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


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ABSTRACT

Hypercontractility of the cardiac sarcomere appears in an adverse pathophysiology of hypertrophic cardiomyopathy and cardiac hypertrophy. To identify therapeutic approaches to address this pathology, we evaluated the effects of CK-3773274, a novel cardiac myosin inhibitor, on cardiac contractility in vivo and on isolated cardiac myofibrils. Male Sprague Dawley rats were anesthetized with inhaled isoflurane (1%–5%) throughout the echocardiogram procedure. Baseline contractility was assessed 1 day prior to CK-3773274 treatment. Animals were orally dosed with vehicle (0.5% hydroxypropylmethylcellulose [HPMC] /0.1% Tween 80) or 2 mg/kg CK-3773274. Left ventricular ejection fraction (LVEF) was evaluated following single dosing. Two hours after dosing, left ventricular dimensions were determined by echocardiography at select time points over 24 hours. EF (% of baseline) was measured as a ratio of LVEF to FS (% of basal) with each myofibril type. Raw ATPase activity was determined with the CK-3773274 and IC100 of 1.07 (nM). CK-3773274 reduced cardiac contractility in vivo in Sprague Dawley rats and beagle dogs in a dose- and concentration-related manner. This study was funded by Cytokinetics, Inc. All authors were employees of Cytokinetics at the time of the study.

INTRODUCTION

1. Hypercontractility of the cardiac sarcomere may be a cause of the impaired cardiac function in the failing heart, potentially underlie severe clinical conditions such as hypertrophic cardiomyopathy (HCM) and cardiac hypertrophy, and maladaptive changes in cardiac contractility and maladaptive remodeling. Hypercontractility of the cardiac sarcomere contributes to cardiac hypertrophy by increasing cardiac load and pressure, which then increases cardiac mechanics.

METHODS

Preparation of Reagents

• Myofibrils were prepared from fast-twitch bovine cardiac, bovine masseter, and rabbit psoas muscle, and slow-twitch bovine cardiac muscle as described in Hwee et al (2015).

ATPase Assay

• Single-myeleleine myofibrils were isolated using a discontinuous sucrose gradient and analyzed for ATPase activity as described in Hwee et al (2015). The ATPase activity of each myofibril type was determined with the CK-3773274 and IC100 of 1.07 (nM). CK-3773274 reduced cardiac contractility in vivo in Sprague Dawley rats and beagle dogs in a dose- and concentration-related manner.

Measure of Cardiomyocyte Contraction and Calcium Transients

• Cardiomyocytes were isolated from cultured bovine cardiac myocytes as described in Hwee et al (2015). Cardiomyocytes, and cardiac myofibrils were measured by edge-detection microscopy and fluorescence microscopy, respectively.

Rat Echocardiographic Assessment

• Adult male Sprague Dawley rats were anesthetized with sodium pentobarbital (50 mg/kg, IP) and ventilated with 100% oxygen. Rats were intubated and placed on a heating pad. Anesthesia was induced with 1%–2% isoflurane and maintained with 0.5%–1.5% isoflurane in oxygen. Intravenous access was achieved via a femoral artery or vein. Echocardiographic imaging was performed using a high-resolution ultrasound (20 MHz) imaging system (GE Vivid 7). Two-dimensional (2D) images were acquired in the parasternal long-axis view. Individual cardiac cycles were analyzed and electrocardiographic (ECG) signals were measured simultaneously. Images were digitized, and analyzed by computer software (Cytel, Inc., South San Francisco, CA) to obtain left ventricular (LV) geometry and function. Fractional shortening (FS) was determined as a ratio of LV end-diastolic volume to LV end-systolic volume. LVEF was calculated as a percentage of baseline. This study was funded by Cytokinetics, Inc. All authors were employees of Cytokinetics at the time of the study.

Dog Echocardiographic Assessment

• Several adult male beagle dogs were anesthetized with sodium pentobarbital (50 mg/kg) and intubated with 100% oxygen. Echocardiographic imaging was performed using a high-resolution ultrasound (20 MHz) imaging system (GE Vivid 7). Two-dimensional (2D) images were acquired in the parasternal long-axis view. Individual cardiac cycles were analyzed and electrocardiographic (ECG) signals were measured simultaneously. Images were digitized, and analyzed by computer software (Cytel, Inc., South San Francisco, CA) to obtain left ventricular (LV) geometry and function. Fractional shortening (FS) was determined as a ratio of LV end-diastolic volume to LV end-systolic volume. LVEF was calculated as a percentage of baseline. This study was funded by Cytokinetics, Inc. All authors were employees of Cytokinetics at the time of the study.

RESULTS

Figure 1. CK-3773274 inhibits the ATPase activity of bovine cardiac myofibrils

• The ATPase activity of CK-3773274 in bovine cardiac myofibrils was measured using a discontinuous sucrose gradient and analyzed for ATPase activity as described in Hwee et al (2015). The ATPase activity of each myofibril type was determined with the CK-3773274 and IC100 of 1.07 (nM). CK-3773274 reduced cardiac contractility in vivo in Sprague Dawley rats and beagle dogs in a dose- and concentration-related manner.

Figure 2. CK-3773274 selectively inhibits the ATPase activity of cardiac, and slow-twitch skeletal myofibrils with the same ATPase activity

• Single-myeleleine myofibrils were isolated using sucrose gradient and an ATPase activity assay as described in Hwee et al (2015). The ATPase activity of each myofibril type was determined with the CK-3773274 and IC100 of 1.07 (nM). CK-3773274 reduced cardiac contractility in vivo in Sprague Dawley rats and beagle dogs in a dose- and concentration-related manner.

Figure 3. CK-3773274 inhibits the ATPase activity of bovine cardiac myofibrils

• The ATPase activity of bovine cardiac myofibrils was measured using a discontinuous sucrose gradient and analyzed for ATPase activity as described in Hwee et al (2015). The ATPase activity of each myofibril type was determined with the CK-3773274 and IC100 of 1.07 (nM). CK-3773274 reduced cardiac contractility in vivo in Sprague Dawley rats and beagle dogs in a dose- and concentration-related manner.

Figure 4. CK-3773274 reduces LVEF in vivo in Sprague Dawley rats in a dose- and concentration-related manner

• LVEF (% of baseline) was measured as a ratio of LVEF to FS (% of basal) with each myofibril type. Raw ATPase activity was determined with the CK-3773274 and IC100 of 1.07 (nM). CK-3773274 reduced cardiac contractility in vivo in Sprague Dawley rats and beagle dogs in a dose- and concentration-related manner.

Figure 5. CK-3773274 reduced FS (% of baseline) in young Beagle dogs in vivo in a dose- and concentration-related manner

• FS (% of basal) was measured as a ratio of LVEF to FS (% of basal) with each myofibril type. Raw ATPase activity was determined with the CK-3773274 and IC100 of 1.07 (nM). CK-3773274 reduced cardiac contractility in vivo in Sprague Dawley rats and beagle dogs in a dose- and concentration-related manner.

Figure 6. CK-3773274 reduced EF (% of baseline) and LVEF (% of baseline) in the young Beagle dogs in vivo in a dose- and concentration-related manner

• EF (% of baseline) was measured as a ratio of LVEF to FS (% of basal) with each myofibril type. Raw ATPase activity was determined with the CK-3773274 and IC100 of 1.07 (nM). CK-3773274 reduced cardiac contractility in vivo in Sprague Dawley rats and beagle dogs in a dose- and concentration-related manner.

REFERENCES


SUMMARY

• CK-3773274 is a novel small molecule that selectively inhibits cardiac myosin ATPase activity and contractility in vivo in Sprague-Dawley rats and beagle dogs in a dose- and concentration-related manner. This study was funded by Cytokinetics, Inc. All authors were employees of Cytokinetics at the time of the study.

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