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 Rationale

CK-2017357 (CK-357) is a small molecule activator of fast skeletal muscle which decreases the rate of calcium release from the regulatory troponin complex thereby sensitizing the sarcomere to calcium. In detergent-permeabilized muscle fibers, this effect results in a leftward shift in the force-calcium relation; muscle fibers produce more force at lower calcium concentrations without a change in maximal force. In intact muscle, the calcium sensitization effect of CK-357 results in increased force generation during submaximal contractions.

A first time in human clinical trial (CY4011A) established the safety and tolerability of CK-357 administered as single oral doses to healthy male subjects. A subsequent follow-on study (CY4011B) was performed to determine if the shift in the force-frequency relation demonstrated preclinically could be recapitulated in healthy volunteers. Confirmation that the mechanism of action translated into humans would lend support for further study of CK-357 in disease settings.

Pre-clinical Findings

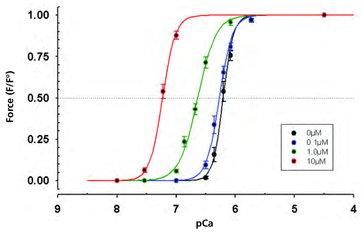
The fast skeletal activator CK-357:

(A) increases Ca^{2+}-sensitivity of isolated, human type IIa, IIx/d muscle fibers.

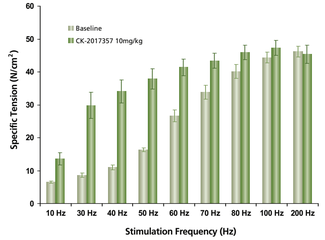
(B) increases force in rat EDL muscle in situ at sub-tetanic stimulation frequencies.

CK-2017357 Sensitizes the Fast Skeletal Sarcomere to Calcium

(A) Human IIa, IId/x Skinned Muscle Fibers



(B) *In situ* Stimulation of Rat EDL via Peroneal Nerve



Dennis Claflin (Univ. of Michigan)

Study Design & Methods

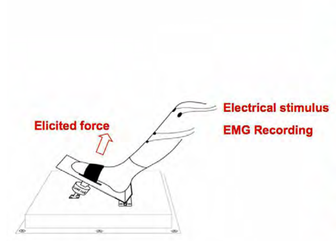
The primary objective was to determine the change in force-frequency profile and its relation to CK-357 plasma concentration when administered orally to healthy volunteers.

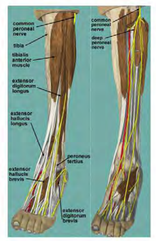
Study Design

- Randomized, double-blind, placebo-controlled, 4-way crossover study with 12 subjects.
- In random order, three single doses (250, 500, and 1000 mg) of CK-357 and placebo administered orally in a liquid suspension formulation with 7 day washout period between dosing.
- Pharmacodynamic effect assessed by transcutaneous nerve stimulation (deep peroneal nerve) to evoke mechanical response of the anterior tibialis muscle.
- Isometric force measured at multiple stimulation frequencies (5, 7.5, 10, 12.5, 15, 17.5, 25 and 50 Hz).
- Each stimulation protocol consisted of three sequences of stimulation trains delivered in mixed order. Trains were 800 ms in duration and separated by approximately 40 s.
- A pre-dose stimulation protocol established the baseline response. Force-frequency response was measured at 1, 3, 5, and 7 hours after dosing with commensurate blood draw to measure CK-357 plasma levels.
- Key eligibility criteria:
  - Healthy male subjects between 18-50 years old
  - BMI of 18.0 to 30.0 kg/m^2
  - Able to comply with and tolerate pharmacodynamic testing procedures

Study Design & Methods (contd.)

Phamacodynamic Effect Assessed by Transcutaneous Stimulation of the Deep Peroneal Nerve to Evoke a Mechanical Response from the Anterior Tibialis Muscle





Analysis and Quality Assessment of Data

- The peak forces *(F)* 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 *(%F)* as well as the difference of summed forces over all frequencies from the summed baseline normalized to the summed baseline *(%ΣF)*.

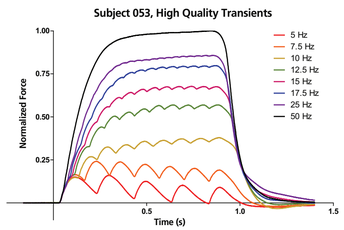
$$\%F = \frac{F - F_o}{F_o} \times 100\% \qquad \%\sum F = \frac{\sum F - \sum F_o}{\sum F_o} \times 100\%$$

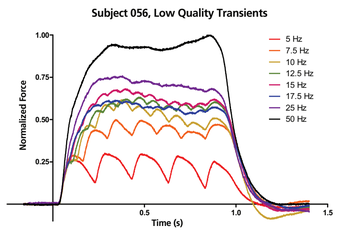
- A dataset containing all data from each subject was assembled and referred to as the **All Data** set.
- A second dataset called the **Over Read** set was assembled by an expert working prior to unblinding of the study using the following quality assessment score:

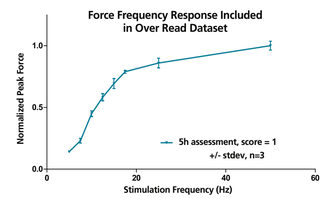
Score Value	Assessment Score Description
1	Ideal, i.e. continuous with canonical force frequency shape
2	Continuous but some spread in replicates, minor non-ideality in force frequency shape
3	Discontinuous, non-ideal force frequency shape
4	Highly discontinuous and underlying traces far from canonical
5	Failure, i.e. measurement could not be completed or data could not be fit to extract force parameters

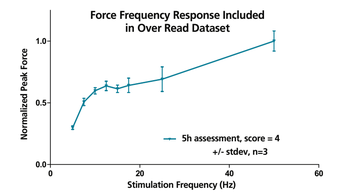
  - Dosing periods for an individual with assessment scores averaging > 2 were rejected in their entirety. Each dosing period was required to have baseline assessment scored at 1 or 2.
  - The Over Read dataset contained data from each subject and 75% of the overall data.
- For each dataset, the placebo-corrected 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.

Example Force Transients from Stimulation of the Anterior Tibialis in Human Subjects







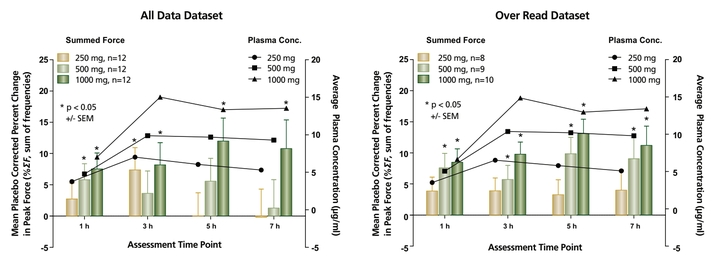


Results

Significant Increases in Placebo Corrected Summed Force Response by Dose

- Both the All Data and Over Read analysis show significant increases in the summed percent change from baseline metric.
- Over Read analysis shows a smoother dose-dependent profile and achieves significance at additional time points for intermediate and low CK-357 doses.
- Plasma concentrations between the All Data and Over Read groups are nearly identical for each dose at each time point.

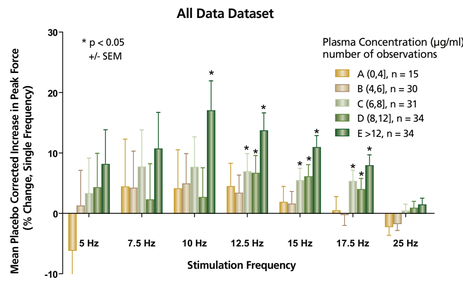
Percent Change in Summed Frequency Peak Force by Dose

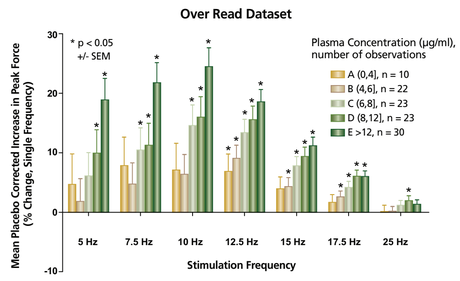


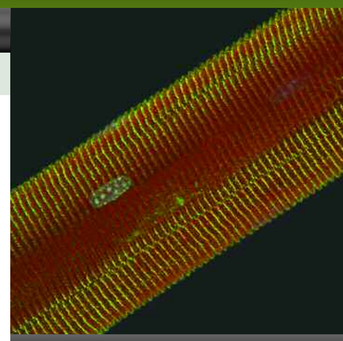
Significant Increases in Placebo Corrected Force Response by Plasma Concentration

- Results are calculated by pooling all time points and binning by coincident plasma concentrations.
- Both the All Data and Over Read analysis show significant increases in the percent change from baseline at low to mid stimulation frequencies at multiple concentration bins.
- The Over Read analysis shows a smoother dose-dependent profile with significance established over a wider range of stimulation frequencies and plasma concentrations.

Percent Change in Peak Force by Plasma Concentration







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. Applying quality metrics to remove inconsistent data prior to analysis resulted in a less variable dataset that showed a smoother dose-dependent response and significant changes at lower plasma concentrations.
3. The mechanism of action of CK-2017357 as demonstrated in pre-clinical models can be translated into statistically significant and potentially clinically important increases in skeletal muscle performance in healthy male volunteers.
4. Further evaluation of CK-2017357 in neuromuscular diseases where neural input is limiting as well as other conditions associated with muscle weakness or fatigue is warranted.

CYTOKINETICS