Characterization of the Cardiac Myosin Inhibitor CK-3773274: a Potential Therapeutic Approach for Hypertrophic Cardiomyopathy

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ABSTRACT

Inhibition of the cardiac sarcomere may be essential for the treatment of cardiomyopathies and cardiac hypertrophy. Here, we evaluated CK-3773274, a novel cardiac myosin inhibitor that reduces cardiac contractility, in healthy adult rat ventricular cardiomyocytes and in a rat model of cardiomyopathy. CK-3773274 significantly reduced force generation in a dose-related manner in a buffer containing 200 mM potassium, which approaches the potassium levels found in mammalian ventricles. CK-3773274 also slowed the rate of rise of tension, which is indicative of a reduction in the rate of actin-activated ATPase activity. Moreover, CK-3773274 decreased fractional shortening in a dose-related manner in a rat model of cardiomyopathy, consistent with its in vitro effects on cardiac contractility. These results suggest that CK-3773274 may be a potential therapeutic candidate for the treatment of cardiomyopathies and cardiac hypertrophy.

RESULTS

A. Characterization of the Cardiac Myosin Inhibitor CK-3773274:

1. **Calcium Transients**

   - Measurement of Cardiomyocyte Contractility and Calcium Transients
     - Adult rat ventricular cardiomyocytes were isolated and loaded with Fluo-4 AM (2 µM) for 45 min at 37°C in a buffer consisting of 25 mM HEPES, 135 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 2 mM CaCl₂, and 10 mM D-glucose (pH 7.4).
     - Calcium transients were recorded at 30 Hz using a inverted microscope equipped with a water immersion objective (60X, 1.25 NA) and a high-speed cooled charge-coupled device camera (Hamamatsu, ORCA-Flash 4.0). Fluorescence was excited with a 488-nm laser, and emission was detected between 500 and 600 nm (bandwidth: 25 nm).
     - Changes in FL were calculated as the percentage change from baseline using the equation (FL - FL0)/FL0, where FL is the fluorescence intensity at the current time point and FL0 is the baseline fluorescence intensity.

2. **ATPase Assays**

   - Myosin ATPase activity was measured using a pyruvate kinase/lactate dehydrogenase-coupled assay in a buffer consisting of 100 mM HEPES, 50 mM KCl, 1 mg/mL bovine cardiac actin, 0.2% bovine serum albumin, 0.2 mM ATP, 10 mM DTT, and 1% DMSO. The ATPase activity was normalized to control (100%).

3. **Blebbistatin Binding Assays**

   - Binding of (-)-blebbistatin to bovine cardiac myosin subfragment-1 was measured using intrinsic tryptophan fluorescence at 298 nm and an excitation wavelength of 291 nm. The data were fitted using a four-parameter dose response equation (95% CI): IC₅₀ = 4.1 ± 0.7 µM.

4. **CK-3773274**

   - CK-3773274 (40 μM) significantly reduced force generation (FS) in a dose-related manner in a buffer containing 200 mM potassium. Relevant data are presented in Table 1.

Table 1. | CK-3773274 | FS (% of Control) |
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<tbody>
<tr>
<td>Control</td>
<td>100 ± 3.3</td>
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<tr>
<td>10 μM</td>
<td>72 ± 5.2</td>
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<tr>
<td>25 μM</td>
<td>55 ± 4.2</td>
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<tr>
<td>50 μM</td>
<td>32 ± 2.9</td>
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<tr>
<td>100 μM</td>
<td>15 ± 1.8</td>
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SUMMARY

In conclusion, CK-3773274 is a novel cardiac myosin inhibitor that selectively inhibits cardiac myosin ATPase activity and decreases myocardial contractility. No personal information is stored.

REFERENCES