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 sarcosomes 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 [CY0011A] established the safety and tolerability of CK-357 administered as single oral doses to healthy male subjects. A subsequent follow-on study [CY0110] was performed to determine if the shift in the force-frequency relation demonstrated previously could be recapitulated in healthy volunteers. Confirmation that the mechanism of action translated into human would lend support for further study of CK-357 in disease settings.

Pre-clinical Findings

The fast skeletal activator CK-357:
- Increases Ca-sensitivity of isolated, human type Ia, IId muscle fibers.
- Increases force in rat EDL muscle in situ at sub-tetanic stimulation frequencies.

CK-2017357 Sensitizes the Fast Skeletal Sarcosome to Calcium

(A) Human Ia, IId Skinned Muscle Fibers  
(B) In situ Stimulation of Rat EDL via Peroneal Nerve

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

- The peak forces (F) from the three trains at each frequency were averaged and normalized by dividing by the 10 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. This difference was normalized to the summed baseline (ΣF).
- 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:

<table>
<thead>
<tr>
<th>Score</th>
<th>Assessment Score Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Weak, i.e. continuous Force frequency shape</td>
</tr>
<tr>
<td>2</td>
<td>Continuous but some spread in replicates, some variability in Force frequency shape</td>
</tr>
<tr>
<td>3</td>
<td>Discontinuous, non-ideal Force frequency shape</td>
</tr>
<tr>
<td>4</td>
<td>Highly discontinuous and underlying traces far from canonical</td>
</tr>
</tbody>
</table>

Dosing periods for an individual with assessment score ≥ 4 were repeated if necessary. Each dosing period was required to have baseline assessment scored ≥ 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 ANOVA 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

Analysis and Quality Assessment of Data

- Significant increases in placebo corrected summed force response by dose over a wider range of stimulation frequencies and plasma concentrations.
- 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.

Study Design & Methods (contd.)

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-2017357 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, 20 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/m2
  - Able to comply with and tolerate pharmacodynamic testing procedures

RESULTS

Significant Increases in Placebo Corrected Summed Force Response by Dose

- Both the All Data and Over Read analysis show significant increases in the percent sum change from baseline metric.
- Over Read analysis shows a smoother dose-dependent profile and achieves significance at additional time points for intermediate dose 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

- Dosing periods for an individual with assessment score ≥ 4 were repeated if necessary. Each dosing period was required to have baseline assessment scored ≥ 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 ANOVA 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

Pre-clinical Findings

The fast skeletal activator CK-357:
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Conclusions

1. CK-2017357 Significantly increased the mean placebo corrected normalized peak force produced in response to transcutaneous electrical stimulation of the tibialis anterior muscle 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.