



A Modified Cysteine Knot Ligand Trap of the TGFβ Superfamily, ACE-083, Increases Muscle Mass Locally in Mice

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Background

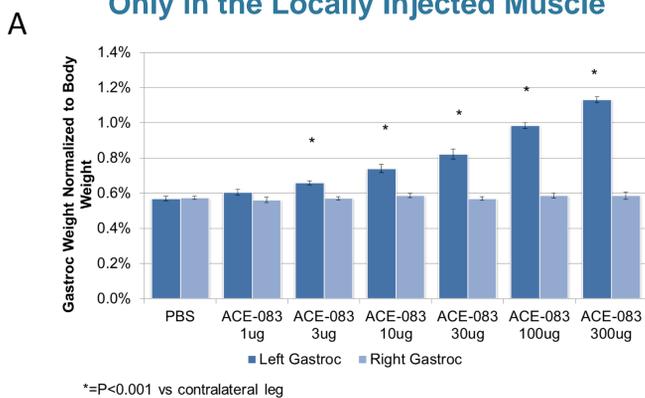
- TGFβ superfamily ligands such as myostatin and activins play a key role in muscle growth and differentiation. Systemic therapeutic agents from this family targeting these ligands have been explored in muscular dystrophies, cancer cachexia and other similar diseases. A number of disorders of skeletal muscle, such as sporadic Inclusion body myositis (sIBM), may lend themselves to treatment with a locally active agent, thus minimizing off-target effects due to systemic inhibition of activins.
- We generated a modified cysteine knot ligand trap of the TGFβ superfamily, ACE-083, which acts locally to increase muscle mass.
- ACE-083 binds to activins and myostatin, among other ligands in the TGFβ superfamily, and inhibits their signaling.
- In the current study we investigated the ability of ACE-083 to increase muscle mass locally using direct injection into the gastrocnemius muscle.

Methods

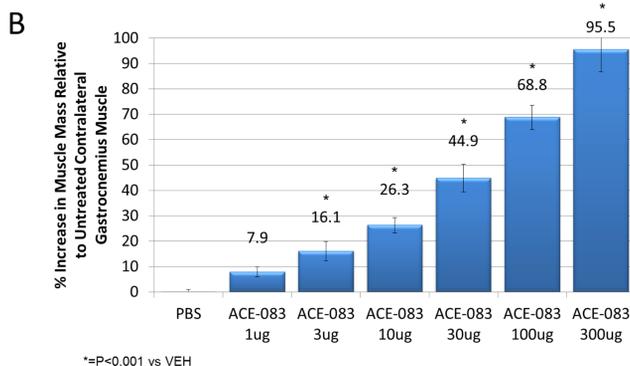
- C57BL/6 mice, 8 weeks of age, received intramuscular injections into the left gastrocnemius muscle of either ACE-083 (1-300 μg, 50 μL, twice weekly) or vehicle (PBS, 50 μL, twice weekly) for four weeks.
- Local administration of 1 to 300 μg of ACE-083 is, approximately equivalent to 0.05 mg/kg to 15 mg/kg body weight.
- FSH levels were measured in terminal serum samples using ELISA

Results

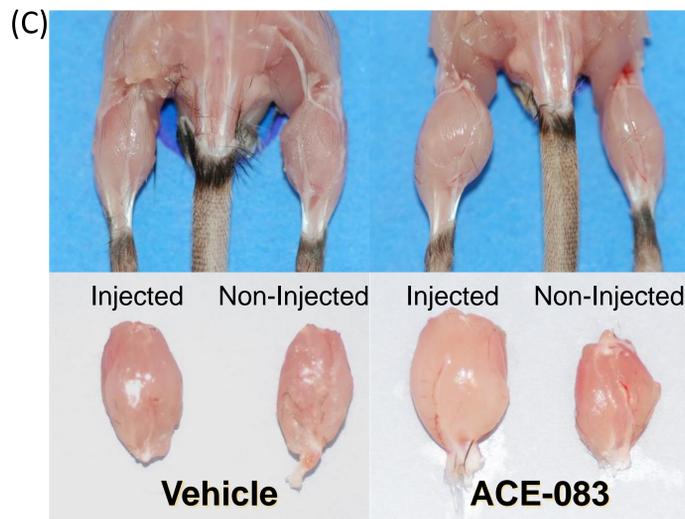
Fig.1 ACE-083 Increases Muscle Mass Only in the Locally Injected Muscle



(A) At necropsy the gastrocnemius muscles from the injected (left) and contralateral, non-injected (right) leg were excised and weighed. ACE-083 injections $\geq 3\mu\text{g}$ increased the left gastrocnemius weight compared to the non-injected contralateral leg ($P<0.001$) as well as compared to the PBS injected left legs ($P<0.01$) in the vehicle group. Gastrocnemius weights have been normalized to the individual body weight for each animal.

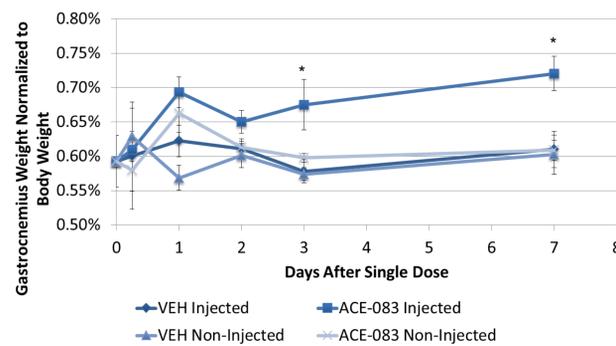


(B) Shown is the percent increase in muscle mass between the injected left gastrocnemius and non-injected contralateral gastrocnemius.



(C) Shown are representative animals and gastrocnemius muscles from the 300μg cohort at the termination of the study.

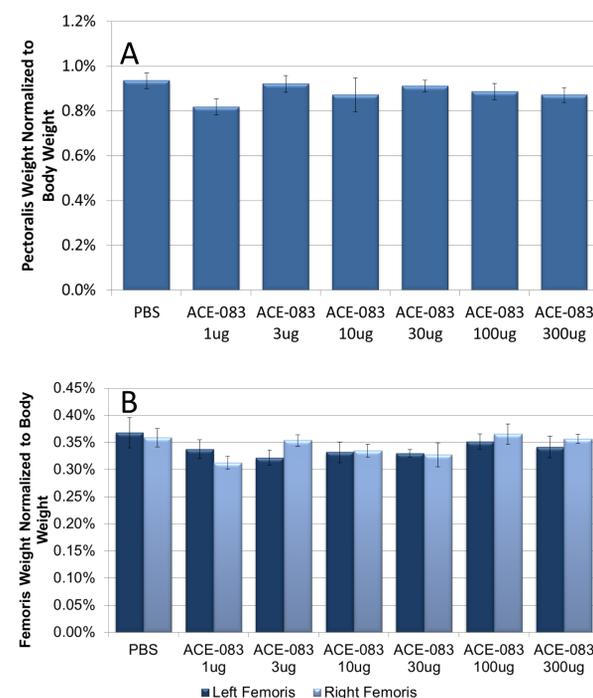
Fig.2 A Single Dose of ACE-083 is Increases Muscle Mass



* $P<0.001$ vs contralateral leg and VEH

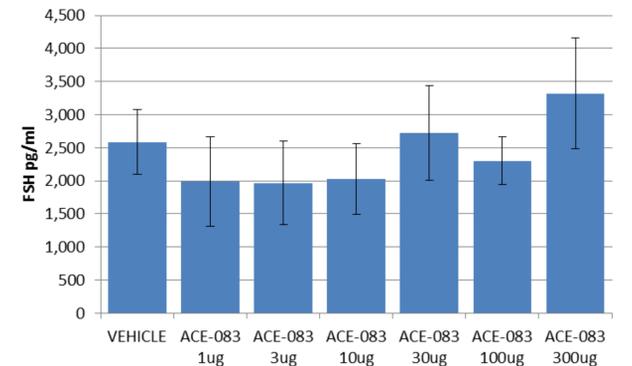
A single dose of ACE-083 (100μg) caused a significant increase in gastrocnemius size 3 days post-injection ($P<0.001$).

Fig. 3 ACE-083 Does Not Affect Non-injected Muscles



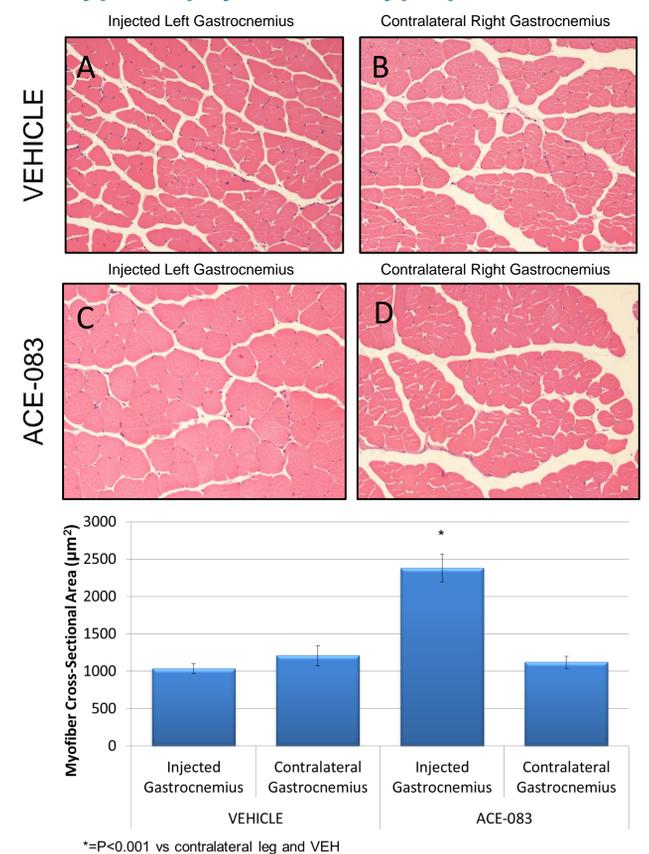
Pectoral and femoris muscles were collected to determine if ACE-083 injected into the left gastrocnemius muscle entered the circulation and affected non-injected muscles. None of the cohorts had a significant increase in (A) pectoral muscle size or (B) femoris size.

Fig. 4 ACE-083 Does Not Affect Serum FSH



There were no significant changes in FSH with ACE-083 treatment injected locally suggesting that ACE-083 did not inhibit systemic activin A activity

Fig.5 ACE-083 Increases Muscle Mass by Hypertrophy without Hyperplasia



* $P<0.001$ vs contralateral leg and VEH

At necropsy the gastrocnemius muscles from the 100μg cohort were excised and analyzed for fiber cross-sectional area at the midline of the gastrocnemius muscle for 200 fibers from each animal ($n=10$). The ACE-083 injected left gastrocnemius muscles (C) had fiber cross-sectional areas that were significantly greater than the contralateral leg (D) as well as the VEH gastrocnemius (A,B) ($P<0.001$).

Conclusions

- These data demonstrate that intramuscular administration of ACE-083 increases muscle mass in the injected muscle and does not affect non-injected muscles.
- The increases in muscle mass were dose dependent
- A single dose of ACE-083 was effective and muscle mass increased 3 days post-injection
- Muscle size was increased by muscle fiber hypertrophy and there is no evidence of hyperplasia.
- Locally injected ACE-083 did not affect systemic activin A activity as monitored by FSH levels
- Local administration of ACE-083 may be useful in the treatment of diseases affecting a select set of muscles such as inclusion body myositis or other muscular dystrophies.

