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Salmeterol enhances the cardiac response to gene therapy in Pompe disease

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ABSTRACT

Enzyme replacement therapy (ERT) with recombinant human (rh) acid α-glucosidase (GAA) has prolonged the survival of patients. However, the paucity of cation-independent mannose-6-phosphate receptor (CI-MPR) in skeletal muscle, where it is needed to take up rhGAA, correlated with a poor response to ERT by muscle in Pompe disease. Clenbuterol, a selective β2 receptor agonist, enhanced the CI-MPR expression in striated muscle through Igf-1 mediated muscle hypertrophy, which correlated with increased CI-MPR (also the Igf-2 receptor) expression. In this study we have evaluated 4 new drugs in GAA knockout (KO) mice in combination with an adeno-associated virus (AAV) vector encoding human GAA, 3 alternative β2 agonists and dehydroepiandrosterone (DHEA). Mice were injected with AAV2/9-CBhGAA (1E + 11 vector particles) at a dose that was not effective at clearing glycogen storage from the heart. Heart GAA activity was significantly increased by either salmeterol ($p < 0.01$) or DHEA ($p < 0.05$), in comparison with untreated mice. Furthermore, glycogen content was reduced in the heart by treatment with DHEA ($p < 0.001$), salmeterol ($p < 0.05$), formoterol ($p < 0.01$), or clenbuterol ($p < 0.01$) in combination with the AAV vector, in comparison with untreated GAA-KO mice. Wirehang testing revealed that salmeterol and the AAV vector significantly increased performance, in comparison with the AAV vector alone ($p < 0.001$). Similarly, salmeterol with the vector increased performance significantly more than any of the other drugs. The most effective individual drugs had no significant effect in absence of vector, in comparison with untreated mice. Thus, salmeterol should be further developed as adjunctive therapy in combination with either ERT or gene therapy for Pompe disease.

1. Introduction

Pompe disease (glycogen storage disease type II; acid maltase deficiency; MIM 232300)) is a devastating myopathy resulting from acid α-glucosidase (GAA; acid maltase; EC 3.2.1.20) deficiency in cardiac and skeletal muscle. Enzyme replacement therapy (ERT) with recombinant human (rh) GAA has prolonged the survival of patients, which has increased the understanding of pathology and extent of disease in infantile Pompe disease. Even in patients with a good response to ERT, residual muscle weakness (neck flexor weakness, dorsiflexor weakness, myopathic facies, ptosis and strabismus) and respiratory dysfunction has been observed in multiple cases [1–3]. Thus, the correction of neuromuscular involvement has not been possible in Pompe disease, despite adherence to standard-of-care ERT.

The paucity of CI-MPR in mammalian adult muscle has underscored the concept that CI-MPR is limiting for ERT in Pompe disease [4,5]; moreover, we have been the first to directly address this problem [6,7]. We demonstrated that increased CI-MPR expression improved efficacy from ERT in GAA knockout (KO) mice, confirming the relevance of CI-MPR expression upon GAA replacement therapy in Pompe disease [7]. Using GAA-KO mice, we showed that clenbuterol, a selective β2 receptor agonist, enhanced the CI-MPR expression in muscle tissues, and increased the efficacy of either ERT or gene therapy in murine Pompe disease [6–8]. The underlying mechanism of clenbuterol's therapeutic action is Igf-1 mediated muscle hypertrophy, which has correlated with increased CI-MPR (also the Igf-2 receptor) expression [9].

In this study we have evaluated 4 new drugs in GAA-KO mice in combination with an adeno-associated virus (AAV) vector encoding human GAA. The dosage for each drug was selected to induce muscle hypertrophy with an associated increased expression of CI-MPR, analogous to clenbuterol's effects [6–8]. Three alternative β2 agonists and dehydroepiandrosterone (DHEA) were tested, given that these drugs were expected to upregulate both Igf-1 and downstream Igf-2R/CI-MPR, similar to clenbuterol [9–11].

2. Results

Mice were injected with AAV2/9-CBhGAApA [1E + 11 vector particles (vp)] at a dose previously found to be partially effective at clearing glycogen storage from the heart following the induction of immune tolerance to GAA [12]. Drugs were dosed continuously at dosages determined from the literature (Table 1). After 18 weeks striated muscles

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Table 1
Small molecule therapies evaluated in combination with gene therapy.

Drug	Dose to induce hypertrophy[a]	Reference
Clenbuterol	6 mg/l	[7]
Fenoterol	30 mg/l	[20]
Formoterol	4 mg/l	[26]
Salmeterol	30 mg/l	[21]
Dehydroepiandrosterone	250 mg/l	[10,11]

[a] Administered in drinking water.

were analyzed for GAA and glycogen content. Heart GAA activity was significantly increased by either salmeterol ($p < 0.01$) or DHEA ($p < 0.05$), in comparison with untreated GAA-KO mice (Fig. 1A). Furthermore, glycogen content was reduced by treatment with DHEA ($p > 0.001$), salmeterol ($p < 0.05$), formoterol ($p < 0.01$), or clenbuterol ($p < 0.01$) in combination with the AAV vector, in comparison with untreated mice (Fig. 1A). In contrast, the AAV vector failed to reduce the glycogen content of heart by itself, in comparison with no treatment. The reduction of glycogen content in absence of significantly increased GAA activity has been observed following the addition of an adjunctive β2 agonist [8]. Of note, glycogen content of the heart and skeletal muscle remained highly elevated in comparison with the nearly undetectable amounts of glycogen observed in the tissues of wildtype mice [13]. The GAA activity and glycogen content of the diaphragm and quadriceps were not affected by any of the treatments (Fig. 1B), consistent with data showing that heart muscle is more responsive to GAA replacement than skeletal muscle [14].

Functional testing was performed subsequently, and the wirehang test at 18 weeks following vector administration revealed that the combination of salmeterol and the AAV vector significantly increased latency in comparison with the AAV vector alone ($p < 0.001$). Similarly, salmeterol with the vector increased latency significantly more than either DHEA ($p < 0.001$), formoterol ($p < 0.05$), fenoterol ($p < 0.05$), or clenbuterol ($p < 0.05$) with the vector (Fig. 2A). No significant difference in body weight was observed between any of the treatments (Fig. 2B).

An important consideration with regard to adjunctive therapy is whether any effects are due to the adjuvant rather than the combined treatment. The most effective individual drugs, salmeterol and DHEA, were evaluated by themselves. No significant effect upon GAA activity of heart, glycogen content of heart, or wirehang latency was observed, in comparison with untreated mice (Fig. 3).

Further evaluation of CI-MPR and LC3 was performed by Western blotting (Fig. 4). Despite evidence that CI-MPR increased in skeletal muscle following β2 agonist administration [6–8], statistically significant increases were not observed in heart or quadriceps following administration of the 4 β2 agonist s in this study for 18 weeks (Fig. 4A–B). However, the abnormally increased LC3-II previously described in the muscle of GAA-KO mice [15] was significantly reduced in heart by administration of each of the 4 β2 agonists (Fig. 4B). Furthermore, administration of propranolol, a beta-blocker that increased the uptake of GAA but reduced efficacy from ERT [16], failed to reduce LC3-II (Fig. 4A,C). Reductions in LC3-II were consistent with reversal of abnormally accumulated autophagosomes previously described in GAA-KO mice [15,17].

3. Discussion

Three alternative β2 agonists and dehydroepiandrosterone (DHEA) were evaluated in combination with gene therapy in GAA-KO mice. These drugs shared the ability to promote muscle hypertrophy, are available in the US, and were well-tolerated in rodent experiments. Mice were injected with AAV2/9-CBhGAA at a dose previously found to be partially effective at clearing glycogen storage from the heart. Heart GAA activity was significantly increased by either salmeterol or DHEA, in comparison with untreated mice. Furthermore, glycogen content was reduced by treatment with DHEA, salmeterol, formoterol, or clenbuterol in combination with the AAV vector. Functional testing with the wirehang test revealed that the combination of salmeterol and the AAV vector significantly increased latency in comparison with

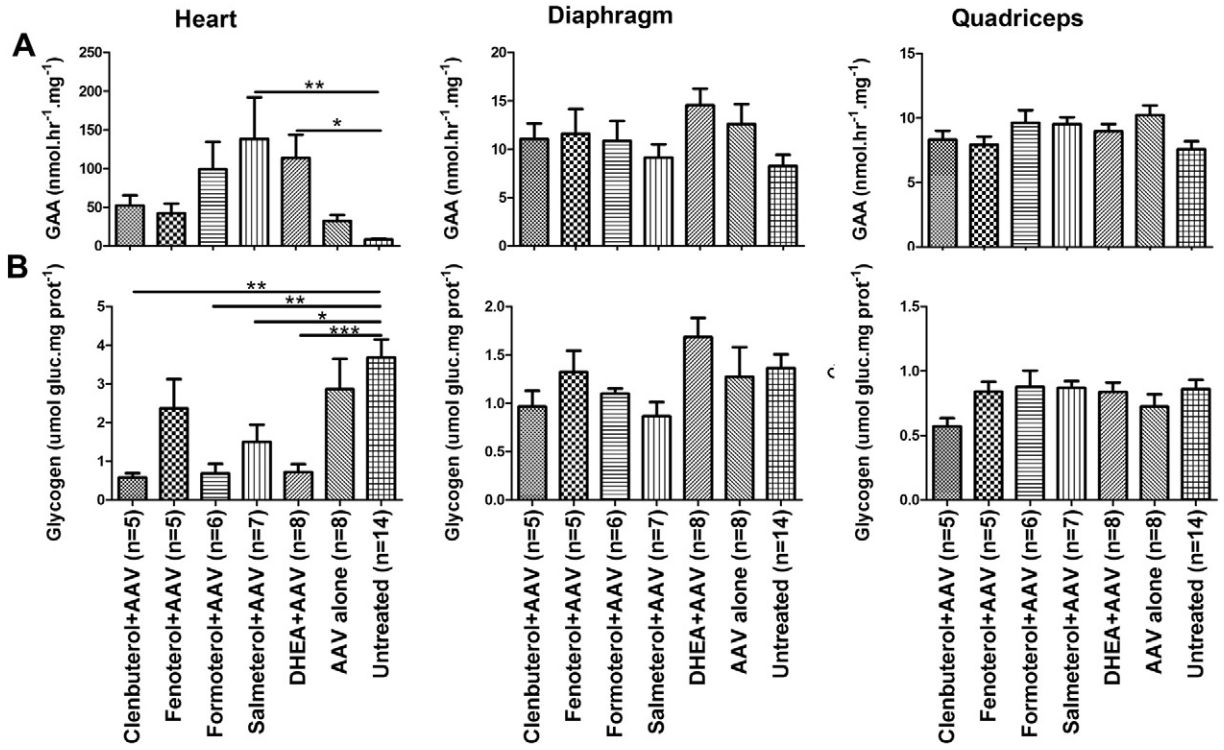

Fig. 1. Biochemical correction of striated muscle following AAV vector administration and adjunctive small molecule therapy. GAA-KO mice were treated for AAV2/9-CBhGAApa (AAV) either alone or in combination with each of the adjunctive drugs (Table 1). Drug was continued for the duration of the experiment. (A) GAA, and (B) glycogen content. Mean ± SEM is shown. $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).