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Therapeutic approaches in Glycogen Storage Disease type II (GSDII)/Pompe disease

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Summary

Glycogen storage disease type II (GSDII)/Pompe disease is an autosomal recessive multi-system disorder due to a deficiency of the glycogen-degrading lysosomal enzyme, acid alpha-glucosidase (GAA). Without adequate levels of GAA, there is a progressive accumulation of glycogen inside the lysosome, resulting in lysosomal expansion in many tissues, although the major clinical manifestations are seen in cardiac and skeletal muscle. Pompe disease presents as a continuum of clinical phenotypes. In the most severe cases, disease onset is in infancy and death results from cardiac and respiratory failure within the first one or two years of life. In the milder late-onset forms, cardiac muscle is spared and muscle weakness is the primary symptom. Weakness of respiratory muscles is the major cause of mortality in these cases. Enzyme replacement therapy (ERT) with alglucosidase alfa (Myozyme$^{@}$, Genzyme Corporation, Framingham, MA) is now available for all forms of GSDII. ERT has shown remarkable success in reversing pathology in cardiac muscle and extending life expectancy in infantile patients. However, skeletal muscle has proven to be a more challenging target for ERT. Although ERT is less effective in skeletal muscle than was hoped for, the lessons learned from both clinical and pre-clinical ERT studies have greatly expanded our understanding of the pathogenesis of the disease. A combination of fundamental studies and clinical follow-up, as well as exploration of other therapies, is necessary to take treatment for GSDII to the next level.

Keywords

enzyme replacement therapy; gene therapy; glycogen storage disease type II; Pompe disease; lysosome

INTRODUCTION

Pompe disease is an autosomal recessive multi-system disorder caused by a deficiency of the lysosomal enzyme, acid alpha-glucosidase (GAA). GAA is the only hydrolase responsible for degrading glycogen to glucose within the acidic milieu of the lysosome. The deficiency of the enzyme results in the accumulation of lysosomal glycogen in multiple tissues, but the clinical manifestations are primarily seen in cardiac and skeletal muscle. Pompe disease belongs to not only the class of glycogen storage diseases (it is referred to as glycogen storage disease type II, GSDII), but also to the group of lysosomal storage disorders. GSD II is a rare disease with an estimated incidence of 1 in 40,000.[1] Clinically, GSDII presents as a wide spectrum of

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phenotypes ranging from the severe rapidly-progressive infantile form to the slowly-progressive relatively mild late-onset form.[2–4] Patients with the most severe infantile form rarely survive past the first two years of life and die from cardiac failure. Adult patients with the mild form experience progressive skeletal muscle weakness without cardiac involvement and eventually succumb to respiratory failure.

There has been a great deal of progress in the last decades in studying GSDII: the natural history of the disease has been elucidated in prospective and retrospective studies, animal models have been developed, and more than 300 variants have been identified in the *GAA* gene. The mutations include the entire range of defects: missense, nonsense, large and small insertions and deletions, and frame-shift mutations. A database containing all the reported mutations and polymorphisms of the *GAA* gene may be accessed at http://www.pompecenter.nl. There is generally a good correlation between the nature of the mutation, the degree of residual enzyme activity, and the severity of the clinical presentation. Infantile patients have either complete or near-complete enzyme deficiency, while late-onset patients retain some residual enzyme activity. The major advance in the field has been the development and manufacturing of recombinant human GAA (rhGAA) for enzyme replacement therapy (ERT). Recombinant hGAA has been produced and purified from Chinese hamster ovary cells (CHO) and in 2006 human acid α-glucosidase (alglucosidase alfa, Myozyme, Genzyme Corporation, Framingham, MA) received broad-label marketing approval in Europe and later in the U.S. This is the first available therapy for GSDII and it represents the first instance of targeting recombinant enzyme to skeletal muscle. This review summarizes the results of the first clinical trials with ERT and the limitations of replacement therapy. Other potential therapeutic approaches will also be discussed.

PATHOGENESIS

Lysosomal abnormalities

The prevailing view of pathogenesis, which has not changed significantly since the 1980's, is that the glycogen-filled enlarged lysosomes eventually rupture releasing toxic contents into the muscle cytoplasm.[5] A recent study has identified several stages of disease progression in skeletal muscle. At the early stage, muscle cells contain small, glycogen-filled lysosomes. This is followed by enlargement of the lysosomes and leakage of glycogen into the cytoplasm in some areas. As the disease progresses, lysosomal rupturing continues until the majority of glycogen is cytoplasmic, replacing the cell's contractile elements.[6] However, this view of pathogenesis may be too simplistic in light of new data concerning additional pathological hallmarks of the disease.

Autophagy

Autophagy, an evolutionarily conserved process that degrades long-lived proteins and damaged organelles, is constitutively active in every cell and is up-regulated under conditions of starvation. Autophagy has been implicated in a number of human diseases, including cancer, neurodegeneration, and lysosomal storage disorders.[7] The failure of productive autophagy in GSDII muscle fibers was first shown in an animal model. In addition to expanded lysosomes, huge areas of cellular debris with a large autophagic component were observed in the core of muscle fibers. The areas of cellular debris, seen in predominantly type IIB myofibers, were associated with resistance to ERT.[8] Analysis of isolated single muscle fibers from patients with GSDII confirmed that autophagic build-up is a prominent feature of the disease in humans as well. In patients, as in the mouse model, the enormous build-up appears to cause greater muscle damage than the enlarged, glycogen-filled lysosomes outside the autophagic regions.[9]

Mitochondrial, structural, and neurogenic abnormalities

Mitochondrial alterations, such as mitochondrial swelling and irregularities of cristae structure, as well as the disintegration of muscle structure, including Z-line streaming and thickening, are observed in the majority of muscle biopsies from patients at any age.[6,10] Morphologically scattered or small groups of angular atrophic fibers are frequently seen in Pompe patients, a pattern which suggests neurogenic atrophy. These findings may be related to glycogen storage in anterior horn cells of the spinal cord, which may lead to motorneuron destruction. The accumulation of glycogen in the spinal cord correlates well with spontaneous activity (positive sharp waves, fibrillation potentials, etc.) detected in many patients by electromyogram.[10] Since glycogen is not completely cleared in the motor neurons in patients on ERT, a potential consequence of the therapy may be development of motorneuron disease.[11]

ENZYME REPLACEMENT THERAPY

Background

Like many other lysosomal enzymes, acid alpha-glucosidase is synthesized in the rough endoplasmic reticulum (RER), where high mannose oligosaccharides are added to the molecule. In the Golgi, the oligosaccharide side chains undergo post-translational modifications by the addition of the mannose-6-phosphate (M6P) recognition marker. The addition of a M6P moiety allows for the recognition of the enzyme by mannose 6-phosphate receptors (MPR), which transport the enzyme to early and late endosomes. Once inside the late endosomes, the receptor-ligand complexes dissociate due to the low pH in these vesicles, and the enzyme is delivered to the lysosome, while the receptors recycle back for the next round of sorting.[12] A portion of the enzyme, which is not bound to the receptors, is secreted, and this extracellular enzyme can be taken up by neighboring cells by cation-independent mannose 6-phosphate receptor (CI-MPR) on the plasma membrane, which directs the endocytosis and transport of the enzyme to the lysosome. In addition to the receptor, numerous proteins are responsible for the delivery of the enzyme to the lysosome.[13] The ability of cells to secrete and uptake lysosomal enzymes was first demonstrated in cross-correction experiments, in which normal cells rescued the phenotype of neighboring cells deficient in a specific lysosomal enzyme.[14] The receptor-mediated uptake of lysosomal enzymes is the fundamental basis of enzyme replacement therapy for GSDII and many other lysosomal storage diseases. In GSDII, the recombinant human enzyme (rhGAA) is an 110kDa precursor containing M6P groups that enable the enzyme to bind the receptor on the cell surface. Once inside the cell, the rhGAA, like the endogenous precursor, is cleaved to yield intermediate forms, followed by conversion to the fully mature lysosomal species.[15,16]

Clinical Trials

Many patients have been receiving rhGAA under different protocols: the expanded access protocol for infantile onset Pompe disease, compassionate use, the protocol for severely affected cases with late onset Pompe disease, etc. Initial trials focused on the severe infantile form of the disease and were expanded to include patients of all ages. Tables 1 and 2 summarize the completed and ongoing trials and the available results.

Studies in infantile-onset patients—The first studies with ERT in infants were conducted by the Rotterdam group using recombinant human α-glucosidase from transgenic rabbit milk.[17–19] Eventually, the production of the milk product was discontinued (the method was not sustainable) and all surviving patients were transitioned to CHO-derived rhGAA, Myozyme. In these open-label studies, four critically ill infants (aged 2.5–8 months; two were less than 3 months of age) were enrolled and treated intravenously (i.v.) at an initial dosage of 15 to 20 mg/kg weekly, which was later increased to 40 mg/kg weekly because the level of enzyme activity in skeletal muscle still remained significantly below normal on the lower dosage.

Increasing the dosage resulted in the normalization of the level of GAA activity, however, muscle glycogen content decreased in only one patient, who was 3 months of age at start.[17,19] All four patients survived beyond the age of one year. [17,18] The short and mid-term reports clearly show that the enzyme was well tolerated and that tissue morphology and motor and cardiac function improved. The effect on the heart was the most significant, with a reduction of the left ventricular mass index (LVMI) in all infants. The most impressive improvement was observed in the two younger patients, who had no significant respiratory problems during the first two years of life.

A long-term follow-up report of these four infants revealed the survival of all beyond the age of four years. The hypertrophic cardiomyopathy diminished significantly during the 84 weeks of ERT. Remarkable progress in motor function was seen in the two youngest infants; they achieved motor milestones that are unmet in untreated infantile GSDII patients. However, one of the two younger patients (2.5 months of age at start) became ventilator dependent at the age of 2 years and died suddenly at the age of 4 years and 3 months after a period of intractable fever, unstable blood pressure, and coma. The two older patients in this study (7 and 8 months of age at start) became ventilator dependent before or soon after the therapy began and stayed completely ventilator dependent.[19]

In 2001, another phase I/II, open-label, single-dose study enrolled three infants (aged 2.5–4 months) with a follow-up period of one year. 5 mg/kg rhGAA was infused i.v. twice weekly.[20] The rhGAA used in this study was purified from genetically engineered CHO cells. All patients survived beyond the critical age of one year. Improvements in pulmonary function were evident within the first two months of ERT. The youngest and least severely affected infant (normal baseline cardiac evaluation despite virtually absent GAA activity) did well on therapy, showed significant improvement in motor function, and began walking independently at 12 months of age. Two other patients had a steady decrease in heart size and maintained normal cardiac function for more than 1 year. Both had some improvement in muscle function, but subsequently deteriorated and became ventilator dependent after episodes of viral pneumonia. In both cases, the decline coincided with the rising titers of antibodies against rhGAA. Data for 16 to 18 months of treatment were reported, at which time all three were alive; however, as of July 2006, only the best responder was still alive.[21]

Two studies from Germany reported the results of ERT phase II clinical trials enrolling two infants (aged 3.1 and 5.9 months) receiving rhGAA from the milk of transgenic rabbits over a period of 48 weeks (40 mg/kg weekly i.v. infusions). There was an overall improvement in left ventricular mass, cardiac function, skeletal muscle function and skeletal muscle morphology. Both infants were not ventilator-dependent at the follow-up period of 10 months and reached a stable cardio-respiratory status throughout the course of the study. The level of GAA activity in muscle increased significantly in both, but glycogen reduction was observed in only one patient, who showed significant improvement of motor function over the course of a 10-month follow-up.[22,23] The current status of these two patients has not been released.

In 2006, a report of the first open-label, multinational, multicenter phase II study with Myozyme was published. This study examined the safety and efficacy of ERT in eight infants (aged 2.7 to 14.6 months, median 4.6 months at ERT start) during a follow-up of 52 weeks.[21] The patients received 10 mg/kg weekly i.v. for the 52-week initial stage, and the surviving patients continued on 10 to 20 mg/kg weekly or biweekly for up to 153 weeks (extension phase). As in all previous studies, the most dramatic effect of ERT was on cardiac muscle. All patients showed improved LVMI. Muscle biopsies were analyzed at baseline, 12, and 52 weeks on ERT by high-resolution light microscopy, digital histomorphometry, electron microscopy, capillary density, fiber type analysis, and confocal microscopy for satellite cell activation. The extent of glycogen clearance varied widely among patients and correlated well with clinical outcome.

Low glycogen levels, mild ultrastructural damage, a high proportion of type I fibers, and young age at baseline were predictors of good histologic response.[6] Six of eight patients were alive after 52 weeks of treatment and five were free of invasive ventilation support. These five patients showed improvement in motor function, and three of them were able to walk independently. Four patients died during the extension phase, bringing the total number of deaths in this study to six. The deaths were attributed to complications of the disease. Median age at death or treatment withdrawal for all patients was 21.7 months, significantly later than would be expected for untreated patients. The two surviving children showed significant reduction in skeletal muscle glycogen level on therapy and were over 3 years of age at the time the study was published.[21] The current status of these two patients has not been released.

Two company-sponsored, multicenter, multinational, open-label, dose-ranging studies of rhGAA safety and efficacy were initiated between 2003 and 2005. These studies had more strict inclusion criteria. The first trial enrolled 18 infants aged 6 months or younger (mean age of 5.3 months at start) with cardiomyopathy and GAA activity of less than 1% normal in fibroblasts. All patients were ventilator free at start of therapy. Patients received i.v. rhGAA at 20 mg/kg (n= 9) or 40 mg/kg (n= 9) every other week. The higher dosage resulted in a greater increase in GAA activity in skeletal muscle. However, this additional increase in GAA activity did not always correlate with glycogen clearance or with clinical outcome. Furthermore, the patients receiving the higher dose tended to have an increased number of infusion-associated reactions. Glycogen level in skeletal muscle, evaluated at week 52 in 17/18 patients, remained stable or decreased in 14 patients. Motor development improved in 13 of 18 patients, as measured by Alberta Infant Motor Scale (AIMS). After 52 weeks of therapy, all 18 patients were alive. Fifteen patients were free of invasive ventilation, 3 of whom required some form of noninvasive ventilation.[24] In the extension phase of the trial, at 121 weeks, 13 patients had survived, of whom 9 were free of invasive ventilator support [P. Kishnani, personal communication].

The second open-label, multicenter study enrolled 21 patients with cardiomyopathy, who were aged 6 months to 3.5 years at the start of therapy. This trial has ended now and the results will soon be published in a peer-reviewed journal. The information on the interim analysis can be found in the reports provided by the European Medicines Agency's website: http://www.emea.europa.eu/humandocs/PDFs/EPAR/myozyme/H-636-en6.pdf. This study showed the benefit of Myozyme in a group of infants with advanced disease, who were followed for an average of two years. After one year, 16 of 21 patients were alive, 10 of whom had attained new motor milestones. As in previous studies, cardiac response was impressive in most patients. Of note is the fact that no reversal of cardiomyopathy was seen in 4 severely affected patients. One of these 4 patients died after 1 infusion and the 3 others died prior to completing 24 weeks of therapy. At baseline, 16 patients were free of invasive ventilatory support, and 7 remained so at study end. At the end of the study, 6 deaths were reported [*Genetic in Medicine*, in press].

Studies in late-onset patients—Information on the efficacy of ERT in late-onset patients remains limited (Table 2). A 3-year follow-up study has been reported for 3 late-onset patients (aged 11, 16, and 32 years).[25] These patients started therapy with rhGAA from milk of transgenic rabbits, but were later transitioned to CHO-derived enzyme (Myozyme). Weekly infusions of 10 mg/kg resulted in only a slight increase in GAA activity in muscle; after 12 to 24 weeks of therapy the dosage was increased to 20 mg/kg weekly. However, even on a higher dosage, the level of GAA activity remained below the normal range and glycogen was only slightly decreased. At baseline all patients were wheelchair-bound and the 2 older patients required ventilator support. After 72 weeks of treatment all patients had stabilized pulmonary function and reported less fatigue. In parallel with these clinical accomplishments, a decrease of the creatine kinase, transaminases and LDH levels was recorded. The distal muscle groups responded better than the proximal muscles. The best clinical response was observed in the youngest patient, who was least affected at start of therapy. This patient performed the 10-meter walk test in 41 seconds at week 84, and in 3 seconds at week 108. The 2 other patients remained wheelchair bound, but they too showed a lower degree of disability and improved quality of life.[25] The stabilization of pulmonary and muscle function as well as the improvement in quality of life during the first 3 years of therapy were maintained throughout the 5 year extension period. The information regarding the extension period was presented by van Capelle et al. at the Fifth Symposium on Lysosomal Storage Disorders (Paris, France; April 10–12, 2008).

An observational, open-label, single center juvenile-onset follow-up study of 3 Pompe patients presenting without cardiomyopathy was reported in 2007. These three patients received the drug under three different protocols with dosages ranging from 10–40 mg/kg every other week. The least affected patient (3 years and 8 months at start) showed significant improvement of muscle function and no regression during 70 weeks of follow-up. The second patient (2 years and 8 months at start) initially showed improved muscle function, motor skills, and motor development, but reached a plateau at around week 114 despite an increase in the drug dose during 140 weeks of follow-up. The third patient (19 years and 9 months at start) had severely compromised skeletal muscle function at baseline and died suddenly after only 20 weeks of ERT.[26]

There are two large clinical trials with Myozyme. Eighteen late-onset patients are being treated in an ongoing open-label study under the expanded access protocol. The results of this trial have not yet been published in a peer-reviewed journal. The information on the interim analysis can be found in the reports provided by the European Medicines Agency's website: http://www.emea.europa.eu/humandocs/PDFs/EPAR/myozyme/H-636-en6.pdf.

Additionally, the first randomized, double-blind, placebo-controlled phase III study, which has enrolled 90 patients over 8 years of age in the United States and Europe, is now being conducted (Late onset treatment study/LOTS). The efficacy is determined by the six minute walk test and the pulmonary function as measured by percent predicted forced vital capacity. The results of this trial have not yet been published in a peer-reviewed journal. The information on the interim analysis can be found in the Genzyme press release at: http://www.amdapompe.org/LOTSpressrelease.pdf.

Since Myozyme received marketing approval, a number of GSDII patients are receiving rhGAA outside the context of company-sponsored clinical trials. The progress of some of these patients on ERT has been published, but much of the information remains unavailable.

Side effects of rhGAA treatment

Myozyme was generally well tolerated. Adverse events on ERT were mostly mild to moderate and were infusion-associated or occurred during the first 2 hours post-infusion. No ERT related death occurred. Immunological responses were seen in the majority of the patients, who developed anti-rhGAA IgG antibodies within the first 3 months of ERT. A summary of the adverse effects experienced by patients on ERT may be found in the EMEA Myozyme scientific report 2006 at http://www.emea.europa.eu/humandocs/PDFs/EPAR/myozyme/H-636-en6.pdf.

Immune response to ERT and CRIM status

Immune response to ERT is seen in the majority of GSD II patients. A subset of patients with no residual GAA protein (**c**ross-**r**eactive **i**mmunological **m**aterial-negative, CRIM-negative) develop a particularly high-titre of anti-hGAA antibodies on ERT. The developments of these antibodies was associated with a poor or short-lived response to ERT.[20,21,24] This phenomenon is paralleled in the Pompe mouse model; the formation of anti-hGAA antibodies in immune-competent GAA knockout mice made long-term ERT studies impossible.[27]

Although no correlation between the outcome of therapy and development of anti-hGAA antibodies was noted in the first Rotterdam study with rhGAA from transgenic rabbit milk, the best response was seen in a CRIM-positive patient.[19] In addition to the inactivation of the enzyme, therapy specific antibodies may interfere with the targeting of the enzyme or lead to adverse effects. Although the CRIM-negative patients seemed to be at a disadvantage, the full implications of the anti-hGAA antibodies are not known and remain to be investigated. In addition to the challenge posed by CRIM-negative status, a number of limitations to ERT have come to light.

Limitations of ERT

Pre-clinical and clinical studies demonstrated that ERT reverses cardiac pathology and significantly reduces mortality in infants, but the effects in skeletal muscle are less than anticipated. Skeletal muscle comprises approximately 40% of body mass, and as such presents a significant challenge for ERT. Other factors besides the sheer mass of muscle include the relatively inefficient system of delivery of rhGAA to lysosomes and the resistance of type IIB muscle fibers to therapy, which has been clearly demonstrated in an animal model. A single study of one infantile-onset Pompe patient has shown that type IIA myofibers do respond to therapy.[28] More studies in humans are needed to evaluate the response of different fiber types to ERT. For instance, it is not known whether the resistance of type IIB myofibers is a feature of human GSDII. The limited glycogen clearance in skeletal muscle may be true of other tissues, such as motor neurons. Persistent glycogen storage in motor neurons may account for the development of neurological symptoms, such as distal foot drop syndrome, in infants who survive longer because of ERT. In addition to the above considerations, the need for life-long infusions, the high cost of the recombinant enzyme, and extremely high doses of the drug (up to ~80-fold higher than those for other lysosomal storage disorders) have stimulated efforts to explore alternative approaches.

EXPERIMENTAL THERAPIES

Gene therapy

Gene therapy for Pompe disease has been explored by several groups. The feasibility of this approach was first shown in *in vitro* studies using retroviral and adenoviral vectors expressing human GAA. The human gene was highly expressed in cultured fibroblasts, myoblasts, and myotubes derived from patients with the disease. Furthermore, once the enzyme was produced, it was secreted into medium and taken up by the neighboring cells through MPR mediated endocytosis, resulting in phenotypic rescue of the non-transduced cells.[29–31]

For *in vivo* studies in GAA-KO mice two gene transfer systems have been used: vectors based on Adenoviruses (Ad) and Adeno-associated viruses (AAV). Ad-based vectors are one of the best characterized gene transfer systems, and they are widely used in basic biology studies. The appeal of the AAV-based therapy lies in the non-pathogenic nature of these viruses, and their ability to infect both dividing and non-dividing cells. A high degree of tropism to skeletal muscle and little immune response make AAV particularly suitable for therapy of muscle disorders. Furthermore, the transgene is integrated into the host genome providing a stable expression of the therapeutic genes.

Skeletal muscle, a major tissue affected by glycogen accumulation, seemed an obvious site for the vector transduction. However, studies with both Ad and AAV vectors quickly demonstrated the limitations of this approach. Intramuscular injection of an Ad vector encoding human GAA into adult KO mice was effective only at the injection site, but not in other distant muscle groups.[32] Similarly, intramuscular and intramyocardial delivery of a recombinant AAV vector containing mouse or human GAA cDNA did not result in phenotypic cross-correction in distant non-injected muscles.[33,34]

In a series of experiments using transgenic GAA-KO mice with tetracycline inducible expression of human GAA in skeletal muscle, it was demonstrated that the skeletal muscle-produced transgenic enzyme, which was turned “on” in adult mice, did not provide any appreciable metabolic cross-correction due to the negligible level of secretion.[35,36] The secretion of the enzyme leading to a systemic effect after unilateral injection in the gastrocnemius muscle of Ad vector encoding hGAA was observed only in GAA-KO neonates.[37]

Unlike skeletal muscle, liver was shown to be an excellent target tissue for the vector transduction. High levels of GAA expression in transduced hepatocytes, achieved by intravenous (iv) rather than intramuscular administration of the viral vectors, resulted in efficient production, secretion, and uptake of the enzyme by skeletal muscle. Reduction of the accumulated glycogen in both cardiac and skeletal muscle was observed within days after a single iv administration of Ad vector encoding hGAA into the GAA-KO mice.[38] This report was the first to demonstrate that liver can serve as a “factory” for the production and secretion of the GAA for metabolic cross-correction of skeletal muscle. Transgenic studies with inducible expression of human GAA in the liver of the GAA-KO mice confirmed that indeed liver is a far better site for the secretion of the enzyme compared to skeletal muscle.[35,36] Even in GAA-KO mice with long-established disease, significant glycogen reduction (84% in heart, 73% in diaphragm, and 46% in quads) was achieved after a single iv injection of Ad-GAA vector.[39]

However, the long-term efficacy of liver targeting of the Ad vector expressing hGAA was hampered by the onset of anti-hGAA antibodies within days of vector injection. The development of neutralizing anti-GAA antibodies correlated with the disappearance of secreted hGAA and gradual accumulation of glycogen in the months following vector administration.[40] Much improved efficacy was achieved in immune-deficient GAA-KO/SCID mice (severe combined immunodeficient GAA double knockout). The secreted hGAA persisted at high levels in plasma for months after i.v. vector injection resulting in improvement of muscle strength and function and reduction, although not complete elimination, of glycogen.[41]

The development of therapy-specific antibodies was also an issue when recombinant AAV vectors were used.[42–45] Humoral immune response to the vector-derived GAA in immune-competent mice prevented any restoration of GAA activity in the affected muscles despite extremely high superphysiologic levels of GAA expression in liver.[42] In contrast, immune-tolerant mice showed significantly increased GAA levels in the heart and skeletal muscles (neonatal administration of the recombinant human GAA was used to induce tolerance to the vector-derived hGAA). The increased levels of GAA activity correlated with reduced glycogen in the heart and diaphragm and improvement of the contractile function of the soleus muscle. Similarly, i.v. administration of AAV-GAA into GAA-KO/SCID mice resulted in high level of hGAA in plasma and correction of glycogen in heart and diaphragm in males, while females had correction only in the heart.[44]

In order to improve the efficacy of the viral vectors and to minimize the immune response, several approaches have been used: modification of the GAA cDNA sequence, different promoters, and different AAV serotypes. Traditional AAV vectors utilizing AAV2 terminal repeat sequences can be efficiently packaged as other serotypes (dozens of serotypes have been isolated).

A replacement of the signal peptide in the human GAA cDNA contained in an AAV vector by human alpha antitrypsin signal peptide resulted in a higher GAA secretion with lower number of viral particles.[46]

Use of a fully deleted adenovirus based vector in which hGAA expression was driven by a nonviral phosphoenolpyruvate carboxykinase (PEPCK)ApoE promoter/enhancer resulted in high hGAA expression level, glycogen reduction, and improved muscle strength in immune-tolerant mice.[47]

Intravenous administration of newer serotypes of AAV, such as AAV8 expressing hGAA under the control of liver-specific promoter (LSP) resulted in evasion of immune response to the produced GAA in immune-competent GAA-KO.[48] Furthermore, the LSP promoter was significantly more efficient compared to a hybrid universal promoter in driving the expression of the hGAA in liver, resulting in normalization of GAA activity and glycogen reduction in cardiac and skeletal muscle (83% decrease in gastrocnemius, 75% in quads). Improved abilities of infection and expression of hGAA from cardiac tissues in vivo was shown for AAV9 serotype.[49]

A single iv administration of a rAAV serotype 1 to neonates resulted in supraphysiologic level of GAA activity in the heart (>64 times normal) and greater than 20% in other tissues. Partial reduction of glycogen was observed in soleus muscle, which showed functional correction despite a relatively low (16% normal) level of enzyme activity.[50] Improved cardiac conductance and a reduction in left ventricular mass, as well as significantly improved diaphragm contractility and ventilatory function, was observed in these mice after 1 year post-treatment.[51]

Finally, AAV-based treatment at a very low number of vector particles was used in combination with ERT to induce tolerance to the recombinant hGAA. A subtherapeutic dose of the AAV vector containing hGAA and the liver-specific promoter injected before initiation of ERT prevented the development of the therapy-specific antibodies in the GAA-KO, resulting in an enhancement of ERT response.[52] Under these conditions, ERT increased GAA activity and reduced glycogen in the heart and to a lesser extent in diaphragm, while the quadriceps were not biochemically corrected. This immunomodulatory role of gene therapy may be particularly valuable for treatment of CRIM-negative infantile Pompe patients.

Both Ad and AAV-based gene therapy have advantages and disadvantages, however, this discussion goes beyond the limits of this review. The reader is referred to recent reviews on the topic.[53–55]

While a gene therapy clinical trial is not in the near future, the findings from these experiments have greatly contributed to our understanding of the complexities in treating skeletal muscle.

Remarkably, even the extremely high persistent levels of enzyme activity in plasma achieved with the most efficient systems of gene therapy (levels which are unrealistic to obtain with ERT) did not lead to a full reversal of pathology in skeletal muscle. Furthermore, the GAA activity in skeletal muscle in many of the gene therapy experiments was near or exceeded normal levels and still failed to rescue muscle pathology. This goes against the prevailing idea that a small increase in residual GAA activity is sufficient to reverse the glycogen accumulation. It is also important to emphasize that the degree of glycogen accumulation in skeletal muscle in severely affected patients is significantly higher than that found in GAA-KO mice.[56]

Enzyme enhancement therapy (EET, chemical chaperone therapy)

EET therapy is based on the ability of pharmacological chaperones/active site inhibitors to rescue mis-folded or unstable proteins from ER-associated degradation by increasing the amount of protein that passes the cell's quality control system. Various inhibitors and derivatives of deoxynojirimycin (DNJ) have been tested in other lysosomal storage diseases.[57] A number of missense mutations found in late-onset Pompe patients result in retention and premature degradation of the GAA precursor in the ER. These mutations may be amenable to chaperone-mediated therapy.

The effects of two imino-sugars, DNJ and its derivative, N-(n-butyl)deoxynojirimycin (NB-DNJ), were investigated in fibroblasts derived from Pompe patients. A significant increase of GAA activity and the amount of the mature form of GAA was observed in fibroblasts from patients carrying L552P and G549R mutations, but not in those carrying several other mutations (A445P; L355P; R375L).[58]

In another study, four mutations were chosen for analysis: Y455F/Y455F, P545L/P545L, 525del/R600C and D645E/R854X. Of these four genotypes two fibroblast cell lines (Y455F/Y455F and P545L/P545L) showed a significant increase of GAA activity when treated with DNJ. These two cell lines were also responsive to NB-DNJ, although the effect of NB-DNJ on Y455F/Y455F subsided quickly after removal of the compound. Brefeldin A, which inhibits protein transport from ER/Golgi to the lysosomes, blocked the corrective effect of NB-DNJ, indicating that this compound indeed facilitated the transport of the mutant enzyme species from ER to lysosomes.[59]

The molecular interaction between imino sugars and Myozyme has been recently investigated. In addition, three-dimensional structural models of the catalytic domain of the enzyme with the imino sugars bound to its active site were constructed. Consistent with biochemical data, DNJ seemed to fit into the active site better than other imino sugars [NM-DNJ, NB-DNJ and NE-DNJ] and had the strongest inhibitory effect on the enzyme.[60] The authors emphasize that the development of new derivatives that bind to mutant α-glucosidases and transport them to lysosomes but do not inhibit the enzyme are required.

Another inhibitor of GAA, D-Glucose, which is actually the product of lysosomal glycogen hydrolysis, has been shown to stabilize the enzyme activity and to increase the production of GAA protein in CHO-K1 expressing cells by preventing the GAA aggregation. Furthermore, D-Glucose increased the residual enzyme activity in fibroblast cell lines from late-onset GSDII patients. However, in these cases the genetic defect was not identified.[61]

Thus, EET therapy may be a promising approach, but its efficacy depends on some residual enzyme activity and may be limited to certain mutations in the GAA gene.

Enhanced delivery of the therapeutic enzyme

Carbohydrate analysis of the rhGAA indicated that the currently available preparations contain a relatively low number of M6P residues, an important recognition marker for the CI-MPR. In an attempt to improve the delivery of the therapeutic enzyme and to facilitate a reduction in the dosage of the drug, a second generation of the rhGAA (neo-rhGAA) with a higher affinity for the CI-MPR was made.[62] This process involves a chemical conjugation to rhGAA of an oligosaccharide ligand bearing M6P residues in the optimal configuration. The resulting modified enzyme had significantly increased affinity for CI-MPR, and it internalized much more efficiently in myoblasts derived from GAA-KO mice. Furthermore, these studies showed greater clearance of glycogen from all affected muscles compared to the currently available drug. It is important to note that a comparable reduction in glycogen levels was realized using an approximately 8-fold lower dose of the neo-rhGAA in the heart and diaphragm and 4-fold lower dose in skeletal muscle.

Similar thinking motivated experiments using hyaluronidase (hyase) in GAA-KO mice to facilitate the delivery of rhGAA to skeletal muscle. Hyaluronidase is known to increase tissue permeability and is currently in clinical use for other disorders. Intraperitoneal injection of hyase prior to ERT increased GAA activity in heart, diaphragm, kidney, and quads.[63]

Nutrition and exercise therapy (NET) in late onset patients

NET is a combination of a high-protein, low-carbohydrate diet and daily conditioning aerobic exercise. This therapy is aimed at minimizing glycogen accumulation, increasing muscle protein synthesis, and increasing the ratio of type I to type II muscle fibers. Late onset patients who complied fully with NET showed much improved prognosis and a significantly slower rate of muscle deterioration.[64–66]

CONCLUSION

ERT has been a major advance in the treatment of Pompe disease. The therapy results in a remarkable reversal of pathology in cardiac muscle in infantile patients, who otherwise would die from cardiac failure. Thus, ERT has changed the natural course of the disease. The greatest success with ERT has been seen when therapy is started early, before irreversible changes occur. However, the success seen in cardiac muscle unfortunately does not fully extend to skeletal muscle, which remains a significant challenge. One reason for the limited success with ERT may be our incomplete understanding of the pathology and the pathogenesis of Pompe disease in skeletal muscle. Recent studies on the potential role of autophagy in GSDII suggest that pathology extends beyond the lysosome. The development of improved therapies would require not only the expansion of our understanding of the basic pathological mechanism but also better exchange of information and cooperation between industry, physicians, and scientists.

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Table 1

Clinical trials of enzyme replacement therapy in infantile-onset Pompe disease

Authors	Year	Design	Treated patients	Study duration	Outcome measures	Primary endpoint	Result
Van den Hout et al. 17–19	2000–4	phase I/II	4	>4y	CF/PF/MF/NF rep. MB	safety/efficacy	3/4 survived >5.5y improved CF/PF/MF/NF
Amalfitano et al.[20]	2001	phase I/II	3	1y	CF/PFMF/NF rep. MB	survival	3/3 survived >1y normal CF and MF
Klinge et al.[22,23]	2005	phase II	2	1y	CF/PF/MF/NF rep. MB	safety/efficacy	survived >21m improved CF/PF/MF/NF
Kishnani et al.[21]	2006	phase II	8	52w	CF/PF/MF/NF	safety/efficacy	4/8 survived improved CF/PF/MF
Kishnani et al.[24]	2007	phase II/III	18	52w	CF/PF/MF/NF	survival/safety/efficacy	18/18 survived improved CF/MF
In press	2008	phase II/III	21	>2y			

CF = cardiac function, FVC= forced vital capacity, GMFM= gross motor function measure, HHD handheld dynamometer, M= months, MB = muscle biopsy, MF = motor function, MRC= medical research council score, NF = neurological function, PF = pulmonary function, rep.= repeated, VT= ventilator time, w= weeks, y= years.

Table 2

Clinical trials of enzyme replacement therapy in late-onset Pompe disease

Authors	Year	Design	Treated patients	Study duration	Outcome measures	Primary endpoint	Result
Winkel LP et al.[25]	2004	phase II	3	3y	MB, PF, HHD, MRC GMFM,	safety/efficacy	3/3 survived improved PF, HHD, MRC
Rossi et al.[26]	2007	phase II	3	20–140w	CF, PF, MF	survival/safety	2/3 survived > 70–140w 2/3 improved CF, PF, MF
Expanded access	ongoing	phase III	18	2–8y	PF, MF	ventilator time, MF	18/18 survived 8/17 reduction of VT 14/18 improvement in MF
LOTS	finished 2007	phase IIIb	90	18m	PF, MF	safety, 6-min. walk, FVC	90/90 survived improved 6-min. walk test improved FVC

CF = cardiac function, FVC= forced vital capacity, GMFM= gross motor function measure, HHD handheld dynamometer, M= months, MB = muscle biopsy, MF = motor function, MRC= medical research council score, NF = neurological function, PF = pulmonary function, rep.= repeated, VT= ventilator time, w= weeks, y= years.