Enzyme Replacement Therapy in Filipino Patients with Gaucher Disease and Pompe Disease

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ABSTRACT

We present three Filipino patients who are on enzyme replacement therapy. Two patients with Gaucher disease have shown significant improvements in the hematologic, skeletal, liver and spleen findings during enzyme replacement therapy. One patient with Pompe disease, although less dramatic, showed notable improvements in fatigue severity scale, movements of some muscle groups, use of mechanical ventilation, and quality of life after two years of enzyme replacement therapy.

Key Words: Gaucher disease, Pompe disease, enzyme replacement therapy

Introduction

Gaucher disease (GD) and Pompe disease (PD) are inherited lysosomal storage disorders. GD is caused by a deficiency of the lysosomal enzyme glucocerebrosidase (acid beta glucosidase), and the resultant accumulation of glucosylceramide (glucocerebroside) in monocytic cells of the reticuloendothelial system.[1] Clinical manifestations include splenomegaly, hepatomegaly, hematologic changes and orthopedic complications. GD is classified into 3 subtypes based on the nature of its effects on the central nervous system. Type 1, the most common variant is non-neuronopathic, type 2 has infantile onset of severe central nervous system involvement and inevitable death in early childhood, and type 3 has onset of central nervous system involvement in adolescence or early adulthood, and shows a more indolent neurologic course.

Pompe disease results from defects in the activity of the lysosomal enzyme acid α glucosidase. The enzyme deficiency leads to intralysosomal accumulation of glycogen in numerous tissues; mostly marked in the cardiac and skeletal muscle. The clinical presentation encompasses a range of phenotypes ranging from a severe classic infantile onset characterized by hypotonia and cardiomegaly to a slowly progressive myopathic late onset disease.[2]

Enzyme replacement therapy (ERT), which addresses the metabolic defect by replacing the deficient or absent enzyme, has revolutionized the treatment and outcome for patients with GD and PD. ERT consists of the intravenous administration of the enzyme produced by recombinant technology. A breakthrough of pivotal importance in ERT was made in early 1990's when the US National Institutes of Health demonstrated that 2-3 mg / kg of mannose terminated, placentally extracted ß glucosidase markedly improved hematologic indices and reduced hepatosplenomegaly in patients with type I GD. The enzyme formulation, marketed as Ceredase demonstrated efficacy but was withdrawn following the introduction of Cerezyme (Imiglucerase), a recombinant human enzyme produced in Chinese hamster ovary cells,[3] The resounding clinical and financial success of Cerezyme stimulated investigators to evaluate Myozyme (Alglucosidase alpha) for Pompe disease and other ERT for the other LSDs.

This paper aims to present two Filipino children with GD and one adult Filipino male with PD who are on enzyme replacement therapy.

Clinical Reports

Gaucher disease

Patient 1 is a female patient, first of twins, born to non-consanguineous Filipino parents. She was well until the onset of abdominal enlargement along with pallor, occasional nose bleeding and bone pains at $2^{6}/_{12}$ years of age. She was diagnosed with acute leukemia and beta thalassemia during various consultations with multiple doctors. Bone marrow aspirate and biopsy, however, revealed hypercellular marrow with numerous histiocytes strongly suggestive of Gaucher disease.

When seen at 3.5 years old, her height of 93 cm and weight of 13.5 kg were both within the 10^{th}-25^{th} percentile for age. She was pale and had hematomas on the lower extremities and petecchiae over the eyelids. The liver was palpable 10 cm below the right costal margin and the spleen was 14.5 cm below the left costal margin. Neurologic examination was essentially normal. Further investigations showed Erlenmeyer flask deformity in both femurs. Liver enzymes were elevated. She had anemia and thrombocytopenia (Table 1).

Enzymatic analysis done on peripheral leukocytes (National Referral Laboratory at Women's and Children's

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Hospital, Adelaide, Australia) showed a level of 390 pmol/min/mg protein (n.v. 600-3200). Molecular analysis showed that the patient was a compound heterozygote. One allele had the common mutation p.L444P and the other allele had the p.P319A mutation. The latter was a single base substitution at c.1072 of exon 8 of the acid β glucosidase gene. Chitotriosidase activity was below the detection limit (National Taiwan University Hospital, Department of Medical Genetics, Taiwan).

She was started on ERT at $3^{8}/_{12}$ years old with Imiglucerase (Cerezyme) at a dose of 60 units/kilogram body weight every 2 weeks. She has tolerated ERT well and there have been no adverse reactions reported. After 5 years of ERT, the liver and spleen sizes have markedly decreased and her hematologic parameters have normalized. Her bony abnormalities however remain, but have not worsened. She has had significant gains in weight and height and there has been a remarkable improvement in her quality of life (Table 1).

On her 5th year of ERT, at 9 years of age, she had 3 episodes of generalized tonic clonic afebrile seizures that were easily controlled by barbiturates. Her EEG and cranial CT scan were normal. She had no pyramidal nor bulbar signs, and no oculomotor involvement was observed. She is currently a grade 1 student and is performing below average. Her psychometric examination revealed that she has mild mental retardation.

Patient 2 is the twin sister of Patient 1. She was evaluated at 3 years of age and noted to have a liver palpable 4.5 cm below the right costal margin and a spleen palpable 5 cm below the left costal margin. There was a hematoma on the lower extremity. Neurologic examination was normal. A presumptive diagnosis of Gaucher disease was made, however, it was only at the age of 4 years that the diagnostic evaluation was completed. By this time, she was anemic and thrombocytopenic, with liver and spleen markedly enlarged to 9 cm and 14 cm below the right and left costal margins, respectively. Liver function tests were normal. Skeletal x-rays showed Erlenmeyer flask deformities in both distal femurs. Bone marrow aspiration showed the characteristic "Gaucher cells". She had a low leukocyte acid β-glucosidase activity of 0.88 nmol/mg/prot/hr (normal range 4.7->5.10, National Taiwan University Hospital, Department of Medical Genetics, Taiwan) compatible with Gaucher disease. Chitotriosidase activity was below the detection limit (National Taiwan University Hospital, Department of Medical Genetics, Taiwan). Prior to the commencement of ERT, supportive medical treatment for hematologic abnormalities and bone pains were given. Three years after the start of ERT at 60 units/kg/day given every two weeks,

Table 1. Hematologic parameters, liver function tests, liver and spleen sizes and skeletal findings in patient 1

	Before ERT	6 mo	12 mo	18 mo	24 mo	3rd year	4th year	5th year
Hemoglobin (g/dL)	68	102	144	166	157	140	154	144
Platelet count (X10 ^{9}/L)	81	22	76	150	184	235	314	355
White blood cell (X10 ^{9}/L)	10.11	5.5	13.85	15.88	9.72	12.12	7.7	9.5
AST (15-37 u/L)	62.37		37	21	25	27	39	
ALT (30-65 u/L)	20.82		31	30	32	31	42	
Liver size (below right costal margin in cm)	11	9.5	6	5	4	3	2	1
Spleen size (below left costal margin in cm)	20.5	16	8	5.5	3	2	2	Not palpable
Skeletal abnormalities	Erlenmeyer flask deformity (EFD)		EFD		EFD	EFD	EFD	EFD

Table 2. Hematologic parameters, liver function tests, liver and spleen sizes and skeletal findings in Patient 2

Parameters	Before ERT	6 mo	12 mo	18 mo	24 mo	3rd year
Hemoglobin (g/dL)	84	132	127	130	139	154
Platelet count (X10 ^{9}/L)	49	112	114	198	257	151
White blood cell (X10 ^{9}/L)	5.8	7.3	4.97	6.7	9.13	7.57
AST (15-37 u/L)	42	38	44			38
ALT (30-65 u/L)	26	40	37			35
Liver size (below right costal margin in cm)	10	4.5	4	3	2.5	2
Spleen size (below left costal margin in cm)	17	9.5	7	3.5	2.5	2
Skeletal abnormalities	Erlenmeyer flask deformity		EFD		EFD	EFD

Table 3. Biochemical and clinical parameters of Patient 3

Parameters	Before ERT	6 months	12 months	18 months	24 months
Creatinine kinase (39-308u/L)	735		499	424	498
AST (15-37 u/L)	104	81	91	86	83
ALT (30-65 u/L)	67	54	77		58
Chest x-ray	Normal				
ECG	Abnormal rhythm RBBB, LVH	Normal rhythm, increased in RBBB, no LVH	Normal rhythm, increased in RBBB		
Echocardiography	Left ventricular ejection fraction 66%, no cardiomyopathy, tricuspid and mitral regurgitation, mitral valve prolapse, pulmonary valve regurgitation	Left ventricular ejection fraction 81%, no cardiomyopathy, no TR,MR,PR, with mitral valve prolapse			Left ventricular ejection fraction 65%, no cardiomyopathy, anterior mitral valve prolapse with mild mitral regurgitation
Number of hours of ventilator use	24 hours	24 hours but tolerates 5 minutes off ventilator	24 hours, tolerates 20 minutes off	24 hours, tolerates 20 minutes off	24 hours, tolerates 25 minutes off

the anemia and thrombocytopenia resolved. Liver and spleen sizes have decreased; however bony abnormalities have remained, but have not deteriorated (Table 2).

She has had a significant improvement in growth parameters. She has tolerated ERT well and there have been no adverse reactions reported. She is currently an average grade 2 student; however, formal psychometric examination showed that she has mild mental retardation (Figures1-2).

Pompe disease

This patient is a 30 year old male born to non-consanguineous Filipino parents after a pregnancy complicated by premature labor during the 6th month age of gestation. He had normal developmental milestones. At 10 years of age, he started to have a clumsy gait, frequent falls and difficulty in climbing stairs. He later developed difficulty in walking. At 12 years of age, he was diagnosed with Duchenne Muscular Dystrophy and was confined to a wheelchair at 16 years of age. He was then brought to the United States for further evaluation where muscle biopsy showed results that were consistent with Pompe disease. At 19 years of age, he started to have respiratory difficulties which led to the use of a Bi-level positive airway pressure (BiPAP) device for 24 hours a day. His echocardiogram showed mitral valve prolapse. His CK, AST and ALT were all elevated. Enzyme assay for acid α glucosidase done on whole blood showed low activity of 13.66 nmol/mg prot/hr (normal range >60, National Taiwan University Hospital, Medical Genetics Department). He was started on ERT with Alglucosidase alpha (Myozyme) at 28 years of age at a dose of 20 mg/kg/day given every two weeks, which he tolerated without the need for pre-medication. After 2 years of ERT, his upper and lower extremities are still weak but there is better movement of both hands. His fatigue severity scale has improved. His Medical Research Council (MRC) scale has remained the same; except for wrist flexion which has slightly improved. Most muscle groups were graded at 1-2 using the scale, and he continues to be wheelchair bound. He can now tolerate room air for 10-20 minutes in a sitting position. Levels of his CK, AST and ALT have improved (Table 3). His energy level has greatly improved as well as his quality of life. He has started to enjoy outdoor activities and gatherings with friends and family and he keeps himself busy with his home business (Figure 3 A-C).

Discussion

Enzyme replacement therapy was started locally in 2003. The enzymes have been acquired through the manufacturer's (Genzyme) International Charity Access Program (ICAP). Patients for therapy are admitted and have the infusions in the hospital. The enzyme solutions are prepared by a pharmacist or a clinical genetics nurse. The infusion and monitoring of the patients are supervised by a physician. The patients are discharged on the same day after review by a metabolic specialist. Actual infusion visits usually take 2-4 hours.

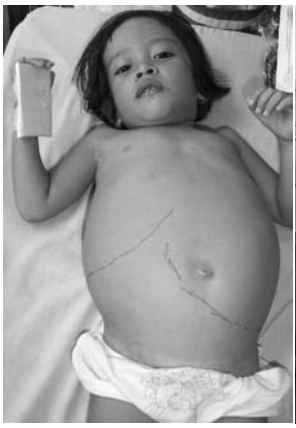

Figure 1. Patient 1 before ERT at 3.5 years old with marked hepatosplenomegaly (with permission).

Figure 2. The twins 4 years after the start of ERT (with permission).

Figure 3. A) Patient with Pompe disease, wheel-chair bound at the time of diagnosis at 16 years of age. B) On Bi-pap machine which he uses 24 hours a day. C) Two years after ERT, patient tolerates 25 minutes off Bi-pap machine (with permission).

Gaucher disease

Gaucher disease is a severe debilitating condition associated with hematologic, skeletal, physical and psychosocial complications. However, ERT has been shown to prevent progressive manifestations of the disease and amelioration of the above features.[4] Our experience with the two patients with GD is consistent with what is reported in the literature.[5,6] With enzyme replacement therapy, the hemoglobin values normalized after 8 months for Patient 1 and after 2 months for patient 2. Platelet counts increased to normal levels after 18 months for patient 1 and 7 months for patient 2. The enlarged liver and spleen have diminished to near normal sizes after 2 years of ERT. The bone disease has persisted but there are no signs of clinical deterioration. We observed a significant gain in weight, an improvement in the quality of life and the absence of therapeutic adverse events for both patients. Occasionally, there have been interruptions in ERT for both patients as drug was not available. The longest time that ERT has been interrupted for both patients was 3 months. During the time that they did not receive the enzyme, no recurrence or worsening of hematologic and bony abnormalities were observed, and liver and spleen sizes have remained stable.

Both patients were classified to have type 1 GD. However, because of the occurrence of seizures in Patient 1 along with the presence of mild mental retardation in both, in the background of the L444P allele which predicts a neuronopathic phenotype of GD, type III GD cannot be entirely excluded.[7] On the other hand, the absence of oculomotor apraxia which is present in most GD type III patients may point against a type III phenotype. Likewise, the seizures and mental retardation may be idiopathic in etiology and may not be part of their primary disease. Full neurologic assessment and regular monitoring are necessary for the twins to better delineate the type of disease they have.

The clinical response of bone disease to ERT has been reported to be favorable with the disappearance of bone crises and new fractures under ERT, and marked reduction in the intensity and frequency of bone pain.[5] However, the response of pre-existing skeletal disease to ERT has remained limited. Significant amelioration of bone lesions has been demonstrated by plain radiography; although the burden of marrow disease remains significant as detected by magnetic resonance imaging (MRI). Studies have shown that a low fat fraction, as assessed by MRI of the lumbar spine, represents a high load of Gaucher cells in the bone marrow and may predict the occurrence of bone complications.[5,8] For our patients, although there was the persistence of the Erlenmeyer flask deformity, there was no history of osteopenia, bone pain or fractures. The status of their skeletal pathology may be accurately determined by MRI for better evaluation of marrow infiltration.

Chitotriosidase is a lysosomal enzyme that originates from Gaucher cells and is closely associated with total body burden of Gaucher cells.[9,10] Persistently high chitotriosidase

levels reflect the presence of a high burden of Gaucher cells, which has been shown to consistently fall after bone marrow transplantation or enzyme replacement therapy in GD patients.[11] However, its use as a clinical biomarker is limited by the observation that 6% of the population lacks activity and 30% carry a mutation in the chitotriosidase gene that results in lower activities.[12] The chitotriosidase activity of both Patients 1 and 2 were not detected. Non-detection of chitotriosidase activity may be associated with a 24 bp duplication polymorphism in exon 10 of the chitotriosidase (*CHIT1*) gene.[12] This polymorphism still has to be detected in our patients and other biochemical markers in the monitoring of GD, such as CCL-18, may have to be used for both of them.

Pompe disease

The clinical presentation of our patient with late-onset Pompe disease is in keeping with other reported cases, wherein most had problems related to limb girdle weakness and walking difficulties. More than half of the patients indicated the onset of problems during childhood, however 28% had delayed diagnosis (between 5-30 years) since Pompe disease may not be readily distinguished initially from other neuromuscular disorders, such as limb-girdle muscular dystrophy.[13] Thus, it is not surprising that it took 6 years before our patient was confirmed to have Pompe disease. Although cardiomyopathy is less frequently seen in older patients, arrythmias have been reported in late-onset Pompe disease similar to what was observed in our patient.[13]

Although ERT in late-onset Pompe disease has led to encouraging results in three patients,[14] the effect on the improvement of skeletal muscle strength and function in our patient has been less impressive. After 2 years of enzyme replacement therapy, our patient's obvious benefit from the treatment are better energy levels and an improved quality of life. Although there was no significant change in muscle function, his pulmonary function is presumably improved as manifested by an ability to tolerate 20 minutes daily, off ventilatory support. The less dramatic response in the proximal muscle groups may be related to the severity of the disease before the start of therapy and the disease duration.

Disease severity in the form of certain variables such as wheelchair use, the need for respiratory support and the number of hours of respiratory support per day, has been correlated with increased disease duration rather than age at symptom onset.[15] The underlying muscle pathology also correlates with the disease severity. Ultrastructural analysis of skeletal muscles in adult onset Pompe disease revealed lysosomal and cytoplasmic glycogen storage, autophagic vacuoles and abnormal mitochondria. Significant glycogen accumulation within the lysosomes causes their rupture and release of glycogen into the cytoplasm could lead to damage of the structure of muscle cells including myofibrils.[16] With ERT, the extent of glycogen clearance varied widely among patients. While age at onset of treatment may be one determinant, the extent and nature of baseline muscle pathology may be an additional factor which may influence response. Patients with more advanced histologic disease or who were older showed less robust histologic and clinical responses in patients.[17] Our patient did not undergo repeat muscle biopsy just prior to ERT. This would have revealed the severity of his muscle histology due to the prolonged glycogen accumulation. However, treatment at the time when his muscle function was virtually poor reflects the extent of muscle damage that may not be easily reversed by ERT. Levels of his AST, ALT and creatinine kinase that have improved but remained elevated may likewise suggest the uncontrolled muscle damage.

Enzyme replacement therapy in infantile Pompe patients has been proven to be safe and effective for the treatment of this disease and treated patients have shown overall improvement in cardiac and skeletal muscle function, and in the histologic appearance of skeletal muscles as early as 5 months after initiation of ERT.[18,19,20] However, in late onset Pompe disease, further studies need to be done regarding its long term efficacy especially for adult patients who started the treatment at the time of advanced disease.

In summary, our two patients with GD have shown significant improvement in the hematologic, skeletal and liver and spleen findings after enzyme replacement therapy accompanied by marked enhancement in the quality of life. This is in keeping with previous reports on the effectiveness of ERT in these organ systems. However, their neurologic status has to be closely monitored; in type III GD, reversibility or prevention of the neurologic complications is a given limitation of enzyme replacement therapy, as enzyme delivery across the blood brain barrier is inadequate.[21]

Follow up of our patient with late-onset Pompe disease should focus on the respiratory and limb girdle muscle function and the capacity to perform daily activities. Attention should also be paid to the natural course of this disease as the efficacy of ERT in this group of patients needs to be elucidated in further follow-up studies. His slow improvement 2 years after ERT suggests that the recombinant acid α-glucosidase may be less robust when treatment is iniated at a more advanced disease stage. This observation emphasizes the value of early recognition and subsequent early treatment of the disorder for a more favorable response to enzyme replacement therapy.

Furthermore, the awareness and understanding of local health professionals about these lysosomal storage disorders should be heightened through education and publicity for prompt diagnosis and early institution of treatment.

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