Alglucosidase alfa: first available treatment for Pompe disease

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Alglucosidase alfa is a recombinant form of acid α-glucosidase that catalyses the complete hydrolysis of its natural substrate, glycogen. It is postulated that administration of alglucosidase alfa will result in clearance of glycogen from lysosomes and will improve muscle function in patients affected with Pompe disease. This review describes the main steps in the history of enzyme-replacement therapy in this specific lysosomal storage disorder and discusses the characteristics of the medicinal product as well as the evaluation of response to therapy.

Keywords: α-glucosidase, enzyme-replacement therapy, glycogen-storage disease type II, lysosomes, muscle, Pompe disease

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Pompe disease, also referred to as glycogen-storage disease (GSD) type II, is the only lysosomal storage disease (LSD) among the different glycogenoses and is caused by defects in the lysosomal acid α-glucosidase (GAA; synonym: acid maltase) gene. This recessively inherited enzyme deficiency results in intralysosomal accumulation of glycogen in most tissues, but symptoms are mainly due to functional impairment of skeletal and heart muscle [1]. Clinically, infants with the classical form of GSD II (namely, Pompe disease) have severe cardiomyopathy leading to death before the age of 2 years. Patients affected with the late-onset form of the disease have symptoms that may first appear from childhood to adulthood with more or less progressive myopathy limited to skeletal and respiratory muscle. Progression to wheelchair is frequent and premature death can occur as a result of respiratory failure.

Pompe disease shares the main general characteristics of the different LSDs. In these disorders, the absence of degradation of one specific substrate leads to severe accumulation of glycosaminoglycans, sphingolipids or glycogen as seen in the mucopolysaccharidoses (MPS), lipidoses or GSD II, respectively. In general, each of these diseases corresponds to a specific enzyme deficiency. Accumulation of biological macromolecules causes a particular cellular dysfunction which is frequently associated with enlarged organs, such as cardiomegaly observed in Pompe disease. Although lysosomal enzymes are ubiquitously expressed, accumulation of macromolecules and associated clinical symptoms are primarily observed in tissues in which the deficient enzyme is actively and specifically involved in the substrate metabolism. Thus, glycosaminoglycans, sphingolipids and glycogen accumulate in cartilage, nervous system and muscle, respectively. This process results in progressive skeletal deformities, neurological disturbances and loss of muscle functions depending on the disease.

Genetically, the global frequency of LSDs as a group is approximately 1 in 5,000–10,000 live births. Most of the genes involved in LSDs have been identified. Different mutations result in a more or less complete absence of enzyme activity and are correlated to various phenotypes. However, the rate of synthesis and influx of the stored macromolecules into the lysosome are probably variable in individuals following genetic background or environmental factors that could also explain the differences in clinical course.

Enzyme-replacement therapy (ERT) in LSD is intended to replace the missing enzyme and has proven to be effective for various diseases. To date, such therapy is still in development for Niemann–Pick disease whereas licensed products are already available for Gaucher disease, Fabry disease, MPS I and more recently for Pompe disease, MPS II and MPS VI. Indeed, Pompe-specific treatment became a real possibility for patients and families in April 2006 when alglucosidase alfa (Myozyme®) was commercially approved in both Europe and the USA. Up to this date, Pompe disease management consisted of multidisciplinary supportive care only. At the beginning, the first attempts at ERT for Pompe disease have failed for two main reasons: absence of sufficient quantities of highly purified GAA available at that time, and failure to recognize the role of mannose-6-phosphate (M6P) receptors in endocytosis and cellular trafficking of lysosomal enzymes. Therefore, no therapeutic effects were

10.2217/14750708.4.3.271 ISSN 1475-0708

seen in the few patients who received GAA-lacking M6P residues extracted from the fungus *Aspergillus niger* [2,3] or purified from human placentas [4]. In the late 1970s, the growing understanding of lysosomal enzyme-uptake mechanisms by the M6P receptor pathway stimulated interest for ERT via tissular delivery of correctly phosphorylated enzymes. In Pompe disease, this approach was developed considerably under the impulse of Reuser's team in Rotterdam between 1984 and 1998 [5]. The first successful experiments showing that the properly processed enzyme can be taken up by the cardiac and skeletal muscle have been performed *in vitro* and *in vivo* (animal) using GAA-containing M6P residues obtained from bovine testis or human urine [6]. Later on, the cloning of the *GAA* gene [7,8] made feasible the large-scale production of the recombinant form of human *GAA* (rhGAA). Two main systems have since been developed in mammalian cells to produce rhGAA, namely GAA production in milk of transgenic animals, and GAA production in Chinese hamster ovary (CHO) cells. The first clinical trials investigating the safety and efficacy of rhGAA were initiated in 1999, with these two biotechnological approaches in parallel. The preliminary studies of ERT using either rhGAA from rabbit milk [9–14] or rhGAA from CHO cells [15,16] have included an overall number of 17 infantile Pompe patients in four main open-label clinical trials. The preparations in these four early Phase I/II and Phase II trials were distinct from alglucosidase alfa (all three enzymes are rhGAA). These studies are now complete and the surviving patients initially treated with the 'pre-Myozyme' products have been transitioned to alglucosidase alfa, the totality of the data clearly showing that ERT prolongs survival and improves cardiac, pulmonary and muscle functions in patients presenting with the infantile-onset form of the disease. More recently, two additional, larger-scale Phase II/III studies have been performed to further evaluate the efficacy of rhGAA. Described below are the results of these two pivotal multinational multicenter trials that have involved 39 rhGAA-naive infants treated exclusively with alglucosidase alfa. Conversely, few data regarding therapeutic effect of ERT in children and adults with Pompe disease have been published to date [17]. Further trials using alglucosidase alfa in patients presenting with the late-onset form of the disease are currently underway and will not be detailed.

Overview of the market

Alglucosidase alfa has been approved for long-term ERT in patients with a confirmed diagnosis of Pompe disease. Clinical diagnosis is established by GAA enzyme measurement in leukocytes, skin fibroblasts, muscle tissue or chorionic villus cells showing varying amounts of residual activity. In some cases this diagnosis is supported by examination of tissue after biopsy showing marked accumulation of glycogen mainly in muscle but also in liver, and by detection of high urine values of the specific tetrasaccharide Glc_4 (Glc α 1–6Glc α 1–4Glc α 1–4Glc). Genotyping may be helpful for carrier testing and prenatal diagnosis.

Pompe disease is clinically heterogeneous and patients are usually classified on the basis of age at onset of symptoms and severity of organ involvement. A broad classification of Pompe disease divides the patients into two subtypes:

- The infantile-onset form of the disease that includes patients presenting with symptoms within the first 12 months of life and is characterized by a rapid fatal progression with cardiomyopathy
- The late-onset form that includes patients presenting with symptoms anytime during early childhood up until adulthood and is characterized by a more slowly progressive myopathy with lack of cardiac involvement

The global incidence of the two Pompe disease phenotypes in Caucasian populations is estimated at 1 in 100,000 live births [1]. As with many genetic diseases, the frequency of the infantile-onset form is higher among some ethnic groups (African–Americans, Chinese).

The *GAA* gene is located on chromosome 17. It is approximately 20 kb long and contains 20 exons. The cDNA for human GAA is approximately 3.6 kb, with 2859 nucleotides of coding sequence predicting 952 amino acids. In order to produce alglucosidase alfa, this gene is inserted into a vector and introduced into the host CHO cells in which, after post-translational modifications, the enzyme is glycosylated at all the potential sites that are used to contain high-mannose chains that are phosphorylated, providing the M6P recognition marker which is essential for targeting to lysosomes.

The phenotypic variation of GSD II is related to different levels of residual GAA activity, which is explained by a high degree of genetic heterogeneity in patients. Infantile-onset patients have undetectable or very low

levels of GAA activity in fibroblasts or muscle tissue obtained by biopsy, whereas adult-onset patients affected with the less severe form of the disease show intracellular GAA activity 10–20% of normal. This is a crucial point when considering ERT in Pompe disease, given the fact that cells from both infantile- and late-onset patients will be corrected with regards to their GAA activity when the enzyme level reaches the critical threshold of approximately 30% of normal. Consequently, complete normalization of the GAA level after treatment is, in practice, probably not strictly necessary in order to observe a reduction in cellular glycogen content with improvement of muscle function, provided trafficking of the therapeutic enzyme is not affected by morphological and structural changes in affected muscle cells (see below).

The principle of ERT in Pompe disease is based on intravenous administration of alglucosidase alfa at regular intervals. The GAA enzyme containing a M6P recognition signal is injected into blood circulation and internalized through the plasma membrane, where it is transported from the extracellular environment to the lysosomal compartment of the cells. Replacement of the missing enzyme has been shown to be safe and efficacious in reducing substrate accumulation of visceral tissues in a variety of LSDs and these data prompt us to consider that ERT will be similarly successful to improve pathological aspects and symptoms in Pompe disease.

However, some potential problems might be encountered with this treatment option. Infused lysosomal enzymes are not capable of crossing the blood–brain barrier and therapeutically efficient amounts of alglucosidase alfa cannot be reached in the brain parenchyma. Fortunately, although lysosomal glycogen is known to be stored in the nervous tissues of affected infants, no CNS manifestations have been reported in Pompe disease to date. Nevertheless, patients with infantile-onset disease treated over the long term may have a risk of developing neurological symptoms, when ERT would significantly prolong their survival. Immune responses to the replacement of GAA can cause hypersensitive reactions (see below). Moreover, circulating antibodies to the administered product could also result in enzyme inactivation and interfere with the effectiveness of the therapy. Finally, the need for lifelong repeated infusions may represent another issue.

Introduction to alglucosidase alfa

Acid hydrolases are ubiquitous components of lysosomes or lysosome-related organelles.

Chemistry

Alglucosidase alfa contains the active ingredient of rhGAA (glucan-1,4-α-glucosidase), which is a monomeric glycoprotein also known as acid maltase or lysosomal α-glucosidase.

Pharmacodynamics & pharmacokinetics

Alglucosidase alfa is a form of rhGAA produced in CHO cells by recombinant DNA technology. The product is synthesized in cells as a catalytically inactive 110 kDa precursor which is identical in amino acid sequence to the commonly occurring human form of GAA. Following *in vitro* and *in vivo* administration of the purified product preparation, the enzyme has the capacity to be internalized, modified by glycosylation and phosphorylation of mannose residues and then targeted to lysosomes via the M6P receptor. There, the glycoprotein is further processed by limited proteolysis, resulting in two mature forms of 76 and 70 kDa. The active enzymes degrade glycogen by cleaving both α-1,4 and α-1,6 glycosidic linkages at acid pH [18].

More than 90–95% of the injected dose goes to the liver, with decreased levels in the heart and skeletal muscle. Alglucosidase alfa administration results in clinical manifestations consistent with hypersensitivity reactions. In patients treated under clinical studies, the pharmacokinetics of alglucosidase alfa is dose-proportional and not affected by the presence of antibodies.

Clinical efficacy

Clinical experience with alglucosidase alfa is based on a number of open-label clinical studies and expanded-access programs, with more than 270 patients being treated worldwide to date. The majority of studies concern patients affected with the infantile-onset form of the disease.

The efficacy of alglucosidase alfa 20 and 40 mg/kg administered every other week was fully examined in infants in two major clinical trials initiated in 2003 and included 39 patients (Study 1: 18 patients; Study 2: 21 patients). Both studies were similar with respect to most of the study design characteristics: randomized, open-label, multicenter and multinational; relatively large cohorts of patients affected with infantile-onset Pompe disease; no history of

previous ERT treatment; comparable efficacy and safety assessments; comparison to an untreated historical control group [19].

In study 1, the patients aged 6 months or less at the onset of treatment had severe GAA deficiency and cardiomyopathy. They were randomly assigned to receive either a dose of 20 mg/kg (n = 9) or 40 mg/kg (n = 9). Efficacy results for the first 12 months of treatment show major clinical benefits with markedly increased survival (all patients survived to the age of 18 months) [20]. All patients with repeated cardiac measurements showed reversal of cardiomyopathy, this positive effect of alglucosidase alfa certainly contributing to the significant increase in lifespan. The majority of patients maintained normal growth and exhibited substantial gains in motor development over the 52-week treatment period. Several patients were able to walk independently by 12 months of treatment. This result illustrates a tremendous positive effect of alglucosidase alfa, considering that untreated infants with Pompe disease never exhibit such an advanced stage of motor development and never achieve ambulation. However, the response in skeletal muscle is variable. This observed variability in motor responses to alglucosidase alfa is not completely explained. A similar proportion of patients in the 20 and 40 mg/kg dose groups demonstrated marked clinical responses with respect to survival, changes in growth, cardiac status and motor functions. As of November 2006, six patients have died, nine out of 12 patients remain alive and free of any ventilator support and nine out of 12 patients demonstrate significant motor gains (seven walkers, two independent sitters).

In study 2, the patients ranged in age from 6 months to 3.5 years at onset of treatment. Consequently, a subgroup of patients were at a very advanced stage of the disease, with ventilator support in five cases. The starting dose was 20 mg/kg in all patients and six who began treatment at 20 mg/kg had their doses increased to 40 mg/kg after 26 weeks of treatment according to the protocol. Efficacy results for the first 12 months of treatment show clinical benefits, even in patients at a severe stage of the disease at initiation of ERT. After 1 year of treatment with alglucosidase alfa, 16 out of 21 patients (76%) were alive, 10 out of 21 patients (48%) acquired new motor milestones and 15 out of 18 patients (83%) showed reversal of cardiomyopathy. As of November 2006, six patients had died, 12 out of 15 patients demonstrated motor response (four walkers, eight able to sit with support or independently). In both studies, results show no alterations at the neuropsychological level, including in the older patients.

The efficacy of alglucosidase alfa 20 mg/kg administered every other week was assessed in an ongoing, open-label, single-center trial in Europe in patients with the late-onset form of the disease, including a small number of patients (n = 5) who ranged in age from 5 to 15 years at onset of treatment (all ambulatory, one required nocturnal noninvasive ventilation). After 6 months of treatment, the results show early signs of benefit on skeletal and respiratory muscle functions, as indicated by an increase in forced vital capacity (three patients) as well as an increase in the distance walked in 6 min (three patients) [Ansvander Ploeg, PERS. COMM.].

Postmarketing surveillance

Genzyme has developed the Pompe Registry, an observational, centralized database to collect anonymous and longitudinal data on Pompe patients [101]. Physicians around the world can access and possibly transfer patient data to the registry online. The system is particularly important in providing natural history and outcomes data, as well as safety and efficacy data concerning patients under therapy.

Safety & tolerability

The safety of alglucosidase alfa has been primarily evaluated in infants and children and remains to be considered in more elderly patients with advanced stages of Pompe disease that may have compromised cardiac and respiratory functions, making them at risk of becoming critically ill in cases of severe, infusion-associated reaction (IAR). Cinical studies have formed the basis for the recommended posology which is 20 mg/kg of body weight administered every other week in both infantile- and late-onset Pompe disease. Transient nephrotic syndrome was observed in one patient who received high doses of rhGAA (10 mg/kg, five-times weekly) [21]. Furthermore, the number of IAR symptoms per patient is higher in those treated with 40 mg/kg in clinical studies. Given that the effects of treatment at 20 mg/kg are similar to those of ERT at 40 mg/kg, this observation suggests that the dosing regimens should remain under a certain level between 20–40 mg/kg.

Alglucosidase alfa is generally well tolerated at doses ranging from 10 mg/kg every week to 40 mg/kg every other week. The major adverse

effects of alglucosidase alfa are allergic-type reactions. They are observed in most treated patients and are highly variable in presentation and severity. The majority of these reactions are reported to be mild or moderate in severity (e.g., cough, rash, discomfort, pyrexia, tachycardia) and managed symptomatically with success. They are more frequent in patients with acute illness, are typically observed during infusion (rather at the beginning) and frequently occur after the fifth to sixth infusion (Personal Data). These reactions are generally dependent on the rate of infusion and resolve without sequelae either after decrease or temporary interruption of infusion and/or with antihistamines and/or corticosteroids and/or antipyretics treatment. However, eight patients treated under clinical studies or in expanded-access programs (age range 5 months to 61 years; one tested positive for anti-rhGAA immunoglobulin [Ig]E) experienced severe anaphylactoid-type IARs, making alglucosidase alfa rather contraindicated in patients with such hypersensitivity reactions to the product. No other serious adverse events have been described in relation to the administration of alglucosidase alfa. Death in treated patients has been considered related to the progression of the disease with cardiorespiratory complications and assessed as clearly unrelated to treatment with alglucosidase alfa.

Of the 18 patients treated with alglucosidase alfa in study 1, 16 developed anti-rhGAA IgG, seroconversion being generally observed after week 4 of treatment. Since most of the IgG-positive patients demonstrated clinical benefit and because only one patient (study 1) who developed high titers of IgG tested positive for *in vitro* inhibitory antibodies at weeks 52 and 64 of treatment [20], the possible role of these circulating antibodies on clinical response is not clearly elucidated. Under the trials, a total of three patients were found positive for anti-rhGAA IgE antibodies. Some patients who experienced an anaphylactic reaction were positive for complement activation or had serum tryptase activity above normal levels.

Discussion

ERT is currently the only treatment option in Pompe disease. Substrate-reduction therapy, which aims to reduce the rate of synthesis of accumulating glycogen and possibly complement ERT, is not yet developed. In the same way, bone marrow transplantation has not been successful and is not recommended in Pompe disease [22].

The first results with alglucosidase alfa are promising, showing that this product is safe and effective but additional data are needed to establish the extent of long-term benefits. The most impressive results have been observed in the severe infantile-onset form of the disease when patients are at an early stage of disease progression and respond very well to alglucosidase alfa, most likely because treatment is started before irreversible damage has occurred to muscle. Conversely, extensive muscle damage at the time of treatment can be beyond repair. In these cases, alglucosidase alfa would be able to stabilize the progression of the disease but is not able to affect muscle regeneration. Beside the early age at onset of treatment, other factors may have effects on outcome of ERT and need to be determined. Outcome remains concerning in some patients (mainly infants) who are poor treatment responders despite early ERT and still have fragile condition that predisposes them to secondary fatal respiratory (and cardiac) complications. Conversely, effectiveness in late-onset Pompe patients has not yet been fully demonstrated, but we can speculate that ERT will probably result in much more progressive improvement of muscle function in this population.

Conclusion

Now that there is a therapy it is crucial to increase awareness of the disease among the medical community. If diagnosed late, Pompe patients are at risk of developing irreversible muscle damage. Thus, early diagnosis by pediatricians or other specialists is essential because it allows not only timely symptomatic intervention but also optimal ERT treatment. However, early identification of this condition remains challenging, since Pompe disease presents as a continuum of phenotypes with a wide spectrum of clinical features.

Surprisingly, more or less severe hypersensitivity reactions have been encountered not only in patients with the infantile form of the disease who carry a null mutation with lack of endogenous GAA production, but also in late-onset patients with some degree of residual enzyme activity that might render them more immunologically tolerant. In most cases, it is possible to continue to administer alglucosidase alfa infusions with adapted monitoring of the patients with concomitant pretreatment medications and/or at lower doses. Additional trials are needed to establish optimal dosing protocols. Furthermore, close monitoring of mental status in infants under treatment

should be continued as it is possible that CNS manifestations, resulting from slowly progressive glycogen accumulation in neurons, may be uncovered over time.

The high costs of treatment may be a problem. It is due to the fact that large-scale production of rhGAA in CHO cell culture systems is expensive and that the recommended dosage is far higher than that used for the treatment in other LSDs. Alglucosidase alfa currently costs approximately US$400,000 per 55-kg patient per year.

Future perspective

Alglucosidase alfa is the first available treatment for Pompe disease but certain limitations of this product in terms of efficacy makes work on a second-generation form of rhGAA and development of gene transfer-based approaches in parallel necessary.

As in many other LSDs, correction of intracellular GAA activity using ERT depends primarily on uptake of the therapeutic enzyme that is mediated by the M6P receptor pathway. There are two types of M6P receptors: cation-dependent and cation-independent, the latter being essential for cellular endocytosis of the enzyme. Remodeling the carbohydrate chains of rhGAA improves its affinity for the cation-independent M6P receptor and the use of such a modified enzyme can represent a feasible approach to enhance both normalization of cellular enzyme level and clearance of glycogen from lysosomes [23].

Until recently, only ERT has been shown to be of any clinical benefit for GSD II. However, different approaches of gene transfer may also offer an alternative that could provide a long-lasting method of GAA delivery to muscle in the future. The advantage of this approach is the possibility to directly transfer the GAA gene to some severely affected and difficult to correct muscles like diaphragm, but also to transduce skeletal muscle or liver, which would be able to secrete the enzyme into plasma in order to obtain a corrective effect in the total body musculature [24]. In this regard, results of ongoing preclinical studies that are testing recombinant adenoviruses or adeno-associated viruses encoding GAA appear to be promising and will be considered with interest for application in humans in the next future [25,26].

It has recently been shown in GAA-knockout mice that trafficking of the therapeutic enzyme appears to be affected by dysfunction of autophagic pathways, especially in type II muscle fibers [27,28]. These results underline the possible role of muscle fiber-type distribution in the individual response to alglucosidase alfa observed in clinical practice and point toward new roads for the development of pharmacological interventions.

Executive summary

- Alglucosidase alfa is the first available treatment for Pompe disease, a rare, progressive and ultimately fatal metabolic disease. It is also the first effective treatment for an inherited muscle disorder.
- Therapeutic correction of the lysosomal GAA deficit is based on lifelong intravenous infusions administered every 2 weeks.
- The natural course of this once lethal disease can be reversed and long-term survival has not yet actually been observed.
- The best therapeutic response is observed in patients treated early, especially in infants. The observed variability in drug response may also be due to genetic background, individual rate of glycogen synthesis or the presence of circulating antibodies.

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