

Treatments for lysosomal storage disorders

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Abstract

There are over 70 human diseases that are caused by defects in various aspects of lysosomal function. Until 20 years ago, the only specific therapy available for lysosomal storage disorders was allogeneic haemopoietic stem cell transplantation. Over the last two decades, there has been remarkable progress and there are now licensed treatments for seven of these diseases. In some cases, a choice of agents is available. For selected enzyme-deficiency disorders, ERT (enzyme-replacement therapy) has proved to be highly effective. In other cases, ERT has been less impressive, and it seems that it is not possible to efficiently deliver recombinant enzyme to all tissues. These difficulties have led to the development of other small-molecule-based therapies, and a drug for SRT (substrate-reduction therapy) is now licensed and potential chaperone molecules for ERT are in the late stages of clinical development. Nonetheless, there is still significant unmet clinical need, particularly when it comes to treating LSDs which affect the brain. LSDs have led the way in the development of treatment for genetic disorders, and it seems likely that there will be further therapeutic innovations in the future.

Introduction

The LSDs (lysosomal storage disorders) are a group of inherited metabolic diseases in which pathology results from the accumulation of undegraded macromolecules within the cell. Although the majority of these diseases are caused by deficiency in the activity of acid hydrolases, others involve the targeting and activation of these proteins or trafficking of molecules in and out of the lysosome. LSDs are extremely variable in their clinical features: the effects of storage depend on the nature of the storage molecule and the cells and tissues in which it accumulates. In many cases, lysosomal storage triggers a complex cascade of downstream events which can eventually affect cells and tissues in which storage itself is not evident and the pathogenesis of these disease is not fully understood.

Over the last 40 years, there has been remarkable therapeutic innovation in the LSD field, and LSDs have become the prototype for developing treatment for genetic diseases. The demonstration by Elizabeth Neufeld that conditioned medium from cells from patients with one LSD could lead to the correction of storage in cells from a patient with a different LSD started this process [1]. This observation led to the elucidation of how enzymes are targeted to the lysosomal compartment by the mannose 6-phosphate receptor and the demonstration that this receptor was also present on the surface of the cell, allowing the uptake of enzyme from outside the cell.

Enzyme-augmentation therapy

The initial approaches to treating LSDs involved attempts to restore degradative activity by providing exogenous enzyme, which could then be taken up into the cell via the mannose 6-phosphate receptor pathway.

Allogeneic BMT (bone marrow transplantation)

The aim of BMT is to use the donor bone-marrow-derived cells as a source of enzyme. In particular, donor macrophages should be able to infiltrate the patients tissues and act as miniature enzyme factories, secreting a cocktail of lysosomal hydrolases which can then correct a variety of lysosomal deficiencies in surrounding host cells [2].

The first BMTs for LSDs were performed in children with Hurler disease [MPS (mucopolysaccharidosis) I] in the early 1980s [3]. Although no formal clinical trial has been conducted, several hundred procedures have subsequently been carried out in this condition, and the place of BMT is now well established. Visceral disease improves. Skeletal disease persists, but may progress more slowly. If transplantation is performed early enough (generally before 2 years of age), then neurological disease may stabilize. This is because the brain is gradually repopulated with microglia of donor origin, which then provide a source of enzyme within the brain, thus circumventing the BBB (blood-brain barrier) which stops circulating enzyme from entering the CNS (central nervous system).

It was hoped that this paradigm would prove successful in a range of other LSDs. Unfortunately this has not turned out to be the case. In MPS II (Hunter disease) and MPS III (Sanfilippo disease), there was no discernable effect of BMT on cognitive decline. In MPS IV (Morquio disease) the skeletal involvement, which is the main feature, does not respond to BMT. There does appear to be a place for

Key words: bone marrow transplantation, enzyme-replacement therapy, lysosomal storage disorder treatment, mucopolysaccharidosis, substrate-reduction therapy.

Abbreviations used: BBB, blood-brain barrier; BMT, bone marrow transplantation; CBS, cystathionine β -synthase; CNS, central nervous system; ERT, enzyme-replacement therapy; GSL, glycosphingolipid; LSD, lysosomal storage disorder, MLD, metachromatic leucodystrophy; MPS, mucopolysaccharidosis; SRT, substrate-reduction therapy.

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BMT in the management of the lysosomal leucodystrophies, MLD (metachromatic leucodystrophy) and Krabbe disease, but only if performed early. This is because it takes from 6 months to 1 year for donor microglia to enter the brain in sufficient quantities to provide metabolic cross-correction and the disease continues to progress throughout this period [4].

Surprisingly, BMT has not established a role in the LSDs which are characterized by visceral disease without skeletal or CNS involvement. To some extent, this has been because these diseases tend to affect patients later on in life. In these older patients, BMT has a much higher morbidity and mortality. In the case of type 1 Gaucher disease, the commonest of the LSDs, the advent of ERT (enzyme-replacement therapy) has removed the need for BMT. In some of the other rarer diseases such as Niemann–Pick type B disease or fucosidosis, where no other treatment is currently available, it may be worth reconsidering BMT, particularly as the procedure itself now appears to be much safer.

Enzyme-replacement therapy

ERT was originally developed for Gaucher disease using glucocerebrosidase purified from human placentas. With the advent of molecular genetics, this was replaced by a recombinant product made in tissue culture. ERT for Gaucher disease has been a great success story. The visceral manifestations of Gaucher disease are primarily due to storage in macrophages. Enzyme administered intravenously is readily taken up by these cells and there is rapid resolution of hepatomegaly and splenomegaly. Haematological parameters improve and patients feel much better.

ERT is not a cure for Gaucher disease; established bone disease does not respond well to therapy and the enzyme does not cross the BBB and cannot affect the neurological manifestations of neuronopathic disease. Patients require lifelong treatment, although experience with a recent global shortage of product has shown that, for many individuals, the intensity of treatment can probably be significantly reduced once the disease has been stabilized. For the vast majority of people with type 1 Gaucher disease, ERT is a life-transforming treatment.

The success of ERT in Gaucher disease led to the development of a similar approach for other LSDs, and licensed products are now available for Gaucher disease, Fabry disease, MPS I, MPS II, MPS VI and Pompe disease. It is fair to say that, although all of these treatments have shown efficacy in clinical trials, none has approached the effect of imiglucerase in Gaucher disease. This is probably because the cell types and tissues involved in these diseases (the heart and kidney in Fabry disease, bone in the MPSs and muscle in Pompe disease) are much less accessible to intravenously delivered enzyme than are macrophages. Many of the manifestations of established disease do not respond to ERT and, in some instances (e.g. renal impairment in Fabry disease), there is continued progression despite therapy. For these conditions, the hope is that very early intervention will

prevent end organ damage and the aim is to identify and treat patients in childhood. It is likely to be many years before we know whether this approach has been successful.

Conventional wisdom suggests that a recombinant protein made by expression of cDNA should be identical with the endogenous 'natural' product. This may be true at the level of amino acid sequence, but will not hold for post-translational modifications, which will be specific to the cell type in which the protein is made. As correct glycosylation is essential for the proper targeting of lysosomal enzymes, the technology used to produce individual products could have a profound effect on their therapeutic efficacy.

The majority of ERT products available are recombinant proteins made in CHO (Chinese-hamster ovary) cell lines. The enzymes are purified from cell culture medium and then treated with exoglycosidases to expose terminal mannose residues on the oligosaccharide chains. Other products are made by overexpressing the native gene in a human cell line in tissue culture, so-called 'gene activation', and these proteins would expect to exhibit a 'natural' pattern of glycosylation. Comparison of crystal structures of recombinant glucocerebrosidase (imiglucerase) and the gene-activated form (velaglucerase alfa) confirm that there are real differences in glycosylation [5] and velaglucerase alfa is taken up more efficiently by cultured macrophages. Velaglucerase alfa has recently been licensed; it remains to be seen whether it will be effective at lower doses than imiglucerase.

A third product, taliglucerase alfa, is also in late stages of clinical development for use in Gaucher disease. This is a recombinant glucocerebrosidase made in carrot cells. Production of recombinant human proteins from plant cells is much simpler than production from animal cell lines. Currently available ERT products are prohibitively expensive, and plant cell technology may lead to substantial cost savings in the future, although the requirement for pharmaceutical companies to recoup development costs and make profits means that drugs for orphan diseases such as the LSDs, where there are only a few tens of thousands of patients in the world, will never be cheap.

One major drawback of the ERT approach is that it has no effect on lysosomal storage in the nervous system. Although work in some animal models has suggested that it may be possible to gain entry of enzyme into the brain if sufficiently large doses are used, there is no evidence to support this approach in humans. A recent clinical trial investigating this approach in MLD was terminated because of a lack of evidence for any effect (<http://clinicaltrials.gov> identifier NCT00633139).

An alternative strategy has been to deliver enzyme by direct injection into the CSF (cerebrospinal fluid) [6]. Results in animal models are encouraging, but this technology has not yet been translated to the clinic and would clearly pose considerable challenges. The lack of efficacy of ERT in treating CNS and skeletal manifestations of disease, the need for regular intravenous infusions and its high cost have driven the search for alternative small-molecule-based approaches to treating LSDs.

SRT (substrate-reduction therapy)

LSDs are characterized by a spectrum of disease severity which correlates, at least in part, with residual enzyme activity. On the whole, only patients with the most severe, early-onset and rapidly progressive disease will have two nonsense mutations and no functional enzyme. The majority of patients will have at least one allele bearing a missense mutation that will code for an enzyme which has some catalytic activity.

In SRT, the aim is to reduce the concentration of the accumulating substrate of a deficient enzyme or process. This approach is familiar to those treating inherited metabolic diseases; it underlies the use of dietary treatment for phenylketonuria and other aminoacidopathies. In LSDs, the strategy has been to reduce the rate of synthesis of the stored macromolecule to a level where the residual degradative enzyme activity can maintain homeostasis [7].

Miglustat is an inhibitor of the ceramide glucosyltransferase which catalyses the first step of GSL (glycosphingolipid) synthesis. It is therefore a potential treatment for a variety of LSDs, including Gaucher disease, Fabry disease and the gangliosidoses, which are devastating neurodegenerative diseases for which no specific therapy is currently available. Miglustat has been demonstrated to clear GSL storage in visceral and CNS cells and to delay symptom onset and increase survival in a variety of mouse models of these diseases [8].

Miglustat was first clinically developed as a second-line therapy in Gaucher disease [9]. Subsequently, a number of trials have been carried out in neurological storage disorders [10–12]. These studies have perhaps told us more about the difficulties of conducting clinical trials in slowly progressive neurological disorders where there is considerable interpatient variability and the natural history of the disease is not well understood [8]. Under these circumstances, it is very difficult to define hard clinical end points which will demonstrate significant change over the relatively short periods over which drug trials are performed. A 12-month placebo-controlled trial of miglustat in Niemann–Pick type C disease (in which secondary storage of gangliosides is important) did, however, demonstrate stabilization of disease progression, and miglustat is now licensed in Europe for the treatment of this disease [11].

Other SRT molecules are now being developed. As well as a second inhibitor of GSL synthesis [13], work has also started using genistein as an inhibitor of mucopolysaccharide synthesis [14,15].

Enzyme-enhancement therapy

In the majority of LSDs and many other inherited metabolic diseases, the disease only becomes clinically evident once residual enzyme activity falls below 15–20%. Another therapeutic approach is to try to enhance the activity of mutant enzyme. Increasing residual enzyme activity by only a few per cent may have profound clinical effects.

This approach is well established for a number of vitamin-dependent enzymes. Homocystinuria, caused by mutations in the gene coding for CBS (cystathionine β -synthase), can be effectively treated with pharmacological doses of pyridoxine (vitamin B₆), which is an essential cofactor for the CBS enzyme. Unfortunately, no suitable cofactors have yet been identified for lysosomal hydrolases.

Chaperone therapy

Like SRT, chaperone therapy exploits the fact that the majority of patients will have at least one mutant allele coding for an intact protein. In many cases, these mutant proteins are unstable; they are recognized by the cell as being defective and are targeted to the proteasome for degradation. The aim of chaperone therapy is to rescue these mutant proteins so that they can be delivered to the lysosome where they can express their residual enzyme activity [16].

The first clinical demonstration of chaperone therapy was in a patient with Fabry disease, where an intravenous infusion of galactose enhanced enzyme activity and led to improvements in cardiac function [17]. Subsequent clinical development has focused on the imino sugar 1-deoxygalactonojirimycin, which has shown good efficacy in a mouse model of Fabry disease and is now in late stages of clinical development [18].

These small molecules are taken orally and should have better biodistribution than recombinant enzyme. However, their effects are not only disease-specific, but also are mutation-specific. In addition, the molecules used tend to bind the active site of the enzyme to be chaperoned and act as inhibitors as well as chaperones. Dosing regimens have to allow time for the pharmacological chaperone to diffuse away from the enzyme once it reaches the lysosome so that it can express its residual activity.

It may be that a number of potential pharmacological chaperones are already available, having been licensed for other indications. A screen of commercially available drugs looking at their ability to enhance the activity of β -hexosaminidase (the enzyme deficient in GM2 gangliosidosis) identified the anti-malarial drug pyrimethamine as a potent pharmacological chaperone [19]. This safe and cheap drug is now being used by clinicians for this devastating and otherwise untreatable condition.

Future developments

Over the last 20 years, we have gone from a situation where there were no specific treatments for LSDs to one where there are three different licensed drugs to treat Gaucher disease (two products for ERT and one for SRT), with a further two in clinical development (one for ERT and one for SRT). Similarly, there are two licensed ERT treatments for Fabry disease, and a pharmacological chaperone is in clinical development. Nonetheless, there is still considerable unmet need. With the exception of type 1 Gaucher disease, currently available treatments are of limited efficacy. The options for treating neurological and skeletal disease are limited. For the

vast majority of the more than 70 LSDs, there is still no specific treatment.

Fortunately, due in part to the ready availability of animal models, many laboratories continue to work on these problems, and there are many other approaches in the pipeline. Inflammation seems to have an important role in the pathophysiology of many of these diseases, and, in mice, the use of commonly available anti-inflammatory agents is synergistic with currently available therapies [20]. Stop-codon readthrough has been successful in mice with MPS I [21]. There are now few mouse models of LSDs which have not been successfully treated with gene and stem cell therapy [22], and these approaches are being adapted to larger animals [23]. It therefore seems likely that LSDs will continue to lead the way in developing therapies for genetic disorders.

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