Direct Activation of Cardiac Myosin by CK-1827452 Improves Cardiac Function in a Dog Heart Failure Model

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Introduction

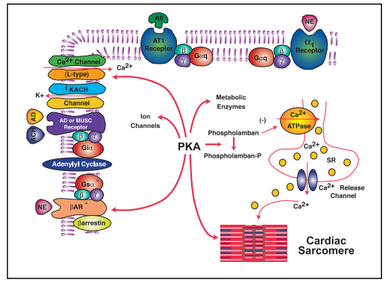
Current inotropes increase intracellular calcium and secondarily increase cardiac contractility. In addition, they increase heart rate, oxygen consumption, the incidence of arrhythmias, and reduce blood pressure. A more direct approach to improving cardiac contractility that may address these liabilities is activation of cardiac myosin itself. We sought to demonstrate the therapeutic hypothesis with the orally bioavailable cardiac myosin activator, CK-1827452.

Therapeutic Hypothesis

Disadvantages of Current Inotropes…

Pleiotropic Actions

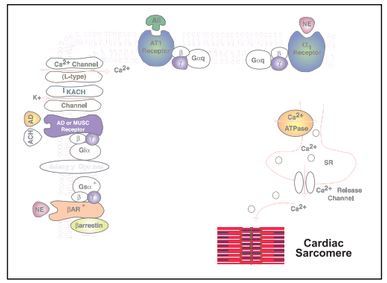
- ↑ Calcium
- ↑ Heart Rate
- ↓ Blood Pressure
- ↑ Oxygen Demand
- ↓ Efficiency
- ↑ Arrhythmias



Advantages of Cardiac Myosin Activators…

Specific Mechanism

- ↔ Calcium
- ↔ Heart Rate
- ↔ Blood Pressure
- ↔ Oxygen Demand
- ↑ Efficiency
- ↔ Arrhythmias



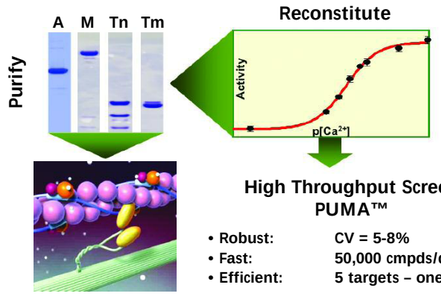
Objectives

- Develop a paradigm for the discovery and optimization of cardiac myosin activators
- Demonstrate that the cardiac myosin activator, CK-1827452, improves cardiac function in a manner consistent with the therapeutic hypothesis

Approach

Discovery and Optimization of Cardiac Myosin Activators

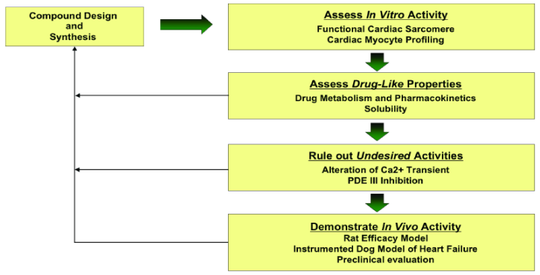
High throughput screening of the cardiac sarcomere

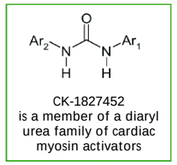


High Throughput Screen
PUMA™

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

Optimization Strategy

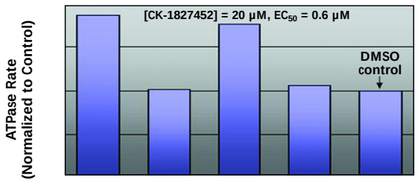




CK-1827452 is a member of a diaryl urea family of cardiac myosin activators

Results

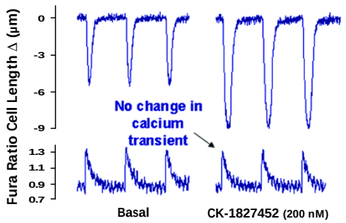
CK-1827452 is a selective cardiac myosin activator



Cardiac S1 myosin and thin filament proteins (actin, troponin complex, and tropomyosin) were prepared from bovine heart or rabbit skeletal muscle. ATPase assays are performed in a kinetic fashion using a NADH coupled enzyme system at p[Ca^{2+}] = 6.75. Rates are normalized to a DMSO control.

Myosin Isoform	Cardiac	Skeletal	Cardiac	Skeletal	Cardiac
Thin Filament Isoforms	Cardiac	Skeletal	Skeletal	Cardiac	Cardiac

CK-1827452 increases myocyte contractility without changing the calcium transient



Cardiac myocytes were isolated from adult Sprague-Dawley rats and used within 5 hours. After isolation they were loaded with Fura-2 AM ester, put into Tyrode buffer containing 1.5 mM Ca^{2+}, and stimulated at 1 Hz. Simultaneous contractility and calcium measurements were performed with a Ionoptix system. 2,3 butanedione monoxime (BDM) is a myosin inhibitor and is used to enable addition of CK-1827452 to the myocytes at high concentrations, improving the stringency of the assay. EC_{20} of contractile response = 0.2 µM.

CK-1827452 (up to 10 µM) does not alter the calcium transient

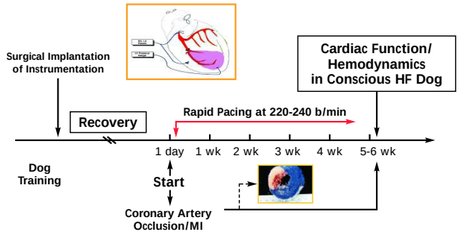
Treatment : Dose	N	Diastolic Ca^{2+} (Fura-2 Ratio)	Systolic Ca^{2+} (Fura-2 Ratio)	T_{75} (seconds)
Untreated	8	0.82 ± 0.01	1.26 ± 0.01	0.32 ± 0.01
CK-1827452 : 0.2 µM		0.84 ± 0.01	1.23 ± 0.01	0.31 ± 0.02
BDM : 10 mM	4	0.87 ± 0.01	1.16 ± 0.03	0.42 ± 0.03
BDM : 10 mM CK-1827452 : 10 µM		0.88 ± 0.02	1.14 ± 0.02	0.41 ± 0.03
BDM : 10 mM	5	0.97 ± 0.02	1.24 ± 0.01	0.26 ± 0.01
BDM : 10 mM Isoproterenol : 5 nM		1.02 ± 0.03	1.45 ± 0.03*	0.14 ± 0.01*

*p < 0.05

CK-1827452 does not inhibit Phosphodiesterase Type III.
(human, derived from platelets)

Test Article	Inhibition (%)
CK-1827452 10 µM	-8
CK-1827452 30 µM	1
IMBX 8 µM	50

CK-1827452 improves cardiac function in a dog model of heart failure

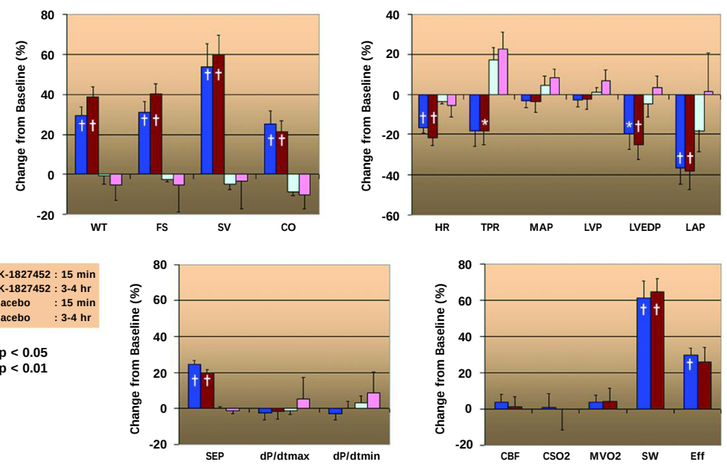


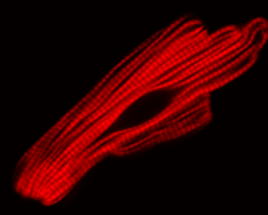
Mongrel dogs were instrumented with pressure transducers in the left ventricle (LV), left atrium, and aorta. Ultrasound crystals are implanted for measurements of LV dimensions and wall thickness. In some dogs, a Transonic flow probe is implanted at the level of the ascending aorta for cardiac output 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-1827452.

	HR (n=8)	MAP (n=8)	LAP (n=7)	dP/dt (n=8)	CO (n=7)	FS (n=8)
HF	149	83	22	1729	1.7	8.8

HR = Heart rate; MAP = Mean Arterial Pressure; LAP = Left Atrial Pressure; CO = Cardiac Output; FS = Fractional Shortening; WT = Wall Thickening; SV = Stroke Volume; TPR = Total Peripheral Resistance; LVP = LV Systolic Pressure; LVEDP = LV End Diastolic Pressure; SEP = Systolic Ejection Period; CBF = Coronary Blood Flow; CSO2 = Coronary Sinus O2 content, MVO2 = Myocardial Oxygen Consumption; SW = Stroke Work; Eff = Cardiac Efficiency

CK-1827452 Improves Cardiac Function and Output, Hemodynamics, and Cardiac Efficiency
(0.5 mg/kg i.v. bolus, 0.5 mg/kg/hr i.v. infusion)





Conclusions

CK-1827452 is a cardiac myosin activator that:

1) **Selectively activates cardiac myosin**
2) **Increases contractility in cardiac myocytes without changing the calcium transient**
3) **Improves cardiac function and output, hemodynamics and efficiency in a dog model of heart failure and fulfills the therapeutic hypothesis**

A first-in-human, Phase I, double-blind, randomized, placebo-controlled, dose-escalation, pharmacokinetic and pharmacodynamic study of CK-1827452 in healthy volunteers started earlier in September.

CYTOKINETICS