Molecular Genetics and Metabolism 105 (2012) 221–227

Contents lists available at SciVerse ScienceDirect

Molecular Genetics and Metabolism

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

Regular Article

β2 Agonists enhance the efficacy of simultaneous enzyme replacement therapy in murine Pompe disease

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ARTICLE INFO

Article history:
Received 7 November 2011
Accepted 7 November 2011
Available online 11 November 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 recombinant human acid α-glucosidase (rhGAA) has improved clinical outcomes in patients with Pompe disease; however, the response of skeletal muscle and the central nervous system to ERT has been attenuated. The poor response of skeletal muscle to ERT has been attributed to the low abundance of the cation-independent mannose-6-phosphate receptor (CI-MPR), which mediates receptor-mediated uptake of rhGAA. Hence the ability of adjunctive therapy with β2-agonists to increase CI-MPR expression in skeletal muscle was evaluated during ERT in murine Pompe disease with regard to reversal of neuromuscular involvement. Mice with Pompe disease were treated with weekly rhGAA injections (20 mg/kg) and a selective β2-agonist, either albuterol (30 mg/l in drinking water) or low-dose clenbuterol (6 mg/l in drinking water). Biochemical correction was enhanced by β2-agonist treatment in both muscle and the cerebellum, indicating that adjunctive therapy could enhance efficacy from ERT in Pompe disease with regard to neuromuscular involvement. Intriguingly, clenbuterol slightly reduced muscle glycogen content independent of CI-MPR expression, as demonstrated in CI-MPR knockout/GAA knockout mice that were otherwise resistant to ERT. Thus, adjunctive therapy with β2 agonists might improve the efficacy of ERT in Pompe disease and possibly other lysosomal storage disorders through enhancing receptor-mediated uptake of recombinant lysosomal enzymes.

1. Introduction

Infantile-onset Pompe disease results from the deficiency of lysosomal acid-α-glucosidase (GAA) and affects the heart and skeletal muscle primarily, causing death early in childhood from cardiorespiratory failure if untreated. Current therapy in the form of enzyme replacement therapy (ERT) has prolonged ventilator-free survival and muscle strength in patients with Pompe disease; however, the limitations of ERT have become increasingly evident and many patients eventually become ventilator-dependent on ERT. The enzyme dosages required for ERT in Pompe disease range up to 100-fold greater than those for other lysosomal disorders, which can be attributed at least in part to the poor uptake of recombinant human (rh) GAA by skeletal muscle associated with low abundance of the cation-independent mannose-6-phosphate receptor (CI-MPR). These limitations must be overcome to address the limitations of replacement therapy, be it ERT or gene therapy. Preclinical and clinical data suggest that paucity of CI-MPR reduced the uptake of GAA by Pompe disease cells [1-3]. The relevance of CI-MPR-mediated uptake during ERT was demonstrated by the increased efficacy of rhGAA that was modified to increase mannose-6-phosphate content in mice with Pompe disease [4,5]. Consistent with a deficiency of the uptake and lysosomal targeting of GAA, Pompe disease patient fibroblasts were found to be deficient in CI-MPR recycling and uptake of rhGAA was impaired [3].

A lack of complete efficacy from ERT has been observed in long term survivors with infantile Pompe disease. Even in patients with a good response to ERT, a residual motor weakness (neck flexor weakness, dorsiflexor weakness, mypathic facies, ptosis and strabismus) has been observed. Respiratory insufficiency is observed especially in those started late [6]. CNS involvement as indicated by the prevalence of sensorineural hearing loss, hypernasal speech, with a flaccid dysarthria and aspiration risk suggestive of bulbar involvement is commonly observed in long term survivors [7]. Strabismus and ptosis have been observed frequently among children with Pompe disease following long-term ERT [8]. Each of these abnormalities demonstrated a lack of complete efficacy from ERT. Patients with late-onset Pompe disease have severe pulmonary insufficiency which may progress to respiratory failure while receiving ERT [9]. 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 [10]. Autopsy of infantile patients has revealed glycogen

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doi:10.1016/j.ymgme.2011.11.005

accumulation in Purkinje cells of the cerebellum, neurons of the cerebral cortex, motor neurons of the spinal cord and in vascular smooth muscle cells of the CNS vasculature, all of which may contribute to the neurological deficits observed in these patients [11].

Dosages for ERT in Pompe disease are up to 100-fold greater than those in other lysosomal disorders, which has been attributed to the large mass of skeletal muscle (~40% of body weight), and to the low abundance of CI-MPR on skeletal muscle. The paucity of 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 [2,12]. We have evaluated the impact of CI-MPR-mediated uptake of GAA upon ERT in CI-MPR knockout (KO)/GAA-KO mice. The essential role of CI-MPR was emphasized by the lack of efficacy for either ERT [13] or gene therapy [14], as demonstrated by markedly reduced biochemical correction of GAA deficiency and of glycogen accumulations in CI-MPR-KO/GAA-KO mice, in comparison with administration of the same therapy in GAA-KO mice. Clenbuterol was previously demonstrated to increase the expression of the insulin-like growth factor 2 receptor (identical to CI-MPR) in muscle of mice [5]. Initial results revealed that high-dose clenbuterol, a β_2-agonist, enhanced CI-MPR expression and increased efficacy from GAA replacement therapy, thereby confirming the key role of CI-MPR with regard to replacement therapy in Pompe disease [13]. Biochemical correction improved in multiple skeletal muscles indicating that adjunctive therapies might enhance the response to ERT in Pompe disease [13].

Clenbuterol treatment clearly enhanced the biochemical correction of muscle from ERT in Pompe disease, which led us to further evaluate the efficacy of β_2-agonist administration in GAA-KO mice. Dose-related side effects have been observed in association with β2-agonist treatment; therefore, we administered clenbuterol at a lower dose in combination with ERT in GAA-KO mice. A second β2-agonist, albuterol, was evaluated similarly at a high dose. Both of these agents increased the clearance of glycogen in the muscle of GAA-KO mice; moreover, the uptake of rhGAA in the cerebral and cerebellar hemispheres of the brain was increased in association with increased clearance of glycogen in the brain of GAA-KO mice. These data further demonstrated the efficacy of adjunctive therapy with selective β2-agonists in a classical lysosomal storage disorder, Pompe disease, and suggested that this strategy might be effective in other lysosomal storage disorders that affect the brain.

2. Materials and methods

2.1. ERT in mice with Pompe disease

Tolerant GAA-KO mice [15] and CI-MPR-KO/GAA-KO mice [13] were administered four weekly doses of 20 mg/kg rhGAA and sacrificed 7 days after the last injection. Selected tissues were collected for GAA enzyme activity and glycogen content analyses. All animal procedures were done in accordance with Duke University Institutional Animal Care and Use Committee-approved guidelines. GAA activity and glycogen content analyses, Rotarod testing, and Western blot detection of CI-MPR was performed as described [13]. Wirehang testing was performed with a 0.5 cm mesh hardware cloth fixed to an 8 by 10 in. frame. Mice were placed on the wire mesh, which was slowly inverted 6 inches over a cage containing paper bedding. The latency, or time until the mouse fell into the cage, was recorded.

2.2. Statistical analyses

Comparison of two groups was assessed by a homoscedastic Student *t*-test. A *p*-value <0.05 was considered to be statistically significant.

3. Results

3.1. Enhanced efficacy from simultaneous ERT and β2-agonist administration: motor function, GAA uptake and glycogen clearance

Two β2-agonists were evaluated in combination with ERT, clenbuterol and albuterol. The dose of clenbuterol was reduced 5-fold from the concentration studied previously [13], to 6 mg/l in drinking water, while albuterol was evaluated at the higher dose (30 mg/l in drinking water). Groups of 3 month-old immune tolerant GAA-KO mice were treated with four weekly doses of rhGAA (20 mg/kg body weight), with or without concurrent β2-agonist treatment. Tolerant GAA-KO mice do not form anti-GAA antibodies or develop hypersensitivity reactions during ERT with rhGAA, in contrast to non-tolerant GAA-KO mice and patients with Pompe disease [2,16].

The efficacy of β2-agonist treatment was demonstrated by an increase in Rotarod and wirehang latency following low-dose clenbuterol treatment. Low-dose clenbuterol significantly increased Rotarod latency following 4 weeks of combined treatment, in comparison with ERT alone (Fig. 1A; $p<0.05$), although the mean latency was significantly elevated at week 0 in the clenbuterol-treated group without reaching statistical significance ($p=0.47$). Low-dose clenbuterol did not significantly increase wirehang latency (not shown). The weight of the gastrocnemius increased in mice treated with combined treatment, in comparison with GAA-KO mice treated with ERT alone (Fig. 1B), which suggested that muscle hypertrophy was stimulated as described by clenbuterol [17].

The efficacy of β2-agonist treatment was further demonstrated by an enhanced biochemical correction of striated muscle. GAA activity was not significantly increased in the heart or skeletal muscle following low-dose clenbuterol treatment (Fig. 1C); however, the glycogen content was reduced significantly in all striated muscles evaluated with the exception of tibialis anterior by clenbuterol treatment, in comparison with ERT alone (Fig. 1D). These biochemical data indicated that clenbuterol treatment increased the clearance of lysosomal glycogen without elevating GAA activity in the muscle homogenate.

Treatment with albuterol increased Rotarod latency only at 5 weeks, in comparison with GAA-KO mice treated with ERT alone (Fig. 2A), which suggested a later-onset effect from albuterol than for clenbuterol at a low (Fig. 1A) or high dosage [13]. Wirehang latency increased significantly following combined treatment, in comparison with ERT alone (Fig. 2B). GAA activity was increased only in the tibialis anterior by albuterol treatment, in comparison with ERT alone (Fig. 2C); however, albuterol significantly reduced the glycogen content in the quadriceps and soleus in comparison with ERT alone (Fig. 2D), despite the lack of increased GAA activity in those tissues (Fig. 2B). These biochemical data were consistent with the effect of low-dose clenbuterol, which increased the clearance of glycogen without significantly increasing GAA activity in the muscle homogenate (Figs. 1C–D).

3.2. Enhanced biochemical correction in the cerebellum following ERT and β2-agonist administration

The effect of β2-agonist treatment was further evaluated by biochemical evaluation of the brain. The cerebral and cerebellar hemispheres were analyzed separately, and a trend toward increased GAA activity was demonstrated in the cerebellum following clenbuterol ($p=0.08$) and albuterol ($p=0.09$) treatment (Fig. 3A). Glycogen content was reduced significantly by either clenbuterol or albuterol treatment in the cerebellum, but not in the cerebrum (Fig. 3B).

Histopathology was performed to examine brain involvement, and glycogen accumulations were detected in the cerebellum with ERT alone (Fig. 4A, arrows). Either albuterol (Fig. 4B) or clenbuterol (Fig. 4C) reduced the glycogen staining in the cerebellum, in comparison