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Promising CNS-Directed Enzyme Replacement Therapy for Lysosomal Storage Diseases

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Lysosomal storage diseases (LSD) are a group of inherited metabolic disorders usually resulting from a deficiency in a single acid hydrolase (Neufeld, 1991). The remaining subsets of LSDs are caused by defects in key lysosomal membrane proteins, proteins involved in lysosomal enzyme trafficking, or lysosomal enzyme activator proteins (Neufeld, 1991). Regardless of the gene defect, most LSDs result in an accumulation of undegraded or partially degraded substrates that leads to the hallmark of LSDs, microscopically demonstrable lysosomal distension. These diseases are usually autosomal recessive in nature but several have an X-linked inheritance pattern. Individually, LSDs are rare but as a group they encompass as many as 50 distinct disorders with a frequency of approximately 1 in 5,000 live births (Meikle et al., 1999). This makes the LSDs one of the most common childhood genetic diseases. In many instances, children are asymptomatic at birth. However, LSDs are progressive in nature and invariably fatal. Since lysosomal enzymes are typically ubiquitously expressed, LSDs can present with a broad spectrum of clinical signs including some or all of the following: organomegally, visual and hearing deficits, cardiac insufficiencies, skeletal defects, immunologic abnormalities, and central nervous system (CNS) involvement (Neufeld, 1991, Marodi et al., 1995, Daly et al., 1999, Gadola et al., 2006). Approximately 75% of LSDs have CNS involvement that manifest as mental retardation, seizures, profound neurodegeneration, behavioral abnormalities, and psycho-motor defects. Due to the debilitating nature of the associated clinical signs and the protective nature of blood brain barrier, the CNS disease remains one of the major challenges when developing therapies for this class of inherited disorder.

Enzyme replacement therapy (ERT) for LSDs is one of the true success stories in modern molecular medicine. The seminal discovery that lysosomal enzymes can be secreted from one cell and taken up by an adjacent cell (originally referred to as “cross-correction) opened up the possibility that this process could be exploited for therapeutic purposes (Neufeld and Fratantoni, 1970). However, the successful application of this process for the treatment of LSDs had to wait until the molecular mechanisms involved in “cross-correction” were dissected. The discoveries of the mannose (Achord et al., 1978) and mannose 6-phosphate (Kornfeld, 1992) recognition systems provided a mechanistic framework by which lysosomal enzymes could be modified for targeted or widespread delivery, respectively. The mannose receptor is localized to the plasma membrane and its expression is limited primarily to cells of the reticuloendothelial (RE) system. In contrast, the mannose 6-phosphate receptor is a

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ubiquitously expressed protein localized to both the endoplasmic reticulum and the plasma membrane.

Once the uptake mechanisms and their respective receptors were determined, properly modified enzyme could be produced and tested clinically. The first successful clinical trial of ERT for a LSD was performed in patients with type I Gaucher disease (Barton et al., 1991, Beutler et al., 1991). Type I Gaucher disease is a LSD caused by the deficiency of acid β-glucocerebrosidase activity and primarily affects cells of the RE system (Beutler and Grabowski, 2001). β-Glucocerebrosidase was modified to expose terminal mannose residues in order to target the enzyme to cells of the RE system. This therapy improves several hematologic parameters, decreases splenomegally, and improves skeletal abnormalities, albeit at a slower rate than the hematologic and splenic improvements. Enzyme replacement therapy for type I Gaucher disease is very effective and is now considered the standard-of-care.

Once ERT for type I Gaucher disease was shown to be safe and efficacious, the field moved quickly to develop ERT for some of the more common LSDs. The major difference between ERT for type I Gaucher disease and other LSDs is that most of the LSDs affect multiple cell types in addition to cells of the RE system. This required enzyme that can be taken up by diverse cell types. It was shown that preparations produced in mammalian cells contained a high proportion of enzyme that contained the mannose 6-phosphate recognition marker. As mentioned above, the mannose 6-phosphate receptor is expressed on most cell types of the body (Kaplan et al., 1977, Sando and Neufeld, 1977, Robbins, 1979, Ullrich et al., 1979, Rome and Miller, 1980). Clinical trials were performed nearly simultaneously for mucopolysaccharidosis type I (MPS I, α-L-iduronidase deficiency) and Fabry disease (α-galactosidase A deficiency) (Kakkis et al., 2001, Eng et al., 2001). Both diseases respond to weekly or bi-weekly intravenous infusions of recombinant enzyme. Enzyme replacement therapy is now available clinically for mucopolysaccharidosis types II (Hunter Syndrome, iduronate sulfatase deficiency) and VI (Maroteaux-Lamy Syndrome, arylsulfatase B deficiency) and Pompe Disease (acid maltase deficiency). Clinical trials are currently underway for Niemann-Pick B (NP-B) disease and mucopolysaccharidosis type IV (MPS IV). Pre-clinical experiments in small animals have also shown promise for ERT in Aspartylglucosaminuria (Kelo et al., 2005), Metachromatic Leukodystrophy (Matzner et al., 2009), Krabbe disease (Lee et al., 2005), Wolman disease (Du et al., 2008), and Sly Syndrome (Sands et al., 1994). One common aspect of all the clinical ERT protocols is that they are approved for mild, non-neuropathic forms of disease. Although ERT can be very effective at treating the systemic disease, lysosomal enzymes do not readily cross the blood brain barrier. Therefore, alternate means for delivering enzyme to the brain will have to be developed.

The article by Dodge et al., in the February issue of *Experiment Neurology* addresses the problem of CNS delivery for lysosomal enzymes (Dodge et al., 2009). In this paper, the investigators sought to treat the CNS manifestations of Niemann-Pick A (NP-A). NP-A is a rare, autosommal recessive LSD that results from a deficiency in the soluble lysosomal enzyme, acid sphingomyelinase (ASM; Schuchman and Desnick, 2001). Acid sphingomyelinase is responsible for the hydrolosis of spingomyelin (SPM; Elleder, 1989; Schuchman and Desnick, 2001). The absence of ASM results in the accumulation of SPM throughout the CNS and viscera. In addition to lysosomal distension resulting in hepatosplenomegaly, NP-A patients suffer from severe cognitive deficits, motor dysfunction, and blindness due to sphingomyelin accumulation in both neurons and glia. Patients are normally diagnosed with NP-A in the first year of life with death occurring by age 3. A less severe form of Niemann-Pick Disease, type B (NP-B), occurs when greater than 10% of residual ASM activity remains causing the viscera to be affected but sparing the CNS.

The creation of mouse models of NP-A (Horinouchi et al., 1995; Otterbach and Stoffel, 1995) has aided in the development of therapies for NP-A and NP-B. The ASM-deficient mouse has been extensively characterized (Horinouchi et al., 1995; Macauley et al., 2008) and recapitulates the clinical course of the disease, especially within the CNS (Sarna et al., 2001; Macauley et al., 2008). Most therapies aimed at treating the systemic aspects of NP-B or NP-A have largely been successful. Notably, considerable success has been achieved with ERT (Miranda et al., 2000) and, as mentioned above, this approach is currently in Phase I clinical trials.

As with other LSDs, treating the CNS manifestations of NP-A continues to be problematic. Previous work in the ASM-deficient mouse utilizing cell-based therapies, such as the intracerebral transplantation of mesenchymal stem cells (Jin et al., 2002) or adult-derived neural progenitors (Shihabuddin et al., 2004), led to improvements in the CNS disease. In both cases, the authors demonstrated that the cells were viable and migrated away from the injection site, providing the CNS with ASM activity and a delay in the onset of disease. However, in both cases, these therapies were partially efficacious, resulted in focal areas of correction, and were not a long-term cure of NP-A. Perhaps the most promising approach has been the use of CNS-directed gene therapy (Passini et al., 2005, 2007; Dodge et al., 2005). These studies demonstrate that various serotypes of AAV vectors expressing ASM are appropriate therapeutic delivery systems for enzymes to the CNS. The vectors showed long-term expression of ASM, axonal transport of both virus and enzyme, reversal of SPM accumulation, decreased neuronal loss, and improvement in motor deficits. Although gene therapy treats the focal areas of CNS pathology (i.e. cerebellum), to date, no therapy has successfully treated the entire CNS. Furthermore, the use of both cell-based therapies and viral vectors are often the subjects of debate due to ethical and safety concerns surrounding their use (see below).

Dodge et al., (2009) utilized the principles of ERT and adapted them for delivery to the CNS. In an attempt to globally treat the CNS in the ASM-deficient mouse, the authors implanted a cannula into the lateral ventricle and slowly infused human ASM (hASM) over the course of 6 hours. This single infusion resulted in detectable levels of hASM in the brains for up to 3 weeks. The spread of hASM throughout the neuroaxis was impressive with areas proximal to the injection site displaying the highest levels of hASM (i.e. forebrain) and a decreasing gradient of enzyme to sites more distal to the infusion site (i.e. cerebellum). Concurrent with the presence of hASM, the authors demonstrate a decrease in the amount of sequestered SPM and cholesterol; most notably in the forebrain. Interestingly, hASM was also detected in the serum. Consistent with this observation, levels of SPM in systemic organs like liver and lung were significantly decreased compared to untreated ASM-deficient animals. After determining that a single infusion successfully lowered SPM levels for 3 weeks post-injection, the authors performed hASM infusions every 2 weeks for 8 weeks. Repeated infusions successfully decreased the SPM load in the brains of ASM-deficient mice for an extended period of time. Furthermore, the treated ASM-deficient mice showed moderate improvements on foot fault tests and gait analysis.

The work by Dodge et al., (2009) provides an alternate approach to the treatment of CNS pathology associated with LSDs. CNS-directed ERT has several advantages. First, unlike other therapeutic strategies, this approach does not require the injection of live cells or viral vectors. Thus, ethical concerns surrounding the use of stem cells or viral vectors are circumvented. There are no concerns about placing a viable cell in the brain without a “kill switch” if an unexpected adverse event occurs. Similarly, in certain cases the use of gene transfer vectors, such as γ-retroviruses (Hacein-Bey-Abina et al., 2003) and adeno-associated viruses (Donsante et al., 2007), have been linked to malignant transformation of transduced cells. One advantage of ERT is that if there is an adverse event, such as a severe immune response, the infusion can

simply be stopped. This gives a patient the option to modify or abandon therapy if the enzyme infusions become problematic.

Second, the authors demonstrate the feasibility of repeated infusions. Although CNS-directed ERT using a single injection has been described previously in the ASM-deficient mouse (Yang et al., 2007) and other models of LSD (Lee et al., 2007; Lonser et al., 2005; Chang et al., 2008), a single injection will not lead to persistent therapeutic levels of enzyme. Dodge et al., (2009) clearly show that the enzyme levels wane after a single injection and SPM begins to reaccumulate relatively rapidly, therefore creating a need for multiple infusions. The data suggest that long-term CNS-directed ERT could be accomplished by performing multiple infusions via cannulation or alternatively, using an implantable pump.

Third, in contrast to other ERT approaches directed to the CNS, Dodge et al., (2009) utilized a slow infusion rate coupled with a relatively long infusion time and large injection volume. This injection protocol is similar to convection-enhanced delivery that uses bulk flow to maximize the spread of therapeutic agents during direct CNS injections (Bobo et al., 1994; Bankiewicz et al., 2000). The authors adapted this approach for an intraventricular infusion, which resulted in enzyme delivery to a greater portion of the brain. The widespread delivery of enzyme to the brain has stymied the field and the approach described by Dodge et al., (2009) gives hope for delivering therapeutic agents to the entire brain and spinal cord.

Fourth, one surprising benefit of CNS-directed infusions was the presence of circulating levels of hASM in the blood as well as clearance of SPM from both the liver and lungs. This novel finding demonstrates that therapies aimed at treating the CNS might be beneficial in mitigating the visceral disease as well. Although the mechanism underlying this finding is unclear, it represents an important observation that warrants further study. Future studies thoroughly investigating the effects of CNS-directed infusions on lysosomal distension and functional deficits associated with the systemic disease will help determine whether this finding has clinical relevance.

Lastly and not surprisingly, Dodge et al., (2009) show that the highest levels of hASM and the greatest decrease in SPM occur near the injection site. This approach might be ideally suited for other LSDs, like Infantile Neuronal Ceroid Lipofuscinosis (INCL, Infantile Batten Disease), where the disease manifests first within the thalamus and cortex (Kielar et al., 2007). Although there is also significant pathology in the INCL cerebellum (Macauley et al., 2009), treatment of the forebrain by this approach could dramatically improve the outcome for these patients. Thus, although enzyme spread throughout the neuroaxis is essential, targeting therapies to the most severely affected brain structures is of paramount importance.

Although there are numerous benefits to the therapeutic approach described by Dodge et al., (2009) there are also questions that remain to be answered before this can be translated into the clinic. Although the authors demonstrate that elevated hASM levels in the CNS correlate with a decrease in SPM and cholesterol, it is not clear whether this correlates with a decrease in neuronal loss, especially in regions that are distant from the injection site. For example, ASM-deficient mice have a severe, time-dependent loss of Purkinje cells in the cerebellum and associated motor deficits as measured in the rotarod behavioral test. Does this approach spare Purkinje cells and improve the motor deficits that have been previously reported in this model (Sarna et al., 2001; Dodge et al., 2005; Macauley et al., 2008)?

Another concern for repeated enzyme infusions is the feasibility of this approach in a larger brain. Since a single cannulation into a mouse brain was not sufficient to completely normalize the SPM levels in the most distal sites (i.e. the cerebellum), the question remains as to whether this approach can be translated to the human brain. Infusions of other lysosomal enzymes both intraventricularly and intraparenchymally are reported in other LSD models (Lee et al.,

2007; Yang et al., 2007; Chang et al., 2008) as well is in human clinical trials for Gaucher (Lonser et al.,2005), however, reports suggest that these therapies are only partially corrective. Thus, for this approach to be successful a second or possibly multiple cannulation sites will be needed when translated to human patients. This obviously increases the risk of complications and possibility of infection.

Alternatively, performing combination therapies might enhance the efficacy of this singular approach. In addition to CNS-directed ERT, a number of labs are exploring other treatments for the CNS disease, which has led to the development of cell-, viral vector-, bone marrow transplant- and small molecule-based treatments (for reviews, see Jeyakumar et al., 2005; Sands and Davidson, 2006). Although no single treatment has “cured” the CNS disease, many approaches yield promising results. Therefore, several labs are using a combination of treatments in an attempt to develop a more synergistic, complete therapeutic strategy. The additive benefit of combination therapy was clearly demonstrated in the MPSVII mouse model when ERT was coupled with bone marrow transplantation (BMT; Sands et al., 1997). Subsequent studies in Sandhoff mice combined substrate deprivation therapy with either BMT (Jeyakumar et al., 2001) or neural stem cells (Lee et al., 2007), which yielded a synergistic benefit in several parameters including lifespan. Additional combination therapies utilizing bone marrow transplantation with either a small molecule (Biswas et al., 2002) or CNS-directed gene therapy (Lin et al., 2007) led to the greatest increase in lifespan to date in a mouse model of Krabbe’s disease. Furthermore, work in NP-A demonstrated that the intravenous and intracerebral injections of either bone marrow-derived cells (Jin and Schuchman, 2003) or gene therapy (Passini et al., 2007) also produces an additive effect. Thus, the efficacy of CNS-directed ERT as described by Dodge et al might be increased with periodic intrathecal enzyme administration (Dickson et al., 2007), systemic or CNS-directed gene therapy (Sands and Davidson, 2006), or the use of small molecules (Schuchman, 2007) that are currently in development.

The work by Dodge et al., (2009) identifies a promising approach and represents an important milestone for the treatment of the CNS disease associated with NP-A disease. It will be important to determine if this approach is equally efficacious in other forms of LSD that have a significant CNS component. This is particularly important when considering diseases where preexisting storage material can be reversed, such as NP-AB (for review, see Schuchman, 2007) and MPS VII (Brooks et al., 2002), as compared to diseases that are more refractory to late intervention, such as Krabbe disease (Lin et al., 2007) and Neuronal Ceroid Lipofuscinosis (Griffey et al., 2004, 2006; Passini et al., 2006; Cabrera-Salazar et al., 2007). Although there are important questions that remain unanswered regarding the ability to translate this approach to humans, chronic or repeated intracerebroventricular infusion of a lysosomal enzyme could be an important component of an overall strategy that combines several modalities.

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