Fast Skeletal Muscle Troponin Activator *tirasemtiv* Increases Muscle Function and Performance in Mouse Models of Spinal Muscular Atrophy

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Abstract

Background: The small molecule *tirasemtiv* is a specific fast skeletal muscle troponin activator that sensitizes the sarcomere to calcium, leading to increased muscle force *in situ* in response to submaximal rates of nerve stimulation and decreased fatigability.

Objectives: The objective of this study was to investigate the effect of *tirasemtiv* on skeletal muscle function in two SMA mouse models with mild and moderate levels of muscle dysfunction and weakness.

Methods: Two SMA mouse models were used in the study: a model corresponding to intermediate SMA and a less severe model corresponding to adult-onset SMA. Both models were evaluated *in situ* for plantarflexor isometric muscle force in response to sciatic nerve stimulation, *in situ* muscle fatigability, and *in vivo* forelimb grip strength and inverted grid hang time.

Results: Intermediate and adult onset SMA mice had lower compound muscle action potentials (CMAP), motor unit number estimation (MUNE) numbers, and hindlimb muscle atrophy. Compared to sibling controls (CON), isometric muscle force *in situ* was significantly lower in both SMA mouse models at all submaximal and tetanic rates of nerve stimulation (10 to 200 Hz) (n=10-15/group, $p < 0.0001$, CON vs. SMA). In the intermediate SMA mice, *tirasemtiv* (10 mg/kg, IP) significantly increased isometric force in response to submaximal (20Hz) nerve stimulation in both female (Vehicle: 37 ± 4.7 mN vs. *tirasemtiv*: 62 ± 7.2 mN, mean ± S.E.M., n=6/group, $p < 0.05$) and male (Vehicle: 24 ± 4 mN vs. *tirasemtiv*: 47 ± 7.9 mN, n=4-5/group, $p < 0.05$) SMA mice. In adult onset SMA mice, *tirasemtiv* (10 mg/kg, IP) significantly increased submaximal isometric force in response to nerve stimulations between 10-60 Hz (n=7-8/group, $p < 0.001$). In both mouse models, *tirasemtiv*-treated SMA mice had higher muscle force under fatiguing conditions induced by repeated nerve stimulation. *Tirasemtiv* (10 mg/kg, PO) significantly increased forelimb grip strength *in vivo* in intermediate SMA mice compared to vehicle (43±3.8 g vs. 52±4.4 g, n=9/group, $p < 0.05$). Adult-onset SMA mice had significantly lower hang time *in vivo* compared to CON mice (CON: 197±23 sec, n=17 vs. SMA: 138±18 sec, n=25, $p < 0.05$). *Tirasemtiv* (10 mg/kg, PO) significantly increased inverted grid hang time in SMA mice (vehicle: 138±18 vs. *tirasemtiv*: 192±34 sec, n=25, $p < 0.05$).

Discussion and conclusions: Intermediate and adult-onset SMA mice exhibited nerve dysfunction, muscle atrophy, and weakness. Single doses of *tirasemtiv* significantly increased submaximal force and fatigue resistance *in situ*, and grip strength and grid hang time *in vivo* in these SMA mice. These results suggest that *tirasemtiv* and other fast skeletal muscle troponin activators may be viable therapeutics for improving muscle function in spinal muscular atrophy.

Introduction

- Spinal Muscular Atrophy is characterized by the degeneration of motor neurons, leading to muscle weakness, atrophy and eventual paralysis
- The small molecule *tirasemtiv* is a specific fast skeletal muscle troponin activator that sensitizes the sarcomere to calcium, leading to increased muscle force *in situ* in response to submaximal rates of nerve stimulation and decreased fatigability
- *Tirasemtiv* has previously been shown to improve muscle function in pre-clinical disease models where neural input is affected, including myasthenia gravis[1] and ALS[2]
- The objective of this study was to investigate the effect of *tirasemtiv* on skeletal muscle function in two SMA mouse models with mild and moderate levels of muscle dysfunction and weakness

1. Russell, *et al.* Nat Med. 2012 Feb 19;18(3):452-5
2. Hwee, *et al.* PLoS One. 2014 May 7;9(5):e96921

Methods

SMA Mouse Models

9-12 month old intermediate SMA and 13-14 month old adult-onset SMA mice were obtained from the laboratory of Dr. Christine DiDonato (Lurie Children's Hospital of Chicago, Chicago, IL). As a confirmation of neuromuscular dysfunction and prior to any muscle function assessment, compound muscle action potentials (CMAPs) and motor unit number estimation (MUNE) numbers were measured in control and SMA mice according to previously described methods.[3,4]

Assessment of Muscle Function *in situ*

Isometric and isokinetic hindlimb muscle force was measured *in situ* in intermediate SMA, adult-onset SMA, and their respective control mice in the presence of vehicle or *tirasemtiv* (10 mg/kg, IP in 50% PEG: 40% Cavitron: 10% DMA) treatment. Mice were placed under anesthesia with isoflurane (1-5%). One incision was made on the mid-thigh region of the right leg to expose the sciatic nerve. An electrode was attached to the sciatic nerve and the foot was taped to a footplate attached to a force transducer. Muscle contractile properties were assessed by applying an electrical current to the nerve and recording the force generated by the muscle via a servomotor. An isometric force-frequency relationship (10-200 Hz, 1ms pulse width, 350 ms train duration) was assessed with the ankle joint at 90° of flexion. An isokinetic force-velocity relationship in response to 30Hz stimulation was assessed over a range from 0 to 20.1 radians/sec. The fatigue properties of the ankle plantar flexor muscles were assessed by 300 repeated nerve stimulations (30Hz stimulation, 3.1 rad/sec, once per 1.5 seconds).

Assessment of Muscle Performance *in vivo*

A blinded cross-over design was implemented to assess the *in vivo* performance of intermediate SMA, adult-onset SMA, and their respective control mice with vehicle and *tirasemtiv* (10 mg/kg, PO in 0.5% HPMC, 0.2% Tween-80) treatment. The cross-over studies were performed over two days, with the order of vehicle or *tirasemtiv* treatment randomized and blinded to the experimenter.

- **Grip Test Protocol**
  Male control and intermediate mice were lowered onto a triangle bar of the grip strength meter until they gripped the bar with their forelimbs or hindlimbs. On each day of testing, mice were dosed 30 minutes prior to assessment with vehicle or *tirasemtiv* (10 mg/kg, PO). Mice were pulled gently backward by their tail until the grip was released. The force gauge of the grip meter was recorded as the maximum force. Grip for both forelimb and hindlimb was measured 3 times in succession.
- **Inverted Grid Hang Time Performance**
  Female control and adult-onset SMA mice were placed on the top surface of a horizontal 0.5 inch wire mesh, which was then rapidly inverted 180 degrees and locked with a metal pin. Latency to fall was recorded. On each day of testing a baseline inverted grid hang time was recorded. Mice were then treated with vehicle or *tirasemtiv* (10 mg/kg, PO). 30 minutes after dosing, mice were again placed on the inverted grid and latency to fall was recorded. Both absolute hang time and the hang time difference from baseline were recorded.

3. Gogliotti, *et al.* Hum Mol Genet. 2013 Oct 15;22(20):4084-1014.
4. Arnold, *et al.* Ann Clin Transl Neurol. 2014 Jan 1;1(1):34-44.

Results

Mouse Characteristics

Intermediate SMA mice

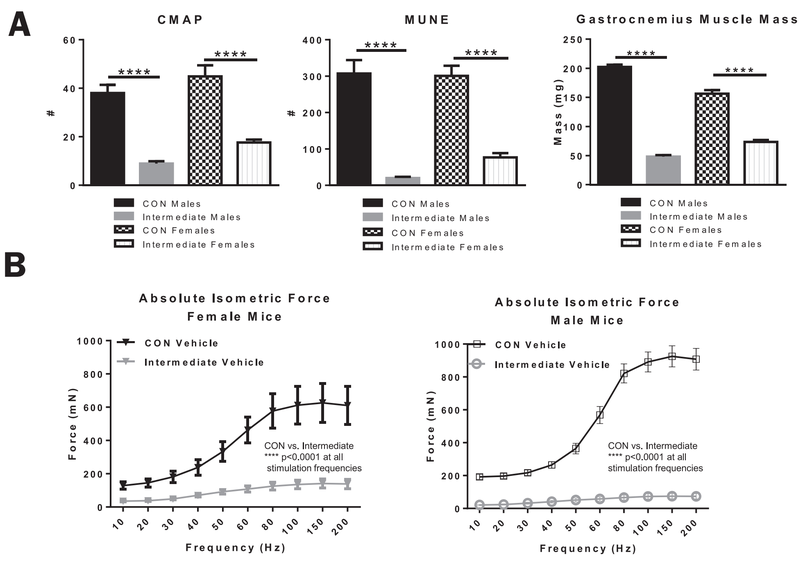


Figure 1. SMA Intermediate mice exhibit neuromuscular dysfunction and reduced muscle force *in situ*. A. At 9-12 months of age, intermediate mice have reduced compound muscle action potentials (CMAP), motor unit number estimation (MUNE) numbers, and hindlimb muscle atrophy. **B.** Compared to control (CON) mice, absolute isometric muscle force is lower at all stimulation frequencies in both female and male intermediate SMA mice. All data are expressed as mean ± SEM. ****$p < 0.0001$

Adult-onset SMA Mice

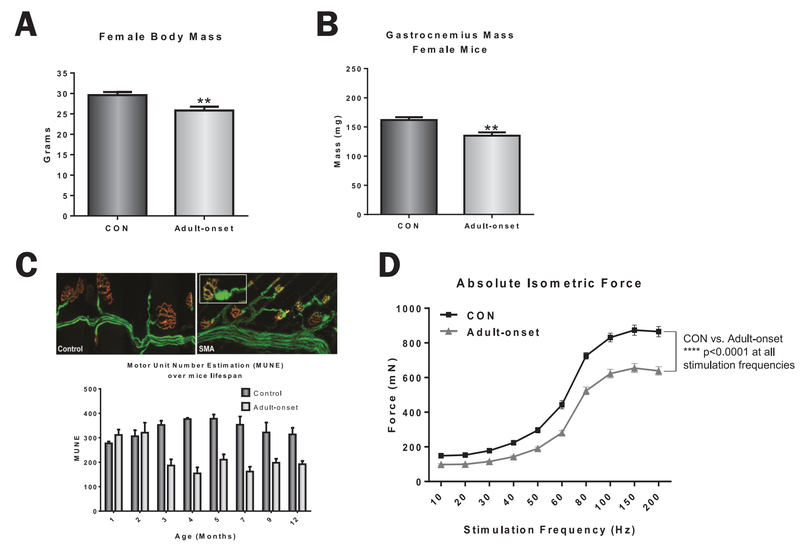


Figure 4. Female adult-onset SMA mice exhibit reduced motor units, muscle atrophy, and reduced muscle force. A. Female adult-onset SMA mice have lower body mass than their CON counterparts. (n=12/15 group) **B.** Female adult-onset mice have lower gastrocnemius mass than their CON counterparts. (n=8-14/ group) **C.** Motor unit number estimation (MUNE) is lower in adult-onset mice from three months of age onward. **D.** Absolute isometric force is lower at all stimulation frequencies in adult-onset mice. (n=12/15 group). All data are expressed as mean ± SEM. **$p < 0.01$.

In Situ Muscle Function

Intermediate SMA mice

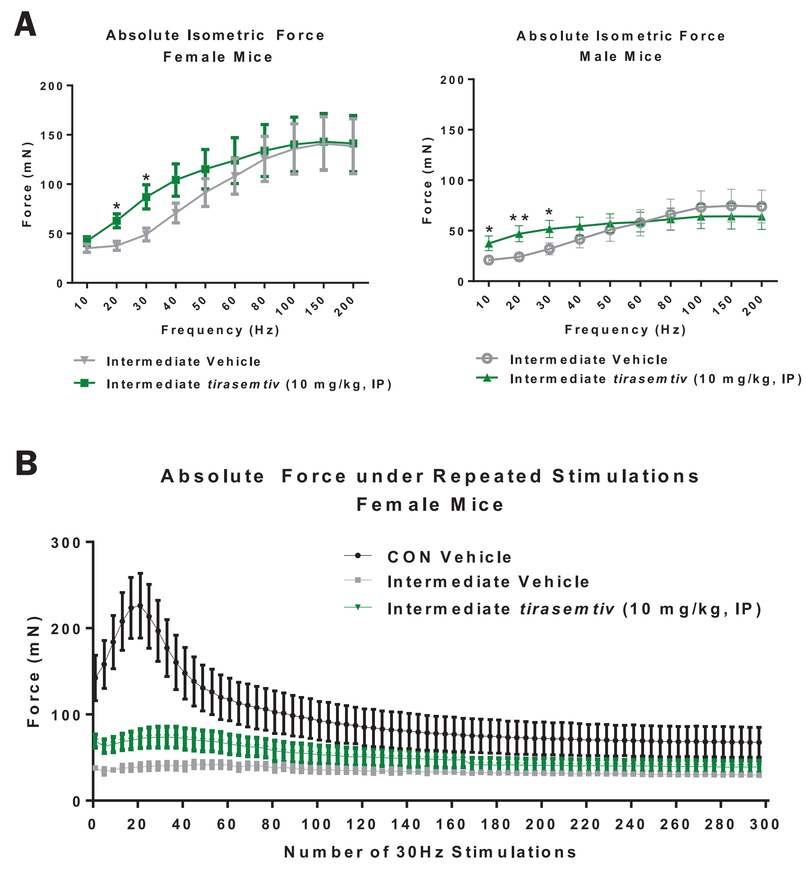


Figure 2. *Tirasemtiv* improves intermediate SMA mouse muscle force and fatigue resistance in response to submaximal nerve stimulation. A. *Tirasemtiv* (10mg/kg, IP) causes a leftward shift in the isometric force-frequency relationship in female and male intermediate mice (n= 3-6/ group) **B.** In response to repeated 30 Hz sciatic nerve stimulation, female intermediate mice produced lower isokinetic force than control mice. *Tirasemtiv* (10 mg/kg, IP) treatment increased the force produced in intermediate mice. **C.** Compared to CON mice, female intermediate mice produced less work in response to repeated nerve stimulations. *Tirasemtiv* (10 mg/kg, IP) treatment significantly increased work in intermediate SMA mice. (n=5-6/ group). All data are expressed as mean ± SEM. **Figure A:** *$p < 0.05$, **$p < 0.01$ Intermediate vehicle vs. *tirasemtiv* at each stimulation frequency.

Adult-onset SMA Mice

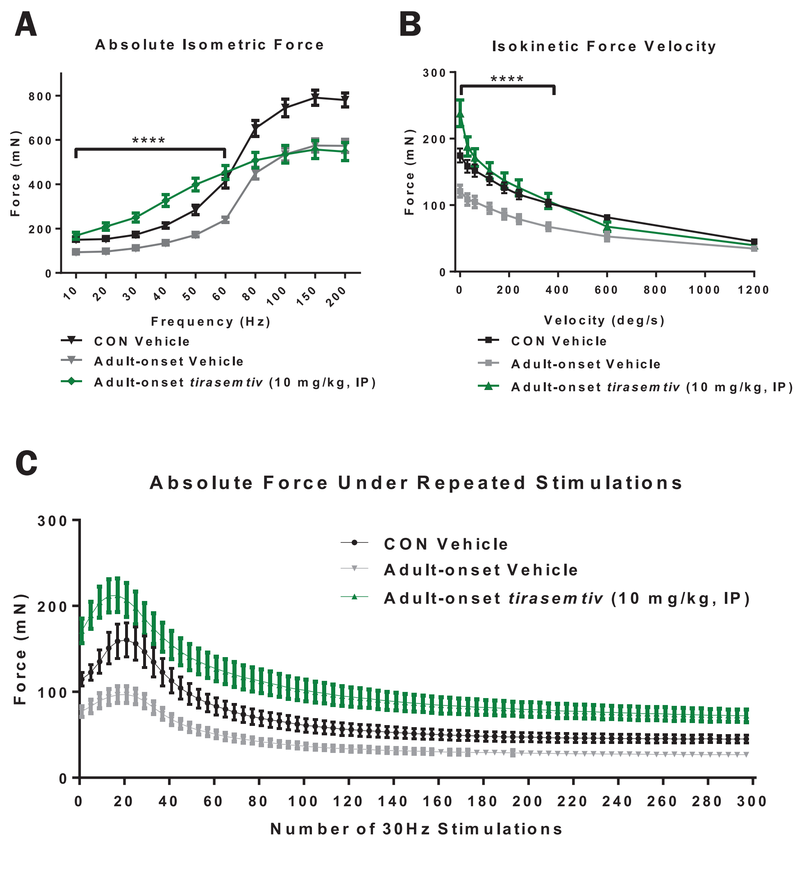


Figure 5. *Tirasemtiv* improves adult-onset SMA mouse muscle force and fatigue resistance in response to submaximal nerve stimulation. A. *Tirasemtiv* (10mg/kg, IP) causes a leftward shift in the isometric force-frequency relationship in adult-onset mice (n= 6-8/group) **B.** In response to 30 Hz stimulation, *tirasemtiv* treatment (10mg/kg, IP) causes an up and rightward shift in the isokinetic force velocity relationship in adult-onset mice (n= 6-8/group). **C.** In response to repeated nerve stimulation, female adult-onset mice produced lower isokinetic force than CON mice. *Tirasemtiv* (10 mg/kg, IP) treatment increased the force production in adult-onset mice. **D.** Compared to CON mice, adult-onset mice produced less work in response to repeated nerve stimulations. *Tirasemtiv* (10 mg/kg, IP) treatment significantly increased work in adult-onset mice (n=6-8/ group). All data are expressed as mean ± SEM. **Figures A and B:** ****$p < 0.0001$. Adult-onset vehicle vs. *tirasemtiv* at each stimulation frequency or velocity. **Figure D:** **$p < 0.01$ ****$p < 0.0001$.

In Vivo Muscle Function

Intermediate SMA mice

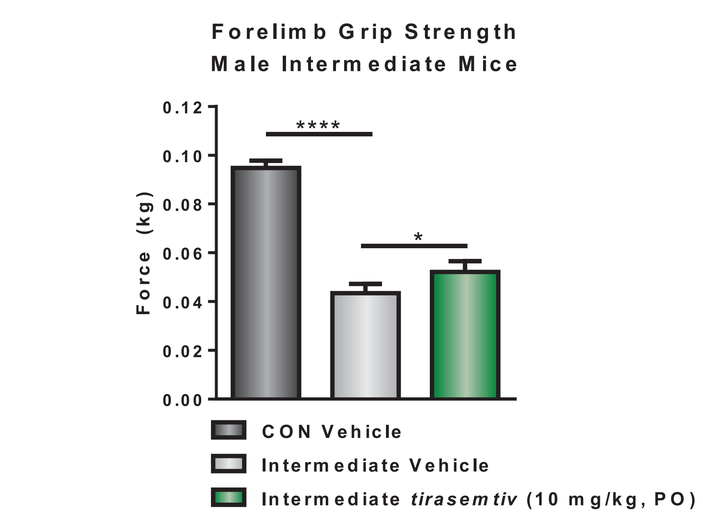


Figure 3. *Tirasemtiv* improves forelimb grip strength *in vivo*. Vehicle-treated intermediate SMA mice had lower forelimb grip strength than vehicle-treated CON mice. *Tirasemtiv* (10 mg/kg, PO) significantly improved forelimb grip strength in intermediate SMA mice compared to vehicle (0.52± 0.044 kg vs. 0.43 ± 0.038 kg, n=9/group). All data are expressed as mean ± SEM. *$p < 0.05$, ****$p < 0.0001$.

Adult-onset SMA Mice

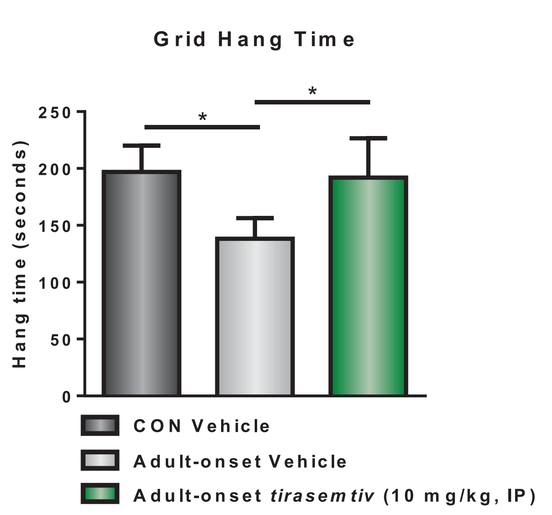
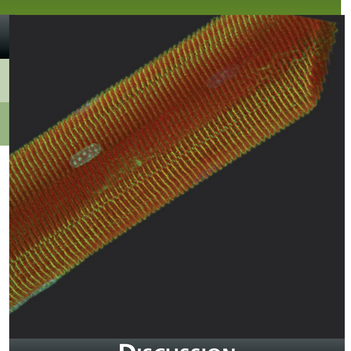


Figure 6. *Tirasemtiv* improves inverted grid hang time *in vivo*. Adult-onset SMA mice had significantly lower hang time *in vivo* compared to CON mice (CON: 197±23 sec, n=17 vs. SMA: 138±18 sec, n=25). *Tirasemtiv* (10 mg/kg, PO) significantly increased inverted grid hang time in SMA mice (138±18 vs. 192±34 sec, n=25). Data is expressed as mean ± SEM. *$p < 0.05$.



Discussion

- **Intermediate and adult-onset SMA mice exhibited nerve dysfunction, muscle atrophy, and weakness.**
- **Single doses of *tirasemtiv* significantly increased muscle force and fatigue resistance *in situ* in response to submaximal nerve stimulation in both SMA mouse models**
- ***Tirasemtiv* improved grip strength *in vivo* in intermediate mice and inverted grid hang time *in vivo* in adult-onset mice.**
- **These results suggest that *tirasemtiv* and other fast skeletal muscle troponin activators may be viable therapeutics for improving muscle function in spinal muscular atrophy**

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