Molecular Genetics and Metabolism 103 (2011) 107–112

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

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

Enhanced efficacy of enzyme replacement therapy in Pompe disease through mannose-6-phosphate receptor expression in skeletal muscle

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ARTICLE INFO

Article history:
Received 12 January 2011
Received in revised form 9 February 2011
Accepted 9 February 2011
Available online 13 February 2011

Keywords:
Mannose-6-phosphate receptor
Enzyme replacement therapy
Acid alpha-glucosidase
Acid maltase
Pompe disease
Glycogen storage disease type II

ABSTRACT

Enzyme replacement therapy (ERT) with acid α-glucosidase has become available for Pompe disease; however, the response of skeletal muscle, as opposed to the heart, has been attenuated. The poor response of skeletal muscle has been attributed to the low abundance of the cation-independent mannose-6-phosphate receptor (CI-MPR) in skeletal muscle compared to heart. To further understand the role of CI-MPR in Pompe disease, muscle-specific CI-MPR conditional knockout (KO) mice were crossed with GAA-KO (Pompe disease) mice. We evaluated the impact of CI-MPR-mediated uptake of GAA by evaluating ERT in CI-MPR-KO/GAA-KO (double KO) mice. The essential role of CI-MPR was emphasized by the lack of efficacy of ERT as demonstrated by markedly reduced biochemical correction of GAA deficiency and of glycogen accumulations in double KO mice, in comparison with the administration of the same therapeutic doses in GAA-KO mice. Clenbuterol, a selective β_2-agonist, enhanced the CI-MPR expression in skeletal tissue and also increased efficacy from GAA therapy, thereby confirming the key role of CI-MPR with regard to enzyme replacement therapy in Pompe disease. Biochemical correction improved in both muscle and non-muscle tissues, indicating that therapy could be similarly enhanced in other lysosomal storage disorders. In summary, enhanced CI-MPR expression might improve the efficacy of enzyme replacement therapy in Pompe disease through enhancing receptor-mediated uptake of GAA.

1. Introduction

Pompe disease (glycogen storage disease type II; acid maltase deficiency; MIM 232300) ranges in severity from a severe, infantile-onset hypertrophic cardiomyopathy to a late-onset myopathy, which is caused by a defect in acid α-glucosidase (GAA) varying from complete to partial deficiency of GAA. Infantile-onset Pompe disease affects the heart and skeletal muscle primarily, and causes death early in childhood from cardiorespiratory failure, if initiation of ERT is delayed or the patient fails to respond to ERT due to high, sustained anti-GAA antibodies [1–3]. However, enzyme replacement therapy (ERT) with recombinant human (rh) GAA has been effective for the long-term only in a subset of patients with infantile-onset Pompe disease.

GAA normally functions as an acid hydrolase that metabolizes lysosomal glycogen, and deficient GAA causes lysosomal glycogen accumulation in virtually all tissues [4]. The availability of ERT with rhGAA has prolonged survival and ameliorated the cardiomyopathy in the majority of patients with infantile-onset Pompe disease [2]. In late-onset Pompe disease the clinical response to ERT has been less dramatic than in the infantile-onset presentation, and ERT has largely resulted in stabilization of the disease process from a pulmonary and motor perspective [5]. Many individuals with late-onset Pompe disease have residual gait abnormalities despite adherence to ERT, indicating a relative lack of response of limb-girdle and leg muscles [5]. Muscle weakness was stabilized by ERT in one small series following 5 patients with juvenile-onset Pompe disease, although only one subject approached the normal range for muscle strength as quantified by hand-held dynamometry following 3 years of ERT [6]. A 3 month trial of ERT in 44 subjects with late-onset Pompe disease demonstrated significant improvement in the 6 minute walk test, modified Gowers' test, and creatine kinase levels, whereas stair climbing and serial arm function tests remained unchanged [5]. Another series of 11 subjects with late-onset Pompe disease was evaluated with dynamometry and quantitative magnetic resonance imaging of leg muscles over the course of 2 years on ERT, and both muscle mass and strength in the anterior thigh improved; however, progression of intramuscular fat accumulation during ERT emphasized the limited efficacy from ERT and the need for early treatment [7]. Taken together, these studies of ERT in juvenile and late-onset

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doi:10.1016/j.ymgme.2011.02.006

Pompe disease emphasized the limited responsiveness of skeletal muscle to the only available therapy.

Documented limitations of ERT in Pompe disease include the requirement for frequent intravenous infusions of high doses of GAA to achieve efficacy, degree of pre-ERT muscle damage, and the possibility of humoral immunity [2,3,8]. The rhGAA doses are markedly higher than those required for ERT in other lysosomal storage disorders, possibly reflecting the higher threshold for correction of GAA deficiency in the skeletal muscle of Pompe disease patients [9]. The paucity of cation-independent mannose-6-phosphate receptor (CI-MPR) in adult mammals' muscle has underscored the concept that CI-MPR is limiting for ERT in Pompe disease. Previously, low levels of CI-MPR were demonstrated in skeletal muscle of GAA-KO mice, specifically in muscles comprised primarily of type II myofibers [10,11]. Further evidence for the importance of CI-MPR expression to ERT in Pompe disease was demonstrated by the increased efficacy of rhGAA modified to increase mannose-6-phosphate content [12–14]. Furthermore, Pompe disease patient fibroblasts were found to be deficient in CI-MPR recycling and uptake of rhGAA was impaired [15]. However, until now the effect of CI-MPR manipulation *in vivo* has never been analyzed in Pompe disease.

In order to gain understanding of the influence of CI-MPR expression upon therapy in Pompe disease, we have characterized muscle-specific CI-MPR-KO/GAA-KO mice, evaluating ERT in these double (D) KO mice and demonstrating impaired responsiveness of skeletal muscle in DKO mice. In order to confirm the relevance of CI-MPR to ERT in Pompe disease, we sought to upregulate expression of CI-MPR in skeletal muscle. The only drug known to have this effect was β_2-agonist therapy with clenbuterol, which increased insulin-like growth factor 2 (IGF-2) receptor (also known as CI-MPR) expression in the masseter muscle of mice, along with IGF-1 and IGF-2 [16]. Therefore, we chose to evaluate the effect of clenbuterol treatment upon receptor-mediated uptake and biochemical correction of skeletal muscle during ERT in GAA-KO mice. The effectiveness of clenbuterol in increasing the response to ERT suggests that this might be valuable as an adjunctive therapy for Pompe disease.

2. Results

2.1. Evaluation of GAA uptake and glycogen clearance in the absence of CI-MPR expression

To understand the role of CI-MPR in recombinant human GAA (rhGAA) uptake and glycogen clearance specifically in Pompe disease, muscle-specific CI-MPR-KO mice were crossed with GAA-KO (Pompe disease) mice [17]. Evaluation of GAA activity demonstrated no significant differences in Pompe disease characteristics between the GAA-KO and the DKO mouse strains (Fig. 1A). GAA-KO and DKO mice were administered four weekly doses of rhGAA (20 mg/kg body weight), and euthanized three days after the last injection to evaluate GAA enzyme activity and glycogen content in the striated muscle. GAA activity analysis demonstrated significantly decreased enzyme levels in the quadriceps of DKO mice following ERT, in comparison with GAA-KO mice ($p = 0.003$; Fig. 1A). However, in the heart increased GAA activity was observed in DKO mice following ERT, in comparison with GAA-KO mice ($p = 0.03$; Fig. 1A). The latter observation suggested an alternative mechanism for uptake of rhGAA in the heart that did not utilize CI-MPR.

The reversal of glycogen storage, or clearance, marks the biochemical correction in Pompe disease. ERT achieved significant glycogen clearance in the heart and quadriceps only in GAA-KO mice, not in DKO mice (Fig. 1B). Furthermore, glycogen content was increased in the heart of DKO mice following ERT, in comparison with GAA-KO mice ($2.1 +/- 0.8$ versus $0.7 +/- 0.4$ μmol glucose/mg protein, respectively; $p = 0.003$). Similarly, resistance to the clearance

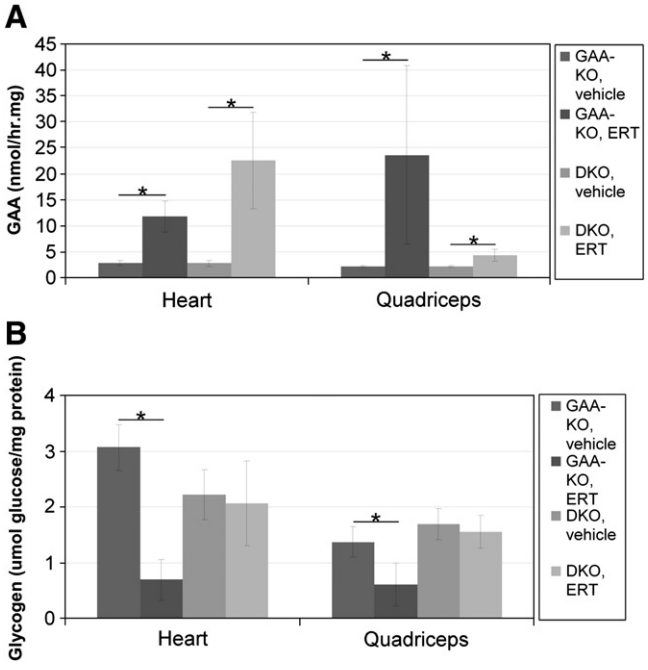

Fig. 1. Impaired rhGAA uptake in DKO mice. The homozygous DKO mice (n = 4) and GAA-KO mice (n = 4) were administered four weekly doses of rhGAA and sacrificed three days after the last injection. (A) GAA enzyme levels and (B) glycogen content were evaluated in the target tissues. Mean +/− standard deviation is shown.

of glycogen was observed in the quadriceps of DKO mice following ERT, in comparison with GAA-KO mice ($p = 0.0002$; Fig. 1B).

The basis for resistance to ERT in DKO mice was further analyzed by Western blot analysis of heart, which revealed more than 90% reduction of CI-MPR in DKO mouse in comparison with GAA-KO mice. In skeletal muscle (quadriceps), DKO mice had 40% decreased CI-MPR expression in comparison with GAA-KO mice (Fig. 2). These data revealed that CI-MPR expression was significantly reduced, establishing a correlation between reduced CI-MPR expression and resistance to glycogen clearance from ERT in striated muscle of DKO mice.

2.2. Enhanced efficacy from simultaneous ERT and clenbuterol administration: motor function, GAA uptake and glycogen clearance

The implication that CI-MPR expression was crucial to both enzyme uptake and glycogen clearance, and intracellular processing of GAA in Pompe disease led us to attempt to manipulate CI-MPR levels in GAA-KO mice. This strategy differs from previous attempts to address the limiting role of CI-MPR expression during ERT in Pompe disease, which relied upon increasing the mannose-6-phosphate content of rhGAA [10,13]. We attempted to increase CI-MPR in skeletal muscle to demonstrate the dependence of biochemical correction upon receptor-mediated endocytosis and uptake of GAA. Therefore, ERT was enhanced by the addition of a drug, clenbuterol, which was previously demonstrated to increase the expression of the IGF-2 receptor; identical to CI-MPR, in the masseter muscle of mice [16]. Groups of 3 month-old GAA-KO mice were treated with four weekly doses of rhGAA (20 mg/kg body weight), with or without concurrent clenbuterol treatment. The efficacy of clenbuterol treatment was demonstrated by an early increase in Rotarod latency in groups of male GAA-KO mice ($p = 0.04$ at week 1), in comparison with GAA-KO mice treated with ERT alone (Fig. 3A). Increased weight gain in GAA-KO mice treated with clenbuterol suggested that muscle hypertrophy was stimulated as described [16], in comparison with GAA-KO mice treated with ERT alone (Fig. 3B; $p < 0.05$ at weeks 1–3).