AHA Keystone, July 2005.

Direct Activation of Cardiac Myosin, A Novel Mechanism for Improving Cardiac Function.

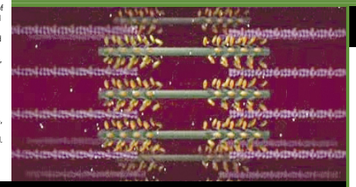
Fady Malik, You-Tang Shen, Tatsuo Katori, Sandra H Sueoka, Robert Anderson, David Cox, Marc Garard, James Hartman, Song-Jung Kim, Erica Kraynack, Alex Kuklov, Ken H Lee, Pu-Ping Lu, Alex Muci, Congrong Niu, Hector Rodriguez, Ion Suehiro, Sheila Sylvester, Todd Tochimoto, Kathleen A Elias, Bradley P Morgan, Roman Sakowicz, David A Kass, Stephen F Vatner, David J Morgans

Current inotropes act upstream of the sarcomere to increase intracellular calcium and secondarily increase cardiac contraction. In addition to effects on contractility, these agents increase heart rate, oxygen consumption, and the incidence of arrhythmias, as well as reduce blood pressure. A more direct approach to improve cardiac contractility that may address these liabilities is activation of the force generating protein, cardiac myosin itself. Utilizing a reconstituted version of the cardiac sarcomere, we screened a small molecule library and identified several chemical classes that activate the cardiac myosin ATPase. One compound class has been optimized extensively using an iterative process guided by biochemical and cellular activity; CK-1213296 is an exemplar of this class. Transient kinetic analysis of the mechanism of action demonstrates that CK-1213296 accelerates the release of phosphate by 2 fold ($EC50 = 2.0 \pm 0.7$ μM) without affecting the ADP release rate, suggesting that CK-1213296 accelerates transition of myosin into the force-generating state without affecting its exit rate. Using Fura-2 loaded primary rat cardiac myocytes, CK-1213296 (0.5 μM) increased cellular contractility by 30.1 ± 3.5% but did not alter peak systolic calcium (Fura-2 ratio = 1.24 ± 0.02 after treatment vs. 1.25 ± 0.01 at baseline, n = 12, p > 0.05). In anesthetized normal rats, infusion (6.3 mg/kg bolus followed by 9.0 mg/kg/hr) of CK-1213296 increased echocardiographic fractional shortening from 47.1 ± 4.5% at baseline to 59.2 ± 4.0% at 30 minutes (n=6, p < 0.01) while vehicle controls did not change significantly (p > 0.05). In a chronically instrumented conscious dog heart failure model produced by myocardial infarction plus tachycardia pacing, infusion of CK-1213296 (8 mg/kg bolus followed by 8 mg/kg/hr, n=7) rapidly increased (p < 0.05) end systolic elastance, regional wall thickness, and fractional shortening by 20±8%, 38±11% and 34±8% respectively. In addition, heart rate and left atrial pressure decreased by 14±2% and 4±1 mmHg while mean arterial pressure was not changed and diastolic function was not impaired. Thus, direct activation of cardiac myosin improves cardiac function in a manner that could be therapeutically useful in patients with heart failure.

Direct Activation of Cardiac Myosin, A Novel Mechanism for Improving Cardiac Function

Fady Malik, You-Tang Shen †, Tatsuo Katori ‡, Sandra H Sueoka, Robert Anderson, David Cox, Marc Garard, James Hartman, Song-Jung Kim, Erica Kraynack, Alex Kuklov †, Ken H Lee, Pu-Ping Lu, Alex Muci, Congrong Niu, Hector Rodriguez, Ion Suehiro, Sheila Sylvester, Todd Tochimoto, Kathleen A Elias, Bradley P Morgan, Roman Sakowicz, David A Kass ‡, Stephen F Vatner †, David J Morgans

†University of Medicine and Dentistry of New Jersey, Newark, NJ; ‡Johns Hopkins University, Baltimore, MD; Cytokinetics, Inc., South San Francisco, CA



Introduction

One therapeutic approach in patients with heart failure has focused on improving the contractile function (inotropy) of the heart. Current drugs, such as β-adrenergic receptor agonists or phosphodiesterase inhibitors, improve cardiac contractility indirectly via second messenger activation which leads to an increase in cardiac myocyte intracellular calcium and secondarily an increase in cardiac contractility. However, these drugs also increase heart rate, oxygen consumption, the incidence of arrhythmias, and at times can cause hypotension. Clinical studies with current drugs have demonstrated that they have significant safety drawbacks, potentially related to their mechanism of action.

Therapeutic Hypothesis

A novel approach to improving cardiac contractility that may address the liabilities of current inotropic drugs is to directly activate cardiac myosin.

Improving cardiac contractility by specifically activating cardiac myosin could offer the following potential advantages over current agents:

- No activation of second messenger signaling
- No increase in cardiac myocyte intracellular calcium
- No increase in heart rate
- No decrease in blood pressure

In addition, this mechanism of action is predicted to minimally impact cardiac oxygen consumption and thus potentially improve myocardial efficiency.

We sought to demonstrate the therapeutic hypothesis with the small molecule cardiac myosin activator, CK-1213296.

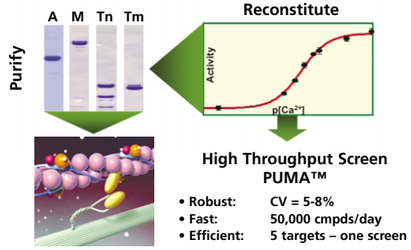
Objectives

- **Develop a paradigm for the discovery and optimization of cardiac myosin activators**
- **Demonstrate that the cardiac myosin activator, CK-1213296, improves cardiac function in a manner consistent with the therapeutic hypothesis**

Approach

Discovery and Optimization of Cardiac Myosin Activators

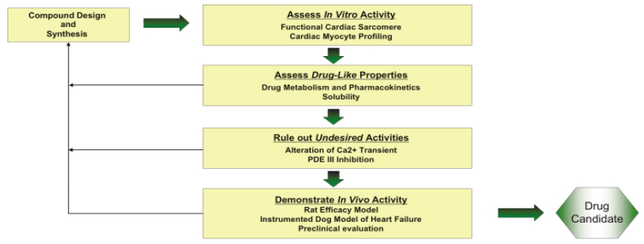
High throughput screening of the cardiac sarcomere



High Throughput Screen PUMA™

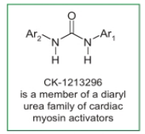
- **Robust:** CV = 5-8%
- **Fast:** 50,000 cmpds/day
- **Efficient:** 5 targets – one screen

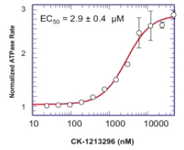
Optimization Strategy



Results

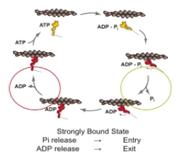
CK-1213296 is a cardiac myosin activator and accelerates actin dependent P_i release



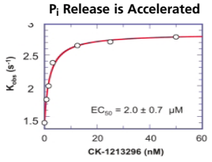


Dose-dependent activation curve of CK-1213296 using a calcium regulated reconstituted sarcomere.

Cardiac S1 myosin and thin filament proteins (actin, troponin complex, and tropomyosin) were prepared from bovine heart. ATPase assays are performed at 50% of maximal calcium activation in a kinetic fashion using a NADH coupled enzyme system. Rates are normalized to a DMSO control.



P_i Release is Accelerated



ADP Release is Unchanged

	Rate (s^{-1})
DMSO Control	118
CK-1213296 (50 μM)	127

Single turnover conditions were employed using as appropriate either phosphate binding protein, modified with the coumarin derivative MDCC, or MANT-ADP as the fluorescent detectors.

CK-1213296 increases myocyte contractility without increasing intracellular calcium

	N	FS%	Systolic Ca^{2+} (Fura Ratio)	Diastolic Ca^{2+} (Fura Ratio)	T75 (sec)
Baseline	7	100	1.24 ± 0.01	0.98 ± 0.01	0.20 ± 0.01
CK-1213296 (1 μM)		169 ± 6	1.20 ± 0.01	0.93 ± 0.09	0.21 ± 0.02

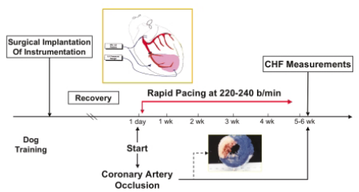
Cardiac myocytes were isolated from adult Sprague-Dawley rats and used for measurement within 5 hours of isolation. Myocytes were loaded with Fura-2 AM ester, put into Tyrode buffer containing 1.5 mM Ca2+, and stimulated at 1 Hz. Simultaneous contractility and calcium measurements were performed using an Ionoptix calcium imaging system.

CK-1213296 increases fractional shortening *in vivo*

	Fractional Shortening (%)		P Value	
	T = 0	T = 30 min	vs. baseline	vs. vehicle
Vehicle	48.0 ± 4.3	51.5 ± 4.1	NS	NA
CK-1213296	47.1 ± 5.4	59.2 ± 4.0	0.0006	0.008

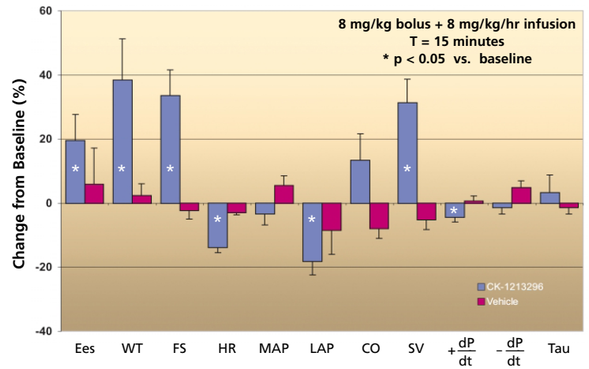
Sprague-Dawley rats were anesthetized with isoflurane gas. CK-1213296 was administered intravenously as a loading dose (6.3 mg/kg) followed by a continuous infusion (9.0 mg/kg/hr). Fractional shortening was quantitated in the parasternal short axis view using 2D echocardiography.

CK-1213296 improves cardiac function in a dog model of heart failure



Mongrel dogs were instrumented with pressure transducers in the left ventricle (LV), left atrium, and aorta. Ultrasound crystals are implanted for measurements of LV dimensions and wall thickness. In some dogs, a Transonic flow probe is implanted at the level of the ascending aorta for cardiac output or an occluder cuff is placed around the IVC to generate PV loops. Heart failure is induced by myocardial infarction using a coronary occluder around the left anterior descending artery followed by continuous rapid ventricular pacing (220-240 bpm) for several weeks. The pacer is turned off just prior to administration of CK-1213296.

	Start	CHF
HR (n=10)	108	134
MAP (n=9)	90	81
LAP (n=9)	4	24
dP/dt (n=10)	2761	1320
CO (n=4)	3.1	1.9
SV (n=4)	28	15
FS (n=10)	12.8	6.7



Conclusions

CK-1213296 is a cardiac myosin activator that:

1) **Accelerates actin dependent P_i release**
2) **Increases contractility in cardiac myocytes without changes in intracellular calcium**
3) **Increases cardiac contractility and stroke volume in a dog model of heart failure and fulfills the therapeutic hypothesis**

Cardiac myosin activators could eventually provide benefit for patients with congestive heart failure.

Cytokinetics