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Gene Therapy for Lysosomal Storage Disorders: Recent Advances and Limitations

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Abstract

Lysosomal storage disorders (LSDs) are a group of diseases with multisystemic features. Current treatments have limitations and gene therapy arises as a promising treatment option. Here, we discuss some of the most recent studies for gene therapy in LSD, vectors used, and outcomes. In particular, the approaches used in animal models aiming to correct the central nervous system, the eye, and the bones are highlighted. Finally, we discuss the recent reports of clinical trials using this technology for these diseases. We conclude that gene therapy for LSD has gathered a substantial amount of evidence from animal models to know its potential and limitations. First evidences from clinical trials using both adeno-associated and lentiviral vectors show that this approach is safe and efficient and therefore could provide an effective treatment for several LSD in the near future.

Keywords

lysosomal storage disorders, gene therapy, vectors, Hurler, clinical trials

Biology of Lysosomal Storage Disorders

Lysosomal storage disorders (LSDs) are a group of about 60 heterogeneous diseases that result in lysosomal dysfunction, usually as a consequence of deficiency of an enzyme required for the metabolism of complex molecules. As a group, these diseases can reach an incidence of about 1:5000 to 1:10 000. Most of the LSDs are autosomal recessive disorders; however, a few are X-linked recessively inherited, such as Hunter syndrome (mucopolysaccharidoses [MPS] II) and Fabry disease.[1]

Storage of undegraded or partially degraded material occurs in the lysosome. The LSD can be grouped into categories, based on the class of the substrate that accumulates, including lipidoses (where storage of lipids occurs), MPS (storage of mucopolysaccharides or glycosaminoglycans), glycogenoses (storage of glycogen), and oligosaccharidoses (storage of small sugar chains). In a general view, these diseases are multisystemic. Clinical findings of several LSDs include organomegaly as well as central nervous system (CNS) dysfunction. These diseases are also known for their progressive nature, with high morbidity and mortality, although there are significant variations between different diseases and even within the same disease.[1,2]

The genes mutated in LSD produce proteins that are important for normal lysosomal and cell function, and therefore, they are ubiquitously expressed. When an enzyme is lacking, the substrate accumulates in all tissues, but mainly in those where the turnover for that particular undegraded substance is higher. Therefore, the storage of the undegraded substrate related to the primary gene defect is believed to be the main cause of the disease phenotype. However, there is more and more evidence that this storage triggers multiple cascades of events that lead to the storage of other compounds (such as secondary accumulation of gangliosides in MPS) and many of the disease manifestations as well.[3]

Principles of Gene Therapy for LSD

Treatment options until a few decades ago for most LSD were restricted to support measures. Specific treatments have been

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Table 1. Specific Treatments Available for Lysosomal Storage Disorders.

Treatment	Summary	Pros	Cons	Examples of Some LSD
ERT	Periodical injection of the missing enzyme	Few adverse reaction Ameliorates visceral disease	Cost Does not cross the BBB (unless applied intrathecally)	MPS I, II, IVA, and VI, Gaucher, Fabry, Pompe, acid lipase deficiency
HSCT	Transplant of hematopoietic stem cells	Corrects the CNS disease and most somatic tissues	Need to find a compatible donor GVHD and other complications	MPS I, MLD, Krabbe, Gaucher
SRT	Drugs that inhibit synthesis of the accumulated substrate	Able to reach the CNS Oral medication	Does not correct the protein defect	Niemann-Pick C Gaucher
Pharmacological chaperones	Drugs that stabilize mutant proteins	Able to reach the CNS Oral medication	Works in particular mutations	Fabry, Tay-Sachs, Sandhoff
Stop-codon read-through therapy	Drugs that can suppress nonsense mutations	Already approved for other diseases Oral medication	Works only in nonsense mutations	MPS I
Gene therapy	Insertion of a functional sequence of the mutated gene	Potential for 1-time treatment	Risk of insertional mutagenesis	MPS IIIA, MPS I, MLD, Sandhoff, Tay-Sachs

Abbreviations: BBB, blood–brain barrier; CNS, central nervous system; ERT, enzyme replacement therapy; GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; MLD, metachromatic leukodystrophy; MPS, mucopolysaccharidosis; SRT, substrate reduction therapy.

developed in the last 2 decades and are available for several LSDs nowadays. Each treatment has its limitations and some are still in development. The main characteristics of currently available treatments of LSD are summarized in Table 1.

The rationale for gene therapy in these diseases is the same of other enzyme-based approaches: the ability of a deficient cell to internalize a lysosomal enzyme from the extracellular media. This enzyme can be originated either from the bloodstream (in case of enzyme replacement) or from a corrected cell (in case of gene therapy and hematopoietic stem cell transplantation [HSCT]). This uptake is a saturable, receptor-mediated process, in most cases by the mannose-6-phosphate receptor.[4]

Based on this, LSDs are considered good targets for gene therapy, despite their multisystemic involvement. The correction of a small percentage of cells results in enzyme secretion into the circulation, and the enzyme can be then internalized by the deficient cells, resulting in biochemical correction even in cells whose DNA was not modified.[5]

In LSD, long-term gene expression is desirable. Therefore, initially most clinical and preclinical trials used viral-based vectors. Nowadays, nonviral integrative approaches are being developed as well. The first studies on skin fibroblasts more than 2 decades ago used retroviruses and showed promising data.[6] However, tests in animal models evidenced that systemic injection of retroviruses would not correct some organs as the brain, as the enzymes cannot cross the blood–brain barrier (BBB).[7] Also, several LSDs involve other organs that are not easily corrected, such as the bone, the joints, and the eye, which evidenced the need for specific approaches. We have recently published a review that focuses on general aspects of gene therapy for MPS.[5] Here, we focus on LSD, specifically the effects of gene therapy on hard-to-correct organs, and the advances in clinical trials recently published for these diseases.

Gene Therapy Approaches Aiming to Correct Visceral Organs

Improvement in visceral organs (liver, heart, lung, and kidneys) is of major importance in LSD. One of the most common findings in these diseases is organ enlargement, especially hepatosplenomegaly. The increase in the size of these organs can alter hematological parameters in patients as well as lead to compression of other organs, such as the lungs, causing respiratory complications as well.[8]

Most gene therapy approaches are able to correct visceral pathology in LSD. Systemic injection of different vectors has shown that most particles are internalized by liver cells and, to a lesser extent, other visceral organs. Even in cases where the liver is the only transduced organ, the enzyme produced in the liver is able to reach the circulation and cross correct other visceral tissues.[9,10]

Based on enzyme replacement therapy (ERT) studies, some potential exceptions to that rule are the heart valves and the podocytes in the kidney. In Fabry disease, the podocytes are especially affected and complete correction is usually not achieved by ERT.[11] Thus, whereas these kidney cells appear to be somewhat refractory to treatment, this limitation is overcome, at least in part, by exposure to higher levels of the enzyme. A study using gene therapy with a pseudotyped rAAV2/8 vector in Fabry mice has shown the presence of high levels of the missing enzyme in renal cells 6 weeks after injection of 2×10^{12} particles and maintained normal levels of enzyme in the kidney even after 60 weeks.[12]

Higher levels of circulating enzyme are also needed to correct the heart valves. These structures are especially affected in different MPS, although other LSDs such as galactosialidosis can also show valve disease.[13] Mucopolysaccharidoses results from deficiency in one of the several enzymes involved in the

Table 2. AAV Gene Therapy in LSD With Brain Involvement: Preclinical Studies.

Disease	Animal Model	AAV Serotype	Route on Injection	Dose	Outcome	Reference
MLD	Mice	AAV 9	IV	2×10^{12}	Improvement in behavior, biochemistry	Miyaki et al[26]
Krabbe disease	Mice	AAV 2/5	Intracranial	2.4×10^{9}	Improvements in biochemistry and histology; neurobehavioral performance and prolonged life span	Lin et al[27]
MPS VII	Canine	AAV 9 or rh10	IV and/or IT	2×10^{13} or 5×10^{12}	Biochemical improvements. IT injection is more effective than IV injection	Gurda et al[22]
GM1-gangliosidosis	Mice	AAV 9	IV	1×10^{11} or 3×10^{11}	Improvements performance in motor function and behavior, enzyme, and GAG clearance	Weismann et al[23]
Sandhoff disease	Feline	AAV 8	Multiple intracranial injections	4.5×10^{11} (Thal), 1.6×10^{11} (DCN), 6.4×10^{11} (ICV)	GAG clearance in cerebral cortex and liver	Gray-Edwards et al[25]
MPS I	Feline	AAV 9	IT	10^{12}	Correction of biochemistry and histology	Hinderer et al[28]
MPS IIIB	Mice	AAV 5, 8, 9, 10	Intracranial	4.8×10^{9}	AAV 8, 9 represent optimal candidates for early gene therapy (biodistribution and transduction efficiency profiles)	Gilkes et al[24]

Abbreviations: AAV, adeno-associated virus; DCN, deep cerebellar nuclei; GAG, glycosaminoglycans; ICV, intracerebroventricular; IT, intrathecal; IV, intravenous; LSD, lysosomal storage disorder; MLD, metachromatic leukodystrophy; MPS, mucopolysaccharidoses; Thal, thalamus.

degradation of glycosaminoglycans, and heart disease is characterized by mitral and aortic valve thickening and regurgitation and aortic dilatation.[14] Recent studies have shown that high levels of circulating enzyme are essential to correct heart valve disease in MPS VII dogs. Treating dogs from birth using a retroviral vector-transduced hepatocytes secreted β-glucuronidase into blood to an average of 3.6-fold normal levels for more than a decade. Retroviral vector (RV)-mediated gene therapy reduced cardiac valve disease in MPS VII dogs, although at later times in life, small abnormalities could be detected in some dogs.[15]

Gene Therapy Approaches Aiming to Correct Brain Disease

Most LSDs have a CNS component, and frequently, it is the most affected system in these disorders. It is known that large molecules such as enzymes cannot freely move from the circulation to the brain cells, due to the BBB. This endothelial occlusive epithelium limits the number of molecules that can reach the brain. Therefore, gene therapy approaches to the CNS need to transduce directly neuron cells, to generate a modified molecule that uses one of the few receptors in the BBB to enter the brain, or to modify cells ex vivo that can then reach the brain after implant.

Several gene delivery systems have been evaluated and showed at least some evidence of CNS correction in animal models of multiple LSD. Both viral and nonviral vectors have been delivered either directly into the CNS or in the circulation. Transplantation of genetically modified stem cells ex vivo has also shown good response.[16]

Due to their characteristic of transducing only cells that are going through division, the use of gamma-retroviral vectors for CNS manifestations is limited. However, a few studies have shown that in animal models, a small percentage of the enzyme crosses the BBB through mechanisms still unknown when very high levels of enzyme are produced by liver cells transduced with retroviral vectors.[17] That scenario is not likely to happen in patients, since upscaling from small mice to bigger mammals usually leads to a significant reduction in serum enzyme levels.[18]

Adeno-associated viral (AAV) vectors are emerging as the in vivo vector platform of choice for neurological diseases. Several studies using different AAV serotypes have been described. These viruses are capable of infecting cells that are not going through division and persist primarily as nonintegrative episomal units, although integration leading to hepatic tumors were reported in mice when neonatal treatment is performed.[19,20]

Adeno-associated virus serotypes 1, 5, 9, and rh.10 are particularly efficient in transfecting neuronal mouse cells after in situ injections. Serotypes 9 and rh.10 can also cross the BBB and transduce CNS cells when applied intravenously in mice and dogs.[21,22]

These vectors were recently used to treat animal models of GM1 gangliosidosis,[23] MPS IIIB,[24] Sandhoff disease,[25] metachromatic leukodystrophy,[26] Krabbe disease,[27] and MPS I.[28] Different doses, routes of delivery, and outcomes are summarized in Table 2. In general, these vectors were very effective in correcting disease phenotype, quickly becoming one of the most promising approaches for the treatment of LSD.

Based on the positive results from the animal models, a phase I/II clinical study in patients with MPS IIIA was conducted. Four patients were treated with intracerebral injections of an AAV rh.10 at a dose of 7.2×10^{11} viral genomes/patient simultaneously over a period of 2 hours. The use of this vector was safe and well tolerated. This study also showed moderate improvement in behavior, attention, and sleep, mainly in the patient treated earlier.[29] An increase in the number of patients is still necessary, but AAV vectors surely arise as a viable and encouraging approach to treat LSD.

Table 3. Lentiviral Gene Therapy in LSD.

Disease	Animal Model	Approach	Route on Injection	Dose	Outcome	Reference
Krabbe disease	Mice	In vivo	Intracerebral	2×10^6 UT	Biochemical and enzymatic correction. Improvement of bone mass and architecture	Lattanzi et al[34]
Pompe disease	Mice	In vivo	Superficial temporal vein	4.5×10^8 to 1.3×10^9 IU/mL	Correction of biochemical abnormalities	Kyosen et al[33]
MPS II	Mice	Ex vivo	IV	2×10^6 cells/mL	Correction of neuronal manifestations by ameliorating lysosomal storage and autophagic dysfunction	Wakabayashi et al[35]
MPS I	Mice	Ex vivo	Tail vein	10^6 cells/mouse	Neurological and skeletal improvement	Visigalli et al[36]

Abbreviations: IU, infectious units; IV, intravenous; LSD, lysosomal storage disorder; MPS, mucopolysaccharidoses; UT, transducing unit.

Other viruses demonstrated efficacy in correcting brain disease in LSD animal models. Notably, the use of lentiviruses to correct both stem cells (ex vivo gene therapy) and quiescent cells (in vivo gene therapy) was described in several diseases, including MPS IIIA, MPS IIIB, and cystinosis, to name a few.[30–33] These vectors can also be injected in situ, as shown for Krabbe mice.[34]

Lentiviruses (Table 3) can be also used to augment the efficacy of existing treatments as shown by studies in both MPS I and MPS II mice. Mucopolysaccharidoses I mice were treated with normal hematopoietic stem cell (HSC) modified by a lentivirus to overexpress alpha-L-iduronidase (IDUA), and it resulted in better correction in organs that are hard to treat by conventional HSCT, such as neurologic and skeletal disease.[36] A recent study demonstrated that although biochemical alterations in the brains of MPS II mice are not fully corrected by regular HSCT, curiously the HSCT modified with a lentivirus to overexpress iduronate sulfatase corrected neuronal manifestations in the brains of MPS II mice.[35]

After preclinical studies, the use of these viruses finally reached the clinical stage. A lentiviral vector was used to transfer a functional Arylsulfatase A (ARSA) gene into HSCs from 3 presymptomatic patients with late infantile metachromatic leukodystrophy. After treatment, the patients showed extensive and stable ARSA gene replacement, no evidence of aberrant clonal behavior in the modified cells, and prevention of disease manifestation for up to 21 months beyond the predicted age of symptom onset.[37] Recently, a report from a phase I trial with this approach was published. The results are quite promising since it showed no serious adverse events in 9 treated children. Furthermore, ARSA levels were restored and 8 patients had prevention of the disease symptoms.[38] Along with AAVs, lentiviruses seem to be currently the 2 best options for gene therapy of LSD.

Gene Therapy Approaches Aiming to Correct Eye Disease

Along with the brain, the eye is also an organ isolated by an occlusive epithelium, the blood–retinal barrier. This structure prevents most substances to cross from the choriocapillaris to the retina.

One of the neuronal ceroid lipofuscinosis type 2 (CLN2) is characterized primarily by progressive loss of neurological functions and vision as well as generalized neurodegeneration and retinal degeneration. A recent study using an intracerebrospinal fluid (CSF) injection of an rAAV-2 in dogs has shown that the degeneration of the retina and loss of retinal function continued to progress, despite an improvement in the ganglion cell layer and in brain disease.[39]

An interesting study using a retroviral vector in MPS I mice reached a somewhat similar conclusion. This vector only transduces liver cells that produce high levels of enzyme (up to 1000-fold normal levels in serum). The authors found that correction of eye disease, as happens to the brain, is only achieved in mice with the highest serum enzyme levels.[40] These 2 studies suggest that intraocular delivery of a vector might be necessary for proper treatment of eye disease in LSD.

Intravitreal injection of AAV vectors is feasible and has been done even in clinical trials for Leber congenital amaurosis, for example. An intravitreal injection of an AAV vector was tested in MPS VII mice. Retinal function was improved, but the efficacy of the treatment depended heavily on parameters related to the injection procedure, such as the injection volume and vector dose. The authors concluded that intraocular AAV-mediated therapy may be efficacious for the retinal disease observed in LSD.[41]

Gene Therapy Approaches Aiming to Correct the Bones

The bones are structures poorly cellularized and vascularized, with a rigid extracellular matrix that does not allow an adequate distribution of enzymes when they are applied intravenously or even in situ. This scenario makes treatment of bone tissue especially challenging, especially because they are responsible for a lot of burden that characterizes these diseases.

Increasing the affinity of the enzyme by modifying its structure was attempted for ERT in animal models, with a certain success.[42] However, gene therapy approaches have not included such modifications so far. Instead of that both transduction of other organs and overexpression of the enzyme or modifications of stem cells with its subsequent reinfusion have been tried.

Although not the only group of LSD with bone involvement, the skeletal disease in the MPS disorders is one of the best studied. Patients with MPS usually present short stature and dysostosis, among other complications. Aiming to correct these characteristics, preclinical studies have shown some progress. Mucopolysaccharidoses VII mice treated either at birth or 7 weeks of age with a systemic lentiviral gene therapy resulted in improvement in parameters of bone mass and architecture as well as biochemical and enzymatic correction. Conversely, growth plate chondrocytes were not responsive to treatment, and vertebral and femoral bone length and growth plate height were not corrected.[43] Neonatal treatment of MPS VII dogs with a retrovirus resulted in high levels of the enzyme in the serum, and despite a positive general clinical effect, treated MPS VII dogs still had hypoplastic ventral epiphyses with reduced calcification in the lumbar spine at 6 months, which evidence that bone and spine disease is not completely corrected in larger animals, even with high enzyme levels in serum.[44] Similar results were also found in another disease, MPS VI, when the feline model was treated with this vector.[10] Based on these results, it becomes evident that even if gene therapy is able to correct a vast number of cells, it will not be able to correct all disease symptoms in this group of disorders, and novel technologies should be considered to treat bone disease.

Concluding Remarks

Gene therapy for LSD has come a long way in the last 30 years. The animal models have been very useful to predict organs that are going to be corrected by each approach and which systems are still refractory to treatment. Central nervous system abnormalities seem to be corrected by specific vectors and injection routes, but new approaches aiming to correct bone and eye disease still need to be developed, because they are important aspects of these disorders. The first clinical studies suggest that AAV and lentiviral vectors have the potential to provide a single-dose, stable, safe, and effective treatment for LSD in the near future.

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