

Therapeutic Options for Tay-Sachs Disease

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ABSTRACT

Tay-Sachs disease is a lysosomal storage disease that currently has no effective treatment. This review focuses on novel therapeutic options for Tay-Sachs disease. The methods of therapy discussed are enzyme replacement therapy and substrate deprivation therapy. In addition, the potential benefits and limitations of each modality are discussed.

INTRODUCTION

Tay-Sachs disease (TSD) is a lysosomal storage disease that is inherited in an autosomal recessive pattern. Lysosomal storage diseases are a group of disorders characterized by deficiency of a specific single lysosomal enzyme, resulting in accumulation of abnormal metabolic products. TSD is characterized by a deficiency in a common lysosomal acid hydrolase, hexosaminidase A (Hex A). An insufficient amount of Hex A causes the accumulation of one of its substrates, GM2 ganglioside. When this accumulation occurs in the neurons of the central nervous system (CNS), the build up GM2 ganglioside leads to diffuse apoptotic cell death. TSD has a variable clinical presentation. It is usually characterized by severe mental retardation and death within two to four years after birth (Chavanay and Jendoubi, 1998). Other symptoms include progressive motor weakness appearing at three to five months of age followed by progressive blindness and decreased attentiveness (Platt and Butters, 1998). Morphologic changes in the neuronal cell population accompany the clinical symptoms. Two well-documented histopathologic findings seen in microscopic studies are ectopic dendritogenesis and axonal spheroid formation (Flax et al., 1998).

Hex A is one of three isoenzymes from the beta-hexosaminidase family. Each isoenzyme within this family represents different association patterns of the alpha and beta subunits. Hex A is an alpha-beta heterodimer, and Hex B is a beta-beta homodimer. A final member, Hex S, is an alpha-alpha homodimer that is thought to have negligible catalytic activity. Two distinct genes, HEX A and HEX B, encode the alpha and beta subunits, respectively (Chavanay and Jendoubi, 1998). TSD is caused by mutations affecting the HEX A gene. At the present time, there is no effective treatment for TSD. The major obstacle in the treatment of TSD is the penetration of the blood-brain barrier by potential therapeutic agents. However, understanding TSD at the molecular level has opened up several therapeutic options. Among these are enzyme replacement therapies and more novel

approaches that attempt to short-circuit the catabolic pathway of GM2 gangliosides.

ENZYME REPLACEMENT THERAPY

Enzyme replacement is a logical approach to treating TSD. However, two factors must be considered if enzyme replacement therapy is to clear stored gangliosides (Chavanay and Jendoubi, 1998). First, a significant amount of Hex A must reach the targeted CNS neurons in a catalytically active form (Flax et al., 1998). Second, the enzyme that reaches the targeted cells must be taken up and incorporated into lysosomes (Platt and Butters, 1998). Many approaches to replacing Hex A are being studied. These include, the use of viral vectors encoding HEX A, the injection of neural progenitor cells expressing functional Hex A, and the use of bone marrow transplantation to provide Hex A through the circulation (Walkley, 1998).

Viral vectors can deliver a specific gene to a host cell. Viruses enter target cells through specific cell surface receptors. Once inside a cell, most viruses will incorporate their genome into the host's genome. The virus uses the cellular machinery of the host to replicate and package new viral particles. Thus, the idea behind viral delivery is to introduce an engineered viral vector into a population of diseased neurons and express the wild-type HEX A gene. The expression of the wild-type alpha subunit would theoretically allow functional Hex A to form and clear the stored GM2 gangliosides. Guidotti et al. (1998) have engineered viral vectors that deliver HEX A to Hex A deficient cells in vitro and in vivo. They have successfully constructed adenoviral and retroviral vectors encoding the human alpha subunit, and have been able to correct Hex A deficient fibroblasts in vitro. This demonstrated that the viral vectors were capable of delivering HEX A and that Hex A deficient cells could either create their own enzyme or be induced to take up recombinant enzyme from the extracellular environment (Chavanay and Jendoubi, 1998). This group has also conducted studies utilizing HEX A deficient mice. These experiments were conducted with adenoviral vectors engineered to express either the human HEX A gene or the HEX B gene. The group found that functional enzyme levels were only restored in vivo with a co-transduction of both HEX A and HEX B adenoviral vectors. This result was probably due to the limited expression of the normal beta subunit in vivo. They also hypothesized that the complete Hex A enzymatic restoration occurred through both direct transduction and secretion-recapture mechanisms. However, these experiments demonstrated

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that neither adenoviral vectors nor secreted Hex A crossed the blood-brain barrier (Chavanay and Jendoubi, 1998). Thus, this therapeutic option would not be applicable to central neurons involved in TSD. However, peripheral neurons involved in TSD might show marked improvement, yet the underlying cause of mortality would remain unaffected.

The use of neural progenitor cells is the latest experimental therapy for a large variety of inherited disorders. In vitro experiments show that these neural stem cells give rise to self-renewing clones of all fundamental neural cell lineages. It has also been shown that these neural stem cells can be genetically engineered to express foreign transgenes in vivo (Guidotti et al., 1998). The idea behind the proposed therapeutic option is to harvest neural stem cells from fetal brain tissue, and engineer these neural stem cells in vitro to express HEX A using retroviral or adenoviral vectors. These newly engineered stem cells could be placed into germinal zones of developing TSD brains. This would allow the engineered stem cells to participate in normal brain development. The only difference would be that these neural stem cells would be able to create functional Hex A. Flax et al. (1998) conducted experiments using human neural stem cells that had been engineered by retroviral transduction to overexpress the Hex A enzyme. These neural stem cells were cultured along with neural cells harvested from a transgenic mouse that had an alpha subunit deficiency. After 10 days, the mutant neural cells were examined. The group found normalized levels of Hex A enzyme in mutant neural stem cells that were cultured with the engineered neural stem cells. These enzyme levels were higher than the untreated controls (Guidotti et al., 1998). Presently, no published studies have incorporated engineered neural stem cells into alpha subunit deficient fetal brains. However, Flax et al. (1998) has shown that neural stem cells can be incorporated along developmental lineages when injected into developing mice brains. These stem cells were identified within the fetal brain using another marker gene (Guidotti et al., 1998).

Another approach to replacing the deficient enzyme in patients with TSD is the use of bone marrow transplantation (BMT). The rationale for the use of this procedure is based on the delivery of the enzyme via donor derived hemopoietic cells to diseased host cells. There is a considerable amount of literature that BMT can correct the enzyme deficiency seen in many storage diseases. This correction is seen in the liver and other visceral organs. There is no evidence that BMT can assist in correcting enzyme deficiencies affecting the CNS. Researchers believe the blood-brain barrier does not allow circulating enzymes to enter the CNS. Feline models of GM2 gangliosidosis show no improvement when a BMT is performed (Platt and Butters, 1998). Another use of BMT for treating storage diseases would be to allow healthy donor microglia to enter the CNS of the recipient where they can secrete Hex A. Microglia are CNS macrophages

derived from the blood monocytes. Researchers believe that these cells could be introduced into the recipient via BMT, cross the blood-brain barrier, and enter the CNS. Within the CNS these microglia would be capable of secreting Hex A. Some studies suggest that microglia can be induced to secrete large amounts of Hex A and cause surrounding cells to uptake the enzyme, while other studies have been shown that microglia do not affect GM2 gangliosidosis despite substantial enzymatic activity being present in the microglia. This finding suggests that TSD affected neurons do not respond to secreted hexosaminidase enzymes in the CNS (Platt and Butters, 1998).

SUBSTRATE DEPRIVATION THERAPY

On the opposite side of the spectrum, another logical way to treat storage diseases would be to decrease the amount of synthesized substrate. This is generally termed as substrate deprivation therapy. In relation to TSD, substrate deprivation would reduce the biosynthesis of gangliosides to match the impaired rate of catabolism. It is hypothesized that subtle changes in the biosynthesis may have a major impact on storage. Similar to the enzyme replacement therapy, substrate deprivation therapy requires two assumptions. First, mammals can tolerate the depletion of gangliosides. Second, there is some residual enzymatic activity present to catabolize the reduced level of substrate. Additionally, any pharmacological treatment for TSD must be able to cross the blood-brain barrier. The choice mode of substrate deprivation would inhibit the transfer of glucose to ceramide. This is a common step in most ganglioside storage diseases and would serve as a generic treatment (Igdoura et al., 1998). Two biosynthetic inhibitors are primarily seen in the literature. These are D,L-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP) and N-butyldeoxynojirimycin (NB-DNJ). Both drugs inhibit the ceramide specific glucosyltransferase that catalyzes the first step in ganglioside biosynthesis. NB-DNJ is an imino sugar and is the N-alkylated derivative of deoxynojirimycin. This compound seems to have several advantages over the PDMP compound as consequently has been studied more extensively (Igdoura et al., 1998).

Recent studies have been conducted using NB-DNJ on knockout mouse models of TSD. Knockout mouse models of TSD have some residual enzyme activity due to the presence of other catabolic enzymes. This feature is necessary, since substrate deprivation must work in conjunction with residual enzyme activity. Platt and Butters (1998) conducted extensive studies using these mouse models and oral administration of NB-DNJ. When this group conducted their study two observations were made. First, NB-DNJ gained access into the brain to a sufficient degree to prevent GM2 ganglioside accumulation. In addition, the number of neurons affected with GM2 ganglioside storage as well as the burden of GM2 gan-

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glioside storage was reduced in TSD mice treated with NB-DNJ when compared to untreated controls (Igdoura et al., 1998). Platt and Butters (1998) showed that by limiting the biosynthesis of the substrate for the defective enzyme one could prevent the accumulation of that substrate. Hence, the concept of substrate deprivation is effective in a mouse model (Igdoura et al., 1998).

The mouse models of TSD used by Platt and Butters (1998) revealed a completely novel approach to therapy. The mouse models of TSD do not exhibit the neurodegeneration that TSD patients do. These mouse models lack the Hex A enzyme, however, they fail to store high levels of the GM2 ganglioside. In fact, the mouse models show abundant conversion of the GM2 ganglioside to the glycolipid, GA2, which can then be acted upon by Hex B enzyme. This enzymatic sequence represents a metabolic bypass of the Hex A defect and allows the mice to avoid neurodegeneration. The enzyme responsible for initiating the bypass sequence is lysosomal sialidase. This enzyme is involved in a complex with beta-galactosidase and cathepsin A (Walkley, 1998). Outside of this complex the lysosomal sialidase enzyme is rapidly degraded. Humans possess minimal lysosomal sialidase activity, unlike the mouse models that possess higher lysosomal sialidase activity. Recent studies conducted by Igdoura et al. (1998) have tried to prove the effectiveness of lysosomal sialidase upregulation in some TSD models. This group isolated TSD neuronal cells. All of the cells were tested to make sure they were devoid of Hex A. The Hex A deficient neurons were transfected with HEX A cDNA. These cells showed clearance of the GM2 ganglioside demonstrating that these cells could react normally to the HEX A replacement. Next, TSD neuronal cells were transfected with lysosomal sialidase cDNA or both lysosomal sialidase and cathepsin A cDNAs. The transfected cells were assayed for GM2 ganglioside clearance. The TSD neuronal cells transfected with both lysosomal sialidase and cathepsin A cDNAs showed almost complete depletion of the stored GM2 ganglioside. In addition, these cells showed a high level of lysosomal sialidase activity, proving that the enzyme was being expressed (Walkley, 1998). Researchers are still determining whether upregulation of lysosomal sialidase in human patients will also require concurrent increase in cathepsin A expression. Another important caveat is active Hex B must be present for lysosomal sialidase bypass to function. The research of Igdoura et al. (1998) seems to be the stepping-stone for a new line of thought. His group

considers that a combination of substrate deprivation and pharmacological upregulation of lysosomal sialidase activity could be a promising TSD treatment modality (Walkley, 1998).

CONCLUSION

TSD and other lysosomal storage diseases represent a tremendous challenge to the medical community. Enzyme replacement therapies were unheard of years ago; especially those that involve genetically engineered viral vectors as opposed to direct administration. Today, only one glycosphingolipid storage disease has been successfully treated with enzyme replacement, namely Type 1 Gaucher disease. Thus, administration of enzyme replacement therapy may still represent a viable therapeutic option for other lysosomal storage diseases like TSD. However, one of the most daunting challenges presented by CNS storage diseases is the designing of a treatment that penetrates the blood-brain barrier. Dr. Frances M. Platt, has conducted abundant research into the use of pharmacological agents that inhibit the biosynthesis of the ganglioside substrate. By preventing the substrate build up, storage is reduced. The most promising aspect of these pharmacological agents is that they penetrate the blood-brain. Finally, mouse models of TSD have allowed scientists to bypass the mechanisms of GM2 ganglioside catabolism. Enzymatic upregulation accompanied with minimal substrate inhibition may be the therapeutic option of the future.

REFERENCES

- Chavanay, C. and Jendoubi, M. (1998) Biology and Potential Strategies for the Treatment of GM2 Gangliosidosis. *Mol. Med. Today*. 4:158-165.
- Flax, D.J., Aurora, S., Yang, C., Simonin, C., Wills, A., Billingham, L., Jendoubi, M., Sidman, R., Wolfe, J., Kim, S., and Snyder, E. (1998) Engraftable Human Neural Stem Cells Respond to Development Cues, Replace Neurons, and Express Foreign Genes. *Nat. Biotechnol.* 16:1033-1039.
- Guidotti, J.E., Mignon, A., Hasse, G., Caillaud, C., McDonnell, N., Khan, A., and Poenaru, L. (1998) Adenoviral gene therapy of the TSD on hexaminase A-deficient knockout mice. *Hum. Mol. Genet.* 8:831-837.
- Igdoura, A.S., Mertineit, C., Transler, J., and Gravel, A.R. (1999) Sialidase Mediated Depletion of GM2 Ganglioside in Tay-Sachs Neuroglia Cells. *Hum. Mol. Genet.* 8:1111-1116.
- Platt, M.F. and Butters, D.T. (1998) New Therapeutic Prospects for the Glycosphingolipid Lysosomal Storage Diseases. *Biochem. Pharmacol.* 56:421-430.
- Walkley, U.S. (1998) Cellular Pathology of Lysosomal Storage Disorders. *Brain Pathol.* 8:175-193.