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The Cardiac Myosin Activator, CK-1827452, Accelerates the Enzymatic Step Gating Entry of Myosin into its Force Generating State

Hector M Rodriguez, Raja Kawas, Fady Malik, Roman Sakowicz, James J Hartman

Cytokinetics, Inc. South San Francisco, CA

Abstract

We have previously identified CK-1827452 as a novel small molecule activator of cardiac myosin, and have now characterized it in steady state and transient kinetic assays to understand its mechanism of action. CK-1827452 increases the steady-state rate of ATP hydrolysis of a bovine cardiac thin filament system reconstituted from purified components as well as bovine cardiac myofibrils, in which the sarcomere structure is intact. Analysis of the individual steps in the enzymatic cycle of cardiac myosin suggests that actin-dependent phosphate release is increased in the presence of CK-1827452 while basal (actin-independent) phosphate release is inhibited. We find no effect on the ATP binding, ATP hydrolysis, and ADP release rates in the presence of CK-1827452. The kinetic analysis suggests that the transition rate of myosin from the weakly to strongly bound state is increased (since phosphate release rate is accelerated) and that there is no effect on the exit from the strongly bound state (since the ADP release rate is not changed). Accelerating the weak to strong transition should increase force production and may underlie its ability to improve cardiac contractility in models of cardiac function, including a dog model of heart failure. In a Phase I clinical study in healthy human volunteers, CK-1827452 also produced dose-proportional increases in cardiac ejection fraction, fractional shortening and ejection time. Currently, CK-1827452 is being studied for use in patients with heart failure.

Materials & Methods

CK-1827452 was synthesized at Cytokinetics Inc. All proteins used were produced by the Biochemistry group at Cytokinetics Inc.

Steady state kinetics

All steady state kinetics measurements were made using an NADH coupled assay system by monitoring the absorbance change at 340 nm in 10mM Pipes, 2mM $MgCl_2$, 1mM DTT pH 6.8 (PM10) buffering system. Assays were run at 25°C unless otherwise specified. All measurements were carried out on a Molecular Devices SpectraMax plate reader.

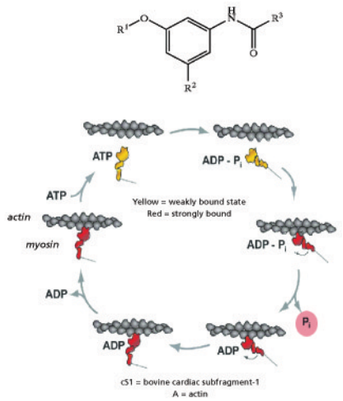
Transient kinetics

Transient kinetic measurements were carried out on a Hi-Tech SF-61DX2 stopped-flow apparatus temperature controlled to 25°C. At least three transient traces were collected and averaged together for each condition. All data analysis was conducted using the KinetAsyst software.

ATP binding to bovine cardiac myosin subfragment-1 (cS1) was measured by monitoring the intrinsic tryptophan fluorescence of cS1. ADP release rates were determined by chasing deacADP from cS1 or acto-cS1 with a high concentration of ATP. ATP stimulated release of cS1 from actin was monitored using pyrene labeled actin and measuring the change in pyrene fluorescence. Phosphate release was monitored using MDCC modified phosphate binding protein (PBP) for cS1 in the presence and absence of bovine cardiac actin (1).

Introduction

There is much interest in small molecule agents that can modulate myosin function. Blebbistatin is an example of one such agent, which specifically inhibits the ATPase activity of type II myosins (2). We discovered a new class of agents that activate the ATPase of myosin and improve contractile function in muscle without increasing the calcium transient (3). Previously we identified a small molecule agent (shown to the right), CK-1213296 (5), that specifically activates the ATPase activity of cardiac myosin by increasing the rate of actin dependent phosphate release (step highlighted in figure to right). We have now characterized the mechanism of action of CK-1827452, a related agent that is currently undergoing clinical trials, using steady state and transient kinetic methods.



CK-1827452 *specifically activates* cardiac myosin

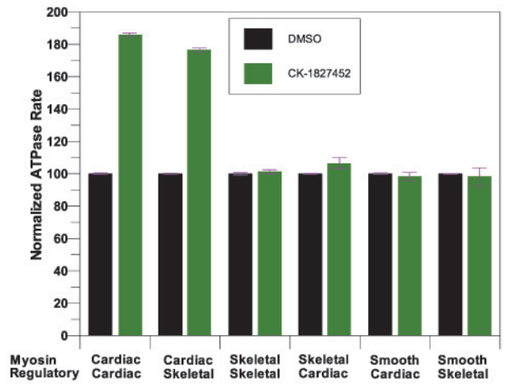


Figure 1. CK-1827452 selectively activates cardiac myosin. Effect of a single dose of CK-1827452 (20 μM) on hybrid cardiac/skeletal Ca-responsive actomyosin systems at pCa=6.75. The top row in the labels corresponds to the myosin subfragment-1 source and the bottom label refers to the source of the thin filament. The cardiac source is bovine, skeletal is rabbit and smooth is from chicken gizzard.

CK-1827452 activates the myofibrillar ATPase but *inhibits* the basal ATPase of cardiac myosin

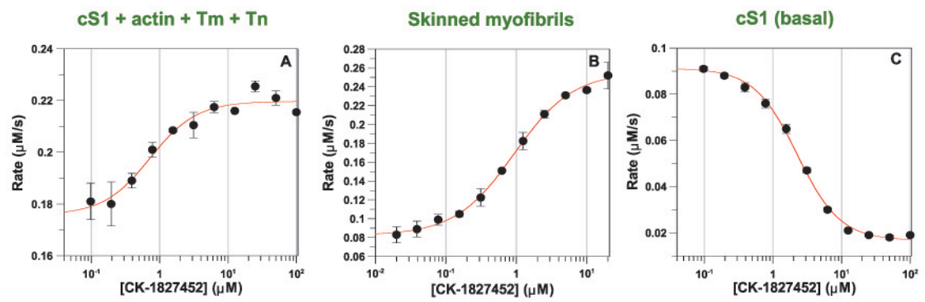


Figure 2. The myosin activator, CK-1827452 activates the cardiac myosin ATPase in complex systems, but paradoxically inhibits the simpler cardiac myosin basal ATPase. Dose response of CK-1827452 in a calcium regulated (A) system (0.5 μM cS1 and 14 μM regulated actin) composed of reconstituted thin filament at 50% calcium activation (soluble regulated system). Skinned (B) myofibrils (1 mg/mL) that are at a calcium concentration to achieve 50% activation. The basal system (C) composed of purified bovine cardiac S1 at 4 μM (basal system). The inhibition of the basal ATPase further demonstrates that CK-1827452 directly interacts with cardiac myosin.

CK-1827452 *accelerates* productive phosphate release while inhibiting non-productive phosphate release

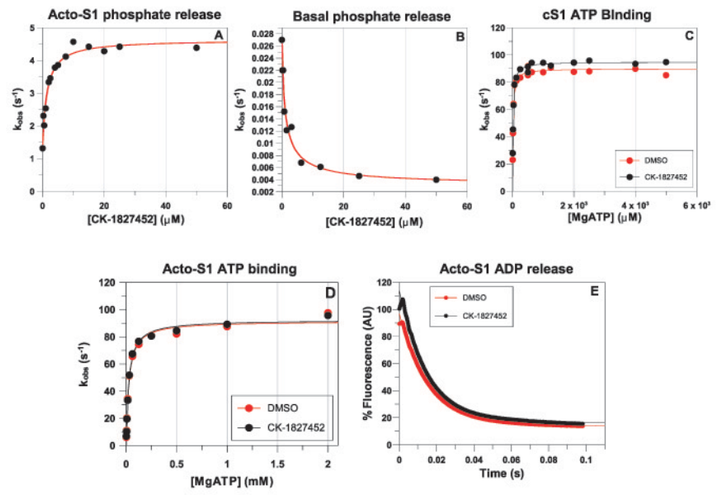


Figure 3. Actin stimulated phosphate release is accelerated up to four fold in the presence of CK-1827452 (EC50=1.4 μM). Basal phosphate release is likely inhibited due to the stabilization of the Myosin-ADP-P_i state. Transient state kinetics of (A) Phosphate release from cS1 under single turnover conditions. (B) Actin independent phosphate release for cS1. (C) ATP binding to cS1. (D) Dependence of pyrene-acto-S1 dissociation rate on the concentration of ATP. (E) Transient kinetic traces of deacADP release from acto-cS1. Concentration of CK-1827452 was kept constant at 50 μM except in phosphate release experiments where the concentration was varied.

CK-1827452 *improves* cardiac function in conscious dogs with congestive heart failure

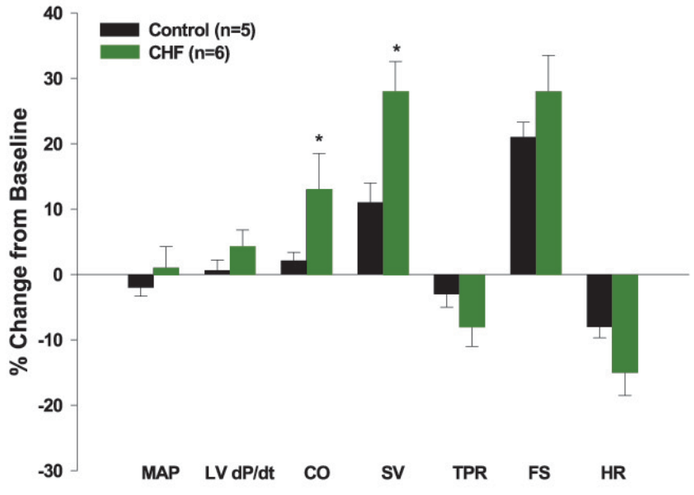
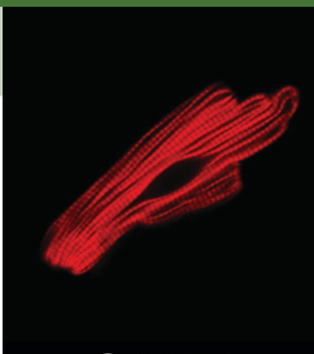


Figure 4. The myosin activator CK-1827452 increases cardiac output and stroke volume in dogs with congestive heart failure. Effects of bolus injection, 0.5 mg/Kg via I.V., of CK-1827452 on mean arterial pressure (MAP), LV dP/dt, cardiac output (CO), stroke volume (SV), total l peripheral resistance (TPR), LV fractional shortening (FS) and heart rate (HR) in control dogs and dogs with congestive heart failure (CHF). Note that CK-1827452 significantly increased CO and SV more in control dogs compared to dogs with CHF. (* $p < 0.05$ control vs. CHF) Data obtained in collaboration with Shen *et al.* (6).



Conclusions

The myosin activator CK-1827452:

- Specifically activates the cardiac myosin ATPase in cardiac myosin systems of increasing complexity.
- Increases the rate of actin stimulated phosphate release and thus entry into the force producing state.
- Inhibits the phosphate release step in the absence of actin.
- Does not affect the rate of ADP release from the actin associated state.
- Improves the cardiac function in animal models of heart failure.
- Has been shown to increase cardiac ejection fraction, fractional shortening and ejection time in healthy humans.

References

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4. Malik *et al.*; United States Patent 6,908,923
5. Rodriguez *et al.*; Biophysical Society, Utah, 2006.
6. Shen *et al.*; Heart Failure Society, Seattle, 2006.

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