectors carrying a β-glucuronidase transgene before performing CNS cell transplantation in mice. Transplanted cells engrafted, migrated, and differentiated into mature neurons and astrocytes. Treated mice showed increased β-glucuronidase activity, decreased lysosomal substrate, and improved cognitive function. Direct cranial injection of neural progenitor cells overexpressing ASM resulted in markedly decreased brain storage of cholesterol and sphingomyelin and cross-correction of host cells in mice with Niemann–Pick disease Type A. Ex vivo gene transfer using multipotent neural cell lines transduced by a retrovirus encoding human β-hexosaminidase, the enzyme deficient in Tay–Sachs disease, demonstrated widespread enzymatic activity in brains of transplanted mice. Wild-type hematopoietic stem cells and cells transduced with a lentiviral vector expressing ARSA were transplanted into a murine model of MLD. Brain microglia and peripheral nervous system macrophages showed extensive reconstitution and increased enzyme activity prevented the development of impaired motor conduction, learning and coordination deficits, and typical neuropathological abnormalities. The ex vivo gene therapy approach had a significantly higher therapeutic impact compared with wild-type hematopoietic cell transplantation, pointing to the potential benefit of overexpression of enzymes in order to maximize benefits. Glial precursors derived from murine embryonic stem cells engineered to overexpress ARSA were transplanted directly into the brain of MLD mice. The glial precursors engrafted into the brain, expressed ARSA, and reduced sulfatide storage, demonstrating that embryonic stem cells may serve as an enzyme-delivery system for lysosomal disorders that affect the CNS.

Krabbe disease is caused by a deficiency of galactocerebrosidase, which leads to the accumulation of psychosine, a toxic glycolipid. Whereas most LSDs result in isolated cellular dysfunction in the context of a relatively normal environment, the presence of psychosine represents a potential hurdle to overcome, because this compound would be expected to damage not only host cells but also any transplanted cells. Neural stem cells, however, appear to have intrinsic resistance to toxic metabolites, making them good candidates for developing therapies for metabolic disorders associated with a damaging cellular environment.

Encouraging results have also been obtained using human oligodendrocyte progenitor cells of fetal and adult origin for treating shiverer mice (a murine model for Krabbe disease characterized by deficiency of myelin basic protein). Oligodendrocyte progenitor cells disperse widely and develop into astrocytes and oligodendrocytes. Adult oligodendrocyte progenitor cells generate more host axons per donor cell than fetal cells, but both adult and fetal-derived oligodendrocyte progenitor cells mediated extensive remyelination in host brain, albeit in different time frames. Adult-derived progenitors achieved widespread myelination within 4 weeks after transplantation; fetal-derived progenitors took longer to achieve widespread myelination, but they exhibited more efficient engraftment and wider dispersion. The extensive emigration demonstrated by the fetal cells and their ability to differentiate into astrocytes and invade gray matter may make them more suited to the treatment of congenital leukodystrophies caused by LSDs than the adult-derived cells.

In 2000, Uchida and coworkers reported the isolation of human CNS stem cells from human fetal brain tissue using cell surface markers and fluorescence-activated cell sorting. The monoclonal antibody 5F3 recognizes the CD133 antigen, a marker that can enrich for human he...
matopoietic stem cells. A novel monoclonal antibody, 5E12, was used in conjunction with 5F3 to isolate a subset of human CD133+ fetal brain cells that have the capacity to generate neurospheres, undergo self-renewal, and differentiate into neurons and glial cells. When transplanted into brains of immunodeficient neonatal mice, human CNS stem cells are able to engraft, proliferate, migrate, and differentiate. Possible therapeutic potential of human CNS stem cells was demonstrated in spinal cord–injured NOD-SCID mice. The stem cells demonstrate long-term engraftment in the spinal cord, as well as the ability to migrate and differentiate into neurons and oligodendrocytes. Engraftment is associated with histological evidence of synapse formation between human CNS stem cells and host neurons and locomotor recovery. Expandable neurosphere cultures of human CNS stem cells grown under defined conditions enable the establishment of cell banks, which are necessary for therapeutic trials. A Phase I study, focusing on safety and preliminary efficacy, of human CNS stem cell therapy for INCL and LINCL is currently underway (ClinicalTrials.gov identifier NCT00337636).

Conclusions

Significant progress has been made toward treating LSDs with brain involvement, but major hurdles still remain. Nevertheless, as has been demonstrated by the continued development of novel therapeutic approaches, the BBB may not be as impenetrable as once imagined. Enzyme replacement therapy, whether by intravenous infusion, gene therapy, or hematopoietic or stem cell delivery, has shown potential to treat pathological conditions of the brain. Small-molecule therapies that readily cross the BBB are also in early trials or in development. Although each therapeutic modality has inherent strengths and weaknesses, perhaps the future of LSD therapy lies in combining different types of treatment. Augmentation of enzyme activity by ex vivo gene therapy before cell transplantation, simultaneous use of stem cells and small-molecule substrate inhibitors, or HSCT or ERT combined with agents that have the potential to disrupt the BBB are attractive strategies. Moreover, identification of affected patients before the onset of symptoms using a tandem mass spectrometry newborn screening approach could also yield significant benefits—treatment could be instituted before irreversible brain damage has occurred. Advances will undoubtedly continue to provide children afflicted by these devastating conditions and those who care for them hope for the future.

Disclosure

Dr. Enns is a consultant to and owns stock in StemCells, Inc. Dr. Huhn is vice president and head of the Neural Program of Stem Cells, Inc.

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Central nervous system therapy for lysosomal storage disorders

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