Editorial

Gene therapy for G_{M1} gangliosidosis: challenges of translational medicine

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G_{M1} gangliosidosis

G_{M1} gangliosidosis is an autosomal recessive lysosomal storage disorder with an estimated incidence of between 1 in 100,000 and 1 in 200,000 (1). Clinical features are predominantly those of a neurodegenerative disorder due to G_{M1} ganglioside deposition in the central nervous system (CNS). Other clinical features include hepatosplenomegaly, coarse facial features and skeletal dysostosis. The underlying biochemical abnormality is a deficiency of β-galactosidase whose level of activity is inversely proportional to disease severity. While the disease is relatively heterogeneous three clinical subtypes are recognised. In the severe infantile form enzyme activity may be 0.07-1.3% of normal and clinical signs emerge between birth and 6 months of age. The clinical signs are a combination of a neurolipidosis (i.e., neurodegeneration and macular cherry-red spots) and a mucopolysaccharidosis (i.e., organomegaly, dysostosis and coarse facial features). The infantile form is characterised by a rapidly progressive course with severe CNS degeneration and death by 1-2 years of age commonly due to aspiration pneumonia or cardiomyopathy. The juvenile form is associated with enzyme activity in the order of 0.3-4.8% of normal and presents between 7 months and 3 years of age. It is characterised by psychomotor retardation with the other features of the infantile form being present in variable degrees. Disease progression is slower than the infantile form and death typically occurs before the second decade of life. The adult form occurs in those with an enzyme activity in the region of 5-10% of normal and is characterised by normal early neurological development with symptom onset between 3-30 years of age. Characteristic features are of a slowly progressive dementia, parkinsonism and dystonia. There is marked phenotypic variability in the adult form and the age of death varies widely. The diagnosis can be made by measuring β-galactosidase activity in peripheral blood leucocytes or by *β-galactosidase* gene (*GLB1*) molecular testing. Over 100 mutations have been reported in GLB1 (1) and there is no clear correlation between genotype and phenotype.

There are currently no effective therapies for G_{M1} gangliosidosis and only supportive treatments can be offered. Bone marrow transplantation for one patient with infantile G_{M1}-gangliosidosis has been performed and this normalised leucocyte β-galactosidase levels but failed to impact on neurological deterioration (2). Laboratory studies have reported attempts to reduce the substrate for ganglioside formation using imino sugars which are competitive inhibitors of the ceramide specific glucosyltransferase that catalyses the first step in glycosphingolipid biosynthesis. This successfully inhibited ganglioside biosynthesis in rodents and reduced the accumulation of gangliosides in the CNS (3,4). This strategy, however, has no effect to increase β-galactosidase activity. Another proposal is to use a chemical chaperone, N-octyl-4-epi-β-valienamine (NOEV), to stabilise the mutant β-galactosidase. A study has demonstrated in a G_{M1} gangliosidosis mouse model treated with NOEV a marked increase in enzyme activity and a reduction in CNS storage of G_{M1} ganglioside with prevention of neurological deterioration (5,6). However, this therapy is dependent on subjects having residual β-galactosidase activity.

Gene therapy as a treatment for G_{M1} gangliosidosis

It is logical that gene therapy be utilised in monogenetic diseases such as G_{M1} gangliosidosis particularly because of its ability to specifically target and correct the underlying molecular abnormality of the disease. By replacing the deficient gene there is also potential for long term efficacy with a single dose. Gene therapy has previously been shown to be effective in a mouse model of G_{M1} gangliosidosis with either intravenous administration of an adenoviral vector or intracerebroventricular (ICV) injection of an adeno-associated virus (AAV) containing cDNA for β-galactosidase (7,8).

While evidence that gene therapy for this condition is effective in a mouse model is a positive step, the mouse brain is 1,000 times smaller than that of a human infant and clearly more studies need to be performed should this therapy ever translate to man. An article published in this journal (9) developed this rodent work further investigating the effect of delivering cDNA for β-galactosidase using an AAV vector in a naturally occurring feline model of G_{M1} gangliosidosis. Of the three recognised clinical subtypes of G_{M1} gangliosidosis, the feline model most accurately portrays the human juvenile-onset form but with a more predictable outcome of death at 8±0.6 months. Three groups were studied: G_{M1} gangliosidosis treated (n=24), G_{M1} gangliosidosis untreated (n=16) and untreated normal cats (n=7). Treated cats received intracranial injections in to the thalamus and deep cerebellar nuclei containing the cDNA for β-galactosidase with one of two vectors; either AAV1 which is commonly used in human trials or a newer serotype AAVrh8. One of three doses were given and all animals were treated before the onset of signs and symptoms.

The effects seen in the treated animals were remarkably beneficial. Firstly of note, despite such localised injection sites there was widespread dissemination of the vector with vector genomes being detected throughout the brain and spinal cord. β-galactosidase activity exceeded that of normal animals, in some areas of the brain activity was as high as 7 times normal. The stored gangliosides in the CNS were substantially reduced, though still remained above normal in some areas of the brain, particularly areas with minimal β-galactosidase activity (temporal lobe and cervical spinal cord). MRI findings correlated with improvement observed with more invasive modalities and as such could be a useful outcome measure if this therapy is translated to man.

There was an impressive improvement in long term clinical effects including mortality with the mean survival of treated cats at the time of publication being >4.7 times that of untreated G_{M1} gangliosidosis cats. Two thirds of treated cats were still alive at the time of publication with the oldest being nearly 53 months of age and treated cats developed subtle or no signs of disease. A quarter of animals responded but in a less dramatic fashion; the reasons for this are not clear but even these "less responsive" animals survived more than twice as long as untreated G_{M1} gangliosidosis animals. While it is the neurological manifestations which predominate, G_{M1} gangliosidosis is characterised by a systemic deficiency of β-galactosidase and as such it is relevant to note that there was some systemic spread following intracranial delivery with vector genomes detected in the liver and β-galactosidase activity in the liver measuring between 0.24-0.38 times that of normal animals (a significant increase compared to untreated G_{M1} gangliosidosis animals). No discernible difference in therapeutic effect was observed between the two vectors but a reassuring dose response was observed.

The study was not blinded which may have influenced some of the more subjective outcomes and in addition there was no sham procedure but the treatment effect was so large it seems unlikely that these factors would have significantly affected the outcome. One cautionary note however, is that half of all treated cats developed seizure activity with a mean onset of 20.1±7.4 months. It is recognised that seizures are a feature of late-stage feline G_{M1} gangliosidosis but it is not possible to rule out that this is a side effect of the therapy because none of the untreated animals with G_{M1} gangliosidosis survived long enough. The first step to understand this may be to deliver this therapy to normal cats and assess whether there is a propensity to seizure activity. These seizures were controlled with medication and one would likely consider that the benefits in terms of clinical outcomes far outweigh this risk, at least in the infantile and juvenile forms of the disease. However, such effects whether they be a consequence of surviving to a late stage of disease or iatrogenic must be acknowledged when considering translation to man.

Challenges for translation of gene therapy to patients with G_{M1} gangliosidosis

While the translation of any treatment from laboratory science to clinical trials is challenging, there are a number of specific issues that arise when developing a therapy such as this. Broadly they consist of selecting an optimal delivery method, demonstrating safety and overcoming financial obstacles.

The choice of vector is an important component of the delivery method (10). AAVs are single stranded DNA viruses from the family parvoviridae and are considered non-pathogenic. There are 13 serotypes identified each with differing affinity to various cell types. Recombinant AAV (rAAV) vectors used in gene therapy exist in the host cell as episomal concatemers with a low risk of insertional mutagenesis. Furthermore, there is evidence for long term gene expression using AAV vectors. These factors make AAV the vector of choice in many current clinical trials of gene therapy. The main limitation of AAVs in the clinical setting is the presence of neutralising antibodies (nAbs) to AAV. Exposure to the wild-type virus in nature does not cause disease but does result in the development of nAbs. These antibodies are believed to significantly impede target cell transduction and patients who have nAbs are excluded from those clinical trials that deliver gene therapy products systemically. The proportion of people with nAbs is dependent on a number of factors but can vary from 20-80%. An additional challenge is selecting the appropriate serotype of AAV to transduce the target organ most efficiently. In the mouse, for example, direct injection of AAV in to the brain demonstrated that different serotypes (1, 2, 5, 6, 8 and 9) varied in their transduction efficiency in different regions of brain (striatum, neocortex and hippocampus) (11). Whether animal data can aid the selection of the most suitable serotypes in man remains to be seen.

G_{M1} gangliosidosis affects multiple organs but it is the CNS involvement which contributes greatest to morbidity and mortality. An ideal delivery method would be an intravenous (i.v.) infusion which corrects the enzyme deficiency throughout the body. AAV9 has been shown to transduce neurons and glia in the CNS following i.v. infusion in rodents, cats and non-human primates. Translation of this route however is problematic due to high doses required for CNS transduction and a systemic administration route would introduce the limitation of nAbs. Therefore, it would seem prudent to target the CNS more directly. The delivery method employed in this study was intracranial injection under general anaesthetic using stereotaxic localisation. Intracranial injections have been performed in clinical trials delivering gene therapy for Parkinson's disease (12) and Alzheimer's disease (13). There appears to be a good safety record however this is still an invasive and lengthy procedure and given that the first patient group likely to be targeted would be the infantile and juvenile forms of the disease, a less invasive delivery route would be preferable. Delivery in to the cerebrospinal fluid (CSF) may be a viable alternative. A study investigating a therapy for spinal muscular atrophy delivered the gene for survival motor neuron protein to non-human primates using AAV9 (14). This study assessed the feasibility of a CSF delivery route, specifically intrathecal injections performed by lumbar puncture into the subarachnoid space of the lumbar thecal sac. There was moderate transduction in the brain, including motor cortex, cerebellum and brainstem. This was more pronounced when the animals were kept in the Trendelenburg position for 5-10 mins (14). This is not an isolated finding since other studies have also demonstrated widespread brain transduction in a non-human primate when AAV9 was injected in to the cisterna magna or lumbar cistern (15,16). The choice of AAV serotype becomes relevant as although AAV9 has been shown to transduce the brain after CSF administration, ICV injection of AAV2, 4 and 5 did not penetrate the brain parenchyma efficiently. A useful experiment to undertake would be to compare an intrathecal route with direct brain injection in a larger animal model of G_{M1} gangliosidosis. An intrathecal route may reduce transduction efficiency but considering the direct injection route achieved supranormal enzyme levels then even less efficient transduction could result in a therapeutic effect with the benefits of a less invasive procedure. An important part of such a study would be to assess if nAbs play a limiting role when using a CSF route. Published data has not fully answered this issue as one study has demonstrated that nAb levels ≥1:200 inhibited CNS gene transfer when AAV9 was injected via the cisterna magna (15) in a different study the highest antibody titre in that group of animals was 1:128 and there was no effect of nAb on CNS transduction and in addition, following therapy nAb concentrations in the blood were high (1:2,048) but no inhibitory factors were present in the CSF (16). These data suggest that if nAbs do inhibit transduction using a CSF route that it would only be of concern in those with very high titres.

Safety assessments are important in all drug development and gene therapy has some specific additional issues. Important lessons have been learned from earlier clinical trials including the first death as a result of a profound immune reaction to an adenoviral vector and the first cases of insertional mutagenesis leading to cancer in children receiving a retroviral vector (17). These issues clearly need to be monitored in all gene therapy trials. In the severe infantile and juvenile forms of G_{M1} gangliosidosis, though an improvement in mortality would clearly be a positive step in this aggressive disease, this should not detract from such important safety concerns.

Economics play a major role in the development of any

new therapy but gene therapy products can be particularly financially demanding. Prior to any first-in-man study new products need to undergo toxicology and dose finding studies, audits of manufacturing practices, stability testing, batch review and regulatory submissions. The costs for such development are great and while common diseases may attract the attention of pharmaceutical industry which can provide the necessary capital, with rare diseases such as G_{M1} gangliosidosis it may be more challenging to find financial support. However, this hurdle is not impossible as demonstrated by the development of the gene therapy product Glybera for an incredibly rare lipoprotein lipase deficiency (18).

McCurdy and colleagues have made great progress on the journey to create a gene therapy product to treat G_{M1} gangliosidosis (9). There are a number of challenges that remain before this can be translated to man but these are not insurmountable and important lessons learned along the way will add to the knowledge pool in this field and may advance gene therapy in other neurological diseases such as Parkinson's disease and Alzheimer's disease which affect a large number of people.

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