Acid Alpha-Glucosidase Deficiency (Pompe Disease)

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Current Neurology and Neuroscience Reports 2007, **7:**71–77
Current Medicine Group LLC ISSN 1528-4042

The development and recent approval of recombinant acid alpha-glucosidase for enzyme replacement therapy have been major milestones in Pompe disease research. Acid alpha-glucosidase is the enzyme responsible for degradation of glycogen polymers to glucose in the acidic milieu of the lysosomes. Cardiac and skeletal muscles are the two major tissues affected by the accumulation of glycogen within the lysosomes. Both cardiomyopathy and skeletal muscle myopathy are observed in patients with complete enzyme deficiency; this form of the disease is fatal within the first year of life. Skeletal muscle myopathy eventually leading to respiratory insufficiency is the predominant manifestation of partial enzyme deficiency. The recombinant enzyme alglucosidase alfa is the first drug ever approved for this devastating disorder. This review discusses the benefits and the shortcomings of the new therapy.

Introduction

Pompe disease owes its name to the Dutch pathologist J.C. Pompe [1], who first described the disease in 1932. This first report on the nosologic entity was a postmortem description of pathology in an infant who died from what was thought to be pneumonia. Two critical observations were made: 1) the disease is a vacuolar myopathy, and 2) there is a massive accumulation of glycogen, primarily affecting cardiac and skeletal muscle. The vacuoles were later identified as lysosomes, and the cause of the glycogen accumulation was found to be a defect in lysosomal acid alpha-glucosidase (GAA) [2]. These early studies established the primary underlying pathology in muscle of Pompe patients, which is accumulation of glycogen in enlarged lysosomes. Recent efforts have brought about the development and production of the recombinant human GAA (rhGAA) for enzyme replacement therapy (ERT).

Pompe disease is one of several lysosomal storage disorders (LSD) being treated by ERT [3•], but it is the only one in this class in which skeletal muscle is the primary target. The success of ERT in other diseases (eg, the non-neuropathic form of Gaucher disease) turned out to be hard to replicate in Pompe disease; the reversal of pathology in skeletal muscle is more difficult than anticipated. The emerging data from the first clinical trials with rhGAA suggested that the most critical factor defining the outcome of therapy is the underlying skeletal muscle pathology at the start of therapy. The view of the pathology as the accumulation of glycogen within swollen lysosomes and probable lysosomal rupture has not changed since the earliest studies, and the literature has been remarkably silent regarding the secondary events that may occur in skeletal muscle as a result of substrate accumulation in the lysosomes. In this review, we focus on the results of the clinical trials, the latest methods for early diagnosis, and the studies in knockout (KO) mouse models that shed new light on the pathogenesis of the disease and the mechanisms of muscle damage.

Clinical Manifestations

Clinical manifestations of the disease depend largely on the level of residual enzyme activity, which, in turn, depends on the nature of the genetic defect. Over 200 mutations scattered across the gene have been found in Pompe patients. The mutations result in various outcomes ranging from a complete lack of the enzyme to a near-normal amount of enzyme with reduced activity. Because the enzyme undergoes complex post-translational modifications while trafficking to the lysosomes, multiple abnormalities along this pathway add to the variability of the level of residual enzyme activity.

The age of onset, organ involvement, and rate of disease progression are heterogeneous [4]. Traditional classification of infantile, juvenile, and adult forms has given way to the view that there is a continuum of disease severity. Recently, a group of experts agreed to classify

the disease into two broad categories: infantile and late-onset forms [5].

The infantile form includes classic and nonclassic variants. Classic infantile disease is a rapidly progressive disorder manifesting as cardiomegaly, hypotonia, or mild hepatomegaly, resulting in death within the first year due to cardiorespiratory failure. In the nonclassic infantile form, the symptoms are still evident in the first year of life but the disease is characterized by a slower progression and less severe cardiomyopathy [6]. A complete or near-complete GAA deficiency is typical for the infantile form [7].

The late-onset form includes childhood, juvenile, and adult-onset variants. Both childhood and juvenile disease present any time after infancy with mild or no cardiac involvement. The adult-onset form usually presents between the second and sixth decade of life and manifests as a slowly progressive skeletal muscle myopathy. The predominant manifestations of the late-onset form include proximal muscle weakness with respiratory muscle involvement. Respiratory failure is the cause of significant morbidity and mortality in this form. Some residual enzyme activity can be found in late-onset patients.

Natural History

Pompe disease is a rare disorder with a total combined incidence estimated at one in 40,000 [8]. The development of ERT underscores the value of studies on the natural history of the disease. First, these data provide a critical reference for the efficacy of ERT. Second, the ability to conduct prospective studies will dwindle as more patients get access to therapy.

A study of the classic infantile form with 20 original cases and 133 cases from the literature showed that the median age of death was 7.7 months for the original group and 6 months for the retrospective study. Motor development in the original group was severely impaired; milestones, such as the ability to turn, sit, or stand, were not achieved or were quickly lost after acquisition. The majority of patients died before 1 year of age (only 5% of the original cases and 8% of all reported cases survived) [9].

When nonclassic infantile patients were included in a retrospective study of 168 patients, slightly different data were obtained [10]. Median age of death was 8.7 months, and the survival rate was 25.7% at 12 months and 12.3% at 18 months. The frequencies of presenting symptoms and signs were similar in both studies. Symptoms included cardiomegaly, cardiomyopathy, congestive heart failure, hypotonia, muscle weakness, respiratory distress, feeding difficulties, failure to thrive, gastroesophageal reflux, and sleep apnea.

Studies of the natural course of late-onset disease demonstrated a significant variability in terms of age of onset, rate of disease progression, development of respiratory problems, wheelchair dependence, and use of respiratory support [11,12]. In most patients, initial symptoms were related to mobility and limb-girdle weakness. Respiratory insufficiency was observed in patients of any age, even in those without limb-girdle weakness [11].

ERT

The concept of ERT for Pompe disease and other LSDs is based on experimental evidence indicating that lysosomal enzymes can be taken up by the cells through receptor-mediated endocytosis. In Pompe disease, the recombinant enzyme is a precursor containing mannose-6-phosphate groups that enable the enzyme to bind the receptor on the cell surface. Cation-independent mannose 6-phosphate receptor (MPR) is responsible for binding and directing the lysosomal enzymes into the endocytic pathway [13]. The receptor-enzyme complexes enter the cells in transport vesicles that fuse with endosomes. The acidic pH of late endosomes induces the dissociation of the complexes; the receptor is recycled back, whereas the enzyme is transported to the lysosomes. Numerous proteins participate in the sorting and trafficking of the lysosomal enzymes [14]. Like the endogenous GAA precursor, the rhGAA is expected to undergo proteolytical cleavage along the transport route to yield intermediate forms followed by conversion to the fully active lysosomal species [15,16].

Clinical Trials

Currently, more than 280 patients in 30 countries are receiving alglucosidase alfa (Myozyme; Genzyme Corporation, Framingham, MA), which is a Chinese hamster ovary (CHO)-derived rhGAA. However, the published data include only the results of the first clinical trials for a small group of infantile and late-onset patients.

Nine patients (six infantile and three late-onset patients) began therapy with a product purified from the milk of transgenic rabbits (trGAA), which was the first clinically applicable rhGAA. Eventually, the production of the milk product was discontinued (the method was not sustainable) and all surviving patients were transitioned to CHO-derived rhGAA.

Of the six severely affected infants who started the therapy on trGAA, four were followed for 3 years. They began therapy at a 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 a lower dosage. Increase in the dosage resulted in the normalization of the level of GAA activity [17,18]. However, glycogen in muscle decreased in only one patient (who was 3 months of age at start) who made remarkable progress and reached milestones never observed in untreated patients. His motor score normalized at the age of 2 years and he remained ventilator-free after 3 years of follow-up [18]. However,

this patient may have regressed because a later observation (Raben, Personal observation) clearly indicates that this child still has a myopathy. 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 one patient (2.5 months of age at start) became ventilator dependent at the age of 2 years and died at the age of 4 years, 3 months after a short period of fever, unstable blood pressure, and coma.

Based on the results of this first study, two other patients (aged 3.1 and 5.9 months at start) began therapy with the trGAA at a dosage of 40 mg/kg weekly. As in the first 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 [19]. In both studies [18,19] anti-GAA antibody did not seem to correlate with clinical decline or lesser effect of therapy.

Data for a 3-year follow-up have been reported for three late-onset patients (aged 11, 16, and 32 years) who started therapy with trGAA [20•]. 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 the start of therapy all the patients were wheelchair bound and two older patients were ventilator dependent. The best clinical response was observed in the youngest patient who was least affected at start of therapy. This patient gained normal muscle strength and function. Two other patients remained wheelchair bound, but they too showed a lower degree of disability and improved quality of life [20•].

Two studies with CHO-derived rhGAA have been reported so far. First, an open-label phase I/II study (5 mg/kg twice weekly) was conducted in three patients (2.5, 3, and 4 months of age at start) with infantile Pompe disease [21]. Prior to ERT, two patients had severe cardiomyopathy typical for classic infantile form. The third patient, who was the youngest at the enrollment and least severely affected, fit the criteria of the nonclassic infantile form because he had less severe cardiomyopathy and normal baseline cardiac evaluation despite virtually absent GAA activity. This patient did well on therapy, showed significant improvement in motor function, and began walking independently at 12 months of age. Two other patients showed steady decrease in heart size and maintained normal cardiac function for more than 1 year. Both patients had some improvement in muscle function, but both 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 [22••].

The second open-label phase II trial with CHO-derived rhGAA was conducted in eight severely affected infantile patients (age 2.7 months to 14.6 months) [22••]. All patients fit the criteria of classic infantile form. They were on 10 mg/kg weekly 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. GAA activity in skeletal muscle increased in all patients. As in all previous studies, the most dramatic effect of ERT was on cardiac muscle. The effect on skeletal muscle, however, was extremely variable. Two patients died during the initial stage, and four additional patients died during the extension phase, bringing the total number of deaths 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.

The safety and efficacy of alglucosidase alfa were assessed in two additional open-label trials. The results of these trials have not yet been published in a peer-reviewed journal; therefore, we will not discuss the details of these studies but present a brief outline and the mortality rates. One study (conducted between 2003 and 2005) involved 18 patients aged 6 months or younger with cardiac hypertrophy who were ventilator free at start of therapy. Within the first 12 months of treatment, there were no deaths. As of July 2006, four patients had died. The second is an ongoing study of 21 patients aged 6 months to 3.5 years at the start of therapy. Five deaths were reported by the end of the 104-week study; one additional death has occurred during the extension phase (Kishnani, Personal communication).

Clinical trials have demonstrated that the strongest and most consistent effect of ERT was on cardiac pathology: all patients showed remarkable decreases in the left ventricular mass index, left ventricular posterior wall thickness, and improvements in cardiac function regardless of disease severity. The reduction in cardiac size has been observed in patients who do not show a decrease in the amount of accumulated skeletal muscle glycogen or improvement in muscle function. Abnormal ECG parameters, which reflect conduction abnormalities and hypertrophic cardiomyopathy, such as shortened PR interval, increased QT dispersion, and large left ventricular voltages, have also been significantly improved on ERT [23]. However, recent data suggest that Pompe patients may be at risk for arrhythmias despite the significant improvement in cardiomyopathy and conduction on ERT [24].

Thus, it is very clear that it is the dramatic effect on cardiac function that results in longer survival of all

patients when compared with the age at death in untreated Pompe patients. However, mortality from disease complications was very high, and only a small subset of patients achieved significant gains in physical performance. The reasons for the variability in skeletal muscle response to therapy remain unclear.

So far, only one study has attempted to correlate the morphology of skeletal muscle and the outcome of therapy. Winkel et al. [25] have examined the morphologic changes in muscle tissues in the first four infantile patients who received the trGAA. The authors came to the conclusion that at each time point muscle pathology correlated with the severity of clinical symptoms, and that the degree of the impairment of muscle function plays the decisive role in the outcome. In the best responder, skeletal muscle striation and architecture were relatively well preserved at start of therapy, and this patient had the least glycogen accumulation [25]. Thus, the important lesson here is that the effect on motor performance is highly dependent on the condition of the patient at start of treatment. Although this study is informative, more details are needed regarding the skeletal muscle pathology and how it correlates with response to therapy. Since this review was submitted, a second study has been published analyzing the morphology of skeletal muscle before and after ERT [26].

Pathogenesis

A lysosomal rupture hypothesis has long been proposed to account for skeletal muscle destruction in Pompe disease [27,28]. The hypothesis, based on electron microscopy observation, suggests that muscle contraction causes enlarged glycogen-filled lysosomes to rupture, resulting in the release of free glycogen and potentially toxic components into the cytosol. Skeletal muscle differs from other cells in that the expanded lysosomes are located in a limited inter-myofibrillar space, thus creating a condition for the physical rupture of the lysosomal membrane. Ruptured lysosomes eventually lead to loss of myofibrillar material and complete fiber destruction [27,28]. If this hypothesis is true, the fibers at the later stages of disease progression would be beyond repair with the current therapeutic approach.

Another pathologic finding in skeletal muscle in Pompe disease has been the presence of areas with large numbers of autophagic vacuoles (AV). This observation was made by Engel [29] as early as 1970; glycogen accumulation was found not only in the lysosomes, but also in AVs with cytoplasmic degradation products. Excessive accumulation of AVs in skeletal muscle was later confirmed by electron microscopy of muscle biopsies from a number of Pompe patients [30–32]. Surprisingly, however, the extent of autophagy, its role in the pathogenesis of disease, and its implications for therapy have been largely ignored.

Autophagy is a highly conserved process of degradation of most long-lived proteins and damaged organelles [33••]. The process starts with the formation of double-membrane vesicles, called autophagosomes, which sequester various constituents of cytoplasm, including glycogen. Autophagosomes fuse with and discharge their content into late endosomes and lysosomes; the autophagosomal membrane and cytoplasmic components are degraded within the lysosomes.

Apart from the increase in the number of AVs, other vesicular compartments linked to lysosomes were found to be affected in Pompe skeletal muscle [34]. Analysis of muscle biopsies from several patients with late-onset disease indicated proliferation of multiple vesicles of the endocytic pathway, such as early and recycling endosomes. Thus, these clinical studies suggest that the abnormalities in Pompe disease go beyond the expansion of the lysosomes. Studies in animal models give insight to the role of these secondary events in the progressive nature of the disease and their effect on the outcome of therapy.

Studies in Knockout Mouse Models

Preclinical studies with ERT in a mouse model of the disease were very consistent with the results of the clinical trials. rhGAA reduced cardiac glycogen to undetectable levels but the reduction of glycogen in skeletal muscle was modest, and some fibers, in particular glycolytic fast twitch type II fibers, showed little or no glycogen clearance [35,36]. The rhGAA was taken up more efficiently by cardiac muscle than by skeletal muscle, but most of the administered enzyme was targeted to the liver.

Analysis of vesicular compartments in myoblasts isolated from the KO mice demonstrated that the cellular pathology in Pompe disease spreads to affect both the endocytic and autophagic pathways as shown by staining with specific markers. Expansion of endocytic and autophagic vesicles profoundly affected the mobility and fusion of these vesicles in Pompe myoblasts [37••,38]. The autophagic vacuoles were easily identified in isolated single myofibers of the KO mice by staining with a specific autophagosomal marker. Furthermore, the entire core of the fibers was filled with multiple vesicular structures of the autophagic and endocytic pathways, and often contained autofluorescent material. This autophagic buildup was limited to therapy-resistant type II fibers [36,37••] and was found in virtually every type II KO fiber, even in young animals. The extent of autophagic buildup was enormous; in some fibers, the volume occupied by the autophagic area reached approximately 40% of the total volume of a particular segment [38]. The large autophagic mass interrupted the contractile proteins in myofibers. Thus, it appears that it is not the primary defect (ie, intralysosomal glycogen accumulation) that is responsible for muscle damage and wasting, but rather the secondary accumulation of what is sometimes referred to as “biologic garbage” [39].

Similar morphologic changes in skeletal muscle fibers were observed in another mouse model of Pompe disease. Hesselink et al. [40,41•] found enhanced deposition of lipofuscin and large areas of centrally localized debris in muscle of the KO mouse. Additionally, our own studies with labeled rhGAA have demonstrated that the bulk of the endocytosed therapeutic enzyme ends up in the autophagic areas within the fibers [42]. Thus, excessive autophagy sets up the conditions for the disruptive buildup and diversion of the therapeutic enzyme away from the lysosomes.

The cause of the excessive autophagy in Pompe muscle is still unclear. Nutritional deprivation is a well-known trigger of autophagy. A hypothetical cascade may involve local glucose starvation (a result of the failure of lysosomal glycogen break-down to glucose) followed by induction of autophagy, which then leads to buildup because of the inability of the lysosomes to fuse with or digest the contents of the autophagosomes.

Excessive and early accumulation of lipofuscin, which normally accumulates in aging post-mitotic cells [43], points to another potential trigger of autophagy: oxidative stress. Oxidative damage in the KO fibers may affect the permeability of the lysosomal membrane, resulting in the leakage of lysosomal components even before the lysosomes become enlarged.

The Diagnosis of Pompe Disease: Latest Advancements

Currently, GAA assay in skin fibroblasts or muscle biopsy remain the standard and most reliable diagnostic methods. GAA has an optimum activity at pH of 3.75 to 4.5, and the enzyme activity is measured using natural substrate, glycogen, or the fluorescent synthetic substrate 4-methylumbelliferyl-alpha-D-glucosidase (4-MU). However, culturing fibroblasts takes weeks and significantly delays the diagnosis. Muscle biopsy is a direct and rapid way of measuring GAA activity and provides a valuable histologic material. However, the risk of anesthesia should be considered in infantile patients. In late-onset cases, the site of muscle biopsy may greatly affect the results of histologic analysis, and some samples may show little or no glycogen accumulation. Mutation detection is still not a trivial procedure, and as such is not suitable for routine diagnostic purposes.

None of these techniques can be used for the mass screening of newborns, which is the most promising strategy for diagnosis of asymptomatic individuals and early therapeutic intervention. Early diagnosis in clinically affected individuals is equally important, because the degree of skeletal muscle involvement at the start of ERT has a major impact on the outcome of therapy. An ideal source of the enzyme for diagnostic purposes would be leukocytes. However, in addition to GAA, leukocytes contain several homologous isoenzymes encoded by different genes: glucosidase II and neutral alpha-glucosidase (with pH optimum of 7.5), and maltase-glucoamylase (with pH optimum of 5 to 5.5). Maltase-glucoamylase poses the greatest problem because it contributes significantly to the total GAA activity measured at acidic pH. Maltase-glucoamylase is expressed in neutrophils but not in lymphocytes [44]. Thus, purified lymphocytes are a better diagnostic material, but potential contamination with neutrophils may result in false-negative results.

Recently, a major effort has been directed toward the development of methods for measuring GAA activity using dry blood spots (DBS), with the idea that these methods can be utilized for newborn screening. Blood spots can be collected by the standard heel-stick procedure or the dropwise addition of whole blood onto filter paper or Guthrie cards, which can be stored for a long period of time and easily transported. Two approaches show promise for the use of DBS for newborn screening.

The first approach is immuno-quantification of GAA protein in DBS [45]. The assay takes advantage of the fact that in Pompe disease, like in most LSDs, the amount of the mutant enzyme is reduced; the exception is a subset of patients who produce a significant amount of protein with reduced activity [46]. A two-tiered screening strategy involving an initial protein determination followed by an immuno-capture enzyme activity assay would address the issue [47].

Another strategy is competitive inhibition of maltase-glucoamylase activity using maltose [48] or acarbose [49]. Maltose has been shown to have much higher affinity to maltase-glucoamylase than to GAA [50]. Direct comparison of the two inhibitors (maltose and acarbose) in DBS extracts using 4-MU showed that acarbose was superior to maltose because there was no overlap in the level of GAA activity between heterozygous and infantile Pompe patients [51]. Acarbose has been successfully used for GAA activity measurement in mixed leukocytes [52] and in lymphocytes [53]. In mixed leukocytes [52], acarbose completely inhibited the maltase-glucoamylase at pH of 4.0 but inhibited the GAA by less than 5%, and there was a clear separation between the Pompe patients and the control group when glycogen was used as a substrate. The two groups were less well separated when 4-MU was used, and the separation was significantly improved by taking the ratio of inhibited over uninhibited activity. Using 4-MU over glycogen is justified to exclude GAA pseudo-deficiency caused by a polymorphism that lowers the activity for glycogen but not for 4-MU [54].

Considering that Pompe disease is a rare disorder, a practical approach would be a multiplex assay for newborn screening of multiple LSDs, especially those for which ERT is available or in development. A multiplex immuno-quantification assay was used in a retrospective study to simultaneously quantify individual proteins for 11 LSDs using DBS [55]. For Pompe disease, the sensitivity was 90% and the specificity was 99%. As

mentioned previously, a subset of Pompe patients with near-normal level of GAA protein would be missed by this method. These patients would, however, be accurately diagnosed by activity assays; multiplex activity assay from a single blood spot has been reported for five LSDs (Fabry, Gaucher, Krabbe, Nieman-Pick A/B, and Pompe disease) [49].

As expected, each of the methods has its shortcomings. The immuno-capture assay is more expensive than the others and the GAA antibodies are not commercially available. The use of inhibitors is very attractive because they are inexpensive and readily available, but for each condition the optimum dosage of inhibitor should be determined so that the right balance between nonspecific and specific inhibition is achieved.

One of the major limitations with screening methods is that they are unable to clearly discriminate between the infantile and late-onset forms [51,52]. Therefore, additional methods (eg, mutation analysis) are necessary for identification of late-onset patients. However, even if the diagnosis is unequivocally late onset, there is no consensus regarding the timing of ERT initiation.

Conclusions

The first clinical trials with rhGAA clearly demonstrated the benefits of ERT in Pompe patients. In all infantile patients studied, the therapy had a dramatic effect on cardiac function, prevented or delayed invasive ventilation, and extended survival. However, the therapy is not yet a cure because only some patients showed significant improvement in motor function. ERT addresses the problem of accumulation of glycogen in lysosomes and it appears to address the problem successfully. Yet, it is increasingly evident that other processes beyond the expansion of the lysosomes (eg, autophagy) affect the outcome of therapy. Better control over secondary events is critical for the success of therapy. Considering the limitations of the current therapy, other therapeutic options, such as enzyme enhancement therapy or gene therapy, should be explored. Regardless of the approach, efforts should be directed toward early diagnosis and intervention before irreversible muscle damage occurs.

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