IUBMB *Life*, 62(1): 33–40, January 2010

Critical Review

Therapy for Lysosomal Storage Disorders

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Summary

In the last years, much progress has been achieved in the field of lysosomal storage disorders. In the past, no specific treatment was available for the affected patients; management mainly consisted of supportive care and treatment of complications. As orphan drug regulations, however, encouraged development of drugs for these disorders by granting marketing exclusivity for 10 years and other commercial benefits, enzyme replacement therapy became available for lysosomal storage disorders, such as Gaucher disease, Fabry disease, mucopolysaccharidoses type I, II, and VI, and Pompe disease. This review will summarize the efficacy and clinical status of hematopoietic stem cell transplantation, enzyme replacement, and substrate deprivation therapy, and describe new therapeutic perspectives currently under preclinical investigations such as chaperone-mediated therapy, stop-codon read-through therapy, and gene therapy.

IUBMB *Life*, 62(1): 33–40, 2010

Keywords lysosomal storage disorder; chaperones; gene therapy; imino sugar; substrate reduction; hematopoietic stem cell transplantation.

LYSOSOMAL STORAGE DISORDERS

The degradation of biological macromolecules such as glycosaminoglycans (GAGs), glycoproteins, and glycolipids takes place in lysosomes that are present in each cell of the organism. These cell organelles enclose a great number of acid hydrolases that after synthesis traverse the Golgi apparatus, where they are modified by the formation of the mannose-6-phosphate (M6P) recognition marker that is essential for uptake into the lysosome via the M6P receptor. In addition to the M6P receptor mechanism, there exist other ways to target proteins to these organelles. A variable portion of newly synthesized lysosomal enzymes is not bound to the M6P receptor and is secreted into the blood stream. These enzymes can be partially taken up again by cells and transported to the lysosomes through M6P-mediated endocytosis where they can fulfill their function. This mechanism is an essential prerequisite for the effectiveness of metabolic cross-correction, for example, by hematopoietic stem cell transplantation (HSCT) or enzyme replacement therapy (ERT).

A defect of the lysosome, most commonly a mutation in genes that encode catalytic enzymes, results in accumulation of compounds that are normally degraded and consequently leads to a storage disorder. Other forms of lysosomal dysfunction that can be responsible for a storage disorder include defects of membrane proteins, errors in enzyme targeting, and defective function of enzyme activators. The mechanisms by which the storage material disturbs cellular function and results in a disease are not well understood until now; they include alterations of intracellular calcium homeostasis, impairment of autophagy, activation of signal transduction by nonphysiologcal substances, inflammation, and others (*1*).

The detection that the metabolic defect of cultured fibroblasts from mucopolysaccharidosis (MPS) patients can be compensated by cross-correction with factors that are secreted by cells not having the same defect lead to the consideration that lysosomal storage disorders should be generally treatable by administration of the intact lysosomal enzyme, a treatment strategy designated as ERT (*2*). In addition to this therapeutic principle that is aimed to remove the accumulated storage material within the lysosome and has been shown to be very effective in Gaucher disease since a long time, there have been developed novel therapeutic approaches using small molecules (imino sugars) that are able to partially inhibit the substrate synthesis and influx into the catabolically compromised lysosome. This therapeutic strategy has been termed substrate reduction therapy. Later on, it has been detected that imino sugars also act as molecular chaperones that can stabilize accurate conformation of improperly folded mutant enzymes. Future therapeutic principles that will be discussed in this review include the use of antiinflammatory drugs, gene therapy, and gene modification by enhanced stop-codon read-through.

Received 8 September 2009; accepted 2 November 2009
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ISSN 1521-6543 print/ISSN 1521-6551 online
DOI: 10.1002/iub.284

METABOLIC CROSS-CORRECTION

Stem Cell Transplantation

The principle of transplantation of hematopoietic stem cells consists of the fact that hematopoietic stem cells of the patient are replaced by cells of a donor. In general, the donor cells are able to build up sufficient amounts of enzyme to correct the deficient activity and to be clinically efficient (*3*). Since the first bone marrow transplantation about 30 years ago, several hundred patients with a lysosomal storage disorder have received a HSCT. Most experience has been gained in treating children with MPS type IH (Hurler disease), and quite good results can be achieved by HSCT if the procedure is performed under the age of 2 years: Engraftment after transplantation results in a rapid decline in GAG excretion, reduction of liver and spleen volume, and improvement of obstructive airway symptoms. The skeleton, however, does not respond as well and patients often need surgical intervention many years after the procedure. Although treated patients often show a significant gain in cognitive function, in others intellectual and developmental deterioration may occur (*4*). If a bone marrow donor cannot be found, cord blood from unrelated individuals can also be used for transplantation (*5*).

HSCT has led to promising results in patients with various lysosomal storage disorders, such as α-mannosidosis (*6*), metachromatic leukodystrophy, and Krabbe disease, provided the transplantation is performed before signs of involvement of the peripheral and/or central nervous system are present (*7*). In MPS I patients, ERT may be applied before HSCT as long as a donor has not been found (*8*).

As most reports on HSCT include only a small number of patients, it is difficult to draw general conclusions about the safety and efficacy of HSCT in lysosomal storage disorders. However, as it can be expected that by using newly developed methods, HSCT will be safer and more widely used; the value of this procedure should be assessed again in the future (*9*).

Enzyme Replacement Therapy

Clinical Efficacy. As mentioned earlier, the fraction of lysosomal enzymes that do not enter the lysosome is secreted from the cell and can be recaptured from other cells by either the M6P or other receptors. This mechanism enables successful treatment with exogenously supplied enzymes.

Type I Gaucher disease, which is characterized by anemia, thrombocytopenia, organomegaly, and skeletal disease, was the first lysosomal storage disorder for which ERT became available. In a clinical trial performed by Barton et al., 12 patients with the nonneuronopathic form (type I) of Gaucher disease received β-glucocerebrosidase that was derived from human placenta and treated with specific glycosidases to expose mannose residues in the oligosaccharide chains, because this enzyme has to be taken up by macrophages via the mannose receptor, and not by the M6P receptor (*10*). Within 1 year, a significant increase in hemoglobin level and platelet count was observed in all patients, and liver and spleen volume decreased. Based on this trial, the enzyme preparation was approved for treatment of patients with Gaucher disease. Some years later, the placenta-derived β-glucocerebrosidase (alglucerase, Ceredase®, Genzyme, Cambridge, MA) was replaced by a recombinant form produced in Chinese hamster ovary (CHO) cells. Also, this enzyme preparation (imiglucerase, Cerezyme®, Genzyme, Cambridge, MA) needed to be modified for targeting mannose receptor sites on macrophages. In the last 10 years, countless publications and reports confirm the positive effects of imiglucerase on hematological parameters and bone disease, and because of its safety and efficacy profile, ERT has become the standard of care for type I Gaucher patients (*11*).

To assess the effect of ERT in patients with the neuronopathic form (type III) of Gaucher disease, Davies et al. reviewed 55 patients from five European countries who had been on ERT for different lengths of time (*12*). From their analysis, the authors came to the conclusion that older patients appeared to remain relatively stable despite a low dose of enzyme. However, there was no clear effect of high-dose ERT in younger patients.

In an attempt to halt disease progression in the acute neuronopathic form (type II) of Gaucher disease, two patients received alglucerase at an early age, 7 months and 4 days, respectively (*13*). However, the treatment could not alter the outcome, both infants died before the 18th month of life. There is now a general agreement that ERT is not indicated in the acute neuronopathic form of Gaucher disease.

A new enzyme preparation is being produced in plant cells and has shown to be well tolerated and safe (*14*). A phase III clinical trial for the recombinant plant cell expressed glucocerebrosidase is currently ongoing. The pharmaceutical company Shire (Shire Human Genetic Therapies, Cambridge, MA) has developed an enzyme preparation that uses gene-activating technologies for production of recombinant glucocerebrosidase (*15*).

In Fabry disease (α-galactosidase deficiency) pain, kidney failure, cardiomyopathy, and cerebrovascular events are the complications that are mainly responsible for morbidity and mortality of this disorder. For patients with Fabry disease, two different enzyme preparations are available: Algalsidase beta (Fabrazyme®, Genzyme, Cambridge, MA), produced in CHO cells, and agalsidase alfa (Replagal®, Shire Human Genetic Therapies, Cambridge, MA), produced in human cell lines. The analysis of latest reports regarding the clinical efficacy and safety of agalsidase alfa in patients with Fabry disease has demonstrated that this enzyme preparation is effective in treating pain and in reducing heart size in patients with Fabry disease, to improve hearing, sweating, and quality of life (*16*). It is able to stabilize the kidney function and slow down the progression of renal failure in patients with end-stage renal disease (*17*).

The clinical efficacy of agalsidase beta has been studied in a double-blind multicenter trial, in which 82 adult Fabry patients with kidney dysfunction were randomly assigned to infusions of agalsidase beta or placebo for up to 35 months (*18*). Data ana-

lysis at the end of the trial has shown that agalsidase beta was able to reduce the frequency and to delay the onset of clinical events. Clinical events encompassed the composite outcome of renal, cardiac, and cerebrovascular events and death.

MPS are multisystemic disorders that affect several organ systems in individually variable degree (*19*). They are caused by deficiency of lysosomal enzymes that are responsible for the stepwise degradation of GAGs. There are 11 known enzyme deficiencies that give rise to seven distinct MPS types. Each of the types is characterized by considerable clinical variability of both the age of onset of symptoms and severity of organ manifestation (*20*). ERT is available now for MPS I, II, and VI (*21–23*). This therapeutic regimen has shown to have a positive effect on joint mobility, lung function, and organomegaly, but it has no influence on the manifestation of the central nervous system and cannot cure the disease.

Pompe disease (α-glucosidase deficiency) is a lysosomal storage disorder characterized by muscle weakness and cardiomyopathy. In 2006 the enzyme preparation alglucosidase alfa (Myozyme®, Genzyme, Cambridge, MA) has got marketing approval after it has been proven to be effective in ameliorating muscle strength and improving heart function. The clinical efficacy of this enzyme preparation could be confirmed by several clinical trials in patients with different ages of onset and disease severity (*24*). In Pompe disease, ERT, however, has its limitations because of unsatisfactory access of recombinant α-glucosidase to the muscle cells and because of the formation of antibodies. To overcome these therapeutic restraints, the development of a more effective enzyme preparation is needed.

Safety and Tolerability. The regular intravenous administration of a protein bears the risk of developing antibodies that may lead to allergic reactions and/or inactivate enzyme activity (*25*). Extensive studies on antibody formation were performed in MPS I patients who have received the recombinant enzyme α-L-iduronidase (IDUA). In the phase III trial of ERT for MPS I, infusion-related reactions that were defined as any adverse event occurring from the beginning of an infusion to the end of the same day have been observed in both, the treated and placebo groups and consisted mainly of flushing, fever, headache, and rash (*26*). To minimize possible infusion-related reactions, the patients were pretreated with an antipyretic and an antihistamine before each infusion. The antibody titers were measured in the patients at the beginning and after several weeks of treatment (*27*). In 5 of 10 patients, increased IgG antibody levels were detected during treatment, but there was no correlation between the occurrence of severity of adverse events and the presence of high antibody titers. The antibodies belonged to the IgG subclass and did not inhibit enzyme activity (*28*). At the end of the study, in most of the patients the titers dropped into the normal range suggesting that the patients developed immune tolerance to the recombinant human IDUA. Similar observations were made in clinical trials of ERT in patients with MPS VI (*29*) and MPS II (*30*).

The formation of α-galactosidase A antibodies was studied in Fabry patients who were treated either with the enzyme agalsidase alfa or agalsidase beta (*31*). After 6 months of therapy, 11 of 16 males developed high titers of IgG antibodies that cross-reacted *in vitro* similarly with both enzyme preparations. In all IgG-negative patients, a significant reduction in urinary globotriasylceramide (Gb_3) was observed, whereas IgG-positive patients showed an increase in urinary Gb_3 during treatment, suggesting a negative effect of these antibodies on clearance of storage material in kidney cells. In some cases, an allergic type reaction to treatment with agalsidase beta has been observed with the presence of IgE in the circulation and/or a positive intradermal reaction (*32*).

In Gaucher disease, much experience has been gained with ERT in a large number of patients. Within the scope of a safety surveillance program, 1,122 Gaucher patients were monitored for the development of antibodies against the enzyme preparation alglucerase (*33*). Seroconversion was detected in 142 individuals (12.8%). In 25% of the seroconverted patients, inhibitory antibodies were found, most of them being transient. In general, the presence of inhibitory antibodies was not associated with a reduction in efficacy of the enzyme preparation.

In Pompe disease, one of the greatest barriers to a successful ERT is the fact that in subjects who are negative for cross-reacting immunologic material, the efficacy is markedly reduced by the formation of high-titer antibodies against human α-glucosidase (*34*). In a mouse model of Pompe disease, it could be shown that the formation of antibodies can be prevented by AAV vector-mediated gene therapy that induced immune tolerance to the infused enzyme (*35*).

Hypersensitivity/anaphylactic reaction against the infused enzyme can be treated by slowing down the infusion rates and premedication with antihistamines and/or corticosteroids. In an ongoing strong immune response, tolerance induction by drugs such as methotrexate or rituximab may become necessary (*25*). In general, however, it can be stated that ERT is well tolerated, and that patients who exhibit seroconversion show a decrease in their antibody titers with time and mostly continue to tolerate the enzyme.

SUBSTRATE REDUCTION

Substrate reduction therapy represents a novel approach for treatment of lysosomal storage disorders. The concept of this therapeutic principle is to reduce the amount of storage material instead of enhancing the activity of the degrading enzymes. The imino sugar *N*-butyldeoxynojirimycin has the ability to inhibit ceramide glucosyltransferase, the enzyme that synthesizes glucosylceramide, the storage compound in Gaucher disease (*36*). To demonstrate safety and efficacy of this substance, a clinical trial was initiated in 28 adult Gaucher patients who were unable or unwilling to receive ERT (*37*). And, based on this trial, *N*-butyldeoxynojirimycin (miglustat, Zavesca®, Actelion Pharmaceuticals, Allschwil/Basel, Switzerland) gained marketing ap-

proval in Europe and in the USA for symptomatic Gaucher patients with mild to moderate clinical manifestation for whom ERT is not an option. Several clinical trials have shown that miglustat did not only improve blood parameters such as hemoglobin and platelet counts but also has a positive effect on bone manifestations (*38*).

Clinically, the lipid storage disorder Niemann-Pick type C (NPC) is characterized by vertical supranuclear gaze palsy, ataxia, dysarthria, seizures, progressive dementia, and cataplexy. NPC results from mutations of either the NPC1 or the NPC2 gene. These genes encode proteins that are responsible for trafficking of unesterified cholesterol between several cell compartments. A defect of one of these genes leads to accumulation of cholesterol, sphingosine, sphingomyelin, and glycosphingolipids. The complex mechanisms, however, that cause the accumulation of heterogeneous substrates in NPC and other lysosomal storage disorders, are not fully understood until now (*39*). And, as miglustat inhibits the synthesis of glucosylceramide, the precursor of glycosphingolipids that accumulate in NPC, this substance was considered as a therapeutic option for NPC patients. A randomized clinical trial was performed in patients aged 12 years or older (*40*). After 1 year of treatment, horizontal saccadic eye movement velocity had improved in patients who received miglustat *versus* those who were treated according to standard care. Also, an improvement in swallowing capacity and a slower deterioration in ambulatory index were seen in the miglustat group. In 2009, miglustat was approved in Europe for the treatment of NPC.

Many other substrate inhibitors have been identified and applied in experiments or in clinical trials. McEachern et al. have found a novel substance (Genz-112638) that—like miglustat—is able to inhibit the synthesis of glucosylceramide and could eventually offer a new therapeutic option for Gaucher patients (*41*).

Rhodamine B has been shown to be an inhibitor of the synthesis of GAGs, the compounds that are accumulating in patients with MPS. In an animal model (mice affected by MPS type IIIA), weekly intravenous application of rhodamine B had a clear beneficial effect on CNS function of the animals, measured by the water cross-maze test (*42*). GAG synthesis can also be inhibited by genistein, an isoflavone extract from soy beans. In an open-label study, Piotrowska et al. have analyzed the effect of genistein on urinary GAG excretion, hair morphology, and behavior in 10 patients, affected by either MPS IIIA or MPS IIIB (*43*). After oral administration of genistein-rich soy isoflavone extract (5 mg/kg/day) for 1 year, a statistically significant improvement in all these parameters was found.

CHAPERONES

Lysosomal enzymes are synthesized and secreted into the endoplasmic reticulum in a largely unfolded state. There exists an efficient cell system that controls that only the properly folded proteins are transported to the Golgi apparatus for further maturation. Misfolded enzymes, however, are degraded by proteasomes. Specific molecules, called "chaperones" (*e.g.*, Bip, heat shock proteins, and calnexin), help the proteins to be folded into the appropriate confirmation. Mutations that affect accurate folding prevent the lysosomal enzymes from reaching their final destination, the lysosome, so that they cannot fulfill their function. However, some mutant enzymes might maintain either full or partial catalytic capability if they could acquire their correct confirmation and consequently would undergo further maturation. And, in lymphoblasts of Fabry patients, it has been demonstrated that a competitive inhibitor of the enzyme α-galactosidase, namely 1-deoxy-galactonojirimycin, at subinhibitory concentrations can act as a chemical chaperone leading to correct confirmation of the mutant enzyme and thereby increasing its catalytic activity (*44*). The concept of using inhibitors as chaperones has been investigated in cultured cells from patients with several lysosomal storage disorders such as Pompe disease (*45*), Gaucher disease (*46*), and Fabry disease (*47*). One of the advantages of pharmacological chaperones is their better biodistribution profile in comparison with recombinant enzymes. In the rat, a wide distribution of the chaperone *N*-butyldeoxynojirimycin (miglustat) in a large number of organs such as the central nervous system, bone, and lung was observed. One of the disadvantages of a chemical chaperone, however, is the fact that its treatment effect is restricted to patients with missense mutations, and the analysis of molecular surveys of a large number of Pompe patients revealed that only 10–15% of these patients are amenable to enhancement therapy (*45*).

GENE THERAPY

ERT has been shown to be of limited efficacy, in particular regarding the effect on bone and brain manifestations. Gene-based therapy may overcome this problem, as it may allow constant delivery of a therapeutic protein to targeted organs, as for example the bone or the brain. Lysosomal storage disorders are excellent candidates for therapy by gene transfer, as they represent generally well-characterized single gene disorders, and in addition, are not subject to complex regulation mechanisms, and an enzyme activity of only 15–20% of the normal level is sufficient for clinical efficacy (*48*). There are two ways to deliver a gene into the organism, the *in vivo* and the *ex vivo* technique.

In Vivo Gene Therapy

To set up a continuous source of enzyme for metabolic correction in peripheral organs, the liver and lung have been used as a depot organ. In animal experiments, several vehicles such as adeno-associated, retroviral, and lentiviral vectors were applied to efficient organ transduction. By this strategy, the liver or lung produced large amounts of therapeutic enzyme that was secreted into the bloodstream and recaptured by the target organs via the M6P receptor. The efficacy of this technique has been demonstrated in several animal models (*49, 50*). The beneficial effect of gene transduction into the liver or other tissues is

restricted to peripheral organs, as the enzyme that is secreted into the circulation will not cross the blood–brain barrier. Therefore, vector delivery systems have been developed for direct *in vivo* gene transfer into the CNS. This procedure has been studied an animal model of late infantile neuronal ceroid lipofuscinosis (LINCL), a neurodegenerative lysosomal storage disorder, caused by mutations of the CLN2 (ceroid lipofuscinosis, neuronal 2) gene. And, in these experiments, it could be demonstrated that administration of an adeno-associated virus gene transfer vector expressing the human CLN2 cDNA into the brain of CLN2 knockout mouse resulted in suppression of the accumulation of autofluorescent material in the CNS (*51*). Based on these animal experiments, a clinical study was initiated in 10 children with LINCL (*52*). In this trial, the adeno-associated virus serotype 2 vector expressing the CLN2 cDNA was administered to several locations in the CNS of the patients. To compare the rate of neurological decline in treated subjects compared with untreated subjects, a neurological rating scale and three quantitative MRI parameters were used. One child died 49 days after the procedure due to intractable status epilepticus, but with no evidence of CNS inflammation. Eighteen months after the surgery, assessment of the neurological rating scale of the treated patients showed a significantly reduced rate of neurological decline compared with the control group. Also, the measured rates of decline of all MRI parameters were slower. Additional studies are necessary to confirm these preliminary results of a gene therapy experiment in humans with a lysosomal storage disorder.

Ex Vivo Gene Therapy

As mentioned earlier, one of the major limitations of ERT represents the blood–brain barrier as it prevents the access of the intravenously administered enzyme to the central nervous system. Hematopoietic cells, however, are able to cross this barrier and can therefore used as vehicles for drugs or genes: After hematopoietic stem cell transplantation, donor-derived cells—in particular from the myelomonocytic lineage—migrate into the brain and differentiate thereafter to form microglia (*53*). By this mechanism, cells from a healthy donor can cross-correct an enzyme defect in neuronal and glial cells. However, despite significant progress in HSCT and the availability of banked umbilical cord blood, this procedure is still associated with significant risks of graft failure or GVHD that can lead to death. This problem may be circumvented by transplantation of autologous hematopoietic stem cells that have been genetically modified to express the missing protein (*ex vivo* gene therapy). The efficacy of *ex vivo* gene therapy has been demonstrated in many experiments on animals, for example, in arylsulfatase A deficient mice. In these animals, hematopoietic stem cells, transduced *ex vivo* with the arylsulfatase A gene, resulted in full reconstitution of enzyme activity. Moreover, this procedure was able to prevent functional deficits such as motor conduction impairment and even could reverse neurological deficits (*54*). From this result, it can be concluded that *ex vivo* human stem cell gene therapy using a viral vector has a significantly higher therapeutic impact than wild-type HSC transplantation, indicating that enzyme overexpression in the transplanted cells plays a crucial role.

Although gene therapy studies performed in animal models are rather promising, many important issues regarding safety and efficacy of this therapeutic strategy need to be addressed before large-scale clinical trials with viral vectors can be initiated. To achieve high enzyme activity, a high level of transgene expression by hematopoietic stem cells might be required, and the integration of a large amount of vectors increases the risk of integrated-dependent adverse events. And, the integration of retroviral and lentiviral vectors close to expressed genes may lead to transcriptional interference between the vector and flanking endogeneous genes (*55*). Such interference my be avoided by using late-generation lentiviral vectors that are self-inactivating with transcriptionally inactive long terminal repeats on transduction and express the transgene from an internal promoter of choice. There is still a long way to go until gene therapy becomes a realistic therapeutic option for patients affected by a lysosomal storage disorder, as transformation of animal studies to clinical trials requires development of large-scale manufacturing and quality assays, rigorous demonstration of safety and efficacy of new gene therapy protocols, and last but not least, the consensus and approval of the scientific and biomedical communities.

FUTURE PROSPECTS OF TREATMENT

As mentioned earlier, inflammation seems to play a significant role in the pathophysiology of lysosomal storage disorders, in particular of GM1- and GM2-gangliosidosis. Based on these considerations, mice affected by GM2-gangliosidosis (Sandhoff disease) were treated with nonsteroidal antiinflammatory drugs (indomethacin, aspirin, and ibuprofen) and antioxidants (L-ascorbic acid and alpha-tocopherol acetate). The treated mice had a significant longer lifespan than the untreated animals and showed a slower rate of disease progression (*56*). This study demonstrates that antiinflammatory and antioxidant drugs may serve as a therapeutic strategy, supplementary to enzyme replacement or enzyme enhancement therapy.

In a large number of patients with a lysosomal storage disorder, nonsense mutations have been identified that lead to a premature stop-codon in the transcribed mRNA and to the synthesis of a truncated and nonfunctional enzyme. It could be demonstrated, however, that certain low-molecular-weight drugs, as for example, gentamicin induced the read-through of premature stop codons, resulting in catalytic activity of the enzyme. And, experimental studies have proven that enhanced stop-codon read-through could be used as a therapeutic approach for different genetic diseases such as cystic fibrosis, Duchenne muscular dystrophy, and ataxia-teleangiectasia (*57*).

In the severe form of α-iduronidase deficiency (MPS IH, Hurler disease), at least 15 mutations have been identified that cause premature stop-codons in the IDUA gene. The capability of gentamicin to suppress the stop-codon has been analyzed in human MPS I fibroblasts carrying different nonsense mutations (*58*). With one exception, gentamicin treatment increased the α-iduronidase activity in all MPS I fibroblasts that were tested. Hence, enhanced stop-codon read-through may be potential treatment strategy for a large subgroup of MPS I patients.

SUMMARY

As each of the therapeutic interventions discussed in this review targets only one aspect of the complex pathophysiology of a lysosomal storage disorder, it seems that by combination of several therapies a better outcome may be achieved than by one therapy alone. In a mouse model of Sandhoff disease, a synergistic effect was observed by combining substrate reduction therapy and bone marrow transplantation (*59*). In a clinical trial, the combination of ERT and substrate reduction therapy was evaluated in patients with the chronic neuronopathic (type III) form of Gaucher disease (*60*). And, from the results of this study, the authors conclude that miglustat in addition to ERT may have positive effects on systemic disease (pulmonary function and chitotriosidase activity) in those patients.

Some limitations of therapeutic interventions are possibly due to the timing of therapy, and early treatment may be more efficient. Therefore, newborn screening programs for lysosomal storage disorder have been developed (*61*). However, as for most of these conditions, a strict genotype–phenotype correlation does not exist, the screening programs will probably not be generally introduced until the phenotype from the newborn result can be exactly predicted.

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