ACE-083, a Locally-Acting TGF-β Superfamily Ligand Trap, Increases Muscle Mass and Strength in a Mouse Model of ALS

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Background

- Myostatin (GDF8) is a member of the TGF-β superfamily and is a known negative regulator of muscle growth
- GDF8 signals through the activin receptor type IIB (ActRIIB) to induce SMAD 2/3 phosphorylation and translocation to the nucleus to regulate gene transcription
- ACE-083 is a locally-acting investigational protein therapeutic that functions as a ligand trap for GDF8 and other negative regulators of muscle mass.
- We have previously demonstrated that ACE-083 induces muscle hypertrophy and strength locally in a dose dependent fashion when injected into target muscles in wild type and *mdx* mice.
- Additionally, the hypertrophy translates into a functional benefit in terms of muscle strength.
- This study was conducted to determine the therapeutic effects of ACE-083 in the SOD1*G93A mouse model of amyotrophic lateral sclerosis (ALS).

Methods

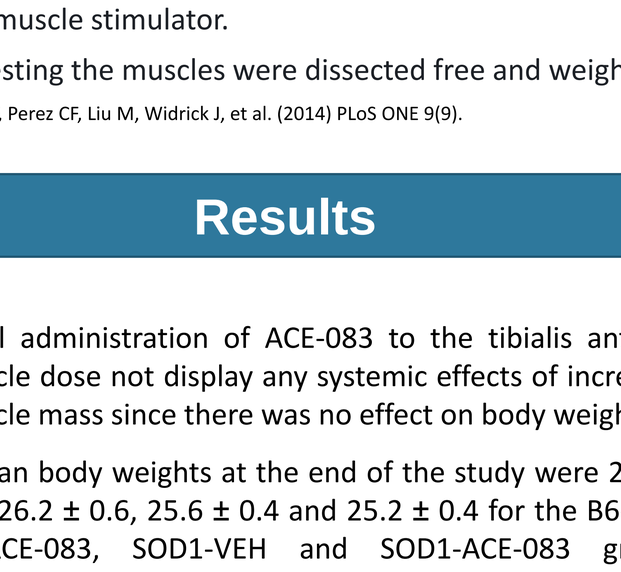
- Twelve week old B6.Cg-Tg(SOD1*G93A)1GUR/J mice (SOD1) or wild type C57BL/6J (B6) mice were used on study (N=10/group).
- Mice received either a vehicle control (VEH) or ACE-083 at 100 µg per dose.
- ACE-083 was administered by direct injection into the right tibialis anterior (TA) muscle twice per week for 4 weeks.
- The physiologic properties of the TA muscle from the right leg (treated side) was tested according to Altamirano *et al*[1]. Briefly, the patellar tendon was fastened to an immobile horizontal support and the distal tendon was attached to the lever arm of a calibrated dual mode muscle lever system. Platinum needle electrodes were inserted behind the knee for stimulation of the peroneal nerve via a bi-phasic muscle stimulator.
- After testing the muscles were dissected free and weighed

[1]Altamirano F, Perez CF, Liu M, Widrick J, et al. (2014) PLoS ONE 9(9).

Results

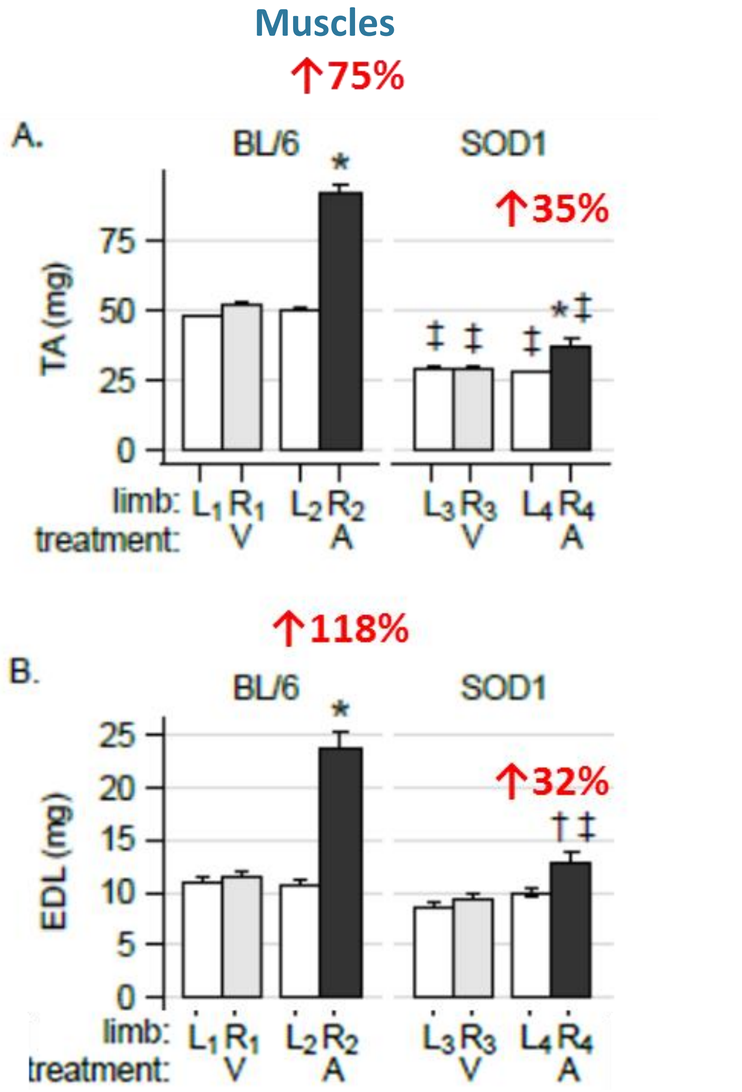
- Local administration of ACE-083 to the tibialis anterior muscle dose not display any systemic effects of increased muscle mass since there was no effect on body weight.
- Mean body weights at the end of the study were 25.9 ± 0.4, 26.2 ± 0.6, 25.6 ± 0.4 and 25.2 ± 0.4 for the B6-VEH, B6-ACE-083, SOD1-VEH and SOD1-ACE-083 groups respectively.
- Administration of ACE-083 increased the TA muscle cross sectional area, indicative of muscle fiber hypertrophy in the injected muscle (Fig. 1).

Fig.1 ACE-083 Has Localized Effect



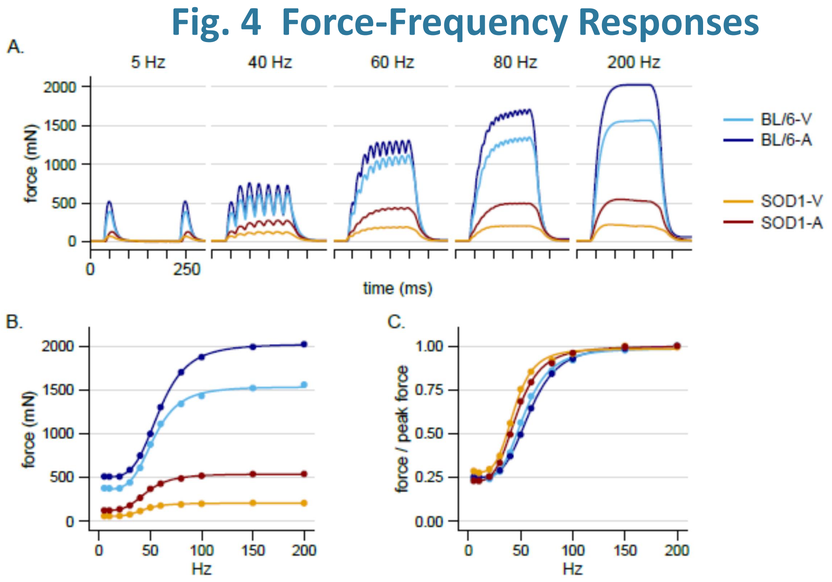
- In normal B6 mice ACE-083 increased the TA muscle mass by 75% compared to the uninjected contralateral TA (L2, Fig. 2A). The adjacent EDL was also increased by 118% compared to the uninjected leg (Fig 2B).
- In untreated SOD1 mice, there was significant muscle atrophy of the TA muscle. ACE-083 treatment increased the TA muscle mass by 35% and the EDL muscle by 32% compared to the uninjected contralateral leg.

Fig.2 ACE-083 Increases Muscle Mass In Injected Muscles



* indicates a significant difference from all other means within the same genotype.
‡ indicates a significant difference vs. BL/10 mice of the same limb/treatment combination.

Fig. 4 Force-Frequency Responses



- Isometric force records from a representative member of each experimental group at different frequencies are shown in Figure 4A. Table 1 contains the data from all animals in each group. The B6 muscles clearly produce greater force than the SOD1 muscles.
- At all stimulation frequencies, B6 muscles produce greater force than SOD1 muscles. ACE-083 treatment increased force in both the B6 and SOD1 muscles.
- When the force was expressed relative to peak force (Figure 3C) there was no change in the shape of the curves in the ACE-083 treated groups compared to the vehicle treated groups in the same genotype.

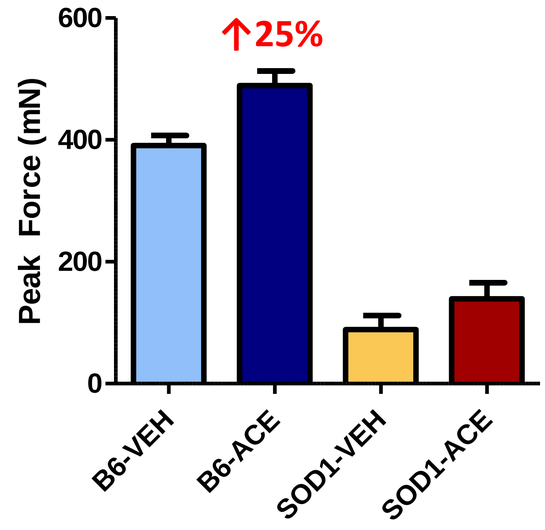
Table 1: ACE-083 Effect TA Muscle Force-Frequency Response

Group	P_{min} (mN)	P_{max} (mN)	K (Hz)	H
B6-V	377 ± 17	1528 ± 36	57 ± 2	4.56 ± 0.08
B6-A	479 ± 22*	2105 ± 60*	64 ± 2	4.98 ± 0.21
SOD1-V	88 ± 23‡	274 ± 70‡	43 ± 4‡	3.71 ± 0.33‡
SOD1-A	140 ± 27‡	469 ± 84*‡	43 ± 2‡	3.65 ± 0.14‡

* indicates a significant difference from vehicle treated group of the same genotype.
‡ indicates a significant difference vs. B6 mice of a similar treatment.
Pmin, minimum estimated twitch force, Pmax, maximum tetanic force, K, inflection point, H, slope

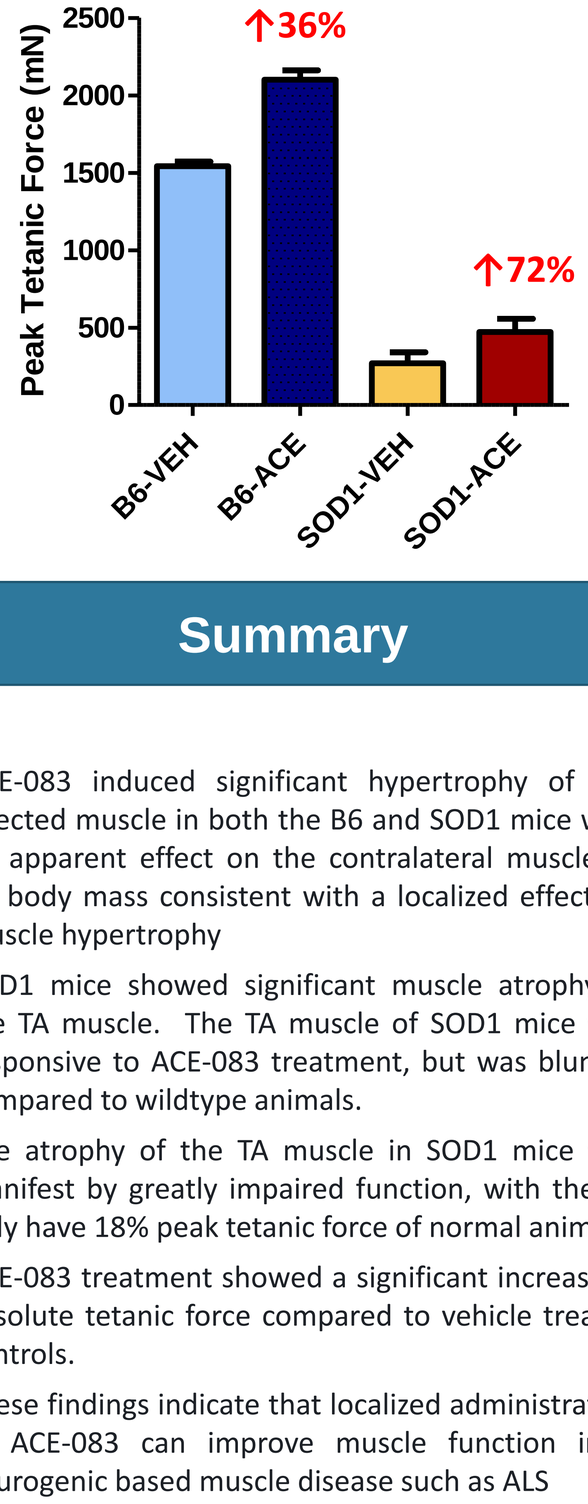
- In B6 mice ACE-083 increased the absolute twitch force by 25% compared to vehicle treated TA muscle (Fig 5).
- The absolute twitch force was significantly lower in the SOD1 vehicle treated mice; ACE-083 treatment of SOD1 mice showed a trend for increased twitch, but it was not significant.

Fig.5 ACE-083 Increased Peak Twitch Force



- ACE-083 treatment increased the peak tetanic force of the TA muscle in B6 mice by 560 mN (36%, Figure 6).
- The absolute peak tetanic force was singificantly reduced in SOD1 mice (18% of B6 mice); ACE-083 treatment increased the absolute tetanic force by 200 mN (72%) compared to the vehicle control.

Fig.6 ACE-083 Increased Peak Tetanic Force



Summary

- ACE-083 induced significant hypertrophy of the injected muscle in both the B6 and SOD1 mice with no apparent effect on the contralateral muscle or on body mass consistent with a localized effect on muscle hypertrophy
- SOD1 mice showed significant muscle atrophy in the TA muscle. The TA muscle of SOD1 mice was responsive to ACE-083 treatment, but was blunted compared to wildtype animals.
- The atrophy of the TA muscle in SOD1 mice was manifest by greatly impaired function, with the TA only have 18% peak tetanic force of normal animals.
- ACE-083 treatment showed a significant increase in absolute tetanic force compared to vehicle treated controls.
- These findings indicate that localized administration of ACE-083 can improve muscle function in a neurogenic based muscle disease such as ALS

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