The Fast Skeletal Troponin Activator, CK-2017357, Improves Resistance to Fatigue in Healthy, Conscious Rats

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Abstract

Fast skeletal muscle troponin activators, such as CK-2017357, amplify the force response of muscle at sub-maximal nerve stimulation frequencies (the force frequency response) in *ex vivo* and *in situ* muscle studies. In addition, they decrease the rate at which muscle fatigues *in vitro* and *in situ* under a number of conditions, including a rat femoral artery ligation model of vascular insufficiency (Hinken et al 2010, Russell et al 2012). We report the effects of CK-2017357 on running fatigue resistance in conscious, healthy rats.

1) Treadmill running time (aerobic). Rats were tested at 30 meters/min until failure; time and distance run were recorded. Oral CK-2017357 at doses of 10 and 20 mg/kg, significantly increased running time on the treadmill by 20% over baseline ($p<0.01$) and 50% over vehicle control ($p<0.01$).

2) Rotarod running time (anaerobic). An accelerating rotarod was ramped between 12-25 revolutions per minute (RPM) over 10 minutes after which animals were allowed to continue at 25 RPM for a further 5 minutes. CK-2017357 increased rotarod running time by up to 2-fold at doses of 1 and 3 mg/kg ($p<0.05$ and $p<0.01$ respectively vs. vehicle control). Similar effects were seen with other fast skeletal muscle troponin activators but not with caffeine, creatine or phosphoserine.

Fast skeletal troponin activators, such as CK-2017357, reduce fatigue in healthy rats in a long distance, endurance assay (treadmill), and in a shorter distance, high-intensity assay (rotarod). Thus, fast troponin activation may be useful in treating disorders that increase susceptibility to muscle fatigue.

Introduction

Muscle fatigue is a reduction in contractile capacity following repeat-use and represents a combination of central fatigue (limitations of the central and peripheral nervous system to sustain activity) and peripheral fatigue (intrinsic loss of muscle function such as a reduced effectiveness of excitation-contraction coupling). Together, these result in reduced muscle performance under fatiguing conditions. Diminished resistance to fatigue is a common symptom of multiple diseases with a broad array of causes. In this context, fatigue is a major factor reducing quality of life in conditions such as ALS, COPD, multiple sclerosis, myocardial infarction, claudication, myasthenia gravis, anemia, and chronic fatigue syndrome.

CK-2017357 is a novel activator of the fast skeletal troponin complex that increases troponin calcium affinity and sensitizes the sarcomere to calcium. As a result, lower calcium concentrations are required to generate equivalent force (Russell *et al.*, 2012). Previous studies have shown that CK-2017357 reduces the fatigability of muscle *in vitro* and *in situ*. We therefore assessed the effect of CK2017357 in two models of fatigue in conscious, healthy rats.

Methods

Animals used in this study were maintained in accordance with the Guide for the Care and Use of Laboratory Animals of the Institute. The local Institutional Animal Care and Use Committee reviewed and approved the protocols related to these rodent models.

Treadmill Running

Animals

Male Sprague Dawley rats (Charles River), 10-12 weeks old, 250-400 g. Rats were acclimated for a minimum of 2 days and weight was measured weekly.

Measurement of Endurance Capacity

The endurance capacity of rats was assessed using a progressive exercise test as previously described (Aaker et al., 1996; Helwig et al., 2002). After familiarization with the treadmill apparatus, rats were run at a treadmill speed of 30 meters per minute (m/min) with a 5% incline. Every 15 minutes, the treadmill speed was increased by 5 meters per minute and the rats continued to exercise until they reached the point of fatigue and were unable to continue exercising (figure 1A). Exercise time was measured in minutes while exercise distance was recorded in meters.

Dosage

The experimental observer was blinded to treatment in all cases.

CK-2017357 was administered via oral gavage 2 hours prior to assessment. Each dose was formulated as a suspension containing 1% hydroxypropyl methylcellulose (HPMC), 0.2% Tween 80, and micronized CK-2017357. Dose volume was 5 ml/kg. Suspensions of CK-2017357 were made up each week and were mixed well before each dose. Vehicle (0.2% Tween 80, 1% HPMC and water) was administered similarly.

Fatiguing Rotarod Test

Animals

Female Sprague Dawley rats (210-260g) were obtained from Charles River Laboratories and acclimated in the test facility for a minimum of six days prior to the start of the study.

Measurement of Endurance Capacity

Rats were assessed according to a protocol developed at Cytokinetics to assess motor coordination under fatiguing conditions (Figure 1B)

All rats were trained the day prior to compound administration. Training consisted of placing the rats on the rotating drum (rod), starting at a low constant speed (10 RPM). The rats were acclimated to walk on the drum for 5 minutes before resting. A second training session of an increasing speed from 14-16 RPM was initiated after all rats in the experimental group had finished the first training session. Those rats that failed to run during the course of the training were removed from the experiment.

On the day of the experiment animals were dosed thirty minutes prior to start of test. The test began with a 5 minute primer session, whereby animals were run at an increasing speed from 14-16 RPM over 5 minutes. Rats were then run at a constantly accelerating rate from 12 RPM to 25 RPM over the course of 10 minutes. Once 25 RPM had been reached, a constant speed of 25 RPM was maintained for an additional 5 minutes. Time to fall was recorded, with the test being terminated at 900 seconds.

Dosage

The experimental observer was blinded to treatment in all cases.

CK-2017357 was administered via oral gavage 30 minutes prior to assessment. Each dose was formulated as a suspension containing 0.2% Tween 80, 0.5% HPMC and water. Dose volume was 5 mL/kg. Vehicle (0.2% Tween 80, 0.5% HPMC and water) was administered similarly.

Creatinine (300mg/kg), Caffeine (10mg/kg) and Phosphoserine (1000mg/kg) were administered in water by oral gavage 60 min, 30 min and 24 hours prior to test respectively.

Treadmill Ramp Protocol

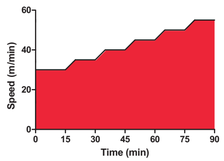

Figure 1A: Treadmill ramp protocol. Animals were run at a speed of 30 m/min with increase in speed of 5 m/min every 15 minutes until fatigue.

Rotarod Ramp Protocol

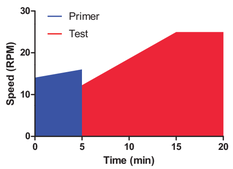

Figure 1B: Rotarod ramp protocol. A five minute primer session was used to improve performance variability, followed by a 15 minute test session that ended with the fall of the animal, or at 900 seconds

Results: Treadmill

Doses of 10 and 20 mg/kg of CK-2017357 resulted in an increase in treadmill running time of 20% over baseline and 50% over vehicle control **(Fig 2A)**

Equivalent increases were seen in distance run **(Fig 2B)**

	Distance (m) Mean ± SEM			Time (Min) Mean ± SEM		
	CK-2017357	Vehicle	P-value*	CK-2017357	Vehicle	P-value*
Predose	1527 ± 127	1330 ± 134	NS	49.3 ± 3.2	44.2 ± 3.6	NS
10 mg/kg	1972 ± 176	1140 ± 129	<0.01	60.7 ± 4.2	38.9 ± 3.6	<0.01
20 mg/kg	1943 ± 205	1281 ± 101	<0.05	59.4 ± 4.7	42.9 ± 2.8	<0.05

*2-way ANOVA vs vehicle control with post-hoc Dunnett's test

Treadmill Running in Rats Administered CK-2017357

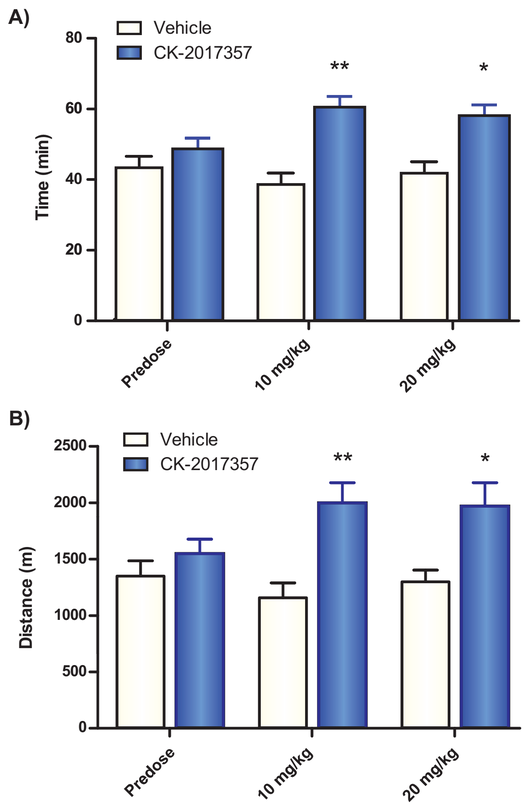

Figure 2:

A) Time on treadmill and **B)** Distance run on treadmill for rats administered oral gavage of CK-2017357 or vehicle (*=P<0.05, **=P<0.01 vs. matched vehicle control by 2-way ANOVA with post hoc Dunnett's)

Results: Rotarod

Rats administered CK-2017357 showed a dose dependent increased in running time on a slowly accelerating rotarod, with 3 mg/kg dose showing more than a doubling of running time at maximum dose tested. **(Fig 3A)**

Dose (mg/kg)	Run time (s) Mean ± SEM	P-value*
Vehicle	169 ± 28	N/A
0.3	301 ± 36	NS
1	334 ± 29	p<0.05
3	389 ± 65	p<0.01

*1-way ANOVA vs vehicle control with post-hoc Dunnett's test

Rats administered compounds previously shown to improve performance in other exercise assays showed no significant difference.

Control treatments were chosen based on associated with the amelioration of central (Caffeine, Davis, 2003) and muscular (Creatine, Boyadjiev, 2007) fatigue as well as phosphoserine, which has been suggested to function in a dual manner (Fanelli, 1976). **(Fig 3B)**

Rotarod Running in Rats Administered CK-2017357

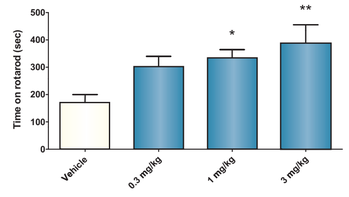

Figure 3A: Time to fall during accelerating rotarod for rats administered CK-2017357 or vehicle (*=P<0.05, **=P<0.01 vs. matched vehicle control by 1-way ANOVA with post hoc Dunnett's test)

Rotarod Running in Rats Administered Other Potential Anti-fatiguing Compounds

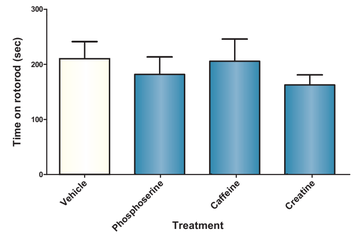

Figure 3B: Effect of compounds previously described to increase fatigue resistance in rodents

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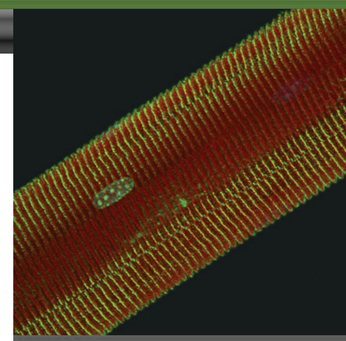

Conclusions

Treadmill Running

Rats showed improvements of 50% in running time compared to controls when administered CK-2017357 at doses of 10 and 20mg/kg.

These data suggest that troponin activators are capable of substantially improving performance in an endurance-type fatigue assay.

Rotarod Running

Rats showed a doubling in running time in the rotarod test at a dose of 3 mg/kg. Potential control anti-fatiguing treatments did not improve performance in this test.

These data suggest that troponin activators are capable of substantially improving performance in an assay that tests motor coordination under moderately fatiguing and increasingly difficult conditions.

Summary

Taken together, these data suggest a role for CK-2017357 and other troponin activators in reducing muscle-related fatigue that may have utility in disease conditions where muscle-related fatigue leads to disability.

Cytokinetics