Robust chemically induced animal model of Gaucher disease for preclinical study.

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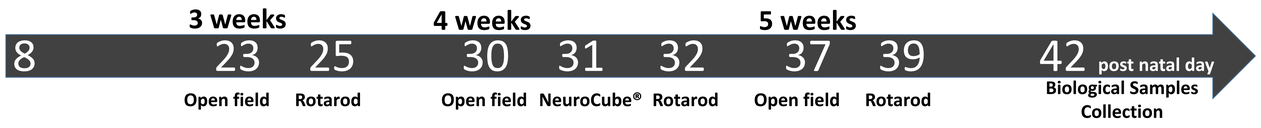
Abstract

Parkinson's Disease (PD) and synucleinophathies are neurodegenerative disorders defined by α-synuclein (α -syn) accumulation. Mutations in the α -syn gene have been demonstrated in vitro and in vivo to accelerate the aggregation and formation of α -syn fibrils, a disease marker. Presence of α -syn positive Lewy bodies were identified in neuropathological analyses of a group of Gaucher disease type I [GD] patients [Sidransky, 2005]. Further human genetic studies have also linked glucocerebrosidase (GCase) gene GBA1 mutations to PD making this mutation the highest genetic risk factor to PD [Sidransky, 2009]. Due to the strong clinical correlation between PD and GD diseases, animal models that demonstrate relevant and robust phenotypes [behavior, mRNA and protein profiling, IHC, …] are of necessity. Genetically modified animal models carrying point mutations in GCase mimicking type 2 and type 3 have been generated targeting neuronal cells [Enquist et al. 2007; Liu et al. 1998]. In the present study, we focus on a chemically induced model consisting in daily injection of the irreversible GCase inhibitor conduritol B-epoxide (CBE) [Vardi et al., 2016). C57Bl6/J mice were dosed daily via intraperitoneal injection at two different doses starting at post-natal day (PND) 8 to 42. Each pup was monitored closely and assessed for motor, coordination and gait functions weekly from 3 weeks of age until study completion. Gait measures were assessed using PsychoGenics' proprietary high throughput gait platform, NeuroCube®. Preliminary assessments of the chemically-induced GD model demonstrate robust, progressive and CBE dose-dependent phenotypic deterioration of responses in motor activity and gait functionality; providing an alternative platform from standard genetically modified rodent models.

Study timeline



Chronic daily CBE dosing from post natal day 8 up to 42
Daily body weight capture and health monitoring

Methods

Animals: C57Bl6/J (Stock #000664) breeders were purchased from Jackson Laboratories (Bar Harbor, ME). Mice were housed at PsychoGenics, received unique identification numbers and housed in polycarbonate OptiMICE ® cages. Animals were paired (WT x WT) and their offspring were enrolled into the study. Due to the breeding design, no genotyping was performed. Offspring received unique identification numbers, were examined, manipulated and weighed prior to study initiation to ensure adequate health and suitability.

During the course of the study, 12 hr / 12 hr light/dark cycles and a room temperature of 20 to 23°C were maintained with a relative humidity maintained around 50%. All mice were housed in an enriched environment. Food (pre-weaning Formulab Diet, #5008 / post weaning #5001) and water were provided ad libitum for the duration of the study. Animals were body weighed daily and checked for survival twice per day. All dosing and assessments were performed during the animals' light cycle phase. At PND 21 surviving WT pups were weaned if at least 6.0g and still gaining body weight. Animals were group housed. Animals received supplementary wet food (BioServ #5001) and hydrogel daily.

Drug preparation: CBE was purchased from Millipore. CBE was formulated at 25 and 37.5mg/kg as per vendor's recommendations.

Study groups: A total of thirty-six mice (n=36) were evenly balanced by gender and body weight between the three (3) treatment groups [PBS, CBE at 25mg/kg or CBE at 37.5mg/kg]. CBE or PBS (vehicle) was dosed once a day via interperitoneally (IP) from post natal day (PND) 8 to PND 42.

Motor paradigms: Open field (OF) test is used to assess both anxiety-like behavior and motor activity. The OF chambers are plexiglas square chambers (27.3 x 27.3 x 20.3 cm; Med Associates Incs., St Albans, VT) surrounded by infrared photobeam sources (16 x 16 x 16). The enclosure is configured to split the open field into a center and periphery zone and the photocell beams were set to measure activity in the center and in the periphery of the OF chambers. Animals having higher levels of anxiety or lower levels of activity tend to stay in the corners of the OF enclosures. On the other hand, mice that have high levels of activity and low levels of anxiety tend to spend more time in the center of the enclosure. Horizontal activity (distance traveled) and vertical activity (rearing) are measured from consecutive beam breaks. Animals were placed in the OF chambers for 30 minutes. Total ambulatory distance, ambulatory distance in center, total rearing, rate of rears in the center, and movement velocity was measured. **Rotarod (RR) test** allows assessment of motor coordination of animal placed on accelerating rod. Accelerating rotarod test (Rotamex, Columbus, OH) was run for 4 min with a speed of 40 rpm. Each mouse received 5 trials with the first 2 trials considered the habituation period which was discarded from the analysis. **NeuroCube®** is an automated behavioral platform that employs computer vision to detect changes in gait geometry and gait dynamics in rodent models of neurological disorders, pain, and neuropathies and extracts gait and non-gait features (Dave et al. Phenotypic characterization of recessive gene knockout rat models of Parkinson's disease. Neurobiology of Disease, 2014). Mice were placed into the NeuroCube® and are given 5 minutes to move freely inside the apparatus for automated gait measures recording. Digital videos were analyzed through computer assisted segmentation algorithms. Fitted parameters were then used to extract clips of motor behavior that were used to extract information about gait geometry and dynamics. Bioinformatics-driven procedures then guide the discrimination probability between treatment groups to determine behavior phenotypes. Data was analyzed via multi-factorial analyses of variance (ANOVA) with a Tukey Post-Hoc

Chronic treatment of CBE impact body weight gain and survival

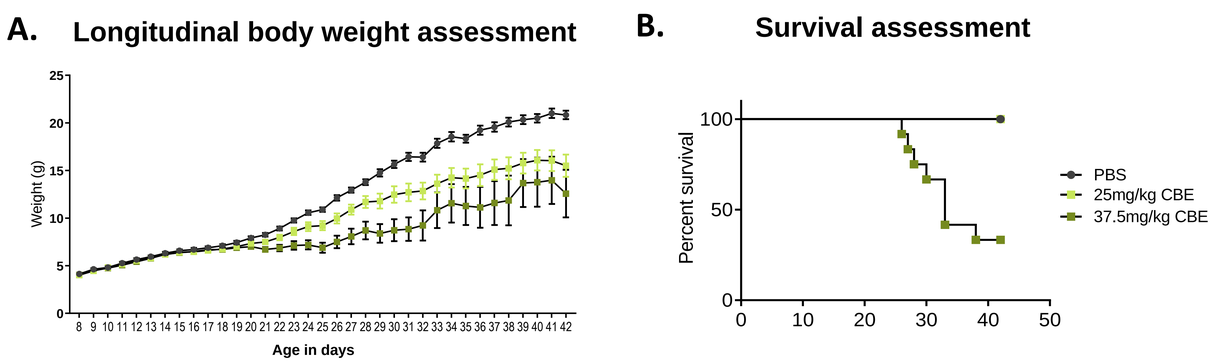


Figure 1. Body weight and survival daily monitoring from PND 8 until study completion: (A.) CBE chronic treatments affect body weight gain in dose dependent matter. (B). Kaplan-Meier survival curve demonstrates a dramatic effect in the 37.5mg/kg CBE treated animals, reaching a 50 percentile at 33 days of age.

Open field

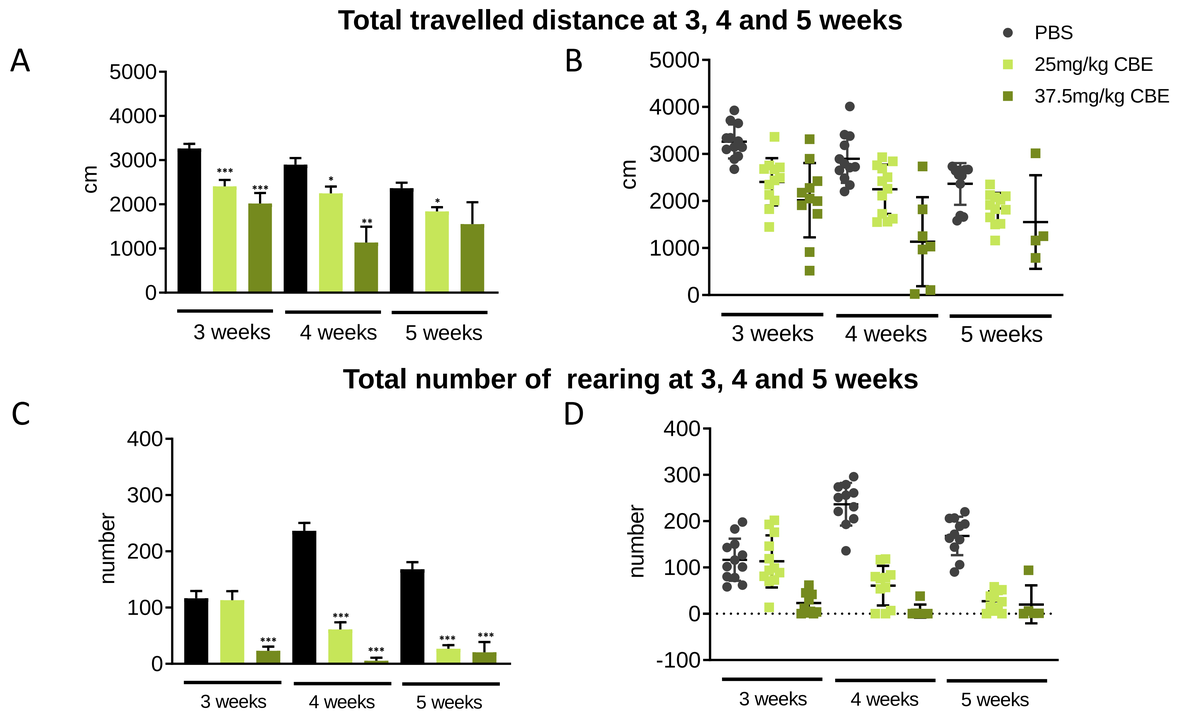


Figure 2. CBE dose dependent reduction in motor activity. Animals were assessed in the Open Field for 30 minutes at 3, 4 and 5 weeks of age. (A) The average Total Distance traveled in a 30-minute session. A CBE dose dependent response is seen with a decrease in horizontal motor activity across all three time points. (B) Total Distance is presented as the average split by individual animal (scatter plot). (C) The average rearing frequency exhibited in a 30-minute session. CBE at 37mg/kg displayed a dramatic reduction in the rearing frequency across all three time points. At 4 and 5 weeks of age, animals treated with CBE at 25mg/kg had significantly lower rearing frequency when compared to PBS treated animals. (D) Rearing frequency is presented as the average split by individual animal (scatter plot). Values presented as mean ± one standard error of the mean (SEM), with n=12 mice per treatment group. Tukey's multiple comparisons statistical analysis were performed compared to vehicle. * <0.05; **<0.01; ***<0.001

Rotarod

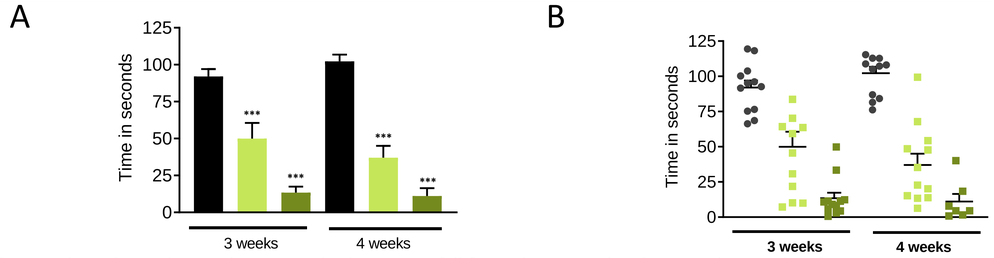
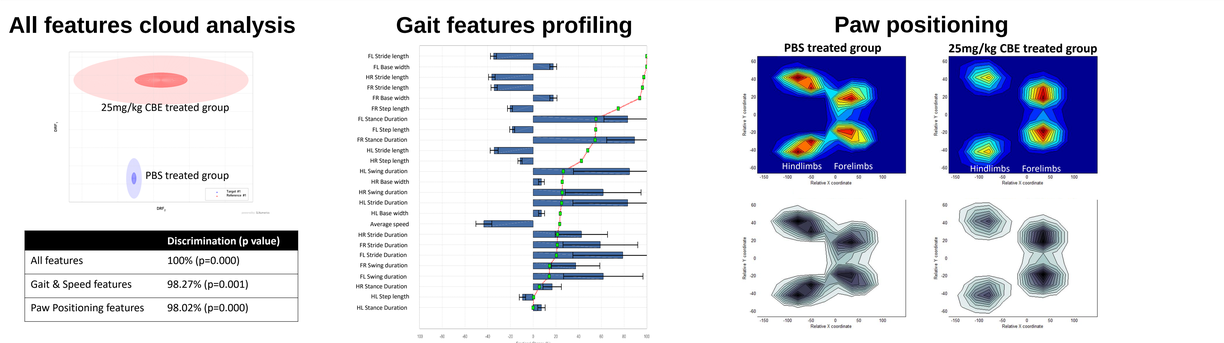


Figure 3. CBE dose dependent reduction in the latency to fall from the rotarod rod at 3 and 4 Weeks of Age. (A) Latency to Fall is presented as the average of three trials. (B) Latency to Fall is presented as the average of three trials split by individual animal (scatter plot). Values presented as mean ± one standard error of the mean (SEM), with n=12 mice per treatment group. Tukey's multiple comparisons statistical analysis were performed compared to vehicle. * <0.05; **<0.01; ***<0.001

NeuroCube®



	Discrimination (p value)
All features	100% (p=0.000)
Gait & Speed features	98.27% (p=0.001)
Paw Positioning features	98.02% (p=0.000)

Figure 4. Dramatic and highly significant gait dysfunction at 4 weeks of age, after 6 weeks of chronic 25mg/kg CBE treatment. Cloud analysis demonstrate a 98.27% discrimination in gait & speed features and 98.02% discrimination in paw positioning features. Gait features profiling highlight stride length and stance duration to be two of the features the most affected.

Summary

We were able to replicate the findings published by Vardi et al., 201. The chemically induced model of Gaucher disease consisting in daily injection of the irreversible GCase inhibitor conduritol B-epoxide (CBE) in C57Bl6/J mice from post-natal day 8 to 42 established a dose dependent phenotype:

- Decreased body weight gain
- Decreased horizontal motor activity, with shorter distances travelled in the open field.
- Decreased motor coordination, with shorter latencies to fall from the rotarod rod
- Decreased survival rate for the 37.5mg/kg group, with 50 percentile reached at PND 33.

Furthermore, the CBE at 25mg/kg treated group showed significant gait impairment at 4 weeks of age in NeuroCube®.

This model has demonstrated robust and reliable phenotype with motor, coordination and gait impairment. To further assess this model, an extensive immunohistochemistry evaluation will be performed.

Acknowledgments:
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