The Fast Skeletal Troponin Activator, CK-2017357, Increases Muscle Function and Survival in SOD1 (G93A) Mice; a Model of ALS

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Objective

To examine the effects of CK-2017357 in SOD1^{G93A} mutant transgenic mice, a model of amyotrophic lateral sclerosis (ALS) in humans

Introduction

1. Transgenic mice carrying ALS-associated mutant human SOD1 genes, including the SOD1^{G93A} mouse, parallel many features of the human disease (Gurney *et al.*, 1994). SOD1^{G93A} mice develop progressive limb and body weakness at approximately 80 days of age, culminating in full limb paralysis, morbidity and death at around 135-140 days (Gurney *et al.*, 1994). The only currently approved medication for ALS patients is *riluzole*, which might extend life on an average of 2 to 3 months, and also extends lifespan in the SOD1^{G93A} mouse (Gurney *et al.*, 1996). However, *riluzole* has not been shown to improve muscle strength or pulmonary function in ALS patients (Miller *et al.*, 2003).
2. CK-2017357 is a novel small molecule activator of the fast skeletal muscle troponin complex (Russell *et al.*, 2012). CK-2017357 slows the rate of calcium release from the regulatory troponin complex of fast skeletal muscle resulting in a sensitization of the contractile apparatus to calcium. In intact skeletal muscle *in vivo*, the drug sensitizes muscle to nerve stimulation and increases force generation at sub-maximal levels of nerve stimulation. In the present studies, SOD1^{G93A} mice were treated with CK-2017357 to investigate its potential effects on fast skeletal muscle function and respiratory function. Survival to a humane endpoint was also evaluated in a post-hoc analysis pooling data from several studies.

Methods

Animals used in this study were maintained in accordance with the Guide for the Care and Use of Laboratory Animals of the Institute (Seventh Edition, National Research Council) and under the supervision of the Cytokinetics Institutional Animal Care and Use Committee.

Transgenic Mice

- B6SJLF1/J mice and SOD1^{G93A} mice over-expressing the human SOD1 gene with mutation G93A were licensed from Northwestern University and obtained from Jackson Labs, Inc at 6-8 weeks of age.

Extensor Digitorum Longus (EDL) muscle for *in situ* analysis

- *In situ* muscle testing occurred at two stages of disease: At 90-100 days of age, when signs of weakness were becoming apparent (weakness and trembling of hindlimbs) or a later stage, 100-110 days where signs of single or dual limb paralysis were evident. Under anesthesia, the EDL muscles were isolated and the distal tendon was cut and tied to the arm of a force transducer (Aurora Scientific, Ontario, Canada).
- Muscle contractile properties were assessed by recording the force generated by stimulation of the peroneal nerve. To measure relative changes in the force-frequency relationship following CK-2017357 dosing, the muscle was stimulated similarly at 30 Hz for the course of the experiment.
- CK-2017357 was administered in solution (50% PEG300/10% EtOH/40% Cavitron® cyclodextrin formulation) as a single slow bolus over a 2 minute period via a catheter in the contralateral femoral artery placed above the aortic bifurcation. CK-2017357 infusions (2, 2, 2, and 4 mg/kg were given at approximately 20 min intervals to achieve a cumulative dose of 10 mg/kg) in order to assess the dose response.

Long-term dosing with CK-2017357

- Six-eight week old SOD1^{G93A} mice were fed Harlan Teklad Rodent Diet 8604 to establish a three week baseline. Animals were then split into two groups: Control and compound-treated 400 parts-per-million (ppm) CK-2017357 formulated into Harlan Teklad Rodent Diet 8604.

Assessment of grip strength

- Hindlimb grip strength was measured by an investigator blinded to whether or not mice were fed control chow or chow containing CK-2017357. Hindlimb grip measurements were acquired in triplicate with a 250 gram Dual Sensor Grip Meter.

Assessment of Animal Viability

- All animals were weighed and assessed for clinical signs once per week using a scoring system composed of three metrics, gait, balance and ability to ambulate, each scored on a five point scale by the observer during the course of the study. Once muscle weakness was evident, animals were weighed and scored more frequently based on the severity of weakness. Humane endpoints used to determine time of euthanasia included weight loss of greater than 20%, dual limb (hind limb or forelimb) paralysis or a low expectation of survival until the next time point. Investigators judging animal viability were blinded to whether or not animals were being treated with control chow or CK-2017357 formulated chow.

Results

Isometric *in situ* Force is Reduced in SOD1^{G93A} Mice

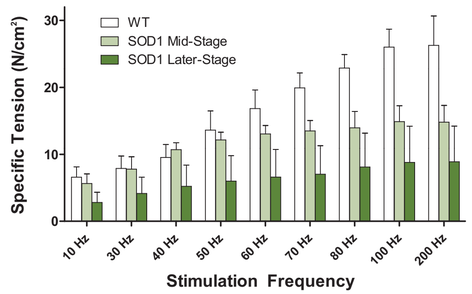

Figure 1:

EDL muscles of mice were stimulated from 10 Hz to 200 Hz (350 ms stimuli) and force recorded. Plotted is the mean specific tension at each frequency (wild-type (WT) B6SJL mice, n=8; mid-stage SOD1^{G93A} mice, n=5; later-stage SOD1^{G93A} mice, n=6. Error bars ± SEM).

Infusion of CK-2017357 Increases *in situ* Force in SOD1^{G93A} Mice Suffering from Neuromuscular Disease

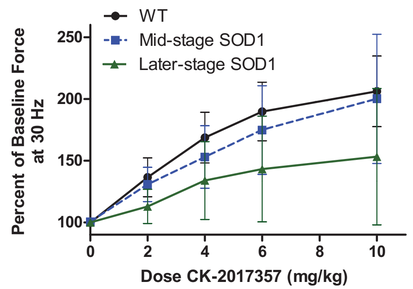

Figure 2:

The EDL muscle was stimulated every 2 minutes with a 30 Hz stimulus (1 ms stimuli, 350 ms duration) via the peroneal nerve. CK-2017357 was then administered as a 2 minute bolus in four cumulative doses up to a total of 10 mg/kg (B6SJL mice, n=8; mid-stage SOD1^{G93A} mice, n=5; later-stage SOD1^{G93A} mice, n=6. Error bars ± SEM). The maximum muscle strength for the mice was: wild-type=26.3 N/cm^2, mid-stage (90-100 days)=14.8 N/cm^2 and later-stage (100-110 days)=8.9 N/cm^2.

References

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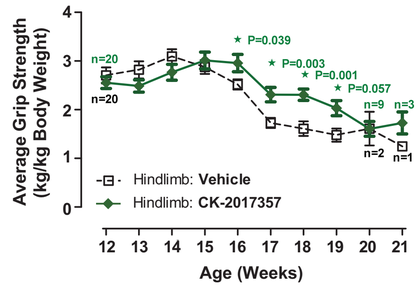
Oral Administration of CK-2017357 Increases Hindlimb Grip Strength in SOD1^{G93A} Transgenic Mice



*Nominal p-values from t-tests at week 16, 17,18 and 19

Figure 3:

Female SOD1^{G93A} mice were fed 400 parts per million (ppm) CK-2017357 or control chow beginning at 11 weeks of age. Hindlimb grip strength was measured in the mice weekly and normalized to body weight.

CK-2017357 Increased Survival Time of SOD1^{G93A} Mice

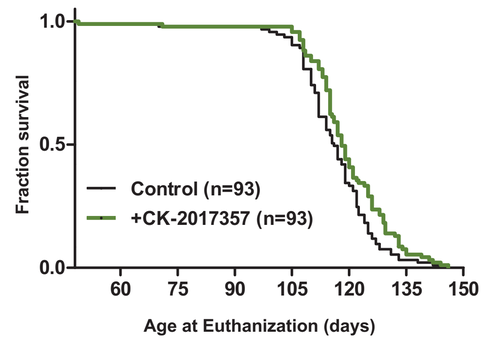
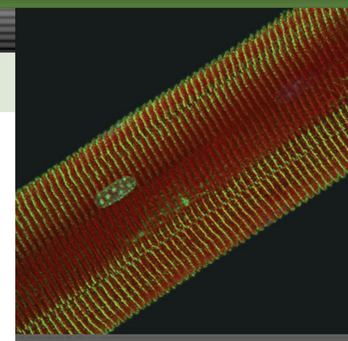


Figure 4:

Pooling data for SOD1^{G93A} transgenic male or female mice fed chow containing CK-2017357 (400 ppm or 600 ppm) beginning at 11 weeks of age. Treated mice survived longer to a humane endpoint longer compared to control chow-fed mice (p=0.029; hazard ratio=0.69). Data presented were pooled from blinded studies conducted in SOD1^{G93A} mice randomized to CK-2017357 or control chow (n = 93 female and male mice treated with CK-2017357; n = 93 control female and male mice).



Conclusions

SOD1^{G93A} mice treated with CK-2017357 exhibited an increase in EDL muscle strength *in situ*.

SOD1^{G93A} transgenic mice treated with CK-2017357 maintained hindlimb grip strength to a greater extent during disease progression.

There appeared to be a delay in the time to a pre-specified humane endpoint in CK-2017357 treated animals compared to the age-matched control mice.

Overall, the current pre-clinical findings support the hypothesis that the fast skeletal troponin sensitizer, CK-2017357, may benefit patients with ALS by increasing force generation in fast skeletal muscle fibers.

CK-2017357 is being evaluated for clinical effectiveness in ALS patients.

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