A Novel Fast Skeletal Muscle Activator, CK-2017357, Improves Muscle Function in a Rodent Model of Myasthenia Gravis

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Introduction

Myasthenia gravis is a chronic autoimmune disease wherein the body produces nicotinic cholinergic receptor antibodies that inhibit transmission at the neuromuscular junction, causing skeletal muscle weakness and fatigue. CK-2017357 is a small molecule activator of fast skeletal muscle that sensitizes the sarcomere to calcium and increases sub-maximal force production. The objective of these studies was to determine if administration of CK-2017357 could increase muscle strength and improve functional capacity in a rodent model of experimental autoimmune myasthenia gravis (EAMG).

Female, Sprague-Dawley rats injected with a single intra-peritoneal dose of inhibitory acetylcholine receptor antibody displayed decreased tension-generating capacity of intact, fast twitch muscle *in situ* and decreased conscious functional capacity assessed by forelimb grip strength. Single doses of CK-2017357 improved conscious grip function in a dose-dependent manner. In addition, CK-2017357 increased force-generating capacity and decreased tension decline during repeated stimulation of intact muscle *in situ*.

Together these data suggest CK-2017357 may ameliorate muscle weakness and fatigue associated with myasthenia gravis to provide a novel therapeutic approach for treatment of patients with this disease.

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Methods

A series of experiments were designed under this study to test the effect of oral doses of CK-2017357 at 5mg/kg, 10mg/kg and 20mg/kg.

All animal procedures were performed under strict compliance with IACUC guidelines. Female Sprague Dawley rats were purchased from Charles River Laboratories (Wilmington, MA). All animals were housed three per cage in a 12-hour light cycle and fed standard chow (Lab Diet 5001) and water *ad libitum*.

Grip strength was measured for all animals prior to antibody injection. Grip strength was measured as previously described by Giardina WJ (Reference 7). After initial grip measurements, all animals received the monoclonal antibody to the acetylcholine receptor (AChRα1/3/5) via intraperitoneal injection. The 20mg/kg cohort received 500ug from lot # I1807 and all others received 750ug of AChRα1/3/5 from lot # I1807 (Santa Cruz Biotechnology, Inc.). Animals had variable responses to antibody exposure so daily clinical observations were performed and all rats were given a clinical score. The clinical scoring system is outlined in Table 1. At 72 hours the myasthenia gravis phenotype was apparent in affected rats. Only animals with a deficit in grip strength (values of 1-2.5kg/kg BW) 72 hours after antibody injection were included in the study.

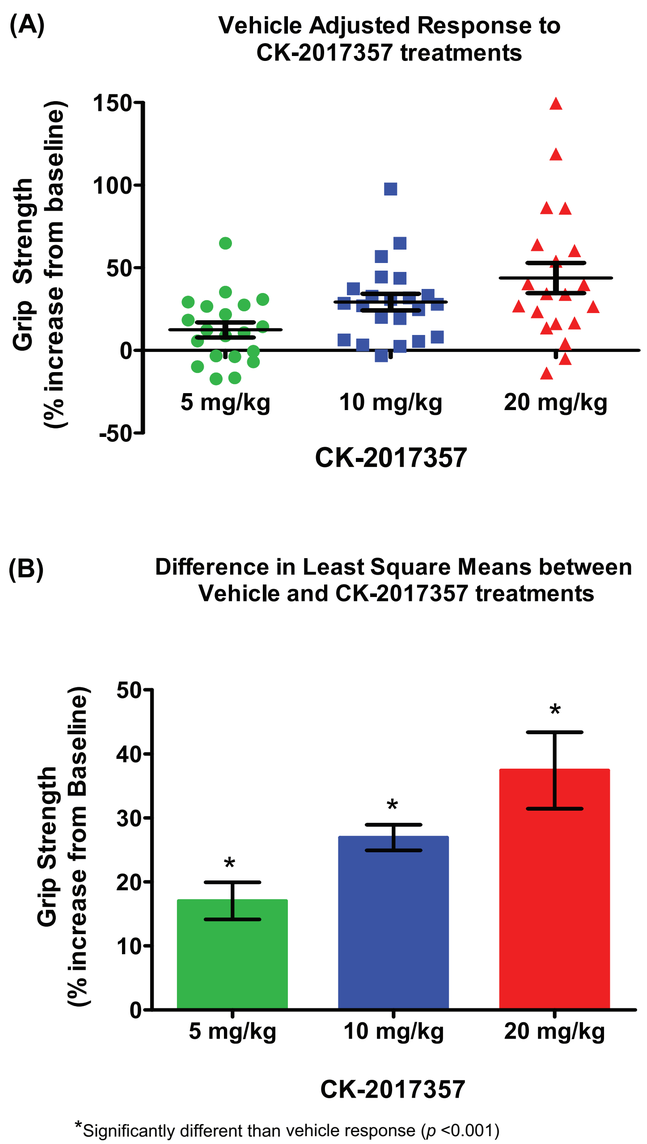
Animals were assigned to groups such that all groups were balanced with respect to average grip strength. Animals were tested on two consecutive days in a cross-over study design. At 72 hours after antibody treatment, half of the animals received an oral dose of CK-2017357, the other half received vehicle. Grip strength was measured by an investigator blinded to study treatment. At 96 hours, all animals received the opposite treatment followed by grip strength measurements.

Grip measurements were assessed twice, once before and once shortly after the expected time of maximum CK-2017357 plasma concentration. Each animal's grip measurements were repeated three consecutive times at each time point and averaged as a single value. In the final analysis, grip strength changes for each individual animal were calculated as the difference between the measurement after vehicle dose to its grip strength after a compound dose.

In order to determine plasma concentrations of CK-2017357 blood samples were collected after grip measurements and analyzed by an in house bioanalytical group. After the 72 hour measurements, blood was collected from the tail vein. After the 96 hour measurements, animals were humanely euthanized and a terminal blood sample was collected by cardiac puncture.

Results

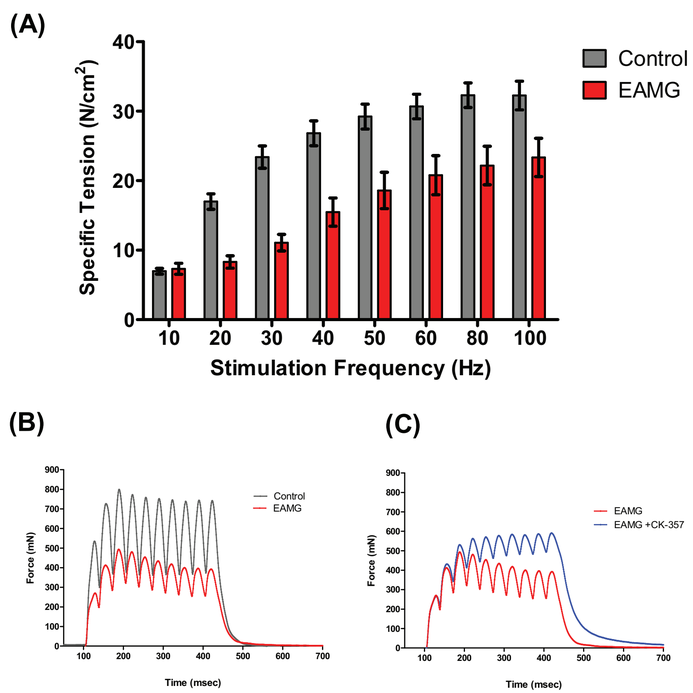
Figure 1: Single doses of CK-2017357 improved conscious grip function in a dose-dependent manner



*Significantly different than vehicle response (p <0.001)

The efficacy of CK-2017357was tested at 5, 10 and 20mg/kg. **(A)** Each rat's grip strength values were normalized against the rat's own baseline grip strength value. Plotted here are normalized individual grip values at 60 minutes after treatment with CK-2017357. **(B)** Difference in Least Square Means between Vehicle and CK-2017357 treatments.

Figure 2: CK-2017357 improves muscle force and reduces fatigability in isolated muscle from animals with EAMG.



The extensor digitorum longus (EDL) of female rats were evaluated *in situ* in order to look at EAMG dysfunction and compound treatment at the isolated muscle level. **(A)** Representative experiments demonstrate the force-frequency relationships for animals with EAMG in relationship to control animals. The EDL was stimulated at increasing frequencies from 10 HZ up to 100Hz. EAMG rats generated lower maximal forces than comparable controls. **(B)** EAMG muscles in females demonstrated lower overall force and a greater time-dependent decrease in force over the course of stimulation (the drop off is referred to as "sag") unlike controls. The EDL was stimulated via the peroneal nerve at 30Hz for 350ms. **(C)** CK-2017357 ameliorates sag in EAMG animals, resulting in better maintained force over the time course of stimulation. This graph demonstrates force production in an EAMG rat before and after treatment with CK-2017357 (3mg/kg intravenously).

References

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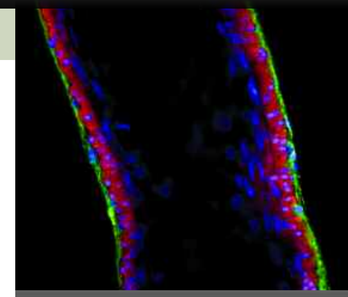

Conclusions

1. Passive-transfer experimental autoimmune myasthenia gravis (EAMG) produces a significant loss of grip strength in Sprague Dawley rats.
2. In this disease model of neuro-muscular dysfunction, isolated muscle demonstrates a loss of muscle strength and an increase in muscle fatigability, replicating hallmark findings found in patients with myasthenia gravis.
3. In this model, the fast skeletal muscle troponin activator, CK-2017357:
   a) Rapidly increased grip strength following oral dosing.
   b) Increased muscle strength *in situ*
   c) Reduced muscle fatigability *in situ*

These findings suggest that sensitization of the troponin complex to calcium, as mediated by the fast skeletal troponin activator, CK-2017357, has the potential to ameliorate the muscle weakness found in myasthenia gravis.

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