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Biomarkers for disease progression and AAV therapeutic efficacy in feline Sandhoff disease

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ABSTRACT

The GM2 gangliosidoses, Tay–Sachs disease (TSD) and Sandhoff disease (SD), are progressive neurodegenerative disorders that are caused by a mutation in the enzyme β-N-acetylhexosaminidase (Hex). Due to the recent emergence of novel experimental treatments, biomarker development has become particularly relevant in GM2 gangliosidosis as an objective means to measure therapeutic efficacy. Here we describe blood, cerebrospinal fluid (CSF), magnetic resonance imaging (MRI), and electrodiagnostic methods for evaluating disease progression in the feline SD model and application of these approaches to assess AAV-mediated gene therapy. SD cats were treated by intracranial injections of the thalami combined with either the deep cerebellar nuclei or a single lateral ventricle using AAVrh8 vectors encoding feline Hex. Significantly altered in untreated SD cats, blood and CSF based biomarkers were largely normalized after AAV gene therapy. Also reduced after treatment were expansion of the lysosomal compartment in peripheral blood mononuclear cells and elevated activity of secondary lysosomal enzymes. MRI changes characteristic of the gangliosidoses were documented in SD cats and normalized after AAV gene therapy. The minimally invasive biomarkers reported herein should be useful to assess disease progression of untreated SD patients and those in future clinical trials.

Introduction

The GM2 gangliosidoses are lysosomal storage diseases caused by inherited deficiency in a hydrolytic enzyme, β-N-acetylhexosaminidase (Hex; EC 3.2.1.52), and clinically characterized by progressive neurological impairment resulting in premature death. Initially, failure to attain developmental milestones or loss of acquired skills signals the onset of clinical disease, which ultimately progresses to loss of all body faculties, a semi-vegetative state, and death by the age of five years. The GM2 gangliosidoses include both Tay–Sachs disease (TSD) and Sandhoff disease (SD), which are almost indistinguishable clinically. Hex functions in the ganglioside degradation pathway by cleaving the terminal N-acetylgalactosamine residue from GM2 ganglioside. Structurally, Hex is comprised of two subunits, α and β, which dimerize to form distinct isozymes. A mutation in *HEXA*, encoding the α subunit, leads to a deficiency of the HexA isozyme (α/β) and results in TSD. A mutation in *HEXB*, encoding the β subunit, leads to a deficiency in both HexA (α/β) and HexB (β/β) and causes SD.

Although mouse models are invaluable in biomedical research, their brain size is >1000 times smaller than an infant (Ohshima et al., 1997; Phaneuf et al., 1996; Casal and Haskins, 2006) and their ~2-year lifespan precludes longer-term studies. Feline models are a logical intermediate between mice and humans because of similarities in brain organization

Abbreviations: AAV, adeno-associated virus; Mann, α-mannosidase; AST, aspartate aminotransferase; βgal, β-galactosidase; Hex, β-N-acetylhexosaminidase; BUN, blood urea nitrogen; BAER, brainstem auditory evoked response; CNS, central nervous system; CSF, cerebrospinal fluid; DCN, deep cerebellar nuclei; ERG, electroretinogram; ICV, intracerebroventricular; LDH, lactate dehydrogenase; NPC, Niemann–Pick C; PBMC, peripheral blood mononuclear cell; SD, Sandhoff disease; TSD, Tay–Sachs disease; Thal, thalamus.

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and neuropathology (Aye et al., 1998; Podell et al., 2000; Cork et al., 1978; Baker et al., 1971). With a brain size 50× larger and more complex than mice, cats are also an important model for therapeutic evaluation (Aye et al., 1998; Podell et al., 2000; Cork et al., 1978; Baker et al., 1971; Vite et al., 2003).

A feline model of SD, first identified in the 1970s (Cork et al., 1977), has been well-characterized (Martin et al., 2004; Cork et al., 1978) and presents an unparalleled opportunity to test therapeutic options for translation to humans. Human TSD and SD are caused by a variety of mutations with varying levels of endogenous protein (Myerowitz, 1997), in contrast to knockout mouse models that express no residual protein (Sango et al., 1995). Feline SD is caused by a 25-base-pair inversion in the terminal exon of *HEXB*, resulting in ~15% of normal protein levels and < 3% of normal enzyme activity, allowing for therapeutic assessment of a severe phenotype in the presence of minimally functional endogenous enzyme (Martin et al., 2004).

Adeno-associated virus (AAV) gene therapy has shown great promise in both murine and feline models of SD. With an untreated life span of ~18 weeks, SD mice treated with bilateral injections of the striatum and deep cerebellar nuclei (DCN) with monocistronic vectors encoding human Hex α- and β-subunits survived up to 2 years, the maximum age permitted by institutional animal welfare mandates (Cachon-Gonzalez et al., 2006, 2012). SD cats treated with the same vectors with bilateral injection to the thalamus alone lived to 7.0 and 8.2 months of age, compared with an untreated life span of 4.5 ± 0.5 months ($n = 11$). Due to a marked humoral immune response to human Hex, SD cats were subsequently treated with feline-specific vectors and lived to 10.4 ± 3.7 months of age ($n = 3$), or 2.3 times as long as untreated cats (Bradbury et al., 2013). While injection to the thalamus significantly increased lifespan, this injection route failed to treat the cerebellum. Additional routes of delivery to treat the cerebellum in SD cats are under investigation. When SD cats were treated by bilateral injection of the thalamus and DCN, Hex activity reached supranormal levels throughout the brain (2.7–to 45-fold normal) and spinal cord (4.2- to 14-fold normal), with reduction of GM2 ganglioside storage by 72–100%. When direct DCN injections were replaced with intracerebroventricular (ICV) delivery via the lateral ventricle, results were similar and have been reported in preliminary form (McCurdy et al., 2013). Results in both murine and feline studies support the therapeutic potential of AAV vectors for SD.

Interpretation of therapeutic efficacy in animal models and human patients would benefit from the establishment of minimally invasive, highly sensitive, reproducible methods of tracking disease progression. Such biomarkers should correlate with results of routine neurological exams and other clinical evaluations, yet provide easy and objective measures of clinical disease. To date, a few prospective biomarkers have been identified in murine models and human SD patients, but as they are technically difficult and time consuming, they have not been adopted into clinical practice (Kodama et al., 2011).

Lysosomal storage diseases (LSD) share many pathophysiologic features (e.g. neurodegeneration), which make biomarkers developed and validated in one LSD potentially useful for others. Elevations of aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) have been noted in serum and CSF of patients with Tay–Sachs disease (Aronson et al., 1958a,b; Bembi et al., 2006) and canine GM1 gangliosidosis (Satoh et al., 2007). After development in a mouse model of Niemann–Pick C (NPC) disease (Lachmann et al., 2004), a fluorescent, acidotropic probe (LysoTracker) has recently been validated as a biomarker in more than 100 NPC patients (te Vruchte et al., 2014). LysoTracker fluorescence increased with expansion of acidic compartments such as lysosomes, correlated with age and disease progression in untreated patients, and decreased in response to substrate reduction therapy.

To date, limited research has been conducted on biomarkers of SD and no results have been reported in a large animal model. In this study we evaluate biomarkers for feline SD and validate these biomarkers after efficacious intracranial AAV gene therapy. To our knowledge, this is the first effort to investigate the validity of a number of minimally invasive measures to track disease progression and therapeutic response after AAV gene therapy in a large animal model of SD.

Materials and methods

Animals and surgery

All animal procedures were approved by the Auburn University Institutional Animal Care and Use Committee. Bilateral injection of the thalamus and DCN were performed according to a previously published protocol (Bradbury et al., 2013). ICV injection was performed under ultrasound guidance to confirm the correct placement of the injection needle. After visualization of the left lateral ventricle with an 8–5 MHz Philips HDI 5000 ultrasound probe (Philips Healthcare, Andover, MA), a single entry site was made through the skull with a 20G spinal needle. Vector was then delivered using a Hamilton syringe (Harvard Apparatus, Holliston, MA) fitted with a 25G non-coring needle (Harvard Apparatus). A total of 200 μL was delivered in 10–15 μL aliquots at a rate of 3–5 μL/s with approximately 1 min between aliquots. Cats were treated at 4–7 weeks of age, prior to symptom onset.

At humane or a predetermined endpoint animals were euthanized by pentobarbital overdose (100 mg/kg) and transcardially perfused with cold, heparinized saline. The brain was divided into coronal blocks of approximately 0.6 cm from the frontal pole through the cerebellum. The right hemisphere of the brain was preserved in optimal cutting temperature (OCT) medium for determination of enzyme activity. The left hemisphere of the brain and all other tissues were formalin fixed or flash-frozen in liquid nitrogen and preserved at −80 °C.

AAV vectors

The feline HEXA/B cDNAs were cloned into an AAV backbone as previously described (Bradbury et al., 2013). Feline HEX transgene expression was driven by the hybrid CBA promoter including the CMV immediate-early enhancer fused to the chicken β-actin promoter (Matalon et al., 2003). The woodchuck hepatitis virus post-transcriptional regulatory element (WPRE) was included for enhancement of gene expression. AAVrh8 vector stocks encoding feline Hex subunits were produced as previously described (Broekman et al., 2006).

Blood and cerebrospinal fluid analysis

Blood was collected from the jugular vein at predetermined time points after tranquilization with dexmedetomidine (0.4 mg/kg IM). Blood for complete blood count was put into EDTA tubes and analyzed using ADVIA 120 Hematology System (Seimens Medical Solutions, Malvern, PA). Blood for serology was placed in heparinized tubes and analyzed using a Cobas C311 chemistry analyzer (Roche Hitachi, Basel, Switzerland, Tokyo, Japan). For quantification of echinocytes, Diff-Quick stained blood smears were scanned on an Aperio ScanScope CS2 (Vista, CA) and 3 40x magnification fields were counted.

CSF collection was performed while under general anesthesia. Cats were anesthetized using dexmedetomidine (0.4mcg/kg IM) and ketamine (10 mg/kg IM), underwent tracheal intubation and anesthesia was maintained using isoflurane (0.5–2%). CSF was collected from the cerebellomedullary cistern as previously described (Cook and DeNicola, 1988). CSF samples were collected and aliquots were analyzed for cytology and total protein. Samples were then immediately centrifuged at 100 ×*g* for 5 min to remove any blood contamination. Samples then underwent one freeze thaw cycle at −80 °C and were analyzed using Cobas C311 chemistry analyzer (Roche Hitachi, Basel, Switzerland & Tokyo, Japan).