Alglucosidase alfa treatment alleviates liver disease in a mouse model of glycogen storage disease type IV

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1. Introduction

Glycogen storage disease type IV (GSD IV) is caused by deficiencies in glycogen branching enzyme (GBE, EC 2.4.1.18), which results in deposition of less-branched, poorly soluble glycogen (polyglucosan) in multiple tissues [12]. Patients with progressive hepatic form of GSD IV often develop irreversible liver cirrhosis [3–5], and liver transplantation is the only treatment option [6–8].

In mammalian cells, the majority of glycogen is degraded in the cytosol by a combined action of glycogen phosphorylase (EC 2.4.1.1) and glycogen debranching enzyme (EC 2.4.1.25; EC 3.2.1.33). However, a small portion of glycogen (approximately 10%) is transported into lysosomes and hydrolyzed by the enzyme acid alpha-glucosidase (GAA, EC 3.2.1.20) [9–11]. In patients with GSD IV, glycogen deposition was observed in both cytosol and lysosomes of affected cells [12], indicating that normal activities of these enzymes may not be sufficient to timely clear this type of insoluble glycogen. Enzyme replacement therapy (ERT) with recombinant human GAA (rhGAA, Alglucosidase alfa) is an FDA approved therapy for Pompe disease where GAA is deficient. We speculate that enhanced GAA activity in lysosomes by rhGAA treatment will accelerate lysosomal glycogen clearance, promote glycogen transfer from cytosol to lysosomes, and thus reduce the overall glycogen deposition in GSD IV. In this study, we tested our hypothesis in a mouse model of GSD IV [13].

2. Material and methods

2.1. Animals and treatment

The GSD IV (Gbe1ys/ys) mouse colony harboring the common Y329S mutation in the Gbe1 gene was kindly provided by Drs. Craig and Akman at Baylor College of Medicine [13,14]. The affected mice have low residual GBE activity and widespread, progressive increase of glycogen deposition in liver, skeletal muscles, and the brain [13]. Alglucosidase alfa (rhGAA) was provided by Roviant Sciences, who purchased from Clinigen CTS Ltd. (Burton-on-Trent, Staffordshire, UK). Male GSD IV mice were intravenously (tail vein) injected with 20 mg/kg (n = 6) or 40 mg/kg (n = 6) rhGAA weekly for 4 weeks starting at age of 10 weeks. Age-matched untreated mice (n = 8) were used as controls (UT). All mice were sacrificed 48 h after the last rhGAA injection following overnight fasting. Blood was collected from the posterior vena cava and plasma was used for testing liver enzyme activities (IDEXX Laboratories, Inc. Westbrook, Maine). Fresh tissue specimens were immediately frozen on dry ice and stored at –80 °C. All animal procedures were done in accordance with Duke University Institutional Animal Care and Use Committee-approved guidelines.

2.2. Measurement of tissue glycogen and GAA activity

Tissue GAA activity was analyzed as described [15]. Glycogen content was measured using a modified method that is suitable for GSD IV tissues [16]. Protein concentration was measured using BCA method.
2.3. Statistics

Data were presented as mean ± standard deviation. The significance of differences from untreated mice (UT) was assessed using two-tailed, equal variance Student t-test.

3. Results

3.1. Uptake of rhGAA by tissues of GSD IV mice

Significant increase in GAA activity was observed in most tissues of GAA-treated mice in a dose dependent manner (Fig. 1A). The greatest increase was found in liver, which had 29 and 48-fold increase over UT controls in the 20 mg/kg dose group and 40 mg/kg dose group, respectively. GAA activity in heart had a 1.7-fold increase in the 20 mg/kg dose group and 2.8-fold increase in the 40 mg/kg group. In quadriceps the increase in GAA activity was negligible at either dosage, while uptake by gastrocnemius was slightly more, with 1.1-fold increase of GAA activity in either treated group. Diaphragm had the highest GAA activity increase among the skeletal muscles, with increases of GAA activity similar to those in heart by the 40 mg/kg treatment.

3.2. Reduction of tissue glycogen accumulation

Significant reduction of glycogen accumulation was observed in liver (−21%) but not in any skeletal muscle of mice treated with 40 mg/kg GAA. The low level of glycogen in heart of this GSD IV mouse model makes it difficult to draw a conclusion for this tissue (Fig. 1B). The 20 mg/kg GAA treatment did not reduce glycogen in any tissue (Fig. 1B).

3.3. Alleviation of liver symptoms by rhGAA treatment

The 40 mg/kg rhGAA treatment lowered liver/body weight ratio from 5.8 ± 0.2% to 5.0 ± 0.2% (p < 0.05; Fig. 1C), and reduced plasma alanine aminotransferase (ALT) from 1029 ± 87 U/L to 650 ± 32 U/L (p < 0.01; Fig. 1D) and aspartate aminotransferase (AST) from 1059 ± 93 U/L to 849 ± 50 U/L (p = 0.074; Fig. 1E), indicating alleviation of hepatomegaly and liver damage.

4. Discussion

Manose-6-phosphate receptor (M6PR)-mediated ERT with rhGAA is an FDA approved therapy for Pompe disease. The pattern of rhGAA uptake by tissues of GSD IV mice (Fig. 1A) was similar to that observed in Pompe disease mice, which correlated well with the relative abundances of M6PR in these tissues [17,18]. Significant biochemical correction of liver glycogen accumulation was achieved by the 40 mg/kg rhGAA treatment, which was accompanied by the reduction of liver size (liver/body weight ratio) and of liver enzymes in serum. Though the 20 mg/kg treatment led to high GAA activity in liver, the reduction of glycogen accumulation was not statistically significant (Fig. 1B). This suggests that the insolubility of GSD IV glycogen makes it highly resistant to rhGAA digestion. Therefore, it is not surprising to see the lack of effectiveness in the skeletal muscles, which showed low uptake of rhGAA (Fig. 1B). Our interpretation is that digestion of insoluble GSD IV glycogen in lysosomes requires highly elevated GAA activity. Clearance of lysosomal glycogen subsequently promotes glycogen trafficking from cytoplasm into lysosomes, and thus reduces the overall glycogen accumulation. It is also possible that the excessive amount of rhGAA in lysosomes led to leakage of the enzyme into the cytoplasm and directly degraded the accumulated glycogen, even though the enzyme is less active in the neutral pH environment than in the acidic lysosome interior [19].

One advantage of treating GSD IV with rhGAA is that, as patients express normal level of GAA, there is unlikely an adverse immune response, which has been a major obstacle in treatment of Pompe disease [20–22]. Our data suggests that rhGAA could be potentially used for liver protection in GSD IV, and possibly in other GSDs that involve liver glycogen storage.
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Conflict of interest

Drs. Baodong Sun and Priya Kishnani have developed the technology that is being used in the study. If the technology is commercially successful in the future, the developers and Duke University may benefit financially. The other authors declare no conflict of interest.

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