

Alglucosidase alfa: Long term use in the treatment of patients with Pompe disease

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Abstract: Pompe disease is a lysosomal storage disorder characterized by muscle weakness and cardiomyopathy. It shows a broad variability regarding the clinical severity as well as the age of onset. In the past, two different recombinant enzyme preparations have been developed for the treatment of Pompe patients: α -glucosidase, produced in rabbit milk, and α -glucosidase, produced in Chinese hamster ovary (CHO) cell lines. The CHO enzyme received marketing approval in 2006 after it was proven to be effective in ameliorating muscle strength and improving heart function. The other has not been approved. The clinical efficacy of this enzyme preparation could be confirmed by several clinical trials in patients with different age of onset and disease severity. Enzyme replacement therapy, however, has its limitations due to unsatisfactory access of recombinant α -glucosidase to the muscle cells and due to the formation of antibodies. To overcome these therapeutic restraints, the development of a more effective enzyme preparation may become necessary.

Keywords: alglucosidase alfa, alpha glucosidase, Pompe disease, enzyme replacement therapy, glycogen storage disorder type II, acid maltase deficiency

Introduction

Pompe disease, also known as glycogenosis type II or acid maltase deficiency, is a lysosomal storage disorder that is caused by the genetic defect of the enzyme α -glucosidase. Deficient activity of this enzyme results in glycogen accumulation in several tissues leading to progressive cardiomyopathy, skeletal muscle weakness, and respiratory insufficiency.¹ Umapathysivam and colleagues demonstrated that α -glucosidase activity and glycogen concentration in skin fibroblasts is clearly correlated with age of onset.² A profound deficiency or even absence of α -glucosidase activity leads to a rapid accumulation in skeletal and heart muscle, resulting in the infantile (early-onset) form of Pompe disease that is characterized by severe muscle weakness, hypertrophic cardiomyopathy, and early death. Patients with a higher residual enzyme activity experience a slower progression of the disease, leading to the juvenile type where muscular weakness and respiratory insufficiency are the leading symptoms. The occurrence of hearing loss has been observed in young children with Pompe disease.³ In the adult form, the first symptoms such as slow, progressive myopathy, mainly in the muscles of the hip, thigh, and spine, and respiratory insufficiency begin at the third decade. The development of spine deformities is very common. In later life, many patients become wheelchair-bound and require artificial ventilation.⁴ In the late-onset form heart involvement is not as frequent as in early-onset phenotypes. In adults, accumulation of glycogen in vascular smooth-muscle may result in intracranial

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aneurysm or arteriopathy, leading to multiple intracerebral embolisms and hemorrhage.⁵

Pompe disease is inherited in an autosomal-recessive manner. The gene encoding acid α -glucosidase is localized on chromosome 17q25 and contains 19 exons. In 2008, 289 sequence variations, 67 nonpathogenetic mutations and 197 pathogenetic mutations have been listed in the Pompe disease mutation database.⁶ The most common mutations in the Dutch population are IVS1 (-13T > G), c.del525, and del exon 18.⁷ The incidence of Pompe disease varies in different ethnic groups between 1:40,000 and 1:50,000.⁷

In the past, no specific therapy was available for patients affected by Pompe disease, and management consisted solely of treatment of complications and supportive care such as nutrition and exercise therapy.⁸

Development of enzyme preparations

Since enzyme replacement therapy has been successfully introduced for patients with Gaucher disease, this principle of treatment has been developed for other lysosomal storage disorders such as mucopolysaccharidose types I, II, and VI and Fabry disease. Decades ago it was recognized that various cell types need specific receptors for uptake of exogenous lysosomal enzymes.⁹ The hepatocyte membrane contains galactose receptors, macrophages require mannose residues for uptake, whereas most cells bind exogenous enzymes via the mannose-6-phosphate (M6P) receptor. This fact explains why the initial trials of enzyme replacement therapy in an α -glucosidase deficient mouse (mouse model for Pompe disease) failed. In these early experiments, a nonphosphorylated human placenta α -glucosidase was used and was shown not to be taken up by the heart and skeletal muscle. Later studies have demonstrated that an enzyme preparation isolated from bovine testes that contained M6P was much more efficient in correcting the biochemical defect in affected tissues.¹⁰

Two different forms of human α -glucosidase that contain M6P have been developed by recombinant techniques: Enzyme produced either in Chinese hamster ovary (CHO) cell lines or in milk of transgenic rabbits. The biochemical and pharmacological properties of both enzyme preparations have been analyzed by McVie-Wylie and colleagues.¹¹ They found that in both enzyme preparations the predominant species was the precursor (110 kDa) form of the molecule that contains M6P in a molar ratio of about 1.2–1.3, whereas human placenta contains mainly the processed 76 kDa form that lacks this essential targeting signal and therefore is not effectively taken up by human fibroblasts. Analysis of

receptor binding and cell uptake, performed in fibroblasts from patients affected by Pompe disease, demonstrated that the uptake of α -glucosidase produced in CHO cells was higher than that of the rabbit milk enzyme. Clearance of muscle glycogen was measured in α -glucosidase knockout mice administered either CHO or rabbit milk α -glucosidase. Both enzyme preparations resulted in a reduction of tissue glycogen content in a dose-dependent manner, although higher levels of enzyme activity were detected in the muscle of mice who received the rabbit milk enzyme when compared to those treated with the CHO enzyme. This discrepancy can be explained by unproductive uptake of enzyme by resident endothelial and fibroblasts rather than by glycogen-containing myocytes. Both the milk enzyme and the CHO cell-derived enzyme were used in clinical trials. Taking into account the limited life expectancy of the disease following diagnosis, a placebo control was included in none of these studies.

Rabbit milk α -glucosidase

The first clinical trial with recombinant human α -glucosidase from transgenic rabbit milk started in 1999.¹² Four patients with the infantile form of Pompe disease received the enzyme once a week at dosages increasing from 15 mg/kg to 40 mg/kg. After 36 weeks of treatment, left ventricular mass index decreased significantly, skeletal muscle morphology and motor function improved. In another trial, four Pompe patients (2.5–8 months of age) were enrolled in a single-center open-label study and treated for at least three years with recombinant α -glucosidase that was purified from the milk of transgenic rabbits. At the beginning of the study a dose of 15 to 20 mg/kg/week was used, and at week 12 the dose was increased to 40 mg/kg/week. The treatment resulted in a dramatic decrease in heart size and improvement of heart function. Signs of regeneration were found in muscle biopsies. All patients survived beyond the age of four years, but one died at the age of four years and three months after a short period of intractable fever. Two infants became ventilator-dependent.¹³

The efficacy of α -glucosidase produced in the milk of transgenic rabbits was also studied in three patients aged 11, 16, and 32 years with late-onset Pompe's disease.¹⁴ In all patients, weekly infusions of this enzyme led to stabilization of lung function and even improvement of skeletal muscle strength in one child.

CHO α -glucosidase Clinical trials

Three infants were included in the first human study with recombinant enzyme from CHO cell lines.¹⁵ Based on the

fact that virtually all patients with Pompe disease die before one year of age, the heart failure-free survival at one year of age was defined as the primary end point. In this trial, patients received the recombinant enzyme twice weekly. The infusions were generally well tolerated. All infants survived the first year of life with well preserved heart function. Muscle strength also improved and a reduction of glycogen accumulation was seen in muscle biopsies.

In an open-label, multinational, multicenter clinical trial, eight infantile Pompe patients were enrolled.¹⁶ After 52 weeks of treatment with recombinant α -glucosidase at a weekly dosage of 10 mg/kg, six of the eight were alive, and five patients were free of invasive ventilator support. Furthermore, an improvement of heart function was observed, and five patients achieved new motor milestones.

Kishnani and colleagues examined the efficacy and safety of recombinant α -glucosidase produced in CHO cells in a large number of severely affected Pompe patients.¹⁷ The study was designed to demonstrate a positive effect on survival compared to a historical control population. This control group consisted of 61 severely affected infants six months old or younger, who were included in a retrospective natural history study.¹⁸ The patients, who were no older than 26 weeks at initiation of the study, received intravenous infusions of the enzyme preparation at 20 mg/kg ($n = 9$) or 40 mg/kg ($n = 9$) every other week. All 18 patients survived to 18 months of age. A statistical analysis performed 52 weeks after the last patient was randomized to the study revealed that treatment with α -glucosidase reduced the risk of death or invasive ventilation by 92% compared to the untreated historical control group. After the end of the study, mean left ventricular mass index had declined from 193.4 g/m² at baseline to 86.8 g/m². Consistent motor and functional gains were observed in 13 of 18 patients measured by the Alberta Infant Motor Scale. During the study, all patients acquired cognitive and social development skills, although generally not as fast as healthy infants of the same age.

In all patients a muscle biopsy was performed at baseline and weeks 12 and 52 in order to assay α -glucosidase activity and glycogen levels. In both dosage groups, a significant increase in muscle enzyme activity from baseline to week 52 could be demonstrated. Fifteen of the 17 patients in whom levels of storage material had been measured exhibited stable or decreased muscle glycogen content (>20% depletion) at the end of the trial as compared to baseline. The 40 mg/kg dose was not superior to the 20 mg/kg dose with regard to efficacy.

In an extension study, 16 of the 18 patients of the 52-week trial continued to receive α -glucosidase at the same dose

to which they were originally assigned.¹⁹ At the age of 24 and 36 months, survival and ventilator use (invasive and noninvasive) were analyzed. At 36 months, the survival rate was 72%, whereas only one of 61 patients in the untreated control group survived to the ages of 24 and 36 months. The invasive ventilation-free survival rate, evaluated as the primary efficacy endpoint, was 66.7% at the age of 24 months and 49.4% at age of 36 months. In contrast, the overall survival rate of untreated patients is much lower.

As a secondary endpoint the proportion of patients who were alive and free from any ventilatory support (invasive or noninvasive) was chosen. As no additional infants needed noninvasive ventilatory support at the ages of 24 or 36 months, the ventilation-free survival rate was the same as the invasive ventilation-free survival rate, namely 66.7% and 49.4%, respectively. Cardiac parameters and motor development were evaluated as in the foregoing 52-week trial: During the extension study mean left ventricular mass continued to decrease and remained stable at the end of the trial. Seven infants gained motor skills and became able to walk at the time of their final assessment. The study was terminated after the enzyme preparation alglucosidase alfa (Myozyme[®]; Genzyme, Cambridge, MA, USA), manufactured in a CHO cell line, was approved for commercial use in 2006.

In order to evaluate whether enzyme replacement therapy with alglucosidase alfa was safe and effective in children with advanced Pompe disease, an open-label, multicenter study was initiated that included 21 infants who were aged 3–43 months at enrollment.²⁰ All patients received a dosage of 20 mg/kg every two weeks initially and after at least 26 weeks of treatment, an increase of dosage to 40 mg/kg every two weeks was allowed if the patient's clinical condition relative to baseline had significantly deteriorated. To compare survival results, the same untreated patients served as a reference cohort that was also used in the first 52-week study.¹⁸ Data analysis at the end of the study revealed that treatment with alglucosidase alfa reduced the risk of death by 79% and the risk of invasive ventilation by 58% compared with the untreated historical group. Echocardiography demonstrated that left ventricular mass index improved or remained normal in all patients evaluated beyond 12 weeks. Thirteen of the 21 patients achieved new motor milestones and five patients were able to walk independently at the end of the study.

The effect of enzyme replacement therapy in late-onset Pompe disease has been investigated in 44 patients with various stages of disease.²¹ Mean age at baseline was 48.9 years and the age range was 21 to 69 years. Standard dosage was 20 mg/kg alglucosidase alfa every other week.

After one year of treatment, a significant improvement of the six-minute walk test could be seen (from 341 ± 149 m at baseline to 393 ± 157 m after one year of treatment). Creatine kinase serum levels showed a significant mean decrease of 10.5%. Lung function remained unchanged.

Safety

In the studies performed by Kishnani and colleagues,^{17,19} 16 of the 18 patients developed immunoglobulin G (IgG) antibodies to glucosidase alfa at different times of the study. Eleven of the 18 patients experienced 224 mild or moderate infusion-associated reactions that were defined as drug-related adverse events. More infusion-associated reactions occurred in patients who received the high dosage (40 mg/kg) than in patients in the 20 mg/kg dose group. Rash, fever, urticaria, and decreased oxygen saturation were the most common infusion-associated reactions. They could be managed easily by stopping the infusion or reducing the infusion rate. All patients who experienced infusion-associated reaction recovered without any sequelae, and no patient had to discontinue treatment because of an adverse event.

Limitations of enzyme replacement therapy

Clearance of glycogen from the skeletal muscle of Pompe patients has been shown to be more difficult than substrate elimination in other lysosomal storage disorders, and several factors contribute to the limited efficacy of enzyme replacement therapy in Pompe disease. At first, muscle cells are separated from the bloodstream by a barrier composed of endothelial cells and ample interstitial tissue, whereas in Fabry and Gaucher disease, the target cells (endothelial cells and macrophages, respectively) are located close to the blood vessels from where they take up immediately the administered enzyme. Furthermore, muscle and heart differ in the response to exogenously administered enzyme due to diversity in the density of the M6P receptor in different tissues. It has been shown that the skeletal muscle contains much less M6P receptors than heart cells.²² Therefore very high amounts of enzyme (up to 40 mg/kg body weight) are necessary to achieve a therapeutic effect not only in cardiac tissue, but also in skeletal muscle. In addition, type II muscle fibers are more resistant to glycogen clearance than type I fibers, most likely because type II fibers contain less proteins involved in endocytosis and trafficking of lysosomal enzymes and do show an increased autophagic activity.²³ One of the greatest barriers to successful enzyme replacement therapy in Pompe disease is the fact that the efficacy is markedly

reduced by the formation of high-titer antibodies against human α -glucosidase in subjects who are negative for cross-reacting immunologic material (CRIM).¹⁷ It can be shown in a mouse model of Pompe disease that the formation of antibodies can be prevented by adeno-associated virus vector-mediated gene therapy that induced immune tolerance to the infused enzyme.²⁴

Some limitations of enzyme replacement therapy in Pompe disease are possibly due to the timing of therapy and early therapeutic intervention has been shown to be more efficient. Therefore, newborn screening programs for lysosomal storage disorder have been developed.²⁵ However, as for most of these conditions a strict genotype–phenotype correlation does not exist, the screening programs will probably not be generally introduced until the phenotype from the newborn result can be exactly predicted.

Perspectives of future therapies

Modification of α -glucosidase

As it has been discussed before, the delivery of α -glucosidase to the target cells depends from the number of M6P residues the enzyme contains. And in order to increase the density of the recognition marker Zhu and colleagues have modified the enzyme by conjugation of a synthetic oligosaccharide containing M6P residues onto recombinant human acid α -glucosidase.²⁶ This modification improved the affinity of the enzyme for the M6P receptor and delivery to the muscle cells. Infusion of the M6P-enriched enzyme into Pompe mice resulted in a significantly higher clearance of glycogen in muscle cells when compared to the unmodified counterpart. A greater improvement in muscle strength was observed. It can be assumed that the modified enzyme may be more effective in the treatment of Pompe patients.

Chaperones

Chaperones are a part of the cell system that has the task to control the quality of newly synthesized proteins by eliminating misfolded or unstable mutant products. Besides the chaperones this machinery involves proteasomes and the ubiquitin system. And in the last years it has been found that iminosugars do not only act as enzyme inhibitors, but also have an effect as pharmacological chaperones.²⁷ In genetic disorders, certain missense mutations and some small in-frame deletions may cause polypeptide misfolding, but may not (or only slightly) impair the functionally essential domains of the mutant protein (the active site, receptor-binding site, etc). Pharmacological chaperones, such as substrate analogues, may facilitate the stabilization of misfolded proteins and

iminosugars such as deoxynojirimycin-analogues function as chaperones.

In summary, small molecule chemical chaperones may be therapeutically useful for various lysosomal storage disorders caused by mutant but yet catalytically active enzymes. Porto and colleagues studied the combined effect of recombinant α -glucosidase and the pharmacological chaperone N-butyldeoxynojirimycin (NB-DNJ) on the efficacy of enzyme activity in cultured Pompe fibroblasts and showed that the chaperone improved enzyme delivery to lysosomes, enhanced enzyme maturation, and increased enzyme stability.²⁸ A similar effect on α -glucosidase activity was also observed in a mouse model of Pompe disease that, in addition to a single infusion of recombinant human α -glucosidase, was treated with oral NB-DNJ.

Conclusion

In summarizing the results of the different clinical trials with recombinant α -glucosidase, it can be concluded that treatment with this enzyme preparation is able to improve cardiomyopathy and motor development. The current enzyme replacement therapy improves quality of life of the patients, but does not cure them. The effect on muscle function is not as satisfactory as to that on the heart because of the variability of skeletal muscle response. In addition, the efficacy of enzyme replacement therapy is reduced by the formation of antibodies, predominantly in CRIM-negative patients. It has also become evident that the outcome is more robust if treatment starts in early life, for which reason several screening programs for Pompe disease will be initiated. In order to overcome these limitations of enzyme-replacement therapy in Pompe disease, many efforts have to be made to modify recombinant α -glucosidase to have better access to muscle cells, to prevent antibody formation, and to develop new drugs such as chaperones that may increase enzyme activity. In the future, gene therapy may be a therapeutic option.²⁹

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References

- van den Hout HM, Hop W, van Diggelen OP, et al. The natural course of infantile Pompe's disease: 20 original cases compared with 133 cases from the literature. *Pediatrics*. 2003;112(2):332–340.
- Umaphysivam K, Hopwood JJ, Meikle PJ. Correlation of acid alpha-glucosidase and glycogen content in skin fibroblasts with age of onset in Pompe disease. *Clin Chim Acta*. 2005;361(1–2):191–198.
- Kamphoven JH, de Ruiter MM, Winkel LP, et al. Hearing loss in infantile Pompe's disease and determination of underlying pathology in the knockout mouse. *Neurobiol Dis*. 2004;16(1):14–20.
- Kishnani PS, Howell RR. Pompe disease in infants and children. *J Pediatr*. 2004;144(5 Suppl):S35–S43.
- Laforet P, Petiot P, Nicolino M, et al. Dilative arteriopathy and basilar artery dolichoectasia complicating late-onset Pompe disease. *Neurology*. 2008;70(22):2063–2066.
- Kroos M, Pomponio RJ, van Vliet L, et al. Update of the Pompe disease mutation database with 107 sequence variants and a format for severity rating. *Hum Mutat*. 2008;29(6):E13–E26.
- Ausems MG, Verbiest J, Hermans MP, et al. Frequency of glycogen storage disease type II in The Netherlands: implications for diagnosis and genetic counselling. *Eur J Hum Genet*. 1999;7(6):713–716.
- Slonim AE, Bulone L, Goldberg T, et al. Modification of the natural history of adult-onset acid maltase deficiency by nutrition and exercise therapy. *Muscle Nerve*. 2007;35(1):70–77.
- Kaplan A, Achord DT, Sly WS. Phosphohexosyl components of a lysosomal enzyme are recognized by pinocytosis receptors on human fibroblasts. *Proc Natl Acad Sci U S A*. 1977;74(5):2026–2030.
- Van der Ploeg AT, Kroos MA, Willemsen R, Brons NH, Reuser AJ. Intravenous administration of phosphorylated acid alpha-glucosidase leads to uptake of enzyme in heart and skeletal muscle of mice. *J Clin Invest*. 1991;87(2):513–518.
- McVie-Wylie AJ, Lee KL, Qiu H, et al. Biochemical and pharmacological characterization of different recombinant acid [alpha]-glucosidase preparations evaluated for the treatment of Pompe disease. *Mol Genet Metabol*. 2008;94(4):448–455.
- Van den Hout H, Reuser AJ, Vulto AG, Loonen MC, Cromme-Dijkhuis A, Van der Ploeg AT. Recombinant human alpha-glucosidase from rabbit milk in Pompe patients [letter]. *Lancet*. 2000;356(9227):397–398.
- Van den Hout JM, Kamphoven JH, Winkel LP, et al. Long-term intravenous treatment of Pompe disease with recombinant human alpha-glucosidase from milk. *Pediatrics*. 2004;113(5):e448–e457.
- Winkel LP, Van den Hout JM, Kamphoven JH, et al. Enzyme replacement therapy in late-onset Pompe's disease: a three-year follow-up. *Ann Neurol*. 2004;55(4):495–502.
- Amalfitano A, Bengur AR, Morse RP, et al. Recombinant human acid alpha-glucosidase enzyme therapy for infantile glycogen storage disease type II: results of a phase I/II clinical trial. *Genet Med*. 2001;3(2):132–138.
- Kishnani PS, Nicolino M, Voit T, et al. Chinese hamster ovary cell-derived recombinant human acid alpha-glucosidase in infantile-onset Pompe disease. *J Pediatr*. 2006;149(1):89–97.
- Kishnani PS, Corzo D, Nicolino M, et al. Recombinant human acid α -glucosidase: Major clinical benefits in infantile-onset Pompe disease. *Neurology*. 2007;68(2):99–109.
- Kishnani PS, Hwu WL, Mandel H, Nicolino M, Yong F, Corzo D. A retrospective, multinational, multicenter study on the natural history of infantile-onset Pompe disease. *J Pediatr*. 2006;148(5):671–676.
- Kishnani PS, Corzo D, Leslie ND, et al. Early treatment with alglucosidase alpha prolongs long-term survival of infants with Pompe disease. *Pediatr Res*. 2009;66(3):329–335.
- Nicolino M, Byrne B, Wraith JE, et al. Clinical outcomes after long-term treatment with alglucosidase alfa in infants and children with advanced Pompe disease. *Genet Med*. 2009;11(3):210–219.
- Strothotte S, Strigl-Pill N, Grunert B, et al. Enzyme replacement therapy with alglucosidase alfa in 44 patients with late-onset glycogen storage disease type 2: 12-month results of an observational clinical trial. *J Neurol*. 2009 Aug 1. [Epub ahead of print].
- Wenk J, Hille A, von Figura K. Quantitation of Mr 46000 and Mr 300000 mannose 6-phosphate receptors in human cells and tissues. *Biochem Int*. 1991;23(4):723–731.
- Raben N, Fukuda T, Gilbert AL, et al. Replacing acid alpha-glucosidase in Pompe disease: recombinant and transgenic enzymes are equipotent, but neither completely clears glycogen from type II muscle fibers. *Mol Ther*. 2005;11(1):48–56.

24. Sun B, Bird A, Young SP, Kishnani PS, Chen YT, Koeberl DD. Enhanced response to enzyme replacement therapy in Pompe disease after the induction of immune tolerance. *Am J Hum Genet.* 2007;81(5):1042–1049.
25. Zhang XK, Elbin CS, Chuang WL, et al. Multiplex enzyme assay screening of dried blood spots for lysosomal storage disorders by using tandem mass spectrometry. *Clin Chem.* 2008;54(10):1725–1728.
26. Zhu Y, Jiang JL, Gumlaw NK, et al. Glycoengineered acid alpha-glucosidase with improved efficacy at correcting the metabolic aberrations and motor function deficits in a mouse model of Pompe disease. *Mol Ther.* 2009;17(6):954–963.
27. Bernier V, Lagacé M, Bichet DG, Bouvier M. Pharmacological chaperones: potential treatment for conformational diseases. *Trends Endocrinol Metab.* 2004;15(5):222–228.
28. Porto C, Cardone M, Fontana F, et al. The pharmacological chaperone N-butyldeoxynojirimycin enhances enzyme replacement therapy in Pompe disease fibroblasts. *Mol Ther.* 2009;17(6):964–971.
29. Ziegler RJ, Bercury SD, Fidler J, et al. Ability of adeno-associated virus serotype 8-mediated hepatic expression of acid alpha-glucosidase to correct the biochemical and motor function deficits of presymptomatic and symptomatic Pompe mice. *Hum Gene Ther.* 2008;19(6):609–621.

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