New Approaches to Correcting Metabolic Errors in Tay-Sachs

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Abstract

Tay-Sachs Disease (TSD) is a neurodegenerative disorder categorized as both a gangliosidosis and a lysosomal storage disease. Tay-Sachs is caused by a deficiency in the enzyme β-hexosaminidase A (Hex A). There is no current remedy available for TSD. However, there have been promising studies and breakthroughs on the use of gene therapy to cure Tay-Sachs, and these studies will be discussed in this review article. Initial progress was made using a cell line model for TSD which led to subsequent studies in HEXA null knock-out mice and mice with Sandhoff disease (a related disease involving the same enzyme). Eventually, researchers were able to transduce the brain of Sandhoff mice correcting the diseases neurological symptoms. Most recently, felines with Sandhoff disease were also successfully treated with gene therapy. Advances made in lifespan, quality of life, and relative safety of the treatment in animal models implies a readiness for human trials. .

Introduction

Background

Tay-Sachs disease (TSD), named for the two physicians who first described it, was discovered by Warren Tay, a British ophthalmologist, and Bernard Sachs, a neurologist from New York City. The disease presents beginning around six months of age with early blindness, severe mental retardation, paralysis, and in most cases death early in childhood before four years of age. It is most commonly seen in children of Eastern European Ashkenazi Jewish descent as well as some French Canadian and Louisiana Cajun populations.[1] TSD is an autosomal recessive disorder caused by a mutation in the gene that encodes for the lysosomal enzyme β-hexosaminidase A (Hex A), *HEXA*.[2]

Metabolic Basis of Tay–Sachs

TSD is classified as both a gangliosidosis and a lysosomal storage disease.[2,3] Gangliosides are the principal glycolipid comprising neuronal plasma membranes. Their surface patterns are created and broken down by various biosynthetic and catabolic pathways. Inherited defects in the catabolic pathways are known to exist in humans. When being recycled, gangliosides are endocytosed and degraded by lysosomes. Defects in different ganglioside hydrolases such as β-galactosidase, β-hexosaminidase and GM2- activator protein (GM2) cause different forms of gangliosidoses. An inherited deficiency in Hex A, which leads to a ganglioside build-up in the patient, is the underlying cause of TSD, a diagnosis of which is confirmed through blood tests for Hex A levels.[1,3]

The specific ganglioside that builds up in TSD sufferers is GM2. GM2 is the target of lysosomal enzyme Hex A. β-hexosaminidase A is a heterodimer of α and β subunits encoded for by the genes *HEXA* and *HEXB* respectively. In TSD it is the *HEXA* gene that is mutated causing the deficiency in Hex A.[2] The most common mutation, a 4-base pair insertion, causes the mRNA transcript to be unstable despite normal transcription.[4] However, other mutations also may cause the protein to be misfolded in the endoplasmic reticulum. This prevents the enzyme from being transported to the lysosome. In the lysosome, without Hex A, GM2 cannot be broken down (Fig. 1). Hex A is responsible for the cleaving of N-acetylgalactosamine from GM2 ganglioside. Without a means to metabolize GM2, it builds up in the cell and disrupts normal cellular function and eventually leads to cell death (Fig. 2). Martino *et al.* clarified the direct link between the lack of Hex A and the build-up of its substrates.[5] The related disorder, Sandhoff disease, is caused by a mutation in *HEXB* which encodes for the β subunit of Hex A. Its symptoms are clinically indistinguishable from TSD.[5] Since they involve the exact same enzyme complex, the two diseases are often studied side-by-side using animal models with Sandhoff disease.
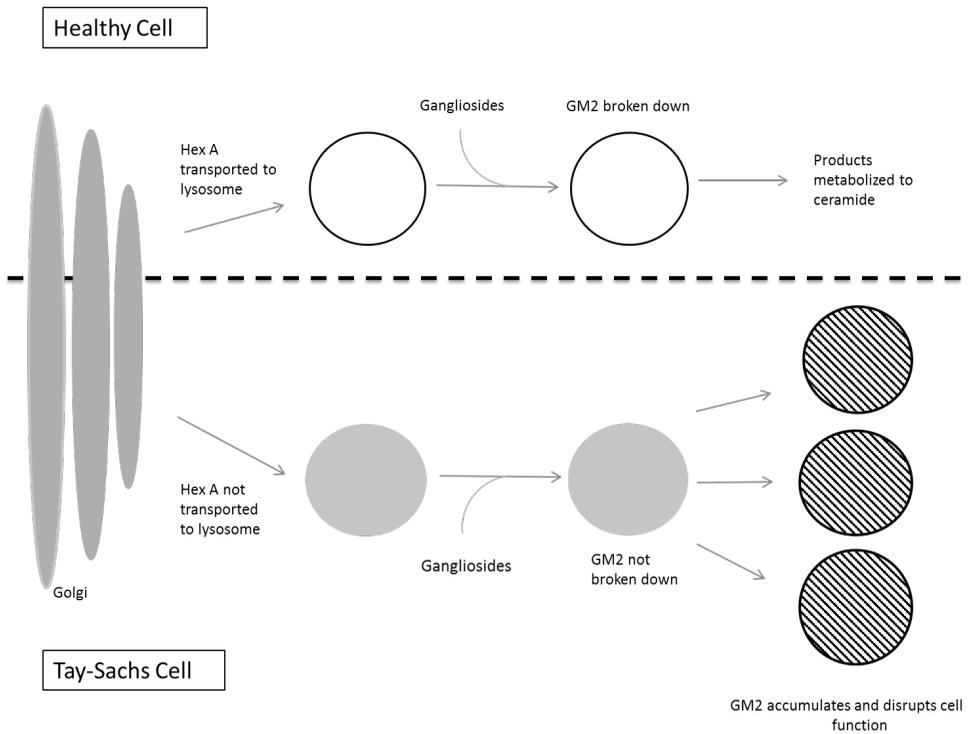
Figure 1. **Diagram showing a comparison of a healthy ganglion cell and one affected by Tay-Sachs disease.** In the healthy cell β-hexosaminidase A (Hex A) is transported from the Golgi apparatus to the lysosome (shown in white) where it breaks down ganglioside G_{M2} (GM2) the products of which are metabolized to ceramide. In the cell affected by Tay-Sachs disease Hex A is either not made or is misfolded which causes it to not be transported to the lysosome (shown in gray). In the absence of Hex A, GM2 accumulates (shown in stripes) and disrupts normal cellular function.
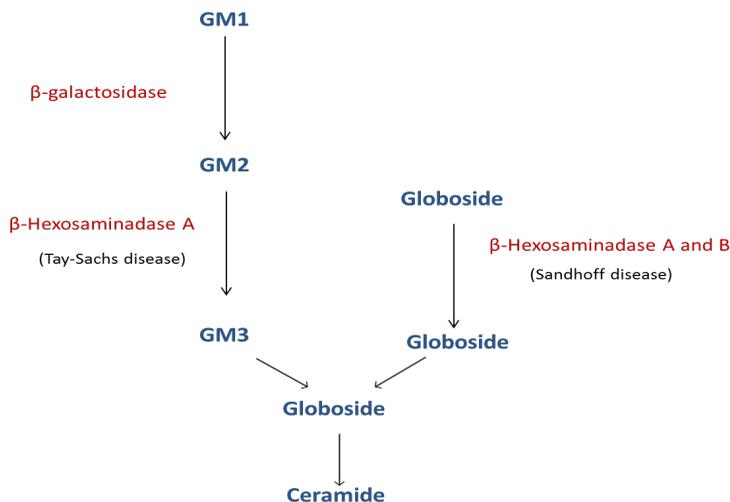
Figure 2. **Metabolic pathways involved in Tay-Sachs disease and Sandhoff disease**. Ganglion cells metabolize gangliosides (GM1, GM2, and GM3) and globosides using enzymes that cleave off various groups from ceramide. A mutated α subunit Hex A causes Tay-Sachs disease, while a mutated of β subunit of Hex A causes the related disease, Sandhoff disease.

Current Management

No cure exists for humans with Tay-Sachs disease. Currently, the best method of prevention is carrier screening. Between 1969 and 1998 1.331 million young adults volunteered for the Tay-Sachs carrier detection test worldwide. During that time period, the number of cases of individuals diagnosed with TSD per year dropped from 50-60 to approximately 2-6 in the US and Canada.[6,7]

Early attempts at intravenous enzyme replacement using purified human Hex A to treat TSD and Sandhoff disease were found to be ineffective.[8] Intracranial fusions and bone marrow transplants also proved to be clinically ineffective. Meanwhile, families and clinicians can only manage the symptoms of the disease. Palliative care includes feeding tubes, range-of-motions exercises and medications for seizures to comfort the patient and ease suffering before death.[9]

Breakthroughs in Gene Therapy

First Steps

In their paper published in 1996, Akli *et al.* describe successfully restoring Hex A activity in fibroblasts (a type of cell commonly found in connective tissue) taken from a human subject suffering from TSD using adenoviral vector-mediated gene transfer.[10] The fibroblasts expressing the recombinant α subunit showed an enzyme activity of 40-80% of normal. These corrected cells secreted up to 25 times more Hex A than the control TSD fibroblasts and showed a normalized degradation of GM2.

Mouse Models

Inspired by this accomplishment, members of the same research team (Guidotti *et al.*) published a paper in 1999 describing their use of gene therapy to treat TSD in Hex A null mice.[11] Their goal was to find an *in vivo* strategy that resulted in the highest Hex A activity in the greatest number of tissues. It should be noted that they used adenoviral vectors carrying human Hex genes. Using vectors carrying the α subunit gene alone brought about only partial correction of Hex A activity. Intravenous co-administration of vectors coding for both α and β subunits of Hex A demonstrated a more successful correction of Hex A levels. The use of both subunits led to high levels of Hex A being secreted by peripheral tissues (heart, liver, spleen, skeletal muscle, and kidney) indicating that exogenous α subunits were unable to dimerize with the low amounts of endogenous β subunits.

The authors contended that while gene therapy would ideally require the transduction of the majority of cells in an organism, this was an unrealistic goal. Therefore, it was their objective to transduce a large number of cells in a large organ causing them to produce and secrete Hex A into the bloodstream in large enough quantities to be taken up by non-transduced cells. They found that injecting the liver brought about transduction of peripheral tissues while injecting other tissue, such as skeletal muscle, did not. The obvious drawback of this study is that Hex A deficiency was not corrected in the brain. Intravenous adenovirus gene transfer does not transduce the brain nor does the secreted enzyme from peripheral tissues cross the blood-brain barrier.

Treating the Brain and Body

In their 2006 paper, Cachon-Gonzalez *et al.* describe their method of transducing neuronal cells with Hex A α and β subunit genes.[12] They used *HEXB* null mice with Sandhoff disease and treated them with stereotaxic intracranial inoculation of recombinant adeno-associated viral (rAAV) vectors encoding both subunits of Hex A, including an HIV tat sequence which greatly increases gene expression and distribution. The authors were able to show that rAAV transduces neurons and promotes the secretion of active Hex A after a single injection. Their evidence indicated a widespread delivery of the enzyme to both the brain and spinal cord. The mice in their study survived for over one year while the untreated mice died before 20 weeks of age. Initially, the motor ability of the treated Sandhoff mice was indistinguishable from the wild-type mice while the untreated mice showed a drastic decline in motor function.

In their follow-up study published in 2012, Cachon-Gonzalez *et al.* used a modified treatment technique yielding more significant results.[13] Again, using rAAV vectors carrying both human Hex A subunits, Sandhoff mice were given intracranial co-injections into the cerebrospinal fluid space and intraparenchymal administration at a few strategic sites. Mice were injected at one month and survived for an unprecedented two years, and the classical manifestations of the disease were completely resolved.

An earlier study using Neimann-Pick disease supports Cachon-Gonzalez's contention that multiple injection sites yield more clinically effective results.[14] Niemann-Pick disease

is another lysosomal storage disease similar to TSD. Passini *et al.* (2007) used mice with this disorder to further investigate gene therapy on lysosomal storage diseases.[14] Using rAAV vectors, they were able to reverse the pathology of the treated mice with both visceral and brain injections. These mice showed superior weight gain and recovery of motor and cognitive functions, when compared to mice treated with either brain or visceral injection alone. The authors assert that this affirms the benefits of multi-site gene delivery strategies in diseases that affect the whole body.

Feline Experiments

Working from the successes of the trials with mice, Bradbury *et al.* conducted studies using felines with Sandhoff disease.[15] A cat's brain is more than 50 times larger than a mouse's, making it a better analog for the treatment of human brains. The researchers first attempted treating two cats with the same vectors used in the mouse studies. These treated cats lived for 7.0 and 8.2 months while untreated cats survived for just 4.1 months. While the two treated cats did show an increase in lifespan, they also showed a pronounced immune response to AAV vector and the human Hex A. Researchers then used AAVrh8 vectors with feline Hex A cDNAs. Three cats were injected with these vectors and survived for an average of 10.4 months. They also found that when phenotypically normal cats were injected with the AAVrh8 vector and examined after 21 months, they showed no sign of vector toxicity.[15]

The cats receiving the AAVrh8 vectors were injected twice. While they showed an increase in lifespan and quality of life, and Hex A activity was increased in most regions of the brain, its activity was minimal in the temporal lobe and cerebellum. Given that the cat brain is about 20 times smaller than a human infant's, the authors suggest that additional injection sites would provide better therapeutic benefits.[15] This assertion accords with the findings of Passini *et al.* in Neimann-Pick mice.

Jacob Sheep as a Potential Model

A line of European Jacob Sheep with a disease similar to TSD has been discovered. The afflicted juveniles show a similar ataxia and early death as other Tay-Sachs animal models.[17] These sheep exhibit a lack of Hex A activity and a missense mutation in the *HEXA* gene has been located.[18] Given that a sheep's brain is even more similar in size and volume to a human brain these sheep are a promising model for the next steps of Tay-Sachs gene therapy research. Researchers have an opportunity with these sheep to further refine injection strategies before human testing.

Conclusions

Human trials are the obvious next step in researching a gene therapy treatment for Tay-Sachs. Promising results have been seen in experiments described above using gene transfer in human Tay-Sachs cells.[12] The results of studies on mice have shown promise in the correction of Hex A expression in the brain and body tissues of mice and felines.[13,14] Many advances have been made in the understanding of implementing gene therapy, spe-

cifically in treatment of gangliosidoses. An understanding of the need to transfer the genes for both subunits of Hex A has been established. Viral-based gene therapy, however, has not been without its problems. Host-induced antibodies and inflammation can limit the use of viral vectors. More specifically, antibodies to adenoviruses exist in most people, which limit reinjections. To combat this, second generation adenoviral vectors have been created with fewer viral genes, which reduce the host's immune response. Vector-related toxicity and immunogenicity related to the remaining viral genes can still present complications.[16] Nevertheless, improvements in the understanding of which vectors and techniques show success in transducing neural cells of the brain in TSD and Sandhoff models have been made. Additionally, these vectors and injection techniques have also not been shown to have deleterious side effects in asymptomatic animal models. Given the progresses made in safety, any remaining risks of gene therapy are outweighed by the severity of TSD. Human trials will hopefully demonstrate a reversal of symptoms in patients giving them a more normal lifespan. In fact, the Tay-Sachs Gene Therapy Consortium, a collaborative of researchers from Auburn University, Boston College, Harvard University/Massachusetts General Hospital, and Cambridge University are attempting to begin these trials by the end of 2014.[19]

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