Challenges in diagnosis and treatment of late-onset Pompe disease

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Purpose of review

The first reports published in 2010 on enzyme replacement therapy in late-onset Pompe disease (LOPD) allow us now to stand back and adapt the strategies. In the meantime, substantial progress has been made in basic and applied research on animal models to enhance the efficacy of treatments. This brief review highlights the new concepts in a contemporary approach.

Recent findings

Interest in LOPD rose since its acknowledgement as a treatable myopathy. New insights from extensive analysis of injurious mechanisms resulted, over the past years, in the development of enzyme replacement therapy and a better understanding of its limits.

Summary

It seems reasonable to consider Pompe disease as a large spectrum of a single ubiquitous lysosomal disease resulting from an enzyme defect, more severe in newborns because of rapid cardiopulmonary and hepatic failures, with a much better prognosis when symptomatic after 12 months. This late-onset form demands therapy to avoid progressive motor disability and pulmonary insufficiency. Diagnosis is easy to confirm through rapid and reliable biochemical tests with sampling of blood dots on filter paper. When started early, treatment would avoid serious irrevocable damage to cells. Increasing precocity of diagnosis and efficacy of treatments are the core challenges for the next few years.

Keywords

autophagy, enzyme replacement therapy, glycogen storage disease type II, Golgi trafficking, lysosomal storage disease, myopathy, Pompe disease

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Introduction

Pompe disease (PD; MIM #232300), [acid maltase deficiency, lysosomal hydrolase acid α-glucosidase (GAA) deficiency, or glycogen storage disease type II (GSD II)] was the first of the lysosomal storage diseases to be identified. It is an autosomal recessive disorder with an estimated prevalence ranging from 1 : 14 000 to 1 : 300 000 depending upon ethnicity [1].

The *GAA* gene, including 20 exons located on chromosome 17q25.2–25.3, is transcribed as a 3.5 kb mRNA encoding a 110 kDa protein precursor [2], which is folded in the endoplasmic reticulum by specific molecular chaperones. After N-glycan processing in the Golgi apparatus, an intermediate molecular form of 90 kDa – including a mannose-6-phosphate (M6P) moiety, which engages the cation-independent M6P receptor (CI-MPR) – is transported into lysosomes through the CI-MPR pathway and proteolytically finalized in the active 76 and 70 kDa enzyme isoforms.

GAA hydrolyses the α-1,4- and 1,6-glycosidic bonds of glycogen to release glucose molecule units. Mutated GAA proteins fail to fold properly and are retro-translocated to the cytosol for degradation. Reduction of α-glucosidase activity below a critical threshold (sensitivity varies depending upon the type of tissue) results in glycogen accumulation in lysosomes, which initiates a cascade of events resulting in cell death. Excessive activation of the autophagic recycling pathway is responsible for most of the alterations observed in the heart, diaphragm, and skeletal muscles resulting in the destruction of the muscle contractile apparatus, atrophy, and functional insufficiency [3].

Pathogenesis

Glycogen accumulation is not *per se* the only cause of cell dysfunction. The knock-out murine model of Pompe disease is essential to understand the changes occurring in the progression of the disorder [4]. Initially, small glycogen-loaded vacuoles appear focally in muscle cells.

Then lysosomes expand and fuse, interfering with the structural organization of the cell. Later, lipofuscin deposits and autophagosomes appear and glycogen becomes dispersed in the cytosol. Lysosomal rupture causes local acidification, which inhibits cellular metabolic processes. This fact and the failure to digest glycogen cause a local starvation, which in turn induces activation of autophagy. The autophagic pathway is however impaired because of the lysosomal dysfunction [5], and aggregates of ubiquinated proteins also accumulate. In mice, fast-twitch type 2 fibers are mostly affected by the autophagic build-up [5]. In humans, fiber-type-specific involvement is less evident.

Moreover, starvation increases proteolysis, immobilization, and aging, favoring atrophic process. The important and elegant works by Raben and collaborators [3,6,7], in mice and human muscle, using immunostaining to distinguish lysosomes from autophagosomes, demonstrated that the autophagic component is prominent in juvenile and adult Pompe disease but is much less in neonatal Pompe disease. This group recently reported, as a surprising finding, that the autophagic component is visible after 6 months' treatment by enzyme replacement therapy (ERT) in infants [8••]. This finding raises the question of whether autophagy may become visible after clearance of glycogen by the therapy or if two different mechanisms result in newborn-onset Pompe disease and late-onset Pompe disease (LOPD). Another consideration may be that increased autophagy in treated patients predicts regeneration.

The presence of multivesicular bodies observed in cultured fibroblasts from patients [9] is a signal of the disruption of the Golgi apparatus system. This is associated with modified distribution of the M6P receptor with increased co-localization of autophagosome markers [9], indicating a change in the trans-Golgi trafficking and stocking of substrate-engulfed lysosomes. This point is important for ERT as integrity of this system is required for the uptake in lysosomes of the recombinant enzyme used.

Clinical features and diagnosis

Pompe disease has been classified depending on the age of onset, severity of organ involvement, and rate of progression. This classification is somewhat artificial and the disease must be considered as a single large spectrum. In general, the earlier the onset of symptoms, the faster is the rate of progression and the worse is the prognosis. Early-onset Pompe disease (EOPD) presents at birth or in the first months of life with profound hypotonia, generalized muscle weakness, feeding difficulties, macroglossia, respiratory distress, cardiomegaly, and hepatomegaly. In untreated infants, death occurs in the first year from a cardiopulmonary failure [10]. At the other end of the spectrum, classical adult-onset Pompe disease presents in the second or third decade, or even later, as a slowly progressive proximal/axial myopathy combined with respiratory symptoms.

Key points

- Pompe disease is clinically a large spectrum of a single ubiquitous lysosomal disease, late-onset Pompe disease (LOPD), featuring in patients with enough residual activity of acid α-glucosidase (GAA) to ensure no lethal cell injuries.
- Diagnosis must be done in LOPD as early as possible with the help of the dot blood sample (DBS) testing, which will allow starting treatment on time and will keep enough capability to drive the enzyme therapy to its lysosomal target thus maintaining regenerative ability of affected tissues.
- Even if efficacy of ERT is currently less evident in LOPD than in EOPD, efficiency and tolerance have now been established through concordant data in all published studies, but have to be valuated in the long term comparing the ability to prevent disabilities in contrast with the long debilitating course of natural history.
- Better knowledge of pathogenic mechanisms, considering their potentiality, would allow adaptation of new therapeutic strategies.

According to the more recent classifications, LOPD is characterized by onset ranging from infancy (>12 months of age) to late adulthood [11–15]. Symptoms are mainly myopathic, associated with mild cardiac or liver dysfunction in child and adolescents.

Typically, patients display weakness in proximal lower limbs and/or lumbar paraspinal muscles with waddling gait. The progression is slow, and the diagnosis may be delayed, often for years. Patients may complain of fatigue, poor performance in sport activities, back pain, and myalgia. The degree of motor disability is related to the duration of symptoms rather than to the age of the patient. Respiratory symptoms are the initial feature in 30% of cases [12] and may involve sleep breathing disorders, morning headache, frequent respiratory infections, excessive daytime sleepiness, exertional dyspnea, or orthopnea. The severity of motor and respiratory manifestations is not correlated: ambulant patients may need ventilation at night whereas those wheelchair bound may have normal pulmonary function [15]. Other findings are ptosis [16], hearing loss [17•], and ectasia of cerebral arteries, which seems to be quite frequent [18], whereas cerebral aneurysms, macroglossia, hypertrophic cardiomyopathy, and hepatopathy are rare. Gastrointestinal manifestations, such as chronic diarrhea, vomiting,

and abdominal postprandial pain, may be present in older patients [19].

Children may present with clinical symptoms resembling muscular dystrophies (progressive hypotonia, motor delay or regression, pseudo calf hypertrophy) [12], whereas in adolescents the main feature may be progressive limb girdle muscle weakness including scapular wigging and/or scoliosis.

Differential diagnosis may include muscular dystrophies, congenital myopathies, Danon disease, metabolic myopthies (other GSD or mitochondriopathies), and spinal muscular atrophy in newborns, and myositis in the elderly.

The diagnosis is simple if several manifestations are present but it may be tricky in the presence of isolated symptoms. Blood creatine kinase levels are usually elevated (twofold to fivefold normal levels) but may be normal in some patients. The electroneuromyogram displays a myogenic pattern. Respiratory function tests may document a restrictive respiratory defect mainly characterized by decreased performance in supine position as compared with upright position. Cardiomyopathy, when present, is characterized on electrocardiogram by high voltage of QRS complex and short PR interval. Computed tomography scan (CT-scan) and MRI with T1-weighted spin echo sequences allow rapid estimation of trophism and detection of specific areas with fatty replacement in muscles. Selectivity and specificity of muscle involvement in Pompe disease have been evaluated only in a few reports [20,21]. In LOPD, paraspinal muscles, psoas, ventrolateral muscles, rectus abdomini, and muscles of the posterior thigh are mostly affected with a symmetric distribution. Calf muscles are usually normal. Selective sparing of the short head of the biceps femori and of the tensor fasciae latae muscles is helpful to differentiate Pompe disease from muscular dystrophies. Glycogen muscle load evaluated by ^{13}C-magnetic resonance spectroscopy [22] is a promising technique for the follow-up of ERT-treated patients.

Muscle biopsy has been considered as crucial for the diagnosis of Pompe disease. Typical findings are the presence of a vacuolar myopathy of lysosomal origin (positive acid phosphatase reaction) with glycogen accumulation on PAS staining. Vacuoles on electronic microscopy contain dense bodies, cytoplasmic debris, and multilamellar structures. Accumulation of autophagic lipofuscin-like material, proliferation of the Golgi apparatus, or abnormal caveolin expression are features highly evocative of the disorder [3,23]. Due to the focal nature of the lesions, histology is not consistent with severity of the disease [24] and is far from unfailing for diagnosis, remaining important for differential diagnosis.

Diagnosis of Pompe disease must be confirmed by biochemical testing. Enzymatic assays [25] can be carried out in muscle homogenates, cultured fibroblasts, or isolated lymphocytes. Simultaneous measurement of another enzyme in the same sample ensures the quality of the sample. GAA activity in EOPD is less than 1% of controls, and is generally higher in LOPD [1,11,26], although there is no real correlation between residual activity and the severity of the disease in patients with LOPD. It is also possible to measure GAA activity in dried blood spots with good precision [27], and fluorometric method has been used for newborn screening [28]. If intermediate or low values are found, results can be confirmed by a second analysis on purified lymphocytes [25].

Demonstrating *GAA* mutations is not required for the diagnosis of Pompe disease, but it is important for prenatal diagnosis (especially in EOPD families). Nevertheless, more than 150 mutations have been identified to date [13,19], and some of them display an ethnic distribution. Genotype–phenotype correlations are not always well defined. Although homozygosity for truncating mutations is almost invariably associated with EOPD, other factors (genetic and environmental) may influence the phenotype associated with hypomorphic alleles. An example is patients with the c.-32–13T>G/null genotype who display markedly different phenotypic severity, unrelated to the degree of residual enzyme activity [29,30]. One of these factors has been recently identified and it is a specific insertion/deletion polymorphism of the angiotensin-converting enzyme [31•].

Treatment

Specific management guidelines have been published [10,32–36]. Their purpose is mainly to define criteria for diagnosis, management of specific symptoms, pretreatment assessment, monitoring of ERT, and enrollment in the Pompe registry (www.pomperegsitry.com). The registry currently includes 742 patients enrolled between 2004 and 2009, 70% being LOPD and 78% having received ERT [37], but no comprehensive information on the efficacy of treatment, tolerance, or progression is available to date from this source.

Until 10 years ago, the management of Pompe disease patients was exclusively based on supportive care. The introduction of ERT has made the multidisciplinary approach even more important. Physical therapy and nutrition remain the mainstay of therapy. Positive effects have been reported in long-term follow-up of adults [38–40], and the association with ERT appears even more promising. Particular attention should be given to calcium intake as osteopenia appears a particularly frequent complication, especially in children [41].

Currently, ERT by recombinant human GAA (rhGAA) is the only treatment approved by authorities for Pompe disease (European Medicines Agency in 2006 and US Food and Drug Administration in 2008). The principle of ERT is to replace the defective enzyme *in situ* by administering intravenously the manufactured rhGAA, which is then delivered to the lysosome through the CI-MPR pathway. The recombinant enzyme (alglucosidase α), produced in Chinese hamster ovary (CHO) cells [42,43], received marketing approval in 2006 (Myozyme). The recommended regimen is 20 mg/kg given every 2 weeks.

In EOPD, the efficacy on cardiac hypertrophy was spectacular [44–47]. Rapid normalization of ventricular function was observed in most cases with lengthening of the PR interval and normalization of ventricles diameters. Consequently, survival was the major outcome in these patients, most of the patients remaining alive as compared with a natural life expectancy of less than 1 year. Several patients were able to sit, stand, and walk, and did not need supportive ventilation. The earlier the onset of therapy, the better was the outcome [1], but not all patients responded equally. Several factors have been invoked. One is the production of antibodies against the recombinant protein. This phenomenon is evident especially in those patients with no GAA activity, developing cross-reactive immunologic material (CRIM) – CRIM-negative status [48]. Another hypothesized condition is that a central nervous system disorder resulting from the enzyme defect does coexist, not initially evident in EOPD, unmasked on increased survival resulting from the treatment, which is not active in the brain, being protected by the blood–brain barrier [49].

In LOPD, efficacy is now established, although it is less striking than in EOPD. Formal clinical studies with 20 mg/kg infusion every 15 days of alglucosidase α were published in 2010. One was a multicenter randomized trial against placebo, conducted for 18 months in 90 ambulatory patients older than 8 years [50••]. The results supported the efficacy of ERT with significant improvement in the primary outcomes: the 6-min walk test and forced vital capacities in upright position. These findings are also supported by several open-label observational studies [51,52,53•].

One study also included an MRI evaluation of the fat deposition in muscle, before and after a 2-year period of ERT [54•]. Although the study confirmed the improvement in the 6-min walk test (11% in the first 6 months, 8% in the later follow-up), fat accumulation in muscles did progress anyway.

Treatment is usually well tolerated and side effects are mostly related to the injection, although some patients have developed anaphylactic reactions against GAA.

There are still several important challenging issues. One is relative to the optimal doses employed in patients, as in the mouse model of Pompe disease up to 85% of the rhGAA was taken up by liver and the dose required to correct the muscle glycogen storage defect completely in this experimental model was much higher, 100 mg/kg [55]. On the contrary, it should be noted that 10 mg/kg weekly was effective in EOPD patients [56]. The second point is regarding the optimal time for treatment in LOPD. In general the best clinical response is in those patients in whom the architecture of skeletal muscle is still preserved, because disruption of the trans-Golgi network in muscle cells in the advanced stages of the disease impairs the lysosomal uptake of rhGAA [9]. On the other hand, the regenerative ability of muscle is questionable after huge disruption. However, assessing the long-term efficacy of ERT in LOPD is a challenge because it is not easy to demonstrate a clear benefit on the slowly progressive motor impairment and respiratory failure in these patients; large cohorts are required to yield statistically significant results. The high costs of a lifelong treatment have to be considered [57•] when starting a cure.

Future developments and challenges

A number of experimental treatments are currently being evaluated. Increasing the affinity of the rhGAA to the CI-MPR is under human experimentation. Concomitant use of small molecule chaperones may enhance the effect of rhGAA by acting on the misfolding of specific mutant proteins (not all missense mutations however respond to such drugs) and increase delivery to lysosomes [58]. Proof-of-concept studies have shown a synergistic effect with ERT *in vitro* and *in vivo* (see [59••] for review), but a phase II clinical trial was stopped after few months. Another strategy in animals aimed at modulating the autophagic buildup: this resulted in enhancement of the effects of ERT [60•].

Substrate reduction therapy is based on the concept that a decrease in substrate synthesis will restore the balance between synthesis and degradation (see [61] for review). In a double knock-out mouse model for *GAA* and glycogen synthetase 1, a significant decrease in lysosomal swelling and autophagic buildup was demonstrated [62].

Gene therapy is another possible approach. Preclinical studies have been conducted testing different vectors. AAV-mediated gene therapy showed ability to restore substantial levels of GAA activity [63]. More recently, the use of lentiviral vectors has also been explored [64], and gel-mediated delivery of AAV1 vectors expressing GAA was reported to improve pulmonary function in mice with Pompe disease, even in advanced stages of the disease [65].

Immune reaction is rare in LOPD. Strategies to counteract the production of antibodies against rhGAA could be used in such conditions, as demonstrated in CRIM-negative newborns [66].

Conclusion

Whatever algorithms would have been recommended for the diagnosis of LOPD, the most important stage when it is clinically suspected is to consider biochemical testing for GAA activity. This is now unproblematic and reliable with easy access through dot blood sample testing. Genetic consideration is not critical to diagnosis, but it is significant for prognosis and genetic counseling.

Data evaluating the efficacy of ERT are accessible. All reports to date are concordant to conclude in functional improvement, modest but promising. On the basis of the knowledge on pathogenicity, efficacy would obviously improve if ERT starts early in the course of the disease, but before housekeeping cell processes result in cell death. Future associated treatment, or improvement in lysosomal uptake of rhGAA, will undoubtedly yield better results.

Acknowledgement

Conflicts of interest

C.D. received research funding for an epidemiologic study on Pompe Disease from Genzyme SA, France.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 513).

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