CK-2017357, A Novel Activator of Fast Skeletal Muscle, Increases Isometric Force Evoked by Electrical Stimulation of the Anterior Tibialis Muscle in Healthy Male Subjects

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Introduction and Study Objective

- CK-2017357 (CK-357) is a a novel, selective small molecule activator of fast skeletal muscle
- CK-357 slows the rate of calcium release from the regulatory troponin complex of fast skeletal muscle and thus sensitizes the sarcomere to calcium[1]
- Pre-clinically, CK-357 increases skeletal muscle force during sub-maximal neural stimulation (i.e., shifts the force-frequency profile up and to the left) and increases the time to fatigue[2]
- The objective of this study was to determine the change in the force-frequency profile of the tibialis anterior muscle and its relationship to the CK-357 plasma concentration after oral administration of CK-357 to healthy male volunteers

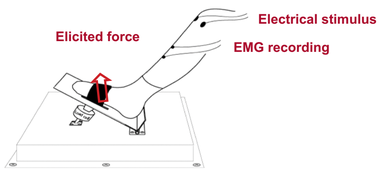
Study Design & Methods

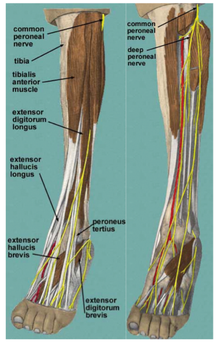
Study Design

- Randomized, double-blind, placebo-controlled, 4-way crossover study
  - In random order, three single doses (250, 500, and 1000 mg) of CK-357 and placebo were administered orally in a liquid suspension formulation
  - Washout period of 7 days between each treatment day
  - Pharmacodynamic effect was assessed by external stimulation of the anterior tibialis muscle over a range of stimulation frequencies (5-50 Hz)
  - Force-frequency response was measured at pre-dose, 1, 3, 5, and 7 hours after dosing during each treatment day; at each assessment time point, the force evoked by each stimulation frequency was normalized to the 50 Hz (~tetany) response
  - N=12 subjects
- Key eligibility criteria
  - Healthy male volunteers between 18-50 years old
  - BMI of 18.0 to 30.0 kg/m^2
  - Able to comply with and tolerate pharmacodynamic testing procedures

Methods: Pharmacodynamic Assessment of Force-Frequency Relationship

- Stimulate a nerve-muscle pair (peroneal nerve, anterior tibialis muscle) via external electrodes
- Measure isometric force at multiple nerve stimulation frequencies
- Voluntary contribution minimized





Anterior tibialis muscle ~ 25% fast (Type II) fibers

Methods: Pharmacodynamic Data Acquisition and Analysis

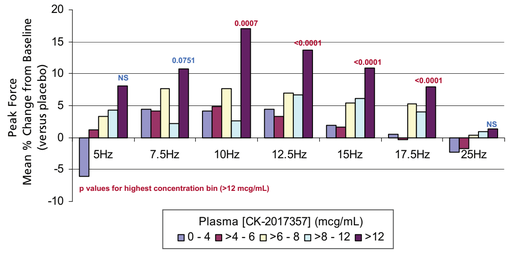
- A pre-dose stimulation protocol was run to establish the baseline response. Stimulation protocols were subsequently run at 1, 3, 5, and 7 hours post-dose.
- Each stimulation protocol consisted of three sequences of eight stimulation trains consisting of 5, 7.5, 10, 12.5, 15, 17.5, 25 and 50 Hz delivered in mixed order. Trains were separated by approximately 40 seconds.
- The peak forces from the three trains at each frequency were averaged and normalized by dividing by the 50 Hz response.
- For each subject at each time point, the percent change in normalized force from baseline was calculated for each frequency.
- Placebo-corrected percent changes from baseline and p-values were calculated for each treatment period using a repeated measures ANCOVA model that included treatment, sequence, and period as fixed effects, baseline as a covariate, and subject as a random effect.

Results

Baseline Characteristics

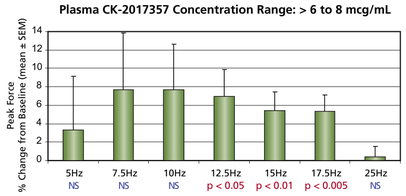
Baseline Characteristic	All Subjects (N=12)
Age, years Mean (SD) Min, Max	 30.4 (7.0) 20, 41
Sex (male), n (%)	12 (100)
Body Mass Index, kg/m^2 Mean (SD) Min, Max	 26.5 (2.7) 20.6, 29.9

Significant Increases in Skeletal Muscle Force at Physiologically-Relevant Stimulation Frequencies: Plasma Mid-Concentration Range



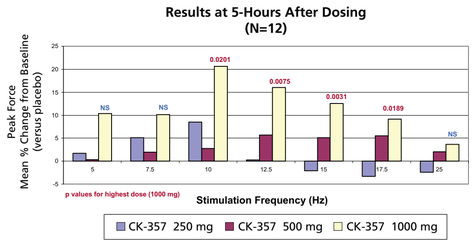
- Peak force normalized to the response at 50 Hz
- Results present placebo-corrected percent change from baseline calculated by pooling all time points and binning by coincident plasma concentration

Significant Increases in Skeletal Muscle Force at Mid-Concentration Range



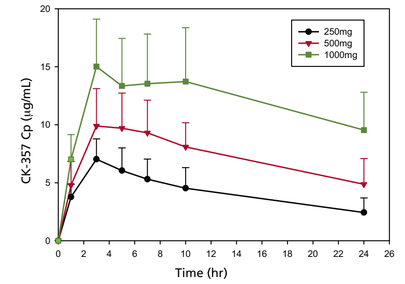
Number of observations = 31 on CK-357; 48 on placebo

Significant Increases in Skeletal Muscle Force by Dose



- Peak force normalized to the response at 50 Hz
- Results present placebo-corrected percent change from baseline at the 5-hour time point

Mean Plasma Concentration – Time Profiles



Summary of Pharmacokinetic Parameters

CK-2017357 Dose (mg)	Pharmacokinetic Parameter Mean (SD) Min, Max			
	C_{max} (µg/mL)	$C_{24\ hr}$ (µg/mL)	$AUC_{24\ hr}$ (hr·µg/mL)	t_{max} (hr)
1000	16.0 (4.1) 9.2, 25.6	9.5 (3.3) 6.1, 16.5	282.0 (83.6) 175.4, 454.8	5.3 (3.1) 3.0, 10.0
500	10.6 (2.9) 7.4, 15.4	4.9 (2.2) 2.1, 9.4	170.0 (53.5) 102.9, 269.0	4.1 (2.0) 3.0, 10.0
250	7.1 (1.7) 4.8, 10.2	2.4 (1.3) 1.0, 5.3	98.8 (36.1) 62.1, 168.4	3.5 (0.9) 3.0, 5.0

Note: Half-life, apparent distribution volume, and clearance could not be adequately calculated from the available data

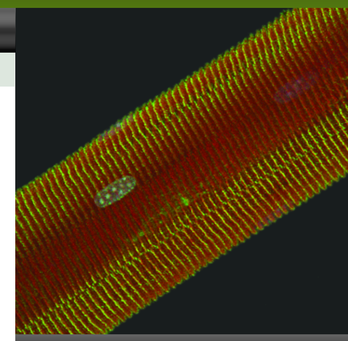
Adverse Events and Other Safety Assessments

- All AEs were mild in severity except for one AE of pharyngitis that was moderate in severity
- No SAEs
- No clinically important changes in range of other safety assessments including clinical labs, vital signs, and ECGs

Adverse Event	Placebo (n = 12)	CK-357 250 mg (n = 12)	CK-357 500 mg (n = 12)	CK-357 1000 mg (n = 12)	Any Treatment (n = 12)
Any Adverse Event	5 (42%)	6 (50%)	10 (83%)	12 (100%)	12 (100%)
Euphoric Mood	0	3 (25%)	6 (50%)	7 (58%)	10 (83%)
Dizziness	1 (8%)	1 (8%)	4 (33%)	6 (50%)	9 (75%)
Somnolence	2 (17%)	3 (25%)	3 (25%)	3 (25%)	6 (50%)
Headache	2 (17%)	0	0	0	2 (17%)
Dermatitis (contact)	0	0	1 (8%)	1 (8%)	1 (8%)
Muscle spasms	0	0	0	1 (8%)	1 (8%)
Nausea	0	0	0	1 (8%)	1 (8%)
Pharyngitis	0	0	0	1 (8%)	1 (8%)
Rhinorrhoea	0	0	1 (8%)	0	1 (8%)

References

1. Kawas R, Russell AJ, Muci A, Morgan B, Malik F, Hartman JJ. *The Small Molecule Skeletal Sarcomere Activator*, CK-2017357, is a Calcium Sensitizer that Binds Selectively to the Fast Skeletal Troponin Complex. Biophysical Society 54th Annual Meeting, San Francisco, CA. February 2010.
2. Russell AJ, Lee K, Jia Z, Muci A, Browne W, Tomlinson M, Hartman JJ, Hansen R, Hinken AC, Albertus D, Claflin D, Morgans DJ., Morgan B, Malik F. *The Fast Skeletal Troponin Activator, CK-2017357, Increases Skeletal Muscle Force* in vitro *and* in situ. 2009 Experimental Biology Conference, New Orleans, LA. April 2009.



Conclusions

1. CK-2017357 significantly increased the mean placebo-corrected normalized peak force produced in response to transcutaneous electrical stimulation of the tibialis anterior muscles of healthy volunteers in a dose-, concentration-, and frequency-dependent manner
2. CK-2017357 was generally well tolerated; all AEs were mild except for one AE of pharyngitis of moderate severity, and no SAEs were reported
3. This study showed that the mechanism of action of CK-2017357 demonstrated in pre-clinical models can be translated into statistically significant and potentially clinically important increases in skeletal muscle performance in healthy human volunteers
4. These results support further evaluation of CK-2017357 in neuromuscular and other diseases associated with muscle weakness or fatigue

CYTOKINETICS