

Enzyme replacement therapy for lysosomal storage diseases

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Purpose of review

Enzyme replacement therapy (ERT) for type 1 Gaucher has been highly successful. ERT is now available for other lysosomal storage disorders (LSDs) but none of these highly expensive treatments has had the same efficacy. This review explores why these newer treatments have failed to live up to expectations and how future products might be made more effective.

Recent findings

In Gaucher, the target cells for ERT are macrophages, which are efficiently accessed by intravenously injected recombinant enzyme. The target tissues in other LSDs receive much lower doses of enzyme and intravenous ERT does not enter the brain at all. Uptake of recombinant enzyme is via the mannose-6-phosphate receptor (M6PR). Recent work has looked at improving the efficiency of enzyme delivery to tissues by altering both the ligand on the infused enzyme and the expression of the M6PR on cells. For delivery to the central nervous system, intrathecal routes of administration have been explored.

Summary

Work in tissue culture and in animal models has shown increased efficiency of enzyme delivery and clinical trials of second-generation products and novel delivery systems are now underway.

Keywords

enzyme replacement therapy, lysosomal storage disorders

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Introduction

Enzyme replacement therapy (ERT) is in principle an attractive approach to the treatment of many inherited diseases, which are often caused by enzyme deficiency. Unfortunately, in most cases, only enzyme synthesized within the cell is correctly processed and targeted; there are no pathways for exogenously administered enzyme to enter the cell and reach the intracellular compartment where it needs to be in order to function. The lysosomal hydrolases, deficiency of which underlies most lysosomal storage disorders (LSDs), are an exception.

Neufeld [1] first demonstrated that co-culture of fibroblasts from patients with different mucopolysaccharidoses (MPSs) led to correction of glycosaminoglycan (GAG) storage in all cells. Glycosylation of lysosomal enzymes allows targeting of both endogenously synthesized and exogenously supplied enzymes to the lysosome by the mannose-6-phosphate receptor [2]. It is the existence of this pathway that makes ERT for LSDs possible.

The first enzyme product to be licensed, alglucerase for the treatment of Gaucher disease, was purified from

human placenta but this was rapidly replaced by imiglucerase, a recombinant version of the glucocerebrosidase enzyme which is deficient in Gaucher disease, and all subsequent ERT products have been synthesized in tissue culture systems.

We now have over 20 years of experience with ERT for Gaucher disease and, for type 1 disease, it is a highly effective treatment [3]. In type 1 Gaucher, intravenously administered enzyme is efficiently delivered to the target cell, the macrophage, and we can easily monitor response by measuring organomegaly and haematological parameters, imaging bone and bone marrow and monitoring well characterized biomarkers. With ERT livers and spleens shrink, cytopenias improve, bone marrow normalizes and, most importantly, patients feel much better.

We now have ERT for five more LSDs: Fabry, MPS I, II, and VI, and glycogen storage disease type II (GSD II, also known as Pompe disease or acid maltase deficiency) (Table 1). Unfortunately, none of these has come close to fulfilling the promise of ERT in Gaucher disease. This review will focus on how and why these new products are less effective than imiglucerase and what

steps are being taken to try to make ERT more efficient in the future.

The response of lysosomal storage disorders to enzyme replacement therapy is determined by the target cell for enzyme delivery

Although they share a basic pathological mechanism, accumulation of undegraded macromolecules within the lysosome, LSDs are clinically remarkably heterogeneous: whatever your clinical subspecialty, at least one LSD is likely to feature in the differential diagnoses you commonly use, albeit somewhere near the bottom of the list. Diseases which are biochemically closely related can affect completely different tissues. Although the basic constituents of every cell in the body may be the same, there are important differences in the proportions of some macromolecules which affect disease expression. In LSDs, the pattern of glycosylation of glycolipids and glycosaminoglycans present in the outer envelope of the cell seems to be particularly important, as many LSDs are caused by deficiency of glycosidase enzymes.

Gaucher and Fabry disease are both glycosphingolipid (GSL) storage disorders. The product of α -galactosidase, the enzyme deficient in Fabry disease, is only a single biochemical step away from glucosyl ceramide, the substrate of glucocerebrosidase, which is deficient in Gaucher. But Gaucher and Fabry are completely different diseases. In type 1 Gaucher disease, missense mutations result in residual glucocerebrosidase activity which is adequate to prevent storage in all cell types except macrophages, which are exposed to a uniquely high glucosyl ceramide load due to phagocytosis of membranes from other, senescent cells. In contrast, Fabry disease is often caused by null mutations in α -galactosidase and storage can be observed in many cell types, although clinical disease is mostly caused by storage in kidney, heart, blood vessels and nerve.

Recombinant lysosomal enzymes injected intravenously are efficiently targeted to reticuloendothelial cells such as macrophages, which spend at least some of their time suspended in the bloodstream. Radiolabelled glucocerebrosidase has been shown to be efficiently taken up by sites of storage in a Gaucher patient, and is targeted to the spleen and liver even in a control subject without a LSD [4]. Similar targeting would also be seen with other injected ERT products. In Fabry disease, intravenous enzyme is efficiently targeted to blood vessels. GL-3 is rapidly cleared from capillary endothelial cells after α -galactosidase injections [5], but it has been much more difficult to demonstrate reversal of storage in other cell types. In a mouse model, after intravenous infusion of α -galactosidase there was 10–100 times as much enzyme

Key points

- The unique cell biology of lysosomal hydrolases allows enzyme replacement therapy (ERT) in lysosomal storage disorders (LSDs).
- ERT for Gaucher disease is very effective but ERT for other LSDs has failed to live up to this promise.
- For ERT to be effective, recombinant enzyme must be efficiently delivered to the target tissues.
- Recent research has led to the development of novel enzymes and new routes of delivery which are now entering clinical trials.

activity detected in liver and spleen as in kidney and heart [6].

The response of the visceral signs of Gaucher disease to ERT is dramatic: in clinical trials, 6 months of treatment led to significant improvements in organomegaly and blood counts. Response in Fabry disease is more subtle and much harder to demonstrate. One enzyme was licensed on the basis of clearance of storage from endothelial cells [5], a surrogate marker, and the other on the basis of improvements in important, but subjective, pain scores [7]. Even with prolonged postmarketing experience, it has been difficult to demonstrate clinically significant improvements in end-organ function, and the emphasis has been on stabilization and slowing of progression [8]. These are important and worthwhile achievements, but ERT has not transformed the lives of patients with Fabry disease in the same way it has those with Gaucher disease.

The fact that intravenously administered enzyme is not efficiently delivered to cells which are not directly exposed to the bloodstream has also influenced the efficacy of ERT in the MPS diseases and GSD II (Table 1). In MPS, ERT leads to a reduction in organomegaly and cardiac and respiratory features. Children show improved growth and the effects on skeletal disease can improve mobility through soft tissue effects, but there is no effect on established deformity [9]. Even in type 1 Gaucher, there are concerns that ERT does not have the same dramatic effects on bony manifestations such as osteoporosis as it does on the other visceral manifestations [10]. It is not entirely clear why this is, but it may be that recombinant enzyme does not penetrate bony tissue well. In GSD II, it is necessary to give an order of magnitude more recombinant enzyme activity than is used in Gaucher for any clinical effects on skeletal muscle to be seen.

In addition, intravenously administered enzyme is entirely excluded from some tissues, notably the brain. The blood brain barrier stops macromolecules diffusing

Table 1 Enzyme replacement therapy for lysosomal storage disorders (LSDs): agents available and their efficacy

Disease	ERT product(s)	Dosing	Endpoints in clinical trials	Efficacy in clinical practice	Unmet needs
Gaucher	Imiglucerase	15–60 Units/kg every other week	Improvements in blood counts, reductions in organomegaly	Resolution of symptoms and most signs of disease, improved quality of life	Cannot treat neuronopathic disease. Effects on bone may not be optimal
	Velaglucerase alfa	15–60 Units/kg every other week	Improvements in blood counts, reductions in organomegaly	Little experience to date but likely to be similar to imiglucerase	
Fabry	Agalsidase alfa	0.2 mg/kg every other week	Improvements in pain scores and decreased use of analgesics. Stabilization of renal function. Reduction in left ventricular hypertrophy. Reduction in GL-3 in plasma, urine and tissue biopsies.	Both products can stabilize renal function and cardiac size, as well as improve pain and quality of life. Some reduction in frequency of occurrence of established renal failure and cardiovascular events	ERT cannot reverse established disease and patients treated as adults continue to suffer significant morbidity and mortality. It is not yet clear whether early treatment can prevent development of significant end-organ involvement
	Agalsidase beta	1 mg/kg every other week	Clearance of GL-3 from endothelial cells, tissue and plasma. Stabilization of renal function		
MPS I	Laronidase	100 units/kg weekly	Improved respiratory function and walking ability. Reduction in hepatomegaly, left ventricular mass and urine GAG	Improvements in hepatomegaly, growth, joint mobility, breathing, and sleep apnea in patients with milder disease phenotypes	Bony disease persists. ERT does not prevent cognitive decline
MPS II	Idursulfase	0.5 mg/kg weekly	Improved respiratory function and walking ability. Reduction in organomegaly, left ventricular mass and urine GAG	Reductions in hepatomegaly with some improvements in mobility and respiratory function	Bony disease persists. ERT does not prevent cognitive decline
MPS VI	Galsulfase	1 mg/kg weekly	Improvements in walking and stair climbing. Reduction of urine GAG	Improved endurance and growth	Lack of effect on skeletal dysplasia and corneal clouding
GSD II	Alglucosidase alfa	20 mg/kg every other week	Infantile-onset form: Increased survival, decreased left ventricular mass. Late-onset form: Improved walking and stabilized pulmonary function	Infantile-onset form: Cardiomyopathy resolves but skeletal muscle involvement progresses. Once patients have been ventilated, they will remain ventilator-dependent. Late-onset form: Some improvements in muscle strength in first year of treatment with subsequent stabilization. Improved wellbeing	Infantile-onset form: Preventing death from cardiac failure may simply leave babies immobile and ventilator-dependent. Some responders who have gained motor milestones may show late, peripheral involvement. Late-onset form: Very high doses needed to see effect on skeletal muscle

ERT, enzyme replacement therapy; GAG, glycosaminoglycan.

from the blood into the central nervous system (CNS) and the neurological manifestations of LSDs cannot be treated by currently available ERTs. Imiglucerase has no effects on neuronopathic manifestations of type 2 and 3 Gaucher disease and ERT is contraindicated in patients with MPS I or II who have cognitive impairment as it will not alter their ultimate prognosis.

Strategies to improve delivery of intravenously administered enzyme replacement therapy to target cells

Receptor-mediated uptake of lysosomal enzymes requires the cell to express the receptor and the recombinant protein to possess the ligand. Initial strategies to improve the efficiency of enzyme delivery have assumed that all cells are able to recapture secreted lysosomal enzymes and have concentrated on modifying the recombinant enzyme to maximize its uptake.

Targeting of enzymes to the lysosome requires proper glycosylation. This is a posttranscriptional process, which is performed differently in different cell types. Enzymes for ERT have until now been made in one of two different ways. Either the relevant gene is cloned and then transfected into a cell line, which was originally derived from the ovary of a Chinese hamster, or the protein is made in a human cell line by over-expression of the native gene. It is reasonable to expect that enzyme produced by these different techniques would have undergone different posttranslational modification and might have different pharmacokinetic properties.

In Europe, two competing enzyme preparations were licensed for the treatment of Fabry: agalsidase alfa (Replagal, Shire Human Genetic Therapies), made in a human cell line, and agalsidase beta (Fabrazyme, Genzyme) made in Chinese hamster ovary (CHO) cells. The licensed dose of agalsidase alfa (0.2 mg/kg fortnightly) is one-fifth of that of agalsidase beta. The rationale behind this difference in dosage was that agalsidase alfa would be more efficiently taken up by target cells due to its authentic glycosylation. In fact, it hasn't been possible to detect significant pharmacological differences between the two preparations. There is very limited clinical data from head to head comparisons, but neither treatment is particularly effective in those with established disease. Patients treated with agalsidase alfa do seem to be less likely to develop antibodies to the infused enzyme, but this may simply be related to the dose rather than an antigenic difference between the products. The cost per patient of the licensed dose is similar, so agalsidase alfa is five times as expensive as agalsidase beta on a mg for mg basis.

Interestingly, velaglucerase, a human cell line derived product, has recently been launched in competition with

the CHO cell-derived imiglucerase for the treatment of Gaucher disease. There are some pharmacological data which suggest that velaglucerase may be more efficiently taken up by macrophages in tissue culture than imiglucerase [11[•]], but the licensed dose of velaglucerase is the same as that of imiglucerase, so the intellectual argument that enzyme made in human cells is significantly different from that made in other cell lines appears to have been abandoned. In clinical practice, the availability of biomarkers such as chitotriosidase means that dose adjustment of ERT in Gaucher is routinely undertaken. If it does turn out that velaglucerase can be given at significantly lower doses than imiglucerase then, as the preparations are similarly priced, it may be possible for funders to make significant cost savings.

In GSD II, delivery of enzyme to skeletal muscle seems to be particularly inefficient. One approach to improving uptake of enzyme would be to optimize the concentration of the M6P ligand on the recombinant α -glucosidase by conjugating extra glycan residues to the protein [12^{••}], and such a second-generation product is in clinical development. An alternative would be to find other ways of binding to the M6P receptor. The M6P receptor is in fact a bifunctional protein and also acts as the insulin-like growth factor II (IGF-II) receptor. By fusing part of the IGF-II molecule to a recombinant lysosomal enzyme, it is possible to target it to the lysosome in a glycosylation-independent manner [13^{••}]. Early clinical trials are currently underway with an IGF-II/GAA fusion protein in GSD II (<http://clinicaltrials.gov/ct2/show/study/NCT01230801?term=BMN701&rank=1>). Both of these approaches may improve the efficiency of uptake of GAA into cells, but neither is muscle specific, and it is therefore still likely to be the case that the majority of infused enzyme will be taken up by macrophages in the liver and spleen.

It may also be possible to influence uptake of ERT by modulating the M6P receptors on the target cell [14^{••}], and this approach could potentially improve tissue specificity. As well as causing muscle hypertrophy, treatment with the β_2 -agonist clenbuterol is known to upregulate the expression of the M6P receptor in skeletal muscle. Treatment of GSD II mice with clenbuterol increased the efficiency of ERT as measured by motor function and glycogen clearance from muscle [14^{••}]. The mechanism of action of β_2 -agonists in GSD II is unlikely to relate solely to their effects on M6P receptor expression, albuterol has been shown to have positive effects on patients in the pre-ERT era [15], and the dosage and route of administration that would be required in humans are not clear, but, as these agents are already licensed, widely used and relatively cheap, it seems likely that clinical studies will be undertaken soon.

Other routes of enzyme replacement therapy delivery

One of the major limitations of intravenously delivered ERT is that it cannot access the CNS and therefore cannot be used to treat the numerous LSDs which primarily affect the brain. Trial of high-dose imiglucerase in type 3 Gaucher disease showed no effects on neurological involvement [16]. Work in mice with metachromatic leukodystrophy suggested that high-dose intravenous enzyme might have an impact on demyelination, perhaps because of breakdown of the blood brain barrier in areas of active disease or because enzyme was being shuttled into the brain inside macrophages. However, a trial in human patients was terminated early because there was no evidence of any effect. There is currently no reason to think that lysosomal enzyme given intravenously can enter the human brain.

In animal models, infusion of enzyme into the cerebrospinal fluid (CSF) has shown promise as a means of delivering ERT to the CNS. Infused enzyme efficiently enters ependymal cells, but is also able to access the brain parenchyma, perhaps by adsorptive transcytosis [17], entering both neurones and glial cells. Intracisternal injection of recombinant *N*-sulphoglucosamine sulphohydrolase (rhSGSH) in dogs with MPS IIIA led to extensive entry of enzyme into the brain via the subarachnoid space. There was limited diffusion of enzyme across the pia mater, but penetration deep into the brain occurred via a perivascular route [18^{••}]. Presymptomatic dogs treated for 3 months with regular intracisternal injections showed improvements in neuropathology and reduction of substrate storage in the brain [19^{••}]. Although technically challenging, this must be a promising route for enzyme delivery in humans with LSDs affecting the brain.

Intrathecal enzyme has been given to treat spinal cord compression in one patient with MPS I [20] and another with MPS VI [21[•]]. Four monthly intrathecal infusions were given via lumbar puncture with some clinical improvement in both cases. There was clearance of GAG from the CSF.

Repeated lumbar puncture is not a practical method for delivering long-term intrathecal ERT. It is, however, possible to insert an intrathecal catheter with a subcutaneous port into the thoracolumbar spine to allow regular intrathecal administration of recombinant enzyme. Long-term studies in monkeys have demonstrated that this approach leads to significant levels of idursulfase activity in the brain [22[•]]. There was some evidence of a transient inflammatory response, but no clinically significant side effects. This system is now being used to assess the effects of intrathecal ERT in

patients with MPS II (ClinicalTrials.gov identifier NCT00920647).

Conclusion

The cell biology of lysosomal acid hydrolases, whereby both endogenously synthesized and exogenously administered enzyme can be targeted to the lysosome, means that ERT is a therapeutic possibility for LSDs. ERT for type 1 Gaucher has been a great success but it has been much more difficult to demonstrate the efficacy of subsequent ERT products. A major reason for this seems to be that, when a recombinant enzyme is administered intravenously, most of it ends up in the reticuloendothelial cells of the liver and spleen. In type 1 Gaucher, this results in enzyme being targeted specifically to the storage cells, but in other LSDs it prevents the vast majority of enzyme from getting to the tissues where it is needed. It seems likely that these treatments would be more effective if enzyme could be more efficiently delivered to the target tissues, and to this end a number of second-generation enzyme products are entering clinical development. Where these involve modification of the ligand on the infused enzyme, it is possible that the efficiency of enzyme uptake will be improved, but it seems unlikely that tissue specificity will be altered, and most of the enzyme will still be taken up in the liver and spleen. Attempts at tissue-specific upregulation of the enzyme receptor, as with the use of clenbuterol in GSD II, may be a better way to improve the specificity of enzyme delivery to target tissues.

These approaches will not help for tissues like the brain which simply cannot be accessed by intravenously delivered enzyme. Here, it is necessary to circumvent the blood brain barrier. In animal models, this can be achieved by using direct injection into the CSF and this approach is now being tried in patients with neurological LSDs.

The great expansion of the number of LSDs which can be treated with ERT continues, with clinical trials in MPS IV and Niemann–Pick type B imminent. This has been driven by the success of ERT in type 1 Gaucher, and the revenue it has raised for the company which developed it. It is clear to all, however, that no subsequent treatments have come close to the efficacy of imiglucerase. These are highly expensive treatments, the value of which is increasingly likely to be challenged by health economists. Both clinicians and the pharmaceutical industry are well aware of the shortcomings of the currently available preparations and it is to be hoped that the current attempts to develop second-generation agents will lead to more effective treatments that will ultimately benefit patients.

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Conflicts of interest

The author has received honoraria and/or nonrestricted research funding from Genzyme, Shire, and Actelion.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 700–701).

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