Published in final edited form as:

Mol Genet Metab. 2020 February ; 129(2): 73–79. doi:10.1016/j.ymgme.2019.10.005.

Evaluation of antihypertensive drugs in combination with enzyme replacement therapy in mice with Pompe disease

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Abstract

Pompe disease is caused by the deficiency of lysosomal acid α -glucosidase (GAA) leading to progressive myopathy. Enzyme replacement therapy (ERT) with recombinant human (rh) GAA has limitations, including inefficient uptake of rhGAA in skeletal muscle linked to low cation-independent mannose-6-phosphate receptor (CI-MPR) expression.

Purpose: To test the hypothesis that antihypertensive agents causing muscle hypertrophy by increasing insulin-like growth factor 1 expression can increase CI-MPR-mediated uptake of recombinant enzyme with therapeutic effects in skeletal muscle.

Methods: Three such agents were evaluated in mice with Pompe disease (carvedilol, losartan, and propranolol), either with or without concurrent ERT.

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AUTHOR CONTRIBUTIONS

Sang-oh Han: Performed research, Analyzed data

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Conflict of interest:

DDK and PSK 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. DDK has received research/grant support from Sanofi Genzyme Corporation in the past, and rhGAA for these studies was supplied by Sanofi Genzyme.

Results: Carvedilol, a selective β -blocker, increased muscle strength but reduced biochemical correction from ERT. Administration of drugs alone had minimal effect, with the exception of losartan that increased glycogen storage and mortality either by itself or in combination with ERT.

Conclusion: The β -blocker carvedilol had beneficial effects during ERT in mice with Pompe disease, in comparison with propranolol or losartan. Caution is warranted when prescribing antihypertensive drugs in Pompe disease.

Keywords

Mannose-6-phosphate receptor; acid alpha-glucosidase; acid maltase; glycogen storage disease type II

INTRODUCTION

Pompe disease is a fatal, degenerative myopathy caused by deficiency of an enzyme called acid α -glucosidase (GAA). In infantile-onset Pompe disease, untreated patients typically die from cardiorespiratory failure before the age of one. Although enzyme replacement therapy (ERT) with recombinant human GAA (rhGAA) has prolonged the survival of infants with Pompe disease [1], effective dosages are up to 100-fold greater than those used in other lysosomal disorders [2]. New therapy for late-onset Pompe disease (LOPD) is needed, because ERT stabilized but did not reverse skeletal and respiratory muscle involvement [3, 4]. One major limitation of ERT stems from the inefficient uptake of rhGAA into skeletal muscle due to low cation-independent mannose-6-phosphate receptor (CI-MPR) expression.

Agents that cause muscle hypertrophy by increasing insulin-like growth factor 1 (Igf-1) expression, such as the β 2-agonists clenbuterol and albuterol, can increase CI-MPR-mediated uptake of rhGAA with beneficial effects on skeletal muscle in Pompe disease. Adjunctive therapy with β 2-agonists improved efficacy from ERT in preclinical experiments and in a Phase I clinical trial in patients with LOPD [5–7]. However, additional agents that increase CI-MPR-mediated uptake of rhGAA might be needed to optimally treat Pompe disease. We hypothesized that agents causing muscle hypertrophy might upregulate CI-MPR expression or increase CI-MPR recycling, and thereby enhance the uptake and lysosomal targeting of GAA. The underlying mechanism of this therapeutic action is Igf-1 mediated muscle hypertrophy, which has been correlated with increased CI-MPR expression [5]. Increased uptake of GAA would enhance biochemical and functional correction in Pompe disease, which can be tested in GAA knockout (KO) mice. We expect that small molecule drugs that induce muscle hypertrophy might be well tolerated and prove effective for clinical use as adjunctive therapy in Pompe disease.

Previously we studied a beta-blocker, propranolol, in GAA-KO mice being treated with ERT, because propranolol blocks the effects of β 2-agonists that are known to enhance ERT on Pompe disease [5–7]. That study confirmed that propranolol could have detrimental effects during ERT [8]. The risk of beta-blockers exacerbating the myopathy in patients with Pompe disease has not been studied, but there are several case reports of beta-blockers causing a myopathy in patients with other conditions [9–11]. Thus, the risk for beta blockade in Pompe disease warrants further study.

This study evaluated three antihypertensive agents in combination with ERT in GAA-KO mice: carvedilol, losartan, and propranolol. The effect of each drug was evaluated with regard to biochemical correction and muscle function, in comparison with ERT alone. The beneficial drug, carvedilol, was further evaluated with regard to their effects on CI-MPR expression and autophagy, because improved uptake and trafficking of GAA to lysosomes might favorably impact the abnormalities of autophagy associated with Pompe disease, in addition to improving biochemical correction and muscle function.

MATERIALS AND METHODS

Animal Studies

All animal procedures were performed under the guidelines of Duke University Institutional Animal Care and Use Committee. Two-month old GAA-KO mice underwent wirehang testing before initiation of drug treatments. The experiments included the following groups of GAA-KO mice that survived to be analyzed, including both males and females of approximately equal number: no treatment control (n=13); ERT alone (n=10); ERT with carvedilol (n=10); ERT with losartan (n=7); ERT with propranolol (n=9); losartan alone (n=6); carvedilol alone (n=10). Mortality occurred as follows: ERT alone (n=2), ERT with losartan (n=3), losartan alone (n=4), ERT with propranolol (n=2).

Drugs were administered as indicated (Table 1). One week following initiation of drug therapy, intravenous rhGAA (20 mg/kg; Genzyme Corporation) was administered weekly through tail-vein injection for 4 weeks. Five days after the last injection of rhGAA, the wirehang test was performed to evaluate muscle function. Two days later mice were euthanized for evaluation of body weight and left ventricular mass (after removing the right ventricle). Skeletal muscle mass was measured using the gastrocnemius of hind limb. Several tissues including cardiac, skeletal muscle, and liver were collected and frozen prior to further evaluation including anti-GAA IgG1 ELISA, GAA activity, and glycogen content as described [5, 12].

Western blotting

Western blots on skeletal muscle were performed to determine the relative protein levels of CI-MPR and LC3. Protein was prepared by tissue homogenization with radioimmunoprecipitation assay (RIPA) buffer followed by microcentrifugation for 20 minutes at 14,000 rpm. Total protein amount of the samples were determined by the Pierce™ BCA protein assay kit (Thermo Fisher Scientific, Inc., Waltham, MA). The desired quantity of protein was mixed with SDS loading buffer followed by boiling 5 minutes. Samples were electrophoresed using either a 4% (for CI-MPR) or 15% (for LC3) SDS-PAGE gel and immediately transferred to a nitrocellulose membrane (Bio-Rad) using transfer buffer 3.03g/l Tris, 14.4 g/l glycine, 7.5% v/v methanol). Following transfer, the membrane was blocked in 5% skim milk in wash buffer (phosphate-buffered saline with 0.5% tween-20, PBST) to block from non-specific binding of antibodies. The membrane was incubated overnight at 4°C with primary antibodies: an anti-CI-MPR rabbit polyclonal antibody (kindly provided by Dr. Peter Lobel; Rutgers University) or anti-LC3 antibody from Sigma (St. Louis, MO). An anti-GAPDH antibody from Cell Signaling Technology

(Danvers, MA) was used as a loading control. After 3 washes with wash buffer, the membrane was incubated with horseradish peroxidase (HRP) conjugated secondary antibodies that were chosen by the species of the primary antibody (5% w/v bovine serum albumin in PBST for 1 hr at room temperature). Following one more wash, blots were developed with Amersham ECL prime Western blotting detection reagent from GE Healthcare (Pittsburgh, PA). Signal intensities were quantified using Image J software (NIH). Finally, the intensity for signals for CI-MPR and LC3 were normalized to the signal for the housekeeping gene, GAPDH.

Cell Studies

For uptake experiments, Pompe fibroblasts (GM04912; Coriell Cell Repository, Camden, NJ) were treated with 50 μ M albuterol and/or 250 nM carvedilol in combination with 30 μ g/mL rhGAA, for 5 h prior to analysis. The extent of enzyme uptake was determined by assaying GAA activity in cell lysates using the fluorescent substrate, 4-methylumbelliferyl- α -glucoside. All analyses were carried out in triplicate within three independent experiments.

Statistical analysis

Data analysis was performed with GraphPad Prism 6 (GraphPad Software, Inc). Multiple comparisons were performed using One Way ANOVA with Dunnett's Multiple Comparison test. The statistical significance of comparisons is indicated as follows (* = <0.05 ; ** = <0.01 , *** = <0.001 , **** = <0.0001). Survival analysis was performed with log-range (Mantel-Cox) test for comparisons between groups.

RESULTS

Four distinct drugs were co-administered with ERT to GAA-KO mice with Pompe disease at the dosages anticipated to induce muscle hypertrophy (Table 1). These four drugs were tested given their ability to increase muscle mass [13–16], which likely stems from upregulation of the Igf-1 axis including Igf-2R/CI-MPR downstream of Igf-1 [17–19]. Mice were evaluated 5 weeks following the initiation of drug therapy and ERT, and compared with mice that received ERT alone or no treatment.

Biochemical analyses of the heart and skeletal muscle revealed that GAA activity in the heart was significantly increased following either losartan or propranolol administration with ERT, in comparison with untreated control mice (Fig. 1A). No drug significantly increased GAA activity in the diaphragm during ERT (Fig. 1B). Either carvedilol or propranolol significantly increased GAA activity in the quadriceps, in comparison with untreated control mice (Fig. 1C). However, only carvedilol administration significantly increased GAA activity in quadriceps, in comparison with ERT alone (Fig. 1C). The latter effect suggested increased GAA uptake in response to carvedilol. Unexpectedly, treatment with each of the three antihypertensives increased the glycogen content of heart, in comparison with ERT alone (Fig. 1A). Propranolol administration uniquely increased the glycogen content of diaphragm, in comparison with ERT alone (Fig. 1B). Furthermore, propranolol administration increased the glycogen content of quadriceps, in comparison with each of the

other treatment groups (Fig. 1C), as previously demonstrated [8]. Of note, either losartan or propranolol administration increased GAA uptake in the heart, while impairing the ability of GAA to reduce glycogen storage in the heart.

The interaction of each agent with ERT was further evaluated by its effect upon weight and muscle mass. Losartan significantly reduced weight gain, in comparison with carvedilol plus ERT, ERT alone, or no treatment (Fig. 2A). Left ventricular weight was reduced for each condition, in comparison with ERT, reflecting the effect of ERT upon cardiac hypertrophy (Fig. 2B). However, left ventricle weight was further reduced by the addition of losartan during ERT, comparison with each other condition (Fig. 2B). The gastrocnemius weight was reduced by losartan administration, in comparison with carvedilol plus ERT, ERT alone, or no treatment (Fig. 2C). Propranolol administration also reduced gastrocnemius weight, in comparison with no treatment (Fig. 2C). Thus, losartan or propranolol administration reduced skeletal muscle weight indicating muscle atrophy in response to these two agents. Furthermore, losartan administration uniquely reduced body and left ventricular weight, suggesting generalized muscle atrophy. Antibody responses can interfere with the benefits of ERT, and therefore anti-GAA IgG antibodies were quantified. Anti-GAA was significantly increased by carvedilol, in comparison with untreated mice (Fig. 2D). However, the presence of increased anti-GAA in the carvedilol groups was not sufficient to prevent the correction of GAA deficiency in the skeletal muscle (Fig. 1C). Overall, the physiologic effects of losartan treatment were potentially harmful, whereas carvedilol improved muscle strength.

Muscle function was evaluated by wirehang testing, and only carvedilol treatment increased the change in holding impulse, in comparison with propranolol plus ERT or no treatment (Fig. 3). Cardiac function was evaluated by monitoring heart rate, blood pressure, and ejection fraction (Fig. 3B–E), which revealed significantly decreased heart rate for mice treated with losartan, in comparison with ERT alone (Fig. 3B). However, blood pressure and ejection fraction were equivalent between all groups (Fig. 3C–E).

The potential effects of agents that interacted with ERT were further evaluated in absence of ERT (Fig. 4). The glycogen content of heart and diaphragm were unaffected by treatment with losartan or carvedilol (Fig. 4A–B). However, losartan treatment increased the glycogen content of the quadriceps, in comparison with carvedilol treatment or no treatment (Fig. 4C). Losartan caused weight loss, in comparison with carvedilol or no treatment (Fig. 4D). Similarly, losartan treatment reduced heart weight in comparison with other treatments or no treatment (Fig. 4E). Unexpectedly, losartan increased the change in the holding impulse, in comparison with no treatment (Fig. 4F). Consistent with the effect of carvedilol with ERT, carvedilol alone also increased the holding impulse (Fig. 4F).

The negative effects of losartan were demonstrated by increased mortality, either alone or in combination with ERT, in comparison with no treatment (Fig. 5). ERT caused increased mortality in GAA-KO mice related to anti-GAA antibody formation and associated hypersensitivity reactions [12, 20, 21]. However, despite the presence of deaths among the ERT only group, mortality was not significantly increased in absence of losartan treatment (Fig. 5).

We next analyzed the steady state level of the CI-MPR in the heart from mice treated with ERT in the absence or presence of carvedilol. As shown in Figure 6, ERT alone or ERT with carvedilol decreased CI-MPR expression in the heart. Western blot analysis of LC3-II levels showed decreased LC3 in mice treated with ERT that was maintained following carvedilol administration (Fig. 6C). However, propranolol prevented the reduction in LC3 observed following ERT (Fig. 6C).

Lastly, we carried out uptake experiments using recombinant human GAA in Pompe fibroblasts to confirm the benefit of combined carvedilol and albuterol treatment. These results demonstrated that enzyme uptake was increased in the presence of 250 nM carvedilol and that this increase was similar to the effect of the β -agonist albuterol (Figure 7). Analogous to propranolol, higher doses of carvedilol ($>5 \mu\text{M}$) were inhibitory to enzyme uptake (data not shown), indicating that the beneficial effects of this drug are dose-dependent in cells. These data support the positive effects seen in mice treated with ERT in combination with carvedilol.

DISCUSSION

In this study, we demonstrated the beneficial effects of carvedilol on enzyme replacement therapy in Pompe mice by enhancing GAA uptake and improving neuromuscular function assessed by wirehang testing. The mechanistic and clinical implications of these findings are discussed below.

Studies in GAA-KO mice have shown that the addition of β 2-agonists (clenbuterol and albuterol) during ERT increased CI-MPR with therapeutic effects in skeletal muscle [5–7]; moreover, the β -blocker propranolol reduced efficacy of ERT in mice with Pompe disease [8]. Carvedilol features biased β 2-blockade that block G-protein signals, but activate β -arrestin signals [22]. Therefore, we hypothesized that carvedilol might have advantages for the treatment of patients with Pompe disease due to the activation of β -arrestin signaling in skeletal muscles, while retaining the ability to block G-protein signaling in heart. In contrast, propranolol completely blocks the effects of β 2-agonists upon skeletal muscle [23], and had detrimental effects during ERT in Pompe disease [8]. In this study the cell based uptake experiments in Pompe fibroblasts confirmed that low doses of carvedilol alone (250 nM) can stimulate enzyme uptake into fibroblasts, which correlated with the increased GAA uptake by skeletal muscle in carvedilol-treated mice. The ability of drugs within the same class (e.g. β 2-agonists or blockers) to exert unique or opposing effects on enzyme uptake or ERT is somewhat surprising from a mechanistic standpoint. Further studies are needed to address the basis for these differences.

Losartan was considered a promising candidate in this study, because it upregulated Igf-1 expression in rodent muscle, and protected against the muscle effects of sarcopenia [14]. However, losartan caused mortality either alone or in combination with ERT, when we administered this treatment to mice with Pompe disease. Beneficial effects from losartan have been reported with regard to protecting against: 1) CCl_4 -induced skeletal muscle injury at the same dose [24]; 2) heart fibrosis in a model for long-term exercise injury [25]; 3) fibrosis in the muscle of mice with Duchenne muscular dystrophy [26] or laminin-deficient

muscular dystrophy [27]. No effect on heart function was detected following losartan treatment, other than a slight decrease in heart rate. Therefore, the toxicity observed following losartan administration in mice with Pompe disease was unexpected and remains unexplained.

While both losartan and carvedilol increased GAA activity in several muscles, an unexpected increase in glycogen content in these tissues was also noted following treatment with these drugs. This suggests that the GAA that is taken up from the bloodstream may not reach the lysosome in sufficient quantity to clear glycogen. These drugs may therefore exert complex effects on intracellular trafficking such that endocytosis of therapeutic enzyme is favored (by enhancing CI-MPR recycling to the plasma membrane or increasing receptor expression) but the movement of enzyme through the endosomal network is impaired. CI-MPR was decreased following ERT alone or ERT with carvedilol, which could reflect internalization and degradation of CI-MPR following the delivery of GAA to the lysosomes. In light of this, it may be possible to combine carvedilol, a selective β -blocker, with additional drugs that increase flux through endosomes to achieve optimal enzyme uptake and glycogen clearance.

Limitations of the current study include the reliance on the GAA-KO mouse model to study drugs that are prescribed to patients with Pompe disease. Furthermore, the β -blockers carvedilol and propranolol unexpectedly had no effect on heart rate, which might effect the dosing regimen or the effect of sedation used during cardiac evaluations in GAA-KO mice. This preclinical study justifies further clinical research to better understand the effects of antihypertensives in Pompe disease.

Valuable information has been obtained by the evaluation of drugs under consideration for clinical use in Pompe disease using the available mouse model. We previously found that propranolol was detrimental in the animal model, interfering with the effect of ERT and causing disease progression [8]. Currently we demonstrated unique toxicity from the administration of losartan in mice with Pompe disease. Carvedilol was well-tolerated, and the ability to use a β -blocker in patients that will not interfere with ERT would be highly valuable for clinical use in patients with Pompe since they often require a β -blocker to mitigate disease-associated hypertension. A clinical trial of carvedilol in patients with Pompe disease should be considered to further evaluate its usefulness.

ACKNOWLEDGEMENTS

This study was supported by the Alice and Y.-T. Chen Pediatric Genetics and Genomics Center, and a grant from the National Mucopolysaccharidoses (MPS) Society to Drs. Steet and Koeberl. Research reported in this publication was supported by the National Center for Advancing Translational Sciences of the National Institutes of Health under Award Number UL1TR001117. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. DDK was supported by NIH Grant R01 HL081122 from the National Heart, Lung, and Blood Institute, and by NIH Grant R01AR065873 from the National Institute of Arthritis and Musculoskeletal and Skin Disorders. DMA was supported by NIH grant HL125905-01 from the National Heart, Lung, and Blood Institute. RS was supported by an NIH Grant R01 GM086524 from the National Institute of General Medical Sciences. GAA-KO mice were provided courtesy of Dr. Nina Raben at the National Institute of Arthritis and Musculoskeletal and Skin Disorders, and rhGAA (Myozyme®; alglucosidase alfa) was provided by Sanofi Genzyme.

REFERENCES

1. Kishnani PS, Corzo D, Nicolino M, et al. Recombinant human acid {alpha}-glucosidase: Major clinical benefits in infantile-onset Pompe disease. *Neurology* 68 (2007) 99–109 [PubMed: 17151339]
2. Desnick RJ. Enzyme replacement and enhancement therapies for lysosomal diseases. *J Inher Metab Dis* 27 (2004) 385–410 [PubMed: 15190196]
3. van der Ploeg AT, Clemens PR, Corzo D, et al. A randomized study of alglucosidase alfa in late-onset Pompe's disease. *N Engl J Med* 362 (2010) 1396–1406 [PubMed: 20393176]
4. Strothotte S, Strigl-Pill N, Grunert B, et al. Enzyme replacement therapy with alglucosidase alfa in 44 patients with late-onset glycogen storage disease type 2: 12-month results of an observational clinical trial. *J Neurol* 257 (2010) 91–97 [PubMed: 19649685]
5. Koeberl DD, Luo X, Sun B, et al. Enhanced efficacy of enzyme replacement therapy in Pompe disease through mannose-6-phosphate receptor expression in skeletal muscle. *Mol Genet Metab* 103 (2011) 107–112 [PubMed: 21397538]
6. Koeberl DD, Li S, Dai J, et al. beta2 Agonists enhance the efficacy of simultaneous enzyme replacement therapy in murine Pompe disease. *Mol Genet Metab* 105 (2012) 221–227 [PubMed: 22154081]
7. Koeberl DD, Austin S, Case LE, et al. Adjunctive albuterol enhances the response to enzyme replacement therapy in late-onset Pompe disease. *FASEB J* 28 (2014) 2171–2176 [PubMed: 24443373]
8. Han SO, Pope R, Li S, et al. A beta-blocker, propranolol, decreases the efficacy from enzyme replacement therapy in Pompe disease. *Mol Genet Metab* 117 (2016) 114–119 [PubMed: 26454691]
9. Teicher A, Rosenthal T, Kissin E, and Sarova I. Labetalol-induced toxic myopathy. *Br Med J (Clin Res Ed)* 282 (1981) 1824–1825
10. Snyder S. Metoprolol-induced polymyalgia-like syndrome. *Ann Intern Med* 114 (1991) 96–97
11. Forfar JC, Brown GJ, and Cull RE. Proximal myopathy during beta-blockade. *Br Med J* 2 (1979) 1331–1332 [PubMed: 519436]
12. Sun B, Bird A, Young SP, et al. Enhanced response to enzyme replacement therapy in Pompe disease after the induction of immune tolerance. *Am J Hum Genet* 81 (2007) 1042–1049 [PubMed: 17924344]
13. Dalla Libera L, Ravara B, Gobbo V, et al. Skeletal muscle myofibrillar protein oxidation in heart failure and the protective effect of Carvedilol. *J Mol Cell Cardiol* 38 (2005) 803–807 [PubMed: 15850574]
14. Burks TN, Andres-Mateos E, Marx R, et al. Losartan restores skeletal muscle remodeling and protects against disuse atrophy in sarcopenia. *Sci Transl Med* 3 (2011) 82ra37
15. Kirk SP, Whittle MA, Oldham JM, Dobbie PM, and Bass JJ. GH regulation of the Type 2 IGF receptor in regenerating skeletal muscle of rats. *J Endocrinol* 149 (1996) 81–91 [PubMed: 8676058]
16. Demling RH. Comparison of the anabolic effects and complications of human growth hormone and the testosterone analog, oxandrolone, after severe burn injury. *Burns* 25 (1999) 215–221 [PubMed: 10323605]
17. Debonneuil EH, Quillard J, and Baulieu EE. Hypoxia and dehydroepiandrosterone in old age: a mouse survival study. *Respir Res* 7 (2006) 144 [PubMed: 17176479]
18. Morales AJ, Haubrich RH, Hwang JY, Asakura H, and Yen SS. The effect of six months treatment with a 100 mg daily dose of dehydroepiandrosterone (DHEA) on circulating sex steroids, body composition and muscle strength in age-advanced men and women. *Clin Endocrinol (Oxf)* 49 (1998) 421–432 [PubMed: 9876338]
19. Matsumoto T, Akutsu S, Wakana N, et al. The expressions of insulin-like growth factors, their receptors, and binding proteins are related to the mechanism regulating masseter muscle mass in the rat. *Arch Oral Biol* 51 (2006) 603–611 [PubMed: 16513081]
20. Raben N, Danon M, Gilbert AL, et al. Enzyme replacement therapy in the mouse model of Pompe disease. *Molecular Genetics and Metabolism* 80 (2003) 159–169 [PubMed: 14567965]

21. Sun B, Kulis MD, Young SP, et al. Immunomodulatory gene therapy prevents antibody formation and lethal hypersensitivity reactions in murine pompe disease. *Mol Ther* 18 (2010) 353–360 [PubMed: 19690517]
22. Kim IM, Tilley DG, Chen J, et al. Beta-blockers alprenolol and carvedilol stimulate beta-arrestin-mediated EGFR transactivation. *Proc Natl Acad Sci U S A* 105 (2008) 14555–14560 [PubMed: 18787115]
23. MacLennan PA, and Edwards RH. Effects of clenbuterol and propranolol on muscle mass. Evidence that clenbuterol stimulates muscle beta-adrenoceptors to induce hypertrophy. *Biochem J* 264 (1989) 573–579 [PubMed: 2481447]
24. Hwang OK, Park JK, Lee EJ, et al. Therapeutic Effect of Losartan, an Angiotensin II Type 1 Receptor Antagonist, on CCl(4)-Induced Skeletal Muscle Injury. *Int J Mol Sci* 17 (2016) 227 [PubMed: 26867195]
25. Gay-Jordi G, Guash E, Benito B, et al. Losartan prevents heart fibrosis induced by long-term intensive exercise in an animal model. *PLoS One* 8 (2013) e55427 [PubMed: 23383330]
26. Lee EM, Kim DY, Kim AY, et al. Chronic effects of losartan on the muscles and the serologic profiles of mdx mice. *Life Sci* 143 (2015) 35–42 [PubMed: 26497927]
27. Vohra R, Accorsi A, Kumar A, Walter G, and Girgenrath M. Magnetic Resonance Imaging Is Sensitive to Pathological Amelioration in a Model for Laminin-Deficient Congenital Muscular Dystrophy (MDC1A). *PLoS One* 10 (2015) e0138254 [PubMed: 26379183]

Figure 1: Assessment of Biochemical Parameters Following Drug Treatment During ERT.

GAA activity and glycogen content were analyzed to evaluate the effect of each drug during ERT in (A) heart, (B) diaphragm, and (C) quadriceps muscle. Mean $\pm$ standard deviation is shown.

Figure 2: Body and Muscle Weight, and Antibody Responses.

Body weight and the masses of cardiac and skeletal muscles were evaluated following drug treatment during ERT. Changes in (A) body weight, (B) left ventricular weights, and (C) gastrocnemius weight. (D) Anti-GAA IgG1 response detected by ELISA. Mean $\pm$ standard deviation is shown.

Figure 3: Muscle Strength and Cardiac Function.

(A) Change in holding impulse during wirehang testing were evaluated following drug treatment during ERT. Cardiac function assessed by (B) heart rate, (C) maximum systolic blood pressure, (D) end diastolic blood pressure, and (E) ejection fraction. Mean $\pm$ standard deviation is shown.

Figure 4: Effects of Drug Treatment in Absence of ERT.

Glycogen content was analyzed to evaluate the effect of each drug without ERT in (A) heart, (B) diaphragm, and (C) quadriceps muscle. Changes (D) in body weight, (E) heart, and (F) wirehang holding impulse. Holding impulse (hang time latency recorded in seconds multiplied by weight in grams) takes into account the generally shorter hang times of heavier mice. Mean $\pm$ standard deviation is shown.

Figure 5: Survival Following Losartan Treatment.

Survival analysis for groups of mice treated with losartan alone (circles), losartan with ERT (squares), propranolol with ERT (triangles), ERT alone (inverted triangles), or untreated (diamonds).

Figure 6: Western blot analysis of CI-MPR and LC3-II in heart.

Western blots of CI-MPR, LC3 and GAPDH from heart homogenates (A). Quantification of CI-MPR (B) and LC3 (C) relative to GAPDH. Four samples from each treatment group were analyzed. The total intensity of the bands was used in the quantification. Mean $\pm$ standard deviation is shown.

Figure 7: Effects of carvedilol on enzyme uptake in Pompe fibroblasts.

The effect of carvedilol (250nM) or albuterol (50μM), on the uptake of rhGAA (30μg/mL) into Pompe fibroblasts was tested following 5 h incubation. The average activity of GAA is plotted. Mean $\pm$ standard deviation is shown.

Table 1:

Small molecule therapies to be evaluated in combination with ERT

Drug	Dose to induce muscle hypertrophy in combination with ERT (reference) ^I
Carvedilol	56 mg/l [24]
Losartan	600 mg/l [27]
Propranolol 170	170 mg/l [30]

^I Administered in drinking water at dosages evaluated in studies documenting muscle hypertrophy or improved cardiac function in rodents.