ACE-083 Increases Muscle Hypertrophy and strength in C57BL/6 Mice

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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 IB (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 acts as a ligand trap for GDF8 and other negative regulators of muscle mass.
• We have previously demonstrated that ACE-083 induces muscle hypertrophy locally in a dose dependent fashion when administered by injection into the muscle.
• This study was conducted to determine if the observed hypertrophy would translate into a functional benefit in terms of muscle strength.

Methods
• Eight week old male C57BL/6 mice were used for this study (N=10/group).
• Mice were divided into 2 groups to receive a vehicle control (VEH) or ACE-083 at 100 µg per dose.
• ACE-083 was administered by injection into the right tibialis anterior (TA) muscle twice per week for 3 weeks.
• After 3 weeks of dosing the physiologic properties of the TA muscle from the right leg (treated side) was tested according to Altamirano et al. Briefly, the patellar tendon was fastened to an immobile horizontal support. 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 muscular stimulator.

Results

Fig. 1 ACE-083 Treatment Increases Muscle Mass in the Injected Tibialis Anterior

As previously observed, ACE-083 treatment did not demonstrate systemic effects since there was no effect on body weight (A) or on the contralateral, uninjected TA muscle (B). ACE-083 injection increased the tibialis anterior (TA) muscle mass by 73% compared to the injected TA muscle of the vehicle treated mice (B). No differences were seen between the uninjected vehicle, injected vehicle or the uninjected vehicle using the cross sectional area (pcSA) of the muscle (determined by the formula pCSA = M/FLWD where M is TA mass, FL is fiber length and D is muscle density) was also increased in the ACE-083 treated muscle compared to the vehicle treated muscle(C). The increase in pCSA was 71%, similar to the change in mass. *p<0.0001. U=Uninjected muscle, I=Injected muscle.

Fig. 2 ACE-083 Increase in Muscle Mass is Due to Hypertrophy

To determine the effect of ACE-083 on the muscle fibers TA muscle sections were stained with an antibody to the sarcoplasmic protein laminin. Representative images from the left (untreated) and right (treated) TA of VEH or ACE-083 treated mice are shown in panel A. The cross sectional area (CSA) of the muscle fibers in the left and right TA of the vehicle treated mice were similar to each other. In contrast ACE-083 injected muscles had fibers that had on average a 78% greater CSA than the uninjected leg and 94% greater than the vehicle treated TA muscle. Fiber cross sectional frequency distributions demonstrated that ACE-083 treated muscle fiber size was increased such that ~70% of fibers were greater than 2000 µm² while on the contralateral, uninjected leg only ~30% were greater than 2000 µm² (C). Uninjected leg, I= injected leg. *p<0.0001.

Fig. 3 ACE-083 Increases Muscle Force

Physiological experiments were performed to determine what effect the ACE-083 hypertrophy had on muscle contractility. Representative data from a single supramaximal pulse to induced a twitch force response is shown in panel A. Panels B-F show superimposed force responses from the same two muscles in response to low-to high-frequency pulse trains to obtain sub-maximal and maximal contractions. B (20Hz), C (50Hz), D (60 Hz), E (80 Hz) and F (200 Hz). Calibration bars for A are 100mN and 100ms while calibration bars for B-F are 500 mN and 10 ms.

Fig. 4 Force-Frequency Responses

The force-frequency relationship of the two groups demonstrate that absolute force was greater for ACE-083 treated muscle at all stimulation frequencies (A). The force at the inflexion point of the curve was 41% greater in ACE-083 vs vehicle treated TA's even though it occurred at the same frequency (65/3 Hz). Plotting the force relative to peak force across the stimulation frequencies demonstrates that the two groups have similar shaped curves indicative of the increase due to the increase in hypertrophy not a change in the intrinsic properties of the muscle.

Fig. 5 ACE-083 Increased Peak Twitch Force

Overall the absolute twitch force from the ACE-083 treated TA group was 52% greater than the vehicle treated TA's (A). The difference between the two groups disappears when the twitch force was normalized to muscle pCSA (B). There was also no difference in the twitch contraction or relaxation times between the two groups (C and D).

Fig. 6 ACE-083 Increased Peak Tetanic Force

The absolute peak tetanic force of ACE-083 treated TA muscle was ~40% greater than the vehicle treated muscle (A). However, since pCSA of the muscle increased by 73% if the force is normalized to cross sectional area the muscle produces about 20% less power would than be expected. This may be due to the rapid nature of the muscle growth and may need to be examined at a later time point. Values are mean ± SEM for 9 VEH and 8 ACE treated mice. *p<0.05.

Summary
• ACE-083 induced significant hypertrophy of the injected muscle with no apparent effect on the contralateral muscle or on body mass consistent with a localized effect on muscle hypertrophy.
• TA hypertrophy was associated with a significant increase in the ability of the muscle to produce force and power. Changes in muscle contractility were due to larger fiber size rather than altered kinetic properties of the muscle.
• The localized muscle hypertrophy and substantial increases in absolute force and power due to ACE-083 treatment are likely to be beneficial therapeutically in diseases with focal muscle atrophy.