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Research Paper

Inspiratory muscle conditioning exercise and diaphragm gene therapy in Pompe disease: Clinical evidence of respiratory plasticity

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ABSTRACT

Pompe disease is an inherited disorder due to a mutation in the gene that encodes acid α-glucosidase (GAA). Children with infantile-onset Pompe disease develop progressive hypotonic weakness and cardiopulmonary insufficiency that may eventually require mechanical ventilation (MV). Our team conducted a first in human trial of diaphragmatic gene therapy (AAV1-CMV-GAA) to treat respiratory neural dysfunction in infantile-onset Pompe. Subjects (aged 2–15 years, full-time MV: n = 5, partial/no MV: n = 4) underwent a period of preoperative inspiratory muscle conditioning exercise. The change in respiratory function after exercise alone was compared to the change in function after intramuscular delivery of AAV1-CMV-GAA to the diaphragm with continued exercise. Since AAV-mediated gene therapy can reach phrenic motoneurons via retrograde transduction, we hypothesized that AAV1-CMV-GAA would improve dynamic respiratory motor function to a greater degree than exercise alone. Dependent measures were maximal inspiratory pressure (MIP), respiratory responses to inspiratory threshold loads (load compensation: LC), and physical evidence of diaphragm activity (descent on MRI, EMG activity). Exercise alone did not change function. After AAV1-CMV-GAA, MIP was unchanged. Flow and volume LC responses increased after dosing ($p < 0.05$ to $p < 0.005$), but only in the subjects with partial/no MV use. Changes in LC tended to occur on or after 180 days. At Day 180, the four subjects with MRI evidence of diaphragm descent had greater maximal voluntary ventilation ($p < 0.05$) and tended to be younger, stronger, and use fewer hours of daily MV. In conclusion, combined AAV1-CMV-GAA and exercise training conferred benefits to dynamic motor function of the diaphragm. Children with a higher baseline neuromuscular function may have greater potential for functional gains.

1. Introduction

Pompe disease is an inherited disorder due to a mutation in the gene that encodes acid α-glucosidase (GAA), the enzyme needed to degrade lysosomal glycogen. In the most severe infantile form of Pompe disease, patients produce no appreciable GAA, which results in profound accumulation of lysosomal glycogen, particularly in cardiac, hepatic, muscular and neural tissue (DeRuisseau et al. 2009; Raben et al. 2002). Infants present weeks to months after birth with failure to thrive, severe hypotonia, macroglossia, and hypertrophic cardiac failure (Raben et al. 2002; van den Hout et al. 2003). This cardiopulmonary failure historically resulted in death in infancy (van den Hout et al. 2003).

An FDA-approved recombinant enzyme-replacement therapy (ERT) intravenously delivers the missing GAA enzyme and has significantly reduced mortality of infantile-onset patients, with accompanying reductions of hypertrophic cardiomyopathy and skeletal muscle glycogenosis (Kishnani et al. 2006; Van den Hout et al. 2000). Despite the cardiac and skeletal muscle improvements, slowly progressive neuromuscular weakness persists (Nicolino et al. 2009; Prater et al. 2012). The phrenic motor system is preferentially affected in Pompe disease, and the majority of infantile-onset survivors have eventually required external ventilatory support (Nicolino et al. 2009; Van den Hout et al. 2004). It is thought that progressive neural pathology contributes to the gradual loss of function, since commercial ERT does not cross the

Abbreviations: ERT, Enzyme replacement therapy; GAA, α-glucosidase; IMST, Inspiratory muscle strength training; LC, Load compensation; MIP, Maximal inspiratory pressure; MRI, Magnetic resonance imaging; MV, Mechanical ventilation.

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blood-brain barrier (Kikuchi et al. 1998; Raben et al. 2003). These data suggest that additional therapies may be needed to address phrenic dysfunction in Pompe.

In an effort to address this phrenic neuropathology, our group has implemented a phase I/II clinical trial of intramuscular gene therapy to the diaphragm, in children with the severe Pompe phenotype (Smith et al. 2013). Our interim results illustrated the safety of the test agent and revealed a potential tidal volume and weaning benefit in the first five ventilator-dependent subjects. In contrast to the observed gains in dynamic respiratory function, we noted there was no change in maximal inspiratory pressure (MIP), a standard clinical estimate of static inspiratory muscle strength. However, static tests do not fully reflect a patient's ability to dynamically shorten and sustain minute ventilation over time.

Tests of dynamic respiratory function are advantageous over static tests because they account not only for tension but also the timing of the respiratory pump contractions. We noted previously that flow and volume compensatory responses to inspiratory threshold loads (termed load compensation, LC) differed between critically ill patients who were able to wean from MV and those who remained ventilator-dependent (Smith et al. 2014). We are also interested in whether LC responses differ in partial and full-time MV users with neuromuscular disease, and whether the LC responses could be used to document a therapeutic effect. Additionally, dynamic magnetic resonance imaging (MRI) of the thorax offers the ability to visualize the actions of the diaphragm and chest wall during breathing. While MRI has documented early, preferential diaphragmatic paresis in adults with Pompe disease (Gaeta et al. 2015), MRI is little-used in children. Finally, the presence of chronic indwelling diaphragm electrodes in one subject offered a unique opportunity to longitudinally measure changes in diaphragm activity after AAV1-CMV-GAA dosing.

Here we report the changes in dynamic respiratory function in children with severe, infantile-onset Pompe disease enrolled in a phase I/II clinical trial of AAV1-CMV-GAA gene therapy to the diaphragm. We hypothesized that, since AAV1-mediated gene therapy may reach phrenic motoneurons via retrograde transduction (Mah et al. 2010), AAV1-GAA would improve result in improved inspiratory flow and volume to a greater degree than exercises and ERT alone. Further, we theorized that children with partial MV dependence would have a higher respiratory function at baseline and therefore have greater potential to improve phrenic neuromuscular performance.

2. Methods

2.1. Design

This was a prospective analysis of the secondary study outcomes from a phase I/II clinical trial of diaphragmatic gene therapy in infantile-onset Pompe disease (NCT: 00976352, IND: BB14131). The Institutional Review Board at the University of Florida approved the study design and procedures. Subjects were enrolled during a Screening visit, and then a period of inspiratory muscle conditioning exercise was implemented as a pre-operative control. Baseline function was recorded the day prior to dosing. After dosing with rAAV1-CMV-GAA, exercise was continued post-operatively. We compared the pre-operative period of exercise, which lasted approximately 90 days, to post-dosing tests conducted every 90 days for one year. The study design and specifics of dosing were published previously (Byrne et al. 2014).

2.2. Subjects

Participants had a confirmed diagnosis of infantile Pompe disease by GAA assay blood test, fibroblast culture or mutational analysis and chronic respiratory insufficiency despite long-term use of ERT. Chronic respiratory insufficiency was defined as a requirement for one or more hours of MV assistance for a minimum of three months prior to enrollment. The first five subjects required 24-hour MV through an invasive airway. Then, when safety reports from the first five subjects were satisfactory (Smith et al. 2013), children with part-time and non-invasive MV interfaces were eligible to participate. Informed consent was obtained from the parents or guardians, and school-aged children provided their assent to participate.

Exclusionary characteristics included a body mass under 10 kg, history of low platelet count or elevated INR, abnormal chemistry profile (elevated transaminases, alkaline phosphatases, bilirubin, or gamma-glutamyl transpeptidase), acute requirement for intravenous antibiotic therapy or corticosteroids, and/or having gene transfer agents six months prior to the study. Participants received ERT infusions every 1–2 weeks for >1 year and remained on ERT throughout the study. Subjects who participated in other therapeutic studies were not eligible.

2.3. Inspiratory muscle conditioning exercises

Upon enrollment, participants were prescribed an inspiratory muscle conditioning exercise program, based upon MIP and their ability to breathe without support. Inspiratory muscle strength training (IMST) exercises were three days per week and consisted of 3–4 sets of 6–10 inspiratory efforts, using a modified threshold-training device (Threshold PEP or Accu-PEEP). The inspiratory port of the training devices remained closed until subjects generated a minimum (threshold) inspiratory pressure. Training devices were connected either directly to the tracheostomy tube, or to a facemask sealed over the mouth and nose, for patients without an invasive MV interface. Subjects trained at the highest tolerated load, where they generated at least 50% of their unassisted tidal volume while maintaining $SpO_2 > 92\%$. Subjects with an MIP of <3 cm H_2O initially used no training device. In addition to IMST, short periods of breathing with reduced or no ventilator support (endurance exercises) were prescribed two days per week for children who used the ventilator full-time. Parents of the participants were instructed in the exercise prescription and provided with a training diary to monitor progress at home. Additionally, investigators communicated regularly with families to monitor compliance, answer questions, and progress training.

2.4. Gene therapy

Study vector was a clinical-grade adeno-associated virus vector serotype 1, with a cytomegalovirus promoter and human GAA cDNA (rAAV1-CMV-GAA) manufactured at The Powell Gene Therapy Center's Human Applications Laboratory, University of Florida. For each subject, three injections of AAV1-CMV-GAA were administered by direct visualization of each hemi-diaphragm into anterior, medial, and posterior regions via video assisted thoracoscopic procedure. Subjects 1–3 received 1×10^{12} vg (low dose), while subjects 4–9 received 5×10^{12} vg (high dose) of vector. Subjects were discharged from the hospital within three days. Further details on vector production, dosing, and safety profile are available in a previous publication (Smith et al. 2013).

2.5. Respiratory outcomes

2.5.1. Resting breathing pattern

Respiratory variables (inspired tidal volume (V_T), peak inspiratory flow (PIF), inspiratory pressure (P_I), inspiratory time (T_I), expiratory time (T_E), and duty cycle (T_I/T_{TOT})) were captured with a respiratory monitor (CO_2SMO, Philips-Respironics, Murrysville, PA) and data acquisition software (Analysis Plus, Respironics Inc., Murrysville, PA). The breathing pattern was recorded for 5 min after the child became acclimated to the testing device, while distracted by a cartoon. Values obtained during the third minute were averaged and reported.