Molecular Genetics and Metabolism 119 (2016) 115–123

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Prospective exploratory muscle biopsy, imaging, and functional assessment in patients with late-onset Pompe disease treated with alglucosidase alfa: The EMBASSY Study

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ARTICLE INFO

Article history:
Received 1 March 2016
Received in revised form 16 May 2016
Accepted 17 May 2016
Available online 19 May 2016

Keywords:
Alglucosidase alfa
Enzyme replacement therapy
Functional effects
Glycogen
Histopathology
Late-onset Pompe disease
Muscle pathology
Pompe disease

ABSTRACT

Background: Late-onset Pompe disease is characterized by progressive skeletal myopathy followed by respiratory muscle weakness, typically leading to loss of ambulation and respiratory failure. In this population, enzyme replacement therapy (ERT) with alglucosidase alfa has been shown to stabilize respiratory function and improve mobility and muscle strength. Muscle pathology and glycogen clearance from skeletal muscle in treatment-naïve adults after ERT have not been extensively examined.

Methods: This exploratory, open-label, multicenter study evaluated glycogen clearance in muscle tissue samples collected pre- and post- alglucosidase alfa treatment in treatment-naïve adults with late-onset Pompe disease. The primary endpoint was the quantitative reduction in percent tissue area occupied by glycogen in muscle biopsies from baseline to 6 months. Secondary endpoints included qualitative histologic assessment of tissue glycogen distribution, secondary pathology changes, assessment of magnetic resonance images (MRIs) for intact muscle and fatty replacement, and functional assessments.

Results: Sixteen patients completed the study. After 6 months of ERT, the percent tissue area occupied by glycogen in quadriceps and deltoid muscles decreased in 10 and 8 patients, respectively. No changes were detected on MRI from baseline to 6 months. A majority of patients showed improvements on functional assessments after 6 months of treatment. All treatment-related adverse events were mild or moderate.

Conclusions: This exploratory study provides novel insights into the histopathologic effects of ERT in late-onset Pompe disease patients. Ultrastructural examination of muscle biopsies demonstrated reduced lysosomal glycogen after ERT. Findings are consistent with stabilization of disease by ERT in treatment-naïve patients with late-onset Pompe disease.

Abbreviations: 6MWT, 6-Minute Walk Test; AE, adverse event; BMI, body mass index; CI, confidence interval; ERT, enzyme replacement therapy; FVC, forced vital capacity; GAA, α-glucosidase; HRLM, high-resolution light microscopy; PFT, pulmonary function testing; GMFCS-E&R, Gross Motor Functional Classification System–Expanded and Revised; GMFM-88, Gross Motor Function Measure-88; GSGC, Gait, Stair, Gower's Maneuver, and Chair; LOTS, Late-Onset Treatment Study; MRI, magnetic resonance imaging; PedsQL, Pediatric Quality-of-Life Inventory; QMFT, Quick Motor Function Test.

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http://dx.doi.org/10.1016/j.ymgme.2016.05.013

1. Introduction

Pompe disease is a rare, autosomal recessive disorder caused by deficiency of lysosomal acid α-glucosidase (GAA), an enzyme that breaks down glycogen in the body [1]. The resulting lysosomal glycogen accumulation, especially in cardiac and skeletal muscle, disrupts muscle function leading to multisystem pathology, disability, and ultimately death [2–4]. The classic infantile form of Pompe disease is rapidly progressive, characterized by cardiomegaly, hypotonia, and death from cardiorespiratory failure in the first year of life [5–7].

Late-onset Pompe disease has a more varied disease course and later manifestation from childhood to adulthood characterized by slowly progressive skeletal myopathy but without the cardiomyopathy typical of infantile Pompe disease [8–13]. Late-onset Pompe disease usually presents with slowly progressive myopathy, predominantly of the proximal muscles in the trunk and pelvic and shoulder girdles, while the degree of respiratory muscle involvement is variable [9]. As skeletal and respiratory muscle weakness progresses, patients often need ambulatory and ventilator assistance. Respiratory failure is therefore a cause of significant morbidity and the most frequent cause of death [8,9,14–17].

Alglucosidase alfa (Lumizyme®/Myozyme®, Sanofi Genzyme, Cambridge, MA, USA) is an enzyme replacement therapy (ERT) for the treatment of Pompe disease that provides patients with exogenous recombinant human GAA [18–20]. In infantile-onset Pompe disease, alglucosidase alfa prolongs overall and ventilator-free survival and improves cardiomyopathy, motor skills, and functional independence [21,22]. In late-onset disease, alglucosidase alfa stabilizes respiratory function and improves mobility and muscle strength [23–25].

Muscle pathology and the pharmacodynamic effects of alglucosidase alfa in clearing glycogen from skeletal muscle have been examined in skeletal muscle biopsies from infantile Pompe patients [26]. Better response to treatment was observed in patients with early-stage cell damage at baseline characterized by predominance of lysosomal glycogen accumulation. Lesser response to treatment was seen in patients with more advanced disease characterized by predominance of cytoplasmic glycogen and ultrastructural damage [26]. Data on muscle histopathology and effects of ERT on glycogen clearance from skeletal muscle in patients with late-onset Pompe disease indicate that there is clinical heterogeneity and variable response to treatment among patients [27–31]. Additional morphologic studies examining pre- and post-ERT muscle biopsies are needed to help determine appropriate timing of treatment initiation for optimal responses for adult patients with Pompe disease. This exploratory study used muscle biopsies, magnetic resonance imaging (MRI) of skeletal muscle, and functional assessments to characterize disease burden and the effects of 6 months of alglucosidase alfa in treatment-naïve patients with late-onset Pompe disease. The results support the proposed biological activity of alglucosidase alfa and characterize its histopathological and functional effects in late-onset Pompe disease.

2. Methods

2.1. Study design

The Exploratory Muscle Biopsy Assessment Study (EMBASSY; NCT01288027, Sanofi Genzyme) was an open-label, multicenter study to evaluate glycogen clearance in muscle tissue samples and imaging assessments collected pre- and post-alglucosidase alfa treatment (20 mg/kg of body weight every other week for 6 months) in treatment-naïve late-onset Pompe disease patients. We also explored possible correlations between glycogen content, MRI, and functional assessments.

Eligible patients were ≥18 years of age with confirmed GAA enzyme deficiency from any tissue source and/or confirmed *GAA* gene mutations without known cardiac hypertrophy. The main inclusion criteria were the ability to walk 50 m without stopping and without an assistive device and forced vital capacity (FVC) in the upright position ≥50% predicted. Exclusion criteria were prior treatment with ERT, need for a wheelchair or invasive ventilation, and formal contraindication to MRI (e.g., pacemaker or implanted ferromagnetic metals).

2.2. Study assessments

The primary endpoint was the reduction in the percent tissue area occupied by glycogen in muscle biopsies from baseline to 6 months. The type of biopsy performed (open or needle) was chosen by the clinical sites based on individual laboratory capabilities and expertise. Biopsies performed at 6 months were performed on the same side of the body, near the original (baseline) site but far enough away so that there would not be any interference from scar tissue at the site of the baseline biopsy. Muscle biopsies were fixed in a glutaraldehyde-based fixative, embedded in epoxy resin, and processed for high-resolution light microscopy (HRLM) and electron microscopy as previously described [26,32]. Muscle glycogen content in HRLM sections was measured by computer morphometry and expressed as "percent tissue area occupied by glycogen" in quadriceps and deltoid muscle biopsies as previously described [32]. As this was an exploratory study, analyses were not blinded. Computer morphometry was used for objective analysis of glycogen. Serial sections from these epoxy resin blocks were prepared for electron microscopy and used to confirm, when necessary, the qualitative observations made on HRLM sections, such as localization of glycogen to the lysosomes or cytoplasm and presence of autophagic debris, fibrosis, and fatty replacement. When feasible, muscle MRI was used to guide the level (i.e., axial slice position) that the biopsy should target in order to capture the least-affected tissue (i.e., avoiding fatty replaced tissue). Secondary endpoints included qualitative histopathological assessment of biopsies, skeletal muscle imaging, and functional assessments.

Skeletal muscle MRI using qualitative T1-weighted imaging in all patients and quantitative T2 and Dixon modalities in a subset of patients was performed at baseline and 6 months. MRIs were read and analyzed by a central laboratory (C.R.I.S., Tournai, Belgium). The T1-weighted data were analyzed using Mercuri grading (1: normal appearance, 2: mild involvement, 3: moderate involvement, and 4: severe involvement) to determine the degree of intact muscle and fatty replacement. The Mercuri grading system provides a qualitative measure of disease involvement. Water T2 imaging provides a quantitative measure of disease activity (e.g., inflammation, sarcoplasmic leakage, cell edema, or necrosis) within muscles, where an abnormal value is defined as >39 milliseconds (ms). T2 determination based on multi spin-echo sequences typically requires knowledge of the radio-frequency transmitter field (B1) spatial deviation in the image, which typically requires an additional acquisition for computing the B1 maps. Muscle T2 can be estimated without this additional acquisition, but at the expense of a loss in precision. Therefore, T2 values (ms) are provided both with and without B1 sorting. In fatty infiltrated muscles, water T2 was separated from the fat signals by tri-exponential fitting of the global signal decay [33]. The percent of fatty infiltration in lower limb muscles was quantified using a 3-point 3D Dixon acquisition. For each subject, the average for each upper (thigh) and lower leg was computed for Mercuri grading, percentage of fat, muscle water, and T2 with and without B1 sorting. Muscle trophicity also was evaluated at the quadriceps level