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 sarcosome to calcium and increases submaximal 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).

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 (AChR/α155) via intraperitoneal injection. The 20mg/kg cohort received 500ug from lot #1887 and others received 700ug of AChR/α155 from lot #1887 (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/g BW) 72 hours after antibody injection were included in the study.

Animals were assigned to groups that all groups were balanced with respect to average grip strength. Animals were tested on two consecutive days in a cross-over 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 following similar grip strength measurements.

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 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

(A) Vehicle Adjusted Response to CK-2017357 treatments

(B) Difference in Least Square Means between Vehicle and CK-2017357 treatments

The efficacy of CK-2017357 was 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.

Table 1: A summary of grip strength measurements in rats treated with CK-2017357 in a dose dependent manner.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Median Grip Force (g)</th>
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<tbody>
<tr>
<td>5</td>
<td>1.5</td>
</tr>
<tr>
<td>10</td>
<td>2.0</td>
</tr>
<tr>
<td>20</td>
<td>3.0</td>
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*Significantly different than vehicle response (p <0.001)

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

(A) Control vs EAMG

(B) EAMG vs EAMG + CK-357

(C) Stimulation Frequency (Hz)

**Conclusions**

1. Passive transfer experimental autoimmune myasthenia gravis produces a significant loss of grip strength in Sprague-Dawley rats.
2. In this disease model of neuromuscular dysfunction, isolated muscle demonstrates a loss of muscle strength and an increase in muscle fatigability, recapitulating hallmark findings found in patients with myasthenia gravis.
3. In this model, the fast skeletal muscle activator, CK-2017357, has the potential to ameliorate muscle weakness found in myasthenia gravis.

REFERENCES