The Fast Skeletal Muscle Troponin Activator *Tirasemtiv* Increases Muscle Function and Performance in the B6SJL-SOD1^{G93A} ALS Mouse Model

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Abstract

OBJECTIVE: To evaluate the effects of *tirasemtiv* on muscle function in a mouse model of ALS.

BACKGROUND: *Tirasemtiv* is a fast skeletal troponin activator that sensitizes the sarcomere to calcium and amplifies the response of muscle to neuromuscular input.

DESIGN/METHODS: Forelimb grip strength in female B6SJL-SOD1^{G93A} transgenic mice was monitored weekly starting at 10 weeks of age (baseline). In order to minimize disease variability, mice were subjected to a battery of functional assessments when their forelimb grip strength declined by 25% from baseline. Mice were dosed once with either vehicle or *tirasemtiv* (10 mg/kg, PO) and forelimb grip strength, grid hang time, or rotarod performance were evaluated. Respiratory parameters of WT and B6SJL-SOD1^{G93A} mice were assessed by whole body plethysmography following treatment with vehicle or *tirasemtiv*-treated (10 mg/kg, PO). In a separate cohort of B6SJL-SOD1^{G93A} mice, isometric extensor digitorum longus (EDL) muscle force output was assessed *in situ* at 90-100 days and 110-115 days of age.

RESULTS: Following a single oral dose (10 mg/kg, PO) of *tirasemtiv,* forelimb grip strength ($p<0.05$), grid hang time ($P<0.01$), and rotarod performance ($p<0.01$) all significantly increased in the B6SJL-SOD1^{G93A} transgenic mice. Compared to vehicle treatment, *tirasemtiv* significantly increased tidal volume in mice breathing room air following a 30 minute hypercapnic challenge ($p < 0.001$). In 90-100 day-old B6SJL-SOD1^{G93A} mice, *tirasemtiv* treatment (10 mg/kg, IV) significantly increased submaximal (30Hz) isometric tension *in situ* from 5.81 ± 0.46 N/cm^2 to 10.8 ± 0.71 N/cm^2 ($p=0.0028$). In 110-115 day-old mice, *tirasemtiv* increased submaximal tension from 3.34 ± 0.73 N/cm^2 to 5.05 ± 1.4 N/cm^2 ($p=0.064$).

CONCLUSIONS: *Tirasemtiv* increased both isometric sub-maximal muscle force *in situ* and *in vivo* muscle performance in B6SJLSOD1^{G93A} mice. *Tirasemtiv* also increased respiratory function. These results indicate that *tirasemtiv* has the potential to improve muscle function and respiration in patients suffering from ALS.

Introduction

Amyotrophic Lateral Sclerosis (ALS) is a debilitating and fatal disease characterized by the selective and progressive loss of motor neurons in the brain and spinal cord leading to atrophy, weakness, and eventually complete paralysis of skeletal muscle. ALS is the most common motor neuron disease in adults, approximately affecting 22,000 individuals in the United States alone *(1)*. Transgenic mice carrying ALS-associated mutant human superoxide dismutase (SOD1) genes, including the B6SJL-SOD1^{G93A} mouse, parallel many features of the human disease *(2)*. B6SJL-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 *(2)*. Many of the histological features of disease in the B6SJL-SOD1^{G93A} mice are similar to those observed in ALS patients, although there appears to be less robust enlargement and sprouting of neighboring motor units towards denervated muscles as compensation for the loss of the primary motor neuron *(3)*.

Tirasemtiv is a novel small molecule activator of the fast skeletal muscle troponin complex. *Tirasemtiv* selectively sensitizes fast skeletal muscle troponin to calcium (Ca^{2+}), and slows the rate of Ca^{2+} release from the regulatory troponin complex of fast skeletal muscle *(4)*. In intact skeletal muscle *in situ*, the compound amplifies the response of muscle to nerve input and increases force generation at sub-maximal levels of nerve stimulation. In the present studies, B6SJL-SOD1^{G93A} mice were treated with single doses of *tirasemtiv* to investigate its potential effects on skeletal muscle function *in vitro* and *in vivo*, including assessments of muscle strength and endurance as well as respiratory function.

Methods

Animals

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.

Functional assessments of muscle performance

Wild-type background strain B6SJLF1/J mice and B6SJL-SOD1^{G93A} mice were received from Jackson Labs, Inc. (strains 100012 and 002726, respectively; Bar Harbor, ME) at approximately seven weeks of age.

Baseline measurements of mouse body weight, forelimb grip strength, grid hang-time and rotarod performance were recorded initially at ten weeks of age and weekly thereafter for the following eight weeks. Once forelimb grip strength declined by 25% (as prespecified by protocol), each mouse performed a battery of functional tests in the presence of *tirasemtiv* or vehicle treatment. *Tirasemtiv* was administered by oral gavage (10 mg/kg dose in 0.5% HPMC/0.2% Tween 80) 30 minutes prior to each test. This dose was expected to provide a peak plasma concentration of approximately 3-4 µg/ml between 20-30 minutes after dosing. The investigator was blinded to treatment and the mice were given the same treatment on successive days, with each assessment occurring on a separate day. Mice were re-randomized to treatment between 25% and 40% test periods. Statistical significance was calculated by t-test between vehicle and treated group at specific animal age and $p < 0.05$ was considered significant.

Forelimb and hindlimb grip measurements were acquired in triplicate with a 250 gram Dual Sensor Grip Meter (DFS-R-250G, Transcat, Inc., Rochester, New York, USA).

A modified cage grid apparatus was designed to allow mice to grab the cage wire mesh grid and then the grid was inverted 180 degrees. The time before the mice dropped off of the grid on to a soft pad below was recorded for each assessment with a maximum time of 300 seconds.

Rotarod performance was evaluated by placing mice on the rotarod (San Diego Instruments, San Diego, CA) at 12 RPM for a maximum of ten minutes, time to fall was recorded.

Respiratory function was assessed in WT and B6SJL-SOD1^{G93A} mice once all motor function tests at 40% decrements were complete. Mice were orally dosed with vehicle or 10 mg/kg *tirasemtiv* and placed in unrestrained whole body plethysmography chambers for 30 minutes of acclimation. Mice were then monitored for 10 minutes at room air, followed by exposure to a 5% CO_2 gas mixture for 30 minutes before being returned to room air for 10 minutes.

Diaphragm contractile force was measured by electrical field stimulation in an organ bath system. Diaphragms from WT and B6SJL-SOD1^{G93A} mice were excised, and placed in a temperature controlled water-jacketed chamber (26-27 °C) containing Krebs-Henseleit Buffer, supplemented with 50mg/L tubocurarine and 50U/L insulin. The force-frequency profile was obtained by stimulating the muscle at frequencies between 10-150 Hz (Grass Stimulator, 0.6 pulse width, 800 ms train duration). *Tirasemtiv* was dissolved in DMSO (1 or 3µM) and directly added into the bath.

In situ muscle testing in a separate cohort of female mice occurred at two stages of disease: at 90-100 days of age, when signs of weakness were becoming apparent (weakness and trembling of hindlimbs) and a later stage, 110-115 days where signs of single or dual limb paralysis were evident. Muscle contractile properties were assessed as described previously *(4)*.

Results

Body mass and muscle performance decreases over time in B6SJLSOD1^{G93A} mice

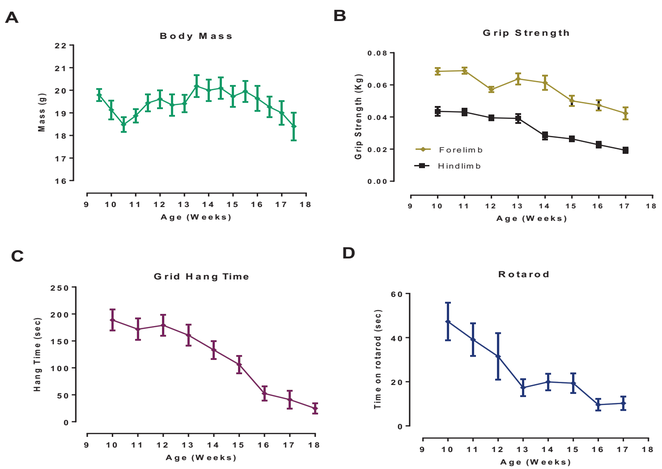


Figure 1: Body mass and muscle performance decreases over time in B6SJL-SOD1^{G93A} mice: A) Body mass, **B)** Forelimb and hind limb grip strength, **C)** Grid-hang time, and **D)** Rotarod performance over 10 to 18 weeks of age. Tests were performed on a weekly basis. Data from mice under assessment with either vehicle or *tirasemtiv* were excluded from these figures for that week. Data are expressed as mean ± SEM. n=24/group.

B6SJL-SOD1^{G93A} mice exhibit significant functional deficits prior to *tirasemtiv* administration

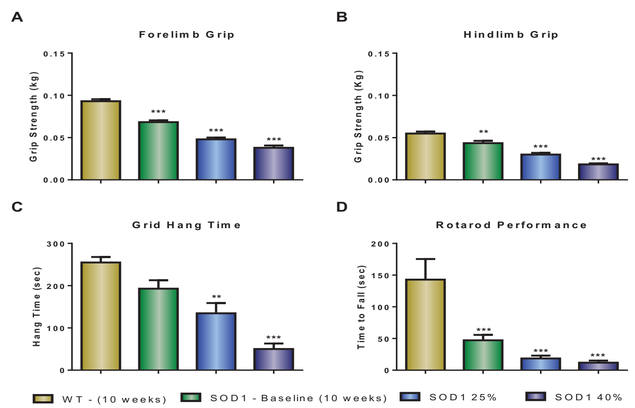


Figure 2: B6SJL-SOD1^{G93A} mice exhibit significant functional deficits prior to *tirasemtiv* administration. Performance values of **(A)** Forelimb grip strength, **(B)** Hindlimb grip strength, **(C)** Hang test performance, and **(D)** Rotarod performance at point of selection for 25% and 40% deficit milestones in forelimb grip compared to the 10-week old baseline. **(E)** Numerical values for WT, 10 week SOD1 baseline, 25% and 40% milestones. Data are expressed as mean ± SEM. n=18-24/group. **= p<0.01, *** = p<0.001 vs. WT by one way ANOVA with post-hoc Tukey's test.

Tirasemtiv increased forelimb grip strength, grid hang time, and rotarod performance in B6SJL-SOD1^{G93A} mice

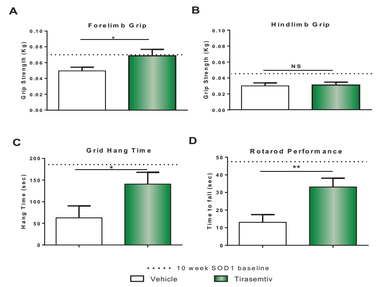


Figure 3: *Tirasemtiv* increased forelimb grip strength, grid hang time, and rotarod performance in B6SJL-SOD1^{G93A} mice. Effect of *tirasemtiv* and vehicle at the 25% (approx. 90-110 days) forelimb grip strength loss milestone. **(A)** Forelimb grip strength, **(B)** Hindlimb grip strength, **(C)** Grid hang time, and **(D)** Rotarod performance. Dotted line shows mean B6SJL-SOD1^{G93A} transgenic mouse performance assay values at baseline (10 weeks of age). Data are expressed as mean ± SEM. n=10-12/group. *= p<0.05, **= p<0.01 vs. WT by one way t-test.

Infusion of *tirasemtiv* increases *in situ* EDL muscle force in WT and B6SJL-SOD1^{G93A} transgenic mice at mid-stage and late stage disease

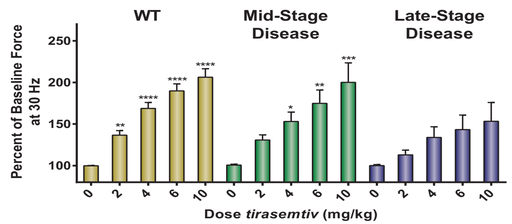


Figure 4: Infusion of *tirasemtiv* increases *in situ* EDL muscle force in wild-type and B6SJL-SOD1^{G93A} transgenic mice at mid-stage (90-100 days) and late stage (110-115 days) disease. The EDL muscle was stimulated every 2 minutes with a 5 Hz stimulus (1 ms pulse width, 350 ms train duration) via the peroneal nerve. *Tirasemtiv* was then administered as a 2 minute i.v. bolus in four cumulative doses up to a total of 10 mg/kg. (WT mice, n=8; mid-stage B6SJL-SOD1^{G93A} mice, n=5; late-stage B6SJL-SOD1^{G93A} mice, n=6 Data are expressed as mean tension ± SEM. *p<0.05, **p<0.01, ****p<0.0001 vs. respective baseline force).

Tirasemtiv increased tidal volume and submaximal diaphragm tension in late stage female B6SJL-SOD1^{G93A} mice

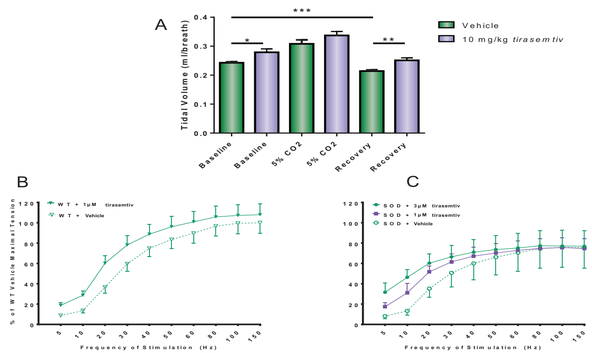
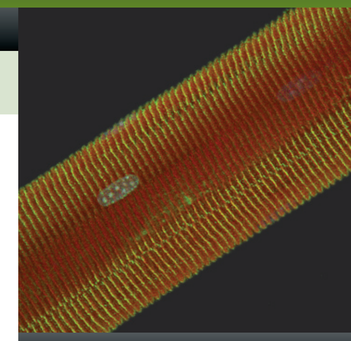


Figure 5: *Tirasemtiv* increased tidal volume and submaximal diaphragm tension in late stage female B6SJL-SOD1^{G93A} mice. Respiratory function and diaphragm tension output were assessed in B6SJL-SOD1^{G93A} mice at 18 weeks of age. (A) B6SJL-SOD1^{G93A} mice dosed with *tirasemtiv* had significantly higher tidal volume than vehicle-dosed mice at baseline and during recovery following a 5% CO_2 exposure. Compared to its baseline tidal volume, vehicle-treated B6SJL-SOD1^{G93A} mice had significantly lower tidal volume during recovery. **(B,C)** Harvested WT and B6SJL-SOD1^{G93A} diaphragms treated with *tirasemtiv* produced greater tension in response to submaximal frequencies of electrical stimulation. Data are expressed as mean ± SEM. N ≥ 5/group.



Summary and Conclusions

A single dose of *tirasemtiv* significantly increases submaximal isometric force, forelimb grip strength, grid hang time, and rotarod performance in a transgenic mouse model (B6SJL-SOD1^{G93A}) of ALS with functional deficits.

Diaphragm force and tidal volume are significantly higher in *tirasemtiv*-treated B6SJL-SOD1^{G93A} mice.

These results support the potential of fast skeletal muscle troponin activators to improve muscle function in neuromuscular diseases.

References

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