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A Hitchhiker's Guide to the Blood–brain Barrier: *In Trans* Delivery of a Therapeutic Enzyme

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Enzyme replacement therapy (ERT) for the systemic disease associated with lysosomal storage disorders (LSDs) has been an unequivocal success.[1] Unfortunately, similar success has not been realized for the central nervous system (CNS) pathology associated with LSDs—in large part because the intravenously administered lysosomal enzymes are unable to cross the blood–brain barrier (BBB). In this issue of *Molecular Therapy*, Meng *et al*. report their achievement of supraphysiological levels of the lysosomal enzyme tripeptidyl peptidase I (TPP1) in the brains of young adult mice with the neurodegenerative LSD known as late infantile neuronal ceroid lipofuscinosis (LINCL; late infantile Batten disease).[2] Remarkably, therapeutic levels of activity in the brain were achieved following an intravenous injection in young adult animals with an intact BBB. This was accomplished via coinjection of TPP1 and a carrier peptide capable of crossing the BBB.[3] The enzyme and carrier peptide were not covalently linked, suggesting that this approach could be useful for a wide range of CNS disorders. Although both the safety and applicability of this approach to other systems must be determined, it could represent an important step forward for the delivery of large molecules across the BBB.

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LSDs comprise a relatively large class of inherited metabolic disorders typically caused by a deficiency in a single lysosomal enzyme. This deficiency results in a progressive intracellular accumulation of undegraded substrates and subsequent cellular dysfunction. Lysosomal enzymes are ubiquitously expressed and are necessary to maintain cellular homeostasis. Therefore, deficiency of a single lysosomal enzyme can affect multiple organ systems and lead to a complicated clinical spectrum. Approximately 75% of LSDs have a clinically significant CNS component.

A serious hindrance to effective treatment of LSDs is that the therapeutic protein must be delivered to many, if not most, cells of the body and then targeted to the correct intracellular compartment, the lysosome. However, in the late 1960s it was discovered that lysosomal enzymes can be secreted from enzyme-sufficient cells and subsequently taken up by enzyme-deficient cells in quantities sufficient to correct the metabolic defect.[4] This process was initially referred to as "cross-correction" and was suggested to be a potential means to treat these diseases. It was subsequently discovered that the mannose and mannose-6-phosphate receptors were responsible for the uptake and lysosomal targeting of exogenous enzyme.[5,6] This knowledge, coupled with advances in molecular biology techniques, allowed for the production of sufficient quantities of properly modified recombinant lysosomal enzymes to treat patients. Intravenous ERT is now the standard of care for Gaucher disease, Fabry disease, Pompe disease, and the mucopolysaccharidoses I, II, and VI. ERT for several other LSDs is also at the preclinical development or clinical trial stage.

If delivered early in life, ERT can effectively treat many, if not most, of the systemic features, including the skeletal and immune defects.[7,8] However, the BBB effectively prevents therapeutic levels of intravenously administered enzyme from reaching the brain. Consequently, ERT has so far proven ineffective for the treatment of the CNS disease associated with LSDs.

Late infantile neuronal ceroid lipofuscinosis is a neurodegenerative LSD caused by a deficiency in the soluble lysosomal enzyme, TPP1.[9] Like other forms of neuronal ceroid lipofuscinosis, LINCL shows the hallmark accumulation of autofluorescent storage material in many cells of the body, including neurons of the brain. The clinical characteristics of LINCL include: progressive motor impairment, cognitive decline, seizures, and premature death typically by 12–15 years of age. Intravenous injection of recombinant native TPP1 has proven largely ineffective in the mouse model of LINCL.[10]

The carrier peptide employed by Meng *et al*.[2] comprised 16 lysine residues followed by the 20 amino acids of apolipoprotein E (K16ApoE) responsible for binding the low-density lipoprotein receptor (LDLR). Interestingly, the authors show that genetic fusion of similar ApoE sequences to TPP1 rendered the enzyme inactive. Delivery of the presumed TPP1-K16ApoE complex to the brain was dependent on dose. Intravenous injection of 17 nmol of TPP1 with 24 nmol of K16ApoE resulted in the highest level of brain activity at two- to eightfold higher than normal. This approach not only delivered relatively high levels of TPP1 to the brain but also resulted in significant biochemical and clinical improvements. Following a single or repeated injection of enzyme and K16ApoE in TPP1-deficient mice, TPP1 was immunolocalized in neurons of the brain and the accumulation of the undegraded storage product was reduced. Intravenous injections of TPP1 alone resulted in no improvement in gait or in life span. By contrast, LINCL mice coinjected with TPP1 and K16ApoE had a nearly normal gait and a significant increase in life span. Importantly, intravenous injection of TPP1 and K16ApoE did

not dramatically alter the systemic delivery of TPP1. Although LINCL is considered a neurodegenerative disease, autofluorescent storage material accumulates in many cells of the body. If treatment were limited to the CNS, it is likely that the systemic pathology would become clinically relevant.

It is presumed that TPP1 interacts with K16ApoE through the polylysine tract and that the 20–amino acid LDLR binding domain of ApoE facilitates the transport of the enzyme across the BBB. Consistent with this hypothesis, Meng *et al.* demonstrate that TPP1 and K16ApoE form complexes *in vitro*.[2] However, the enzyme can be transported across the BBB even if K16ApoE is not premixed with the enzyme but is injected immediately before or after TPP1. These data suggest that multiple mechanisms may be at work. Clearly more research is necessary to understand the mechanism(s) by which K16ApoE facilitates transport of large molecules across the BBB.

Although this method was capable of delivering supraphysiological levels of TPP1 to the brain of young adult animals, there was significant toxicity associated with bolus injections of high doses of K16ApoE. Understanding the mechanism by which K16ApoE exerts its toxic effects is critical before this approach can be considered for human application. However, the toxicity can be decreased or virtually eliminated by decreasing or splitting the dose or decreasing the infusion rate.

Numerous attempts have been made to deliver therapeutic levels of lysosomal enzymes to the CNS of animal models of LSD. Lysosomal enzymes have been delivered directly to the CNS by intrathecal injection.[11] However, there are significant risks associated with lifelong repeated intrathecal injections or with semi-permanent implantable pumps. Intravenous delivery of therapeutic levels of lysosomal enzymes to the CNS would certainly pose fewer risks. Other studies have demonstrated that therapeutic levels of a lysosomal enzyme can be delivered to the CNS following intravenous injection in small animal models of LSD. Injection during the newborn period—during which the BBB is not completely formed and the expression of the mannose-6-phosphate receptor is relatively high in the brain—prevented the accumulation of storage material in the brains of mice with mucopolysaccharidosis VII.[7,12,13] It was shown in the same animal model that intravenous injection of very high doses of enzyme also results in significant delivery to the CNS.[14] Although these are potentially viable approaches, most affected children are not identified at birth, and it is likely that repeated injections of high-dose enzyme would have immunological consequences. Removing the carbohydrate recognition markers from β-glucuronidase dramatically increased the circulating half-life in mucopolysaccharidosis VII mice and enhanced the delivery of enzyme to the brain.[15] Unfortunately, this same approach has thus far not been successful with other lysosomal enzymes.[10,16] It was recently demonstrated that the LDLR-binding domain from apolipoprotein B increased transcytosis of sulfamidase across the BBB and increased activity in the brains of mucopolysaccharidosis IIIA mice to 10–15% of that observed in normal mice.[17] This level of activity was sufficient to significantly decrease storage material and improve behavioral performance.

These approaches designed to increase delivery of a lysosomal enzyme to the brain all have merit and should be pursued. However, none of the studies described above have achieved supraphysiological levels of enzyme in the brain following an intravenous injection in a young adult animal. In fact, most studies were able to achieve only a fraction of normal levels. Meng *et al.*[2] have shown that high levels of enzyme can be delivered to the brain following intravenous injection of TPP1 and the carrier peptide, K16ApoE. They have shown that delivery to the CNS is dose-dependent, that the CNS disease can be significantly decreased, and that animals can tolerate repeated injections. Because transport across the BBB occurs *in trans*, this approach is ideally suited for proteins that cannot tolerate modification with additional amino acids. Similarly, the *in trans* function of this system might make it applicable to CNS diseases for which it would be beneficial to deliver a large molecule or protein across the BBB. Although critical questions remain regarding the mechanism of action and safety, Meng *et al.* performed an important proof-of-principle study demonstrating that this approach holds promise as a less invasive method of treating a CNS disease.

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