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Gene Therapy for Lysosomal Storage Diseases (LSDs) in Large Animal Models

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Abstract

Lysosomal storage diseases (LSDs) are inherited metabolic disorders caused by deficient activity of a single lysosomal enzyme or other defects resulting in deficient catabolism of large substrates in lysosomes. There are more than 40 forms of inherited LSDs known to occur in humans, with an aggregate incidence estimated at 1 in 7,000 live births. Clinical signs result from the inability of lysosomes to degrade large substrates; because most lysosomal enzymes are ubiquitously expressed, a deficiency in a single enzyme can affect multiple organ systems. Thus LSDs are associated with high morbidity and mortality and represent a significant burden on patients, their families, the health care system, and society. Because lysosomal enzymes are trafficked by a mannose 6-phosphate receptor mechanism, normal enzyme provided to deficient cells can be localized to the lysosome to reduce and prevent storage. However, many LSDs remain untreatable, and gene therapy holds the promise for effective therapy. Other therapies for some LSDs do exist, or are under evaluation, including heterologous bone marrow or cord blood transplantation (BMT), enzyme replacement therapy (ERT), and substrate reduction therapy (SRT), but these treatments are associated with significant concerns, including high morbidity and mortality (BMT), limited positive outcomes (BMT), incomplete response to therapy (BMT, ERT, and SRT), life-long therapy (ERT, SRT), and cost (BMT, ERT, SRT). Gene therapy represents a potential alternative, albeit with its own attendant concerns, including levels and persistence of expression and insertional mutagenesis resulting in neoplasia. Naturally occurring animal homologues of LSDs have been described in all common domestic animals (and in some that are less common) and these animal models play a critical role in evaluating the efficacy and safety of therapy.

Keywords

cat model; dog model; fucosidosis; glycogen storage; lysosomal storage disease; mannosidosis; mucopolysaccharidosis

Lysosomal Storage Diseases

Lysosomal storage diseases (LSDs[1]) are characterized by disruption of normal lysosomal function and subsequent accumulation of incompletely degraded substrates. Over 40 characterized genetic conditions are classified as LSDs (Scriver et al. 2001). Inheritance is autosomal recessive, with the exception of the two X-linked diseases, mucopolysaccharidosis (MPS[1]) II and Fabry disease, neither of which has a natural animal model. Lysosomal enzymes are post-translationally modified in the Golgi apparatus by the

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[1]Abbreviations used in this article: AAV, adeno-associated virus; CNS, central nervous system; IDUA, α-L-iduronidase; LSD, lysosomal storage disease; M6P, mannose 6-phosphate; MPS, mucopolysaccharidosis

addition of mannose 6-phosphate (M6P[1]) and delivered to the lysosome by M6P receptor-mediated transport. Most LSDs are caused by loss of normal function of a specific lysosomal acid hydrolase, which degrades large complex substrates in a stepwise fashion, with each step requiring the action of the previous hydrolase to modify the substrate; if one step in the process fails, further degradation ceases and the partially degraded substrate accumulates.

LSDs are not homogeneous as not all disorders are caused by a defect in the coding sequence of a single hydrolase. Modifications and activator proteins are necessary for normal function of certain hydrolases, which can be defective. For example, a mutation in the post-translational addition of the M6P moiety to most hydrolases results in low intracellular activity of many enzymes, with concomitant high serum activities, and is the defect in the severe LSD known as mucolipidosis II (ML II, or I-cell disease; reviewed in Kornfeld and Sly 2001). A cat model of ML II exists (Bosshard et al. 1996; Mazrier et al. 2003) but has not yet been used in gene therapy trials.

The lysosomal accumulation of partially degraded substrate affects the architecture and function of cells, tissues, and organs. The pathogenesis of many of the metabolic alterations are not completely understood, such as the mechanisms of inflammation in synovial joints and the central nervous system (CNS[1]). The clinical course of these diseases is chronic, progressive, and very often lethal before or during early adulthood. Rarely, the accumulated substrate may be cytotoxic, as in galactocerebrosidosis. The signs associated with LSDs are often neurological, but they can also be multisystemic, with skeletal, CNS, cardiovascular, and ocular system involvement. Frequently, the clinical signs are related to increased cell size; but scientists are just beginning to understand the pathogenesis of the skeletal disease. In many syndromes with CNS dysfunction, the neurons are swollen and lysosomes contain lamellar inclusions. The pathogenesis of the CNS lesions includes, but is not limited to, the development of meganeurites and neurite sprouting with synapse formation, which appears to be correlated to primary or secondary alterations in ganglioside degradation (Siegel and Walkley 1994; Walkley 1987, 1998; Walkley et al. 1988, 1990, 2000). Microglia activation and inflammation also occur in the CNS in multiple LSDs (Jeyakumar et al. 2003; Ohmi et al. 2003).

Therapy for most LSDs requires the provision of normal enzyme to the lysosomes of abnormal cells. In a process critically important to therapy, a proportion of the M6P-modified enzyme may leave the cell via secretory granules, enter the extracellular fluid, and be taken up by other cells by the M6P receptor (M6Pr) present in the plasma membrane of most cells (Distler et al. 1979; Kaplan et al. 1977; Natowicz et al. 1979). Researchers first demonstrated these normal physiological processes of mannose 6-phosphorylation, release, and receptor-mediated uptake of lysosomal enzymes using media from cultured fibroblasts (Fratantoni et al. 1968; Neufeld and Fratantoni 1970). Providing normal enzyme to abnormal cells in a patient permits that enzyme to be taken up by the plasma membrane receptor, resulting in delivery of the normal enzyme to the lysosome where it can catabolize stored substrate. A general rule for these diseases is that early treatment improves the outcome, which argues for the screening of newborns for LSDs.

The following sections provide brief descriptions of current approaches for delivering normal enzyme to abnormal cells.

Parenteral Injection of Purified Recombinant Enzyme (Enzyme Replacement Therapy, ERT)

Injection of purified recombinant enzyme, which is usually produced in Chinese hamster ovary cells, has been the standard therapy for nonneuronopathic Gaucher patients for more than a decade and is approved or under evaluation for treatment of Fabry disease, Pompe

disease, and MPS I, II, and VI (Breunig and Wanner 2008; Clarke 2008; Harmatz et al. 2008; Koskenvuo et al. 2008; Parini et al. 2008; Rohrbach and Clarke 2007; Rossi et al. 2007; Wraith 2001). The efficacy of ERT has been tested in various animal models including MPS VII mice (Sands et al. 1994; Vogler et al. 1993, 1996), MPS I dogs (Kakkis et al. 1996) and cats (Kakkis et al. 2001), MPS VI cats (Brooks et al. 1997; Byers et al. 1997, 2000; Crawley et al. 1996), and glycogen storage disease in Japanese quail (Kikuchi et al. 1998; Yang et al. 1998). Researchers have evaluated enzyme derived from rabbit milk (Raben et al. 2003) and from Chinese hamster ovary cells (Kakkis et al. 1996, 2001).

Heterologous Bone Marrow Transplantation (BMT)

This procedure has been performed for decades (reviewed in Brochstein 1992; Haskins 1996; Haskins et al. 1977; Hoogerbrugge and Valerio 1998; Krivit et al. 1999; O'Marcaigh and Cowan 1997) and provides both normal bone marrow and bone marrow–derived cells, which release normal enzyme continuously. Importantly, normal monocytes may cross the blood brain barrier and become microglia, secreting enzyme for uptake by neurons. Individual reports of BMT in animals include fucosidosis dogs (Taylor et al. 1992), mannosidosis cats (Walkley et al. 1994), MPS VI cats (Dial et al. 1997; Norrdin et al. 1993, 1995), and MPS VII dogs (Sammarco et al. 2000). A newer approach uses hematopoietic stem cells derived from placental cord blood rather than bone marrow and, with early implementation, has demonstrated clinical benefit in a variety of LSDs (Martin et al. 2006).

Use of a Viral Vector or Other Gene Transfer Agent to Transfer a Copy of the Normal Enzyme's cDNA to a Patient's Own Cells

This procedure enables the cells to act as a source of normal enzyme and is defined as gene therapy (reviewed in Barranger and Novelli 2001; Cabrera-Salazar et al. 2002; Caillaud and Poenaru 2000; Cheng and Smith 2003; Eto and Ohashi 2002; Gieselmann et al. 2003; Ioannou 2000; Kaye and Sena-Esteves 2002; Poenaru 2001; Watson and Wolfe 2003; Yew and Cheng 2001). The amount of normal enzyme released by successfully transduced cells will in large part affect the degree of correction—the more enzyme secreted, the more available for uptake by diseased cells.

Animal Models of LSDs

One of the most valuable uses of animals with homologous human genetic diseases is in tests of new therapies, and the monogenic inborn errors of metabolism are particularly attractive targets for gene therapy. The major hurdles for successful gene therapy are (1) the difficulty of obtaining adequate levels of gene product in specific tissues such as the CNS, (2) maintaining in vivo expression, and (3) regulating gene expression. Large species such as the dog and cat are more similar to human patients than mice in terms of their heterogeneous genetic background, size (which is more suitable for surgical manipulations and clinical evaluations, including imaging), physiological and pathological measurements, and longevity (which permits assessment of the long-term consequences of gene therapy over years). These advantages, along with the accumulated background of physiological and clinical veterinary knowledge in these species, make them more analogous to the human patients that will eventually be the recipients of gene therapy. In addition, because large animal models are discovered due to their clinical phenotype, they have disease, in contrast to some knockout or point mutation mouse models that may have histological lesions but lack clinical disease such as seen in the Tay-Sachs, Fabry, cystinosis, and Gaucher mice (Cherqui et al. 2002; Cohen-Tannoudji et al. 1995; Ohshima et al. 1997; Phaneuf et al. 1996; Xu et al. 2003), or that die within days of birth such as the Types 2 and 3 Gaucher mice (Liu et al. 1998; Xu et al. 2003).

Several animal species were diagnosed clinically and pathologically with an LSD before investigators defined the cause of this group of diseases by the deficiency in activity of a lysosomal enzyme. Because of the distinctive central and peripheral nervous system lesions, one of the first of these identified diseases was globoid cell leukodystrophy in Cairn and West Highland white terriers (Fankhauser et al. 1963), two breeds of dog now known to share a mutation (Victoria et al. 1996) that apparently originated in the 19th century before the two breeds were clearly developed. The first etiologically defined LSD identified in nonhumans was GM1 gangliosidosis in a Siamese cat in 1971 (Baker et al. 1971). Since then, researchers have recognized naturally occurring LSDs in the mouse, rat, dog, cat, goat, cow, sheep, pig, guinea pig, emu, quail, flamingo (Haskins and Giger 2008), and black bear (EH Kolodny, New York University, personal communication, February 2008). Breeding colonies exist for many LSDs of various species.

Gene Therapy and LSDs

Gene therapy is an attractive approach to treat many LSDs for several reasons. The phenomenon of cross correction obviates the need to transfer genes to all cells, allowing for the possibility that a small percentage of transduced cells can be therapeutic at the organ or whole animal level. Also, the amount of enzyme needed in the lysosome for phenotypic correction of an individual cell is only a small percent of normal. For many tissues, circulating enzyme from any transduced somatic cell type should be sufficient to target other deficient cells, permitting uptake and correction of storage. However, because of the blood brain barrier, somatic cells that produce and secrete large enzyme proteins may not target the CNS. This is critical as at least 75% of all LSDs have significant CNS lesions. Intracerebral gene therapy represents a promising approach for the treatment of CNS disease as it has the potential to provide a permanent source of the deficient enzyme on the parenchymal side of the blood brain barrier. Direct intracranial injection of viral gene vectors has resulted in reduced lysosomal storage and functional improvement in some large animal models of LSDs (see below). Another approach to the CNS has been to provide a high dose of serum enzyme that appeared to cross the blood brain barrier in adult MPS VII mice (Vogler et al. 2005). Gene therapy experiments in MPS I and VII dogs have supported this approach as dogs with high serum enzyme activity had more improvement in CNS storage than did those with less activity (Traas et al. 2007; Haskins, unpublished data)(see below).

Feline Mucopolysaccharidosis I

The enzyme that is deficient in activity and therefore produces this MPS disorder is α-L-iduronidase (IDUA[1]) with storage of the glycosaminoglycans dermatan and heparan sulfates (Haskins et al. 1979, 1983). The mutation results from a three base pair (bp) deletion, leading to the loss of a conserved aspartate residue (He et al. 1999). The MPS I cats have facial deformity, lameness, corneal opacity, and cardiac murmurs. In an initial experiment that treated 12 neonatal MPS I kittens with a retroviral (RV) vector containing the canine IDUA cDNA (the feline cDNA was unavailable), all kittens had serum expression up to eight times normal (Liu et al. 2003). However, the serum activity dropped to untreated affected levels by 60 days after treatment. One kitten that died at 10 days had liver activity almost three times normal, but liver biopsies from the remaining kittens at 90 days had no detectable IDUA activity. A subsequent study reported a CTL response to transduced hepatocytes (Ponder et al. 2006), which explained the loss of expression. An additional two kittens then received the same vector as well as four intravenous injections of CTLA4-Ig, which prevented the CTL response. These cats have expressed IDUA for 3 years and have shown improvement in facial dysmorphia, corneal clouding, and mobility (Haskins and Ponder, unpublished data).

Canine Mucopolysaccharidosis I

Dogs with MPS I are similar to the MPS I cats but have more significant skeletal disease (Shull et al. 1984; Stoltzfus et al. 1992). The disorder results from a null mutation, a G>A transition in the donor splice site of intron 1, creating a premature termination codon at the exon-intron junction (Menon et al. 1992). Six MPS I dogs were treated with in vitro RV vector–transduced bone marrow cells and developed an antibody response, and two dogs that were retreated had an anamnestic response (Shull et al. 1996). Three MPS I dogs were treated by intramuscular gene therapy by plasmid injection or injection of ex vivo RV vector transduction of myoblasts. However, an immune response to the vector and/or enzyme in the null mutant MPS I dog also abrogated these therapies (Lutzko et al. 1999a). Autologous marrow cells modified in vitro by an RV vector also failed due to an apparent humoral response to the enzyme and serum components of the culture media (Lutzko et al. 1999b). To try to avoid the immune response, in vitro IDUA-transduced MPS I marrow cells were transplanted into preimmune, midgestation fetal MPS I pups. Neither IDUA activity nor proviral-specific transcripts were detected in the blood or marrow leukocytes of any of the pups and all died at 8 to 11 months of age from complications of MPS I disease, with no evidence of amelioration of the clinical phenotype. However, there was no evidence of an immune response to the enzyme (Lutzko et al. 1999b).

Neonatal MPS I dogs were injected intravenously with an RV vector containing the canine IDUA cDNA (Traas et al. 2007). This resulted in stable serum IDUA activity of 28-fold normal for up to 1.8 years, which likely was primarily from secretion by transduced liver cells. RV vector–treated dogs had decreased severity and/or incidence of hernias, chest deformities, facial dysmorphia, corneal clouding, valvular heart disease, and aortic dilation. A marked reduction in lysosomal storage in the brain of these dogs may have been due in part to expression from the LTR of the vector in cells in the brain or to high serum activity permitting distribution across the blood brain barrier. Radiographic features of the skeleton revealed less improvement in the cervical spine than other synovial joints (Herati et al. 2008), similar to observations in MPS VII dogs (Herati et al. 2008) and MPS VI cats (Haskins, unpublished data).

The efficacy and safety of stereotaxic injection of recombinant adeno-associated viral (AAV) vectors coding for IDUA were tested in MPS I–affected dogs. Because, as seen above, the dogs produce antibodies against the protein, intracerebral vector injections were combined with an immunosuppressive regimen. The vector genome was widely distributed in the brain of immunosuppressed dogs and prevented glycosaminoglycan and secondary ganglioside accumulations. However, in dogs with only partial immunosuppression, vector injection was associated with subacute encephalitis (Ciron et al. 2006).

An alternative approach to somatic gene therapy is to deliver a therapeutic protein by implanting “universal” recombinant cells in alginate microcapsules that provide immunological protection from graft rejection. This strategy was used to deliver IDUA in the MPS I dog (Barsoum et al. 2003). Canine kidney cells were genetically modified to express canine IDUA and then enclosed in alginate-poly-L-lysine-alginate microcapsules of about 500 μm in diameter. The encapsulated cells were implanted into the brain under stereotaxic guidance. The brains were monitored with computed tomographic scans before and after surgery and examined biochemically and histologically after sacrifice. Delivery of IDUA in plasma, cerebrospinal fluid, and various regions of the brain was extremely low but detectable. However, an extensive inflammatory reaction limited the success of this approach.

Feline Mucopolysaccharidosis VI

The cause of MPS VI is the deficient activity of N-acetylgalactosamine 4-sulfatase (arylsulfatase B, ASB) and is associated with storage of dermatan sulfate (Haskins et al. 1979a; Jezyk et al. 1977). The severe form of feline MPS VI results from a point mutation that causes a leucine to proline substitution at amino acid residue 476 (L476P) (Yogalingam et al. 1996). Affected cats are dwarfed and have facial deformity, corneal opacity, lameness, joint swelling and stiffness, pectus excavatum, and fusion of the cervical and lumbar spine. Fibroblast-mediated in vitro gene therapy was evaluated in the MPS VI cat (Yogalingam et al. 1999). RV vector–transduced cultured skin fibroblasts overexpressing ASB were implanted under the renal capsule of three MPS VI kittens at 8 to 16 weeks of age. A transient low level of enzyme activity was detected in peripheral blood leukocytes shortly after implantation but was lost 3 to 8 weeks after implantation. Long-term biochemical and clinical evaluation revealed no benefit from the therapy (Yogalingam et al. 1999). A similar clinical and biochemical outcome was found using RV vector–transduced cultured autologous bone marrow cells. Interestingly, engraftment of marked cells persisted despite the lack of conditioning (Simonaro et al. 1999).

A protocol to target the retinal pigment epithelium in MPS VI cats entailed unilateral subretinal or intravitreal injection of AAV vector containing feline ASB cDNA into the eyes of affected cats (Ho et al. 2002). Contralateral eyes received AAV vector with the green fluorescent protein (GFP) reporter gene as a control. Based on ophthalmoscopy and histological analyses, GFP was evident as early as 4 weeks and persisted through the latest time point (11 months). As retinal function is normal in MPS VI cats, there was no evaluation of the electroretinograph. However, histological evaluation revealed that the untreated and control GFP-treated diseased retinas contained massively hypertrophied retinal pigment epithelium cells typical of MPS VI, while the eyes treated subretinally with the ASB-expressing AAV vector had minimal cytoplasmic inclusions and consequently were not hypertrophied.

In a preliminary experiment, three neonatal MPS VI kittens received intravenous injections of an RV vector containing the feline ASB cDNA (Haskins, unpublished data). Serum ASB activity has remained stable for more than 3 years at 5- to 16-fold normal levels, the facial dysmorphia typical of the disease has been reduced, and after more than a year corneal clouding improved. However, the cervical spine lesions are not significantly improved. Synovial joint lesions are particularly resistant to enzyme replacement or gene therapy, perhaps because the articular cartilage is poorly vascularized and chondrocytes are embedded in a dense extracellular matrix. Glycosaminoglycan storage in animal models of the MPS disorders has previously been shown to lead to inflammation and apoptosis in cartilage and the findings have been extended to show that synovial fibroblasts and fluid displayed elevated expression of numerous inflammatory molecules, including several proteins important for lipopolysaccharide signaling (Simonaro et al. 2008). The expression of tumor necrosis factor, in particular, was elevated up to 50-fold. Studies of the pathogenesis of the synovial joint lesions may lead to alternate approaches to gene therapy beyond supplying the normal enzyme, such as modulation of the expression of cytokines, cathepsins, or matrix metalloproteinases.

Neonatal MPS VI cats have also received injections of an AAV vector expressing feline ASB under the control of liver-specific, muscle-specific, or universally active promoters (Tessitore et al. 2008). Systemic or intramuscular administration of AAV led to therapeutic levels of circulating ASB, resulting in skeletal improvements and a significant decrease in glycosaminoglycan storage, inflammation, and apoptosis. However, widespread dissemination of vector occurred after intramuscular AAV administration, resulting in secretion of therapeutic levels of ASB with the use of the universally active, but not the

muscle-specific, promoter, suggesting that transduction of extramuscular sites rather than enzyme secretion from muscle occurred after the muscle injection.

Canine Mucopolysaccharidosis VII

Mucopolysaccharidosis VII, caused by deficient activity of β-glucuronidase (GUSB), was first described in a child in 1973 (Sly et al. 1973). The disease has multisystemic manifestations including organomegaly and skeletal, CNS, cardiovascular, and ocular abnormalities. The importance of MPS VII models in therapy stems from a number of factors, including the early identification, characterization, and establishment of breeding colonies of animals with the disease. The first animal model described was in German shepherd dogs in 1984 (Haskins et al. 1984), just a decade after the identification of the human disorder. Murine and feline MPS VII were subsequently described (Birkenmeier et al. 1989; Fyfe et al. 1999; Schultheiss et al. 2000) and colonies established. For all three models, the cDNA sequences are known, the mutations have been identified (Fyfe et al. 1999; Ray et al. 1998; Sands and Birkenmeier 1993), and all have a clinical phenotype that is similar to that of children with the disease.

Mucopolysaccharidosis VII has become a research paradigm for LSD therapy because GUSB has the following properties that make it extremely useful in the assessment of gene targeting and therapy:

- Transduced cells release large amounts of it.
- Very high levels appear to have few if any serious side effects (Vogler et al. 2003), negating the need for regulated expression.
- Histochemical staining permits the direct visualization of the enzyme's location and an estimate of the amount of GUSB activity.
- The heat stability of the enzyme is significant but variable among species, allowing experiments in normal dogs and cats using the very thermostable human enzyme.

In addition, affected animals have a clear and striking phenotype, with severe clinical signs of skeletal, ocular, and cardiovascular disease and clear histological lesions in the CNS.

The models also have the following drawbacks:

- MPS VII is one of the rarest of the LSDs in humans.
- Due to the severe nature of the orthopedic and ocular disease, and shortened life span, a clear and unambiguous evaluation of the pathogenesis and therapeutic efficacy of the CNS disease alone in dogs and cats is difficult.
- As with all LSD models, direct extrapolation to other LSDs is limited because each has its own set of idiosyncratic therapeutic challenges.

But relative to the advantages, these are minor issues and any perusal of the literature will reveal the extensive use of the MPS VII models, especially the mouse, in therapeutic evaluations.

Insights from experiments in MPS VII mice led to the testing of intravenous RV vectors with and without hepatocyte growth factor in neonatal dogs, and these tests indicated that the natural hepatocellular division in the canine neonatal period was sufficient for significant RV integration (Xu et al. 2002). This preliminary step was followed by neonatal intravenous RV gene therapy in dogs with MPS VII, yielding impressive results (Mango et al. 2003; Ponder et al. 2002; Sleeper et al. 2004). The series of canine experiments involved treatment of 2- to 3-day-old pups with the canine cDNA; the dogs have had stable serum GUSB

activity of between 40% and 6000% of normal for over 8 years. The M6P moiety was present in approximately 30% of the serum enzyme, a percentage equivalent to normal dogs, indicating a significant level of enzyme available for cross correction. Important clinical signs of disease, such as cardiac abnormalities, were absent or minimal (Sleeper et al. 2004). There was a marked improvement in the dogs' growth, and the dysostosis multiplex, a hallmark of MPS VII, was improved in many long bones (Mango et al. 2003; Ponder et al. 2002), but not the vertebrae (Herati et al. 2008). The dogs have remained ambulatory to beyond 8 years of age (unlike most affected dogs, which are unable to stand or walk by 6 months of age), corneal clouding has been absent or very mild, and the males are fully fertile. In addition to the transduction of up to 20% of hepatocytes, this treatment transduced hematopoietic stem cells, as peripheral white blood cells have remained vector- and GUSB expression–positive for 8 years. CNS lesions were significantly improved in a dog with eight-fold normal serum activity (Haskins and Ponder, unpublished data), an outcome reminiscent of the adult MPS VII mouse ERT experience (Vogler et al. 2005).

Canine Globoid Cell Leukodystrophy

A canine model of Krabbe disease, which is characterized by a genetic deficiency in galactocerebrosidase (GALC) activity, occurs in West Highland white and Cairn terriers (Fankhauser et al. 1963; McGowan et al. 2000; Wenger et al. 1999). The mutation is an A to C change at bp 473 (Victoria et al. 1996). The CNS and peripheral nervous system are affected early and severely by progressive demyelination due to the accumulation of small amounts of psychosine. This is the only LSD with a natural mouse (Suzuki and Suzuki 1995) and nonhuman primate model (Baskin et al. 1998) in addition to the dog model. In preliminary experiments, the direct intravenous injection of an RV vector with the canine GALC cDNA into neonatal globoid cell leukodystrophy puppies resulted in little serum or peripheral white blood cell activity and no clinical improvement (Haskins and Wenger, unpublished data).

Feline α-Mannosidosis

Mannosidosis is a glycoprotein storage disease caused by a deficiency of lysosomal acidic α-mannosidase and it results in the accumulation of mannose-rich oligosaccharides (Jezyk et al. 1986; Vite et al. 2001). The mutation in the feline model is a 4 bp deletion, leading to a frame shift and premature termination codon (Berg et al. 1997). Affected cats exhibit generalized action tremors, intention tremors of the head and neck, loss of balance, nystagmus, spinal ataxia, and dysmetria, and have widespread neuronal storage. All signs are progressive and euthanasia is usually performed at 18 to 20 weeks of age for humane reasons. Heterologous bone marrow transplantation yielded dramatic results in the treatment of the CNS disease in cats with mannosidosis, even though therapy began when mild clinical signs were present (Walkley et al. 1994). This result raised the possibility of a significant therapeutic effect in the CNS using gene transfer, avoiding the complications of bone marrow transplantation. Results of brain-directed AAV vector–mediated gene therapy for 8-week-old α-mannosidosis cats showed marked and impressive improvement in brain α-mannosidase activity, myelination abnormalities, and neuronal swelling, as well as in magnetic resonance imaging abnormalities, clinical signs of disease, and life span (Vite et al. 2005).

Canine α-Fucosidosis

Canine α-fucosidosis, another glycoproteinosis, results from a 14 bp deletion at the end of exon 1, leading to a frame shift and premature stop codon in the transcript of the canine gene for α-fucosidase (Healy et al. 1984; Kelly et al. 1983; Skelly et al. 1996). This model has been treated by heterologous bone marrow transplantation, which showed efficacy for the CNS disease with the administration of therapy at an early age (Taylor et al. 1992).

However, transplantation of irradiated affected dogs with in vitro RV vector–transduced allogeneic autologous bone marrow resulted in early graft failure (Ferrara et al. 1997).

Canine Glycogen Storage Disease Ia

Glycogen storage disease Ia in the Maltese dog due to defective glucose-6-phosphatase (G6Pase) activity is similar clinically, biochemically, and pathologically to the human disease (Brix et al. 1995; Kishnani et al. 2001; Walvoort et al. 1982). The cause is a point mutation that produces a methionine to isoleucine substitution at codon 121 (Kishnani et al. 1997). Affected puppies exhibited tremors, weakness, and neurologic signs when hypoglycemic, as well as postnatal growth retardation and progressive hepatomegaly. Biochemical abnormalities included fasting hypoglycemia, hyperlactacidemia, hypercholesterolemia, hypertriglyceridemia, and hyperuricemia. Histologically, tissues from affected puppies showed diffuse, marked hepatocellular vacuolation. Intravenous administration of AAV vector containing the canine cDNA to three neonatal affected dogs resulted in reduction of liver glycogen following hepatic expression of canine G6Pase (Beaty et al. 2002). Two months after AAV vector administration, one affected dog had normalization of fasting glucose, cholesterol, triglycerides, and lactic acid. Six weeks after vector administration, the level of vector DNA signal in each dog varied from one to five copies per cell, consistent with variation in the efficiency of transduction and histological improvements in the liver.

In a separate experiment using an AAV vector containing a small human G6Pase transgene, three GSD Ia dogs survived more than 11 months (Koeberl et al. 2008). Urinary biomarkers, including lactate and 3-hydroxybutyrate, were corrected by G6Pase expression solely in the liver, where glycogen accumulation fell almost to the normal level.

Conclusions

LSDs are a set of diseases that respond to treatment by gene transfer technology because a subset of a patient's cells can be transduced and the relevant enzyme can be secreted into the extracellular fluid, transported to nontransduced cells by the circulation, taken up by distant cells, and directed to the lysosome where the substrate can be degraded. However, while most of the more than 40 LSDs share this basic therapeutic approach, each disorder has its own set of characteristics dependent on the properties of the enzyme involved and the substrate(s) that accumulate. Thus, testing the various vector systems and evaluating the outcomes can best be done in animal models for each disorder. While mouse models are invaluable for the initial evaluation of gene therapy, the use of large animals with these natural diseases is a critical step in moving toward clinical trials. In particular, dogs and cats with LSDs have a heterogeneous genetic background, a size suitable for surgical manipulations and clinical evaluation over time in individuals, and a longevity that permits assessment of the long-term expression and risks of gene therapy over years. Currently, at least 18 LSDs have been described in dogs and cats (Haskins and Giger 2008), and 13 exist in research colonies available for gene therapy trials.

The future of therapy for LSDs is likely to involve multiple approaches, but all will depend on early diagnosis, increasing the need for newborn screening for these genetic diseases. While early gene therapy in MPS VII dogs achieved very high serum activity by 1 week of age, with a dramatic improvement in many clinical signs, it did not prevent all manifestations. The CNS and synovial joints remain sites that are difficult to reach by systemic therapy, and the pathogenesis of the lesions in these tissues, such as the development of inappropriate neurite synapses and defects in collagen biosynthesis, is challenging. Multiple approaches using different vectors, different transgenes, local

administration, and a combination of therapies will continue to rely on the use of large animal models of LSDs to lead the way.

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