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Review

Pathogenesis and therapies for infantile neuronal ceroid lipofuscinosis (infantile CLN1 disease)☆

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ABSTRACT

The neuronal ceroid lipofuscinoses (NCL, Batten disease) are a group of inherited neurodegenerative diseases. Infantile neuronal ceroid lipofuscinosis (INCL, infantile Batten disease, or infantile CLN1 disease) is caused by a deficiency in the soluble lysosomal enzyme palmitoyl protein thioesterase-1 (PPT1) and has the earliest onset and fastest progression of all the NCLs. Several therapeutic strategies including enzyme replacement, gene therapy, stem cell-mediated therapy, and small molecule drugs have resulted in minimal to modest improvements in the murine model of PPT1-deficiency. However, more recent studies using various combinations of these approaches have shown more promising results; in some instances more than doubling the lifespan of PPT1-deficient mice. These combination therapies that target different pathogenic mechanisms may offer the hope of treating this profoundly neurodegenerative disorder. Similar approaches may be useful when treating other forms of NCL caused by deficiencies in soluble lysosomal proteins. Different therapeutic targets will need to be identified and novel strategies developed in order to effectively treat forms of NCL caused by deficiencies in integral membrane proteins such as juvenile neuronal ceroid lipofuscinosis. Finally, the challenge with all of the NCLs will lie in early diagnosis, improving the efficacy of the treatments, and effectively translating them into the clinic. This article is part of a Special Issue entitled: The Neuronal Ceroid Lipofuscinoses or Batten Disease.

1. Introduction

Infantile neuronal ceroid lipofuscinosis (INCL, infantile Batten disease, or infantile CLN1 disease) is a rapidly progressing lysosomal storage disorder (LSD) caused by defects in the gene coding for palmitoyl protein thioesterase-1 (*CLN1*) [42]. This soluble lysosomal enzyme is responsible for cleaving long-chain fatty acid residues from cysteine residues on a multitude of protein targets [26,27,41]. In the absence of PPT1 activity, undegraded substrates accumulate in both CNS and systemic tissues. Infantile CLN1 disease can be distinguished ultrastructurally from other forms of NCL by the accumulation of granular osmiophilic deposits in the CNS and in cultured fibroblasts. A hallmark of the NCLs, including infantile CLN1 disease, is the progressive accumulation of autofluorescent material (lipofuscin), most notably in the nervous system, but which is also found in some other tissues [33]. Profound neuronal degeneration, cortical thinning, and overall brain atrophy are prominent features of infantile CLN1 disease [14,15]. The mass of an affected child's brain at autopsy may be only 50% of a comparably aged normal child.

Children with infantile CLN1 disease generally develop symptoms around 18 months of age, which include visual defects and blindness, motor and cognitive deficits, seizures and ultimately early death [36,46]. No treatment or cure is currently available for these children.

Two mouse models of infantile CLN1 disease were developed that are completely deficient in PPT1 activity [13,18]. These animals recapitulate many features of the human disease including progressive neurodegeneration, cortical thinning, brain atrophy, autofluorescent accumulation, retinal dysfunction, spontaneous seizures, motor deficits and shortened lifespan [1,11,13]. These mice are accurate phenocopies of the human disease and are valuable tools for studying disease pathogenesis and testing treatment strategies.

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The development of treatments for infantile CLN1 disease has been greatly accelerated by the availability of accurate mouse models. Unfortunately, few single therapies have provided any extension in lifespan, and as described below, any improvements observed using single therapies have been rather modest. However, emerging data from combination therapies for various LSDs, and in infantile CLN1 disease in particular, suggest that this approach offers much promise for treating this intractable disease.

2. Enzyme replacement therapy

Enzyme replacement therapy, or ERT, is a conceptually simple method for treating an enzyme deficiency. Enzyme replacement therapy takes advantage of the fact that lysosomal enzymes can be secreted by cells and taken up by neighboring cells through a receptor-mediated process; either the mannose-6-phosphate or the mannose receptor systems [20]. This process was originally referred to as 'cross-correction' [31]. Recombinant enzyme that is post-translationally glycosylated and contains terminal mannose or mannsose-6-phosphate residues will be endocytosed by cells *in vivo* following an injection. Enzyme replacement therapy is relatively effective at treating the systemic disease associated with several other lysosomal storage diseases following intravenous injection. This approach had not, until recently, been attempted in infantile CLN1 disease due to its predominantly neurological pathology. Lysosomal enzymes, in general, do not cross the blood brain barrier (BBB) effectively. Although some systemically delivered recombinant human PPT1 (rhPPT1) does appear to cross the BBB in the mouse model of infantile CLN1 disease, this was in very small amounts [17]. Nonetheless, intravenous rhPPT1 delivery was tested for tolerability, tissue distribution, and efficacy in *Ppt1*$^{-/-}$ mice. Levels of PPT1 activity reached near normal levels in the visceral tissues of treated *Ppt1*$^{-/-}$ mice. As expected, levels of PPT1 activity in the brain remained quite low, although at high dose a small amount of activity was seen at 2 h post-injection. Unfortunately, PPT1 activity was undetectable in the brain by 24 h post-injection [25]. However, systemically ERT might be able to reduce the disease burden in visceral organs and thus have a positive impact on disease course. Indeed, the animals receiving weekly intravenous injections of rhPPT1 from birth had some improvements in neuropathology and significant autofluorescent clearance in visceral tissues [25]. Importantly, these animals also had a modest, but significant increase in lifespan from 236 to 271 days, and a similar improvement in motor function. Although intravenous delivery of ERT will likely be ineffective as a stand-alone therapy for infantile CLN1 disease, it may be very effective at treating the systemic disease as part of a combination approach including CNS-targeted therapies. Alternatively, chronic delivery of recombinant enzyme to the CNS through an implantable pump or periodic intrathecal injections might provide significant benefit to the CNS. This approach has resulted in improvements in the CNS in animal models and is being tested in children with different lysosomal enzyme deficiencies [6,7,16,23,28,43].

3. Bone marrow transplantation

Bone marrow transplantation (BMT) or hematopoietic stem cell transplantation (HSCT) has each been employed to treat LSDs for decades. The efficacy of this approach varies with respect to the specific lysosomal storage disease, as well as the age when the transplant is initiated. Like ERT, this approach largely relies on the phenomenon of cross-correction [31]. It is believed that a small number of normal donor cells of hematopoietic origin can migrate into the brain and supply enzyme to a large number of deficient host cells. Unfortunately, both pre-clinical and clinical experience with BMT alone has proven ineffective for infantile CLN1 disease [5,21,22,29]. In fact, not only is there no increase in lifespan but also there is a deterioration in motor function in PPT1-deficient mice treated with BMT during the neonatal period [29].

4. Gene therapy

Intracranial gene therapy was first attempted in *Ppt1*$^{-/-}$ mice utilizing a first-generation adeno-associated virus (AAV) vector (AAV2) injected in two locations per hemisphere in the forebrain [10]. A reduction in autofluorescent storage material, as well as increase in the brain mass and cortical thickness were reported. Further investigation demonstrated that CNS-directed gene therapy is more efficacious when the cerebellum is targeted along with the forebrain [12]. Significantly greater enzyme activity and reductions in autofluorescent accumulation, as well as increased in brain weight were observed with this improved strategy. Importantly, the combination of forebrain and cerebellar injections resulted in improvements in motor function and decreased seizure activity. Unfortunately, there was no significant increase in lifespan. In a related study, *Ppt1*$^{-/-}$ mice were injected intravitreally with the same AAV2-PPT1 vector [11]. The infantile CLN1 disease mice receiving eye-directed gene therapy demonstrating marked improvements in both retinal pathology and function. Interestingly, intravitreal injections allowed for anterograde axonal transport of PPT1 activity into the brains of treated mice and decreased neurodegeneration throughout the optic tracts [11]. This approach, perhaps combined with direct intracranial injections could provide more widespread delivery of PPT1 activity to the CNS. This is vital in a disorder such as infantile CLN1 disease in which CNS pathology is widespread.

A recent study in PPT1-deficient mice demonstrated that bone marrow transplantation can synergize with CNS-directed gene transfer to greatly enhance the efficacy of either approach [29]. As mentioned above, clinical experience has demonstrated that the NCLs are refractory to BMT [5,21,22]. This was confirmed in the mouse model of INCL, since *Ppt1*$^{-/-}$ mice receiving BMT had identical lifespans as untreated animals (median ~35.5 weeks). Treatment with a second-generation AAV vector (AAV2/5) alone resulted in a median lifespan of ~54 weeks. This clearly demonstrates the value of increased PPT1 distribution and expression. Interestingly, and quite surprisingly, animals receiving both AAV2/5-mediated, CNS-directed gene therapy and BMT had a median lifespan of ~74 weeks. This increase in lifespan is coupled with significant improvements in motor function, with combination-treated animals performing nearly normally until ~56 weeks of age. Consistent with the improved lifespan and behavioral measures, significant improvements in histological markers of disease were also observed. Although the mechanism of synergy between gene therapy and BMT is not currently known, this strategy holds promise for the treatment of this invariably fatal neurodegenerative disease.

5. Neuronal stem cells

The advancements in recent years in stem cell technology have raised the possibility that neuronal stem cell transplantation might be a viable treatment strategy for infantile CLN1 disease. Stem cells isolated from the human central nervous system have been cultured, purified and banked [38,40]. These cells were successfully transplanted into the brains of immunodeficient NOD-SCID mice, which will not reject the human cells [38]. When normal (PPT1-positive) human neuronal stem cells were transplanted into PPT1-deficient mice on the NOD-SCID background, the cells engrafted and migrated widely throughout the brain [39]. Interestingly, these cells provided sufficient levels of PPT1 activity to decrease autofluorescence, delay loss of host neurons and improve motor function. These pre-clinical data were used to support a phase one neuronal stem cell clinical trial in infantile CLN1 disease and late infantile CLN2 disease children. Although some safety concerns remain, neuronal stem cell therapy may be an appropriate treatment for infantile CLN1 disease and other LSDs.