Current therapies for the soluble lysosomal forms of neuronal ceroid lipofuscinosis

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Abstract

The NCLs (neuronal ceroid lipofuscinoses) are the most common inherited paediatric neurodegenerative disorder. Although genetically distinct, NCLs can be broadly divided into two categories: one in which the mutation results in a defect in a transmembrane protein, and the other where the defect lies in a soluble lysosomal enzyme. A number of therapeutic approaches are applicable to the soluble lysosomal forms of NCL based on the phenomenon of cross-correction, whereby the ubiquitously expressed mannose 6-phosphate/IGF (insulin-like growth factor) II receptor provides an avenue for endocytosis, trafficking and lysosomal processing of extracellularly delivered enzyme. The present review discusses therapeutic utilization of cross-correction by enzyme-replacement therapy, gene therapy and stem cell therapy for the NCLs, along with an overview of the recent progress in translating these treatments into the clinic.

Introduction

Collectively, the NCLs (neuronal ceroid lipofuscinoses) (also known as Batten disease) are a family of at least ten genetically separate neurodegenerative LSDs (lysosomal storage disorders). They are clinically characterized by loss of vision and seizures alongside an accumulation of autofluorescent lipofuscin. Although the mutated gene is specific to each form of NCL, these mutations broadly fall into two categories, causing either a mutation of a transmembrane protein or a mutation of a soluble lysosomal enzyme. The history and pathology of NCLs is covered in greater detail, both in this issue of Biochemical Society Transactions [1] and elsewhere [2]. For the soluble lysosomal enzyme deficiencies, it is possible to utilize the normal lysosomal enzyme trafficking system and the phenomenon of cross-correction to target therapies to deficient cells. These mechanisms have been more thoroughly reviewed previously [3,4] and are therefore discussed only briefly. In short, lysosomes will endocytose molecules from the extracellular milieu via a receptor-mediated pathway. The two cell-surface receptors that facilitate uptake into the lysosome are the mannose 6-phosphate/IGF (insulin-like growth factor) II receptor or the mannose receptor. These receptors subsequently mediate endocytosis and trafficking of extracellular enzyme to the lysosome, where the enzyme can perform its designated catalytic function. Lysosomal enzymes are also secreted into the extracellular space and, as well as re-uptake into the same cell, can also be taken up by neighbouring cells. Thus cross-correction provides us with the ability to extend the therapeutic effect more widely, spreading beyond an individual cell. This effectively means that it is only necessary to correct the defect in a subset of cells which can secrete the missing enzyme to cross-correct other cells.

ERT (enzyme-replacement therapy)

Via the principle of cross-correction, it stands to reason that exogenous administration of enzyme should have a therapeutic effect. This has been effectively demonstrated for the non-neuropathic forms of Gaucher disease, mucopolysaccharidosis I and Fabry disease, with ERT available for clinical treatment [5–8]. In vitro experiments have demonstrated proof of principle of ERT for the NCLs. Fibroblasts cultured from a human LINCL (late-infantile NCL) patient readily endocytosed exogenous recombinant human TPP-1 (tripetidyl peptidase I), leading to a restoration of enzyme activity and a reduction in subunit c accumulation [9]. In order to determine the levels of enzyme required which would be therapeutically effective, Sleat et al. [10] made hypomorph LINCL mouse models (CLN2-deficient) which express differing residual levels of TPP-1. A restoration of activity to 3% of normal levels in the CNS (central nervous system) was sufficient to delay disease onset, and increasing this to 6% led to a prolonged lifespan approaching that of unaffected mice. Although the ideal scenario would be to restore enzyme levels completely, these findings illustrate how, via cross-correction, even relatively low levels of enzyme may be therapeutic. Nevertheless, with the main therapeutic target being the brain, it will still be necessary...
to overcome the significant obstacle presented by the blood–brain barrier. A simple approach to achieve this is by using the cerebrospinal fluid as a delivery route [11]. TPP-1 delivered intraventricularly via osmotic pumps to LINCL mice spread to both hemispheres of the brain, ameliorated the tremor phenotype and reduced neuropathological hallmarks of astrogliosis and autofluorescent storage body accumulation. Although this study demonstrated that it is possible to bypass the blood–brain barrier with ERT, clinical application would require regular infusions of enzyme which can lead to tolerization, as well as being prohibitively expensive. Therefore, in the last two decades, there has been a lot of interest in the applicability of gene therapy for the LSDs, with a particular focus on the NCLs.

**Gene therapy**

Gene therapy for LSDs represents a highly attractive strategy for long-term therapy. By introducing a functional copy of the mutated gene into the host CNS, it may be possible to obtain long-term expression of the functional enzyme. Alongside this, cross-correction would allow for widespread migration of the enzyme, mitigating the need to transduce every deficient cell. The first demonstration of gene therapy in NCL was provided by Sands and colleagues, who delivered intracranial injections of AAV2 (adeno-associated virus serotype 2) expressing human recombinant PPT1 (palmitoyl protein thioesterase 1) into a mouse model of INCL (infantile NCL) [12]. Following these neonatal injections, PPT1 activity was detected in the vicinity of the injection sites, restored to approx. 15% of normal levels. This restoration of enzyme resulted in a reduction in autofluorescent storage, alongside histopathological improvements such as an increase in brain weight, volume and thickness. As well as these pathological improvements, gene therapy resulted in functional benefits. Intraventricular injection of AAV2–PPT1 into INCL mice resulted in overexpression of PPT1 activity and a restoration of retinal function [13]. Interestingly, PPT1 activity was also detected in the brain of injected INCL mice, suggesting that either the enzyme or AAV2 had undergone anterograde axonal transport. Regardless of the method, this transported enzyme was able to reduce the level of neurodegeneration in the CNS, raising the possibility of utilizing the visual pathway as a less-invasive route to deliver therapy to the brain compared with direct intracranial injections. Notable functional improvements were also observed on the Rotarod, pole and ledge behavioural tests, as well as on electroencephalography activity [14]. However, despite these behavioural benefits, there was no increase in the lifespan of AAV2–PPT1-treated INCL mice compared with untreated INCL mice.

Using a similar paradigm, Crystal and colleagues have carried out therapeutic studies in LINCL mice using an AAV2–TPP1 vector [15,16]. Similar to the previous experiments, AAV2–TPP1 cross-corrected human LINCL fibroblasts [15] and reduced autofluorescent storage in LINCL mice following intracranial injection [16]. The AAV2–TPP1 vector has also been assessed for pre-clinical safety and efficacy in rats and non-human primates [15,17]. On the basis of these findings, a Phase I clinical trial was approved and started recruitment of patients in 2004 [18]. Ten patients with LINCL received multiple injections of AAV2-expressing human CLN2. One patient died from a serious adverse effect related to status epilepticus, whereas four other patients mounted a mild humoral immune response to the vector. MRI (magnetic resonance imaging) of the treated patients suggested a reduction in the rate of disease progression, supported by assessments on the Hamburg LINCL neurological rating scale [19].

Although promising, these initial studies have illustrated that gene therapy requires further refinement. In particular, the failure of AAV2-mediated therapy to improve the lifespan of mouse models of NCL and other LSDs needs to be addressed. It appears that the two factors that have prevented this are (i) choice of vector, and (ii) time of therapeutic intervention. AAV2 was considered to be the vector of choice for these studies, as it was one of the first AAV vectors to be characterized with a high transduction efficiency and low immune tolerance. In the intervening years, a number of second-generation AAV vectors have been discovered, which all provide higher levels and a wider efficacy of transduction. A range of second-generation AAV vectors expressing human CLN2 have been evaluated in the LINCL mouse [20]. Of these, the most efficient was AAVrh.10, a serotype derived from the rhesus monkey, which provided marked functional improvement and increased lifespan of LINCL mice by 1 month over that of untreated mice. This AAVrh.10–CLN2 vector has been used to address the issue of determining the optimal time for therapeutic intervention. Following direct injection of the therapeutic vector to the CNS of LINCL mice at 2 days, 3 weeks and 7 weeks of age, mice treated as newborns had a survival advantage of 376 days, compared with 277 days and 168 days for 3- and 7-week-old treated mice respectively, demonstrating a clear benefit to early therapeutic intervention [21]. On the basis of these impressive results, AAVrh.10–CLN2 has been approved for use in a Phase I clinical trial, which has recently begun recruiting patients, and is due to start imminently.

The fact that these diseases occur as a result of a single gene defect raises the possibility of prenatal screening and potential in utero therapy. Early presentation of fetal inflammation in a large animal model of NCL further advocates prenatal intervention [22]. Prenatal gene therapy offers several potential advantages for the CNS: long-term expression of the gene product in post-mitotic cells, alongside the potential for transduction of stem or progenitor cells; induction of immune tolerance to the expressed protein; and finally the potential to treat the disease at the earliest possible timepoint before any irreversible damage may have occurred [23]. A single intracranial injection of non-integrating lentivirus into embryonic day 14 pups resulted in widespread bilateral transduction of the CNS compared with the localized transduction observed following an adult intracranial injection [24], with sustained long-term
expression observed at 1 year post-injection to date (A.A. Rahim, A.M. Wong, S.M.K. Buckley, J.D. Cooper and S.N. Waddington, unpublished work). One vector which shows particular promise for CNS treatment is AAV2/9, owing to the unique ability to cross the neonatal and adult blood–brain barrier and transduce CNS cells following intravenous injection, making it a prime candidate for non-invasive treatment [25]. Therapeutic use of AAV2/9 has recently shown considerable efficacy in a mouse model of spinal muscular atrophy [26]. Using these new vectors as an early therapeutic intervention may also lead to synergistic benefits. We hypothesize that prenatal injection of these second-generation viral vectors into the CNS bloodstream will result in greater efficiency and spread of transduction, possibly throughout the body. This would be highly advantageous for treatment of the global genetic defect in Batten disease. Therefore prenatal gene therapy presents an intriguing means to obtain global correction with a minimal number of injections.

Stem cell therapy

The promise of stem cell therapy has led to a great degree of interest for neurological disorders, including the LSDs [27,28]. In theory, stem cell therapy may be therapeutic in two ways. The prime method of therapy would be to use the stem cells to produce and deliver the enzyme that is lacking throughout the brain via cross-correction. Alternatively, depending on the stage of disease progression, stem cells could integrate into the diseased CNS and replace lost cells by appropriate differentiation. Ideally, these two approaches would complement one another, with the stem cells adjusting accordingly to the surrounding disease environment.

The eventual clinical tool for clinical use is human stem cells, but initial pre-clinical work to date has been carried out in mouse models, which raises the issue of xenograft immune rejection. In order to remove this complication, Tamaki et al. [29] back-crossed INCL mice on to the NOD (non-obese diabetic)–SCID (severe combined immunodeficiency) background [29]. These INCL/NOD–SCID mice received a transplant of purified huCNS-SCs (human CNS stem cells) at a neonatal time point. In vitro co-culture experiments demonstrated that huCNS-SCs produced and secreted PPT1. Following transplantation, huCNS-SCs were found to survive and migrate extensively through the INCL brain as well as undergo site-specific differentiation into neurons, astrocytes and oligodendrocytes, although the majority of cells remained in an undifferentiated state. Despite this, the engrafted cells secreted PPT1, leading to a therapeutic reduction in autofluorescent lipofuscin accumulation, neuroprotection of host CA1 neurons and delay in the rate of motor decline. These results suggest that the therapeutic effect of stem cell transplants are mediated primarily by secretion of PPT1 and cross-correction, rather than by replacement of lost cells.

Following on from this promising pre-clinical study, an open-label Phase I clinical trial was carried out using these huCNS-SCs in INCL and LINCL patients by StemCells, Inc. Six patients (two with INCL and four with LINCL) underwent huCNS-SC transplantation. All patients were at a moderate-to-severe stage of disease progression at the time of transplantation and received multiple grafts into cerebral parenchyma and lateral ventricles of up to 1 billion cells. To date, one patient has died as a result of severe status epilepticus, although a brain autopsy revealed no toxic effects relating to the transplant, and some evidence of huCNS-SC migration and engraftment. The remaining five patients are currently undergoing long-term follow-up studies, with no signs of any adverse effects related to the transplant, suggesting that the huCNS-SCs and transplantation procedure have an acceptable safety profile and that the human CNS is able to tolerate a high dose of exogenous stem cells. In advance of a subsequent clinical trial, it will be important to characterize the therapeutic efficacy of the huCNS-SCs in the LINCL mouse model in order to determine how this particular disease milieu affects stem cell integration, migration and differentiation in comparison with the INCL environment. As the majority of gene therapy trials published to date have been carried out using the LINCL mouse model [15–17,20–21], this study would also allow for an informed comparison between gene and stem cell therapy.

As mentioned above, a major issue with these stem cell transplantation studies is the use of human stem cells in the mouse CNS, with all of the related immune rejection problems. Utilizing a mouse stem cell line in a mouse model of NCL would closely mimic the proposed clinical approach. To investigate this, we have recently begun transplantation of MHP36 neural stem cells, a well-characterized stem cell line derived from embryonic mouse hippocampus, into INCL mice. MHP36 cells have demonstrated therapeutic efficacy in models of stroke, cardiac arrest and Huntington’s disease [30–32]. An initial graft of MHP36 cells into the CNS of symptomatic INCL mice appears to result in an immediate reduction of autofluorescent storage and migration towards the VPM (ventral posteromedial)/VPL (ventral posterolateral) thalamic nucleus and the subventricular zone, regions of disease-specific neurodegeneration and neurogenesis respectively (A.M. Wong and J.D. Cooper, unpublished work). It will be important to determine how the progressing disease environment of the INCL and LINCL brain alters transplanted stem cell fate, particularly the presymptomatic onset of inflammation and synaptic alterations in advance of neuronal loss [33,34].

Chaperones

The therapies mentioned so far all revolve around the concept of reintroducing functional enzyme into the NCL brain. To date, there are currently 48 disease-causing mutations documented for INCL and 72 for LINCL (NCL Resource, http://www.ucl.ac.uk/ncl/). A small minority of these variant forms occur as a result of a missense mutation, which codes for a misfolded enzyme. For these rare variants, it may be possible to use pharmacological chaperones to correctly
fold the protein and increase enzyme activity. This has been demonstrated previously in other LSDs, namely Gaucher disease, Fabry disease and GM1 gangliosidosis [35–37]. The first application of pharmacological chaperones in INCL has recently been reported [38]. Chaperones restored PPT1 enzyme activity 2-fold in lymphoblasts isolated from INCL patients with residual enzyme activity, but had no effect on INCL patient lymphoblasts with inactive enzyme. Given that the two most common mutations of INCL present with no enzyme activity, either as a result of a missense or nonsense mutation, and the same is true for LINCL (http://www.ucl.ac.uk/ncl/), chaperone therapy may only prove to be a niche treatment for particular rare forms of NCL.

**Summary**

Over the last two decades, our understanding of the soluble lysosomal forms of NCL has moved from pre-clinical studies to two complete Phase I clinical trials, with Phase II efficacy trials likely to take place in the near future. Although these developments are promising, there are still many hurdles that need to be surmounted. Even if we can treat CNS pathology, the genetic defect is prevalent throughout the body, with potential downstream consequences. It may be the case that a combination of the therapies outlined in the present paper may be necessary for an optimal clinical outcome. It should also be mentioned that none of these therapies would be ideally suited for the more common transmembrane forms of Batten disease such as JNCL (juvenile NCL). Indeed, although promising progress has been made, there are still many issues that will need to be addressed before effective treatments for these disorders become available.

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