Presence of Neuromuscular and Pulmonary Deficits in a Mouse Model of Pompe Disease

Jill Dalton, Matthew Abernathy, Marci Harter, Jonelle May, Theodore Baird
MPI Research, Mattawan, MI, USA

INTRODUCTION

Pompe disease is an autosomal recessive genetic disorder caused by a mutation in the Gaa gene which results in a deficiency or dysfunction of the lysosomal hydrolase acid alpha glucosidase (Gaa). Alpha glucosidase is responsible for the breakdown of cellular glycogen. Deficiency of Gaa produces accumulation of lysosomal glycogen in multiple tissues, most commonly cardiac and skeletal muscle tissues, and the build-up of glycogen in these tissues causes progressive muscle weakness, leading to complications within the heart, skeletal muscles, and nervous system. The mice used in this study were a Gaa knockout strain (B6;129-Gaa^{tm1Rabn}/J) which mimics Pompe disease. The objective of this study was to evaluate the effects of glycogen accumulation on neuromuscular and pulmonary function in Gaa knockout mice using standard safety pharmacology methodology. Wild-type mice were used in this study as controls. The mice were evaluated over an 8-week period for the presence of neuromuscular and pulmonary deficits. Neuromuscular evaluations were performed during Weeks -1 and 8 and included grip response and hindlimb splay measurements. Pulmonary function was also evaluated using whole-body plethysmography during Weeks -1, 2, 4, and 8. The Gaa knockout mice demonstrated a significant deficit in both neuromuscular measurements compared to wild type controls; however, only grip response demonstrated a further decline during Week 8. The Gaa knockout mice also exhibited differences in respiratory function as compared to the Wt animals. The effects on these standard functional endpoints further support the use of Gaa knockout mice as an appropriate model for Pompe disease, while providing insight into the neuromuscular and pulmonary changes that may be expected when this model is used in drug safety evaluations.

OBJECTIVE

To determine the utility of standard safety pharmacology methodology when used in conjunction with specialized animal models by employing functional neuromuscular and pulmonary evaluations in Gaa knockout mice.

METHODOLOGY

Fifteen Gaa knockout mice (B6;129-Gaa^{tm1Rabn}/J; Gaa) and five wild-type mice (C57BL/6J; Wt) per sex were used as the test and control groups, respectively. All animals were 15 weeks of age at the start of testing and were evaluated over an 8-week period for the presence of neuromuscular and pulmonary deficits.

Study Design

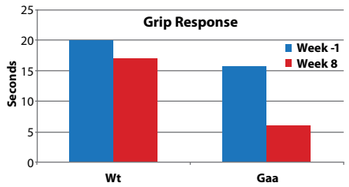
Group Number	Treatment	Number of Animals	
		Male	Female
1	Wt	5	5
2	Gaa	15	15

Neuromuscular evaluations were performed during Weeks -1 and 8 and included forelimb and hindlimb grip strength, measured via grip response using an inverted screen test (Kim, *et al.*) and hindlimb splay (Edwards and Parker). Pulmonary function was evaluated using whole-body plethysmography using PLY4211 mouse plethysmograph chambers during Weeks -1, 2, 4, and 8. All animals were acclimated to the plethysmograph chambers for at least 1 hour and data were collected for 10 minutes thereafter. All animals were observed for morbidity, mortality, injury, and the availability of food and water twice daily. Body weights were measured weekly. Food and water were not available to the animals during the respiratory recording sessions. Statistical analysis was performed using a Repeated Measures Analysis of Variance (RMANOVA) using the mixed model analysis procedure (Littell, *et al.*) within the SAS/STAT System (SAS) software.

RESULTS

Neuromuscular Evaluations

1A



1B

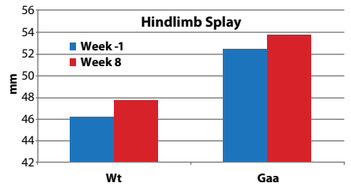


Figure 1. Neuromuscular differences between Wt and Gaa mice over time. Gaa mice demonstrated a decrease in both grip strength and an increase in hindlimb splay pretest as compared to Wt controls. A further decline in grip strength was noted for the Gaa mice at Week 8 (1A), while the difference in hindlimb splay between the two groups remained consistent at Week 8 (1B).

Body Weight

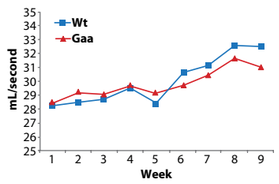
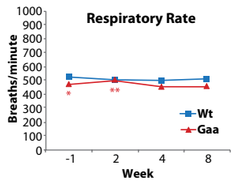


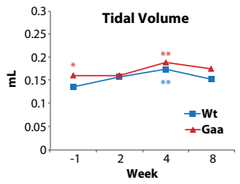
Figure 2. Mean body weight changes over time in Wt and Gaa mice. Body weights were similar at Week 1 and did not vary appreciably over the course of the study.

Respiratory Evaluations

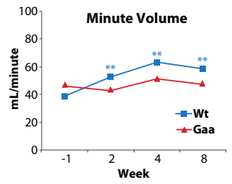
3A



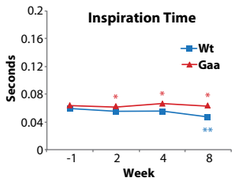
3B



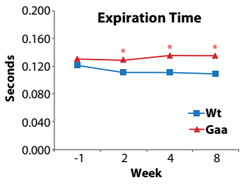
3C



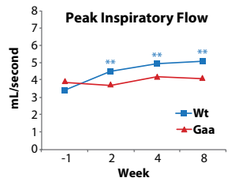
3D



3E



3F



RESULTS CONTINUED

3G

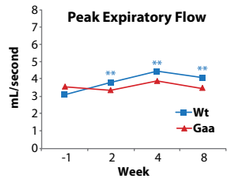


Figure 3. Mean respiratory changes over time in Wt and Gaa mice. The Gaa group exhibited lower respiratory rates (3A) and higher tidal volumes (3B) as compared to the Wt controls over the course of the study. Significant increases in minute volume (3C), peak inspiratory flow (3F), and peak expiratory flow (3G) were observed over the 8-week period, as compared to pretest for the Wt group only. Significant increases in inspiration time (3D) and expiration time (3E) were observed for the Gaa group over the 8-week period, as compared to Wt group.

* Significantly different from Wt ($p<0.05$); ** Significantly different from Week -1 ($p<0.05$)

CONCLUSIONS

- Gaa knockout mice demonstrated a significant deficit in both neuromuscular measurements compared to wild-type controls; however, only grip strength demonstrated a further decline during Week 8.
- No meaningful differences in body weight were noted between the Wt and Gaa groups over the course of the study.
- The Gaa group generally exhibited lower respiratory rates and higher tidal volumes as compared to the Wt controls over the course of the study; however, the magnitude of the difference between the two groups was minimal and remained relatively constant over the 8-week period.
- Increases in minute volume, peak inspiratory flow, and peak expiratory flow were observed over the 8-week period, as compared to pretest for the Wt group only, indicating that the normal increases in these parameters observed for the Wt animals were not occurring in the Gaa animals.
- Increases in inspiration time and expiration time were observed for the Gaa group over the 8-week period, as compared to Wt group, indicating that the normal progressive decreases in these parameters observed for the Wt animals were not occurring in the Gaa animals.
- **These results support the use of the Gaa knockout mouse as an appropriate model for Pompe disease and provide insight into the neuromuscular and pulmonary changes that may be expected when this model is used in drug efficacy/safety evaluations. Further, the effects on these functional endpoints demonstrate the utility of standard safety pharmacology methodology used in conjunction with specialized animal models.**

REFERENCES & ACKNOWLEDGEMENTS

1) Kim ST, Son HJ, Choi JH, Ji IJ, Hwang O. "Vertical grid test and modified horizontal grid test are sensitive methods for evaluating motor dysfunctions in the MPTP mouse model of Parkinson's disease." *J Brainres*. 2010; 1306:176–183.

2) Edwards PM, Parker VH. "A simple, sensitive, and objective method for early assessment of acrylamide neuropathy in rats." *Toxicol Appl Pharmacol*. 1977; 40:589–91.

3) Littell RC, Milliken GA, Stroup W, Wolfinger RD, Schabenberger O. SAS System for Mixed Models, 2nd ed.. Cary (NC): SAS Institute Inc.; 2006.

The authors gratefully acknowledge the assistance of the MPI Research Telemetry Group and the Safety Pharmacology Technical Staff.