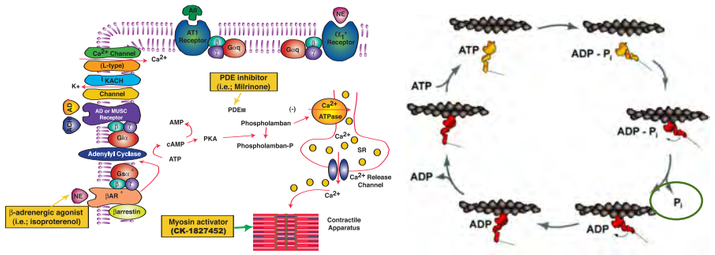
Abstract #1728

In Vitro and *In Vivo* Efficacy of the Cardiac Myosin Activator CK-1827452

RL Anderson, SH Sueoka, HM Rodriguez, KH Lee, DR Cox, R Kawas, BP Morgan, R Sakowicz, DJ Morgans Jr, F Malik, KA Elias

Cytokinetics, Inc. South San Francisco, CA

Introduction

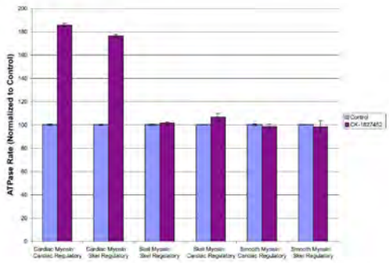


Contraction of the cardiac muscle is controlled by cycles of calcium release and uptake which in turn activate and deactivate access of cardiac myosin motor proteins to calcium-regulated actin-tropomyosin-troponin filaments. Traditional inotropic agents used in treating acute decompensated heart failure increase cellular contractility by increasing the calcium transient. β-adrenergic agonists activate β-adrenergic receptors resulting in an increase in cAMP and activation of the PKA signaling cascade. Numerous proteins are phosphorylated including phospholamban that results in an increase of the calcium transient and thus contractility. Phosphodiesterase (PDE) inhibitors increase the cAMP concentrations by slowing cAMP degradation also resulting in a calcium transient increase. PDE inhibiting compounds result in increased mortality in clinical trials likely due to altering the calcium transient (Packer et al., 1991; Cohn et al., 1998).

A novel approach to improving cardiac contractility is to directly activate the force-generating enzyme cardiac myosin without altering the calcium transient. We have previously reported on a class of small molecule agents, directly stimulating activity of the myosin ATPase in the cardiac sarcomere (Rodriguez et al., 2004). *In vitro* enzymatic assays demonstrate that myosin activators accelerate the rate limiting step of the myosin enzymatic cycle (green circle, right figure above), reported by phosphate release, almost 2-fold. This portion of the cycle constitutes transition from the weakly to the strongly filament-bound state of myosin. Thus, by reducing the time spent in the weakly bound state, myosin activators shift the myosin enzymatic cycle in favor of the strongly bound, force producing state.

Results

CK-1827452 is a selective cardiac myosin activator



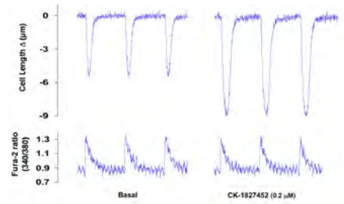
In biochemical mix and match preparations, the myosin ATPase rate is increased only in the presence of cardiac myosin demonstrating that the activity of CK-1827452 is 1) cardiac myosin specific and 2) not acting via the regulatory apparatus. Experiments shown performed at pCa^{2+} = 6.75.

Methods

Biochemical assays: Cardiac S1 myosin and thin filament proteins (actin, troponin complex, and tropomyosin) were prepared from bovine heart or rabbit skeletal muscle. Smooth muscle myosin was prepared from chicken gizzard. ATPase assays were performed in a kinetic fashion using NADH coupled enzyme system at pCa^{2+} = 6.75. Rates were normalized to a DMSO control.
Myocyte contractility experiments: Ventricular myocytes were isolated from adult male SD rats (275- 325g) using a collagenase digestion procedure and used within 5 hrs of isolation. Myocytes were warmed in perfusion chambers, perfused with Tyrode buffer and field stimulated at 1 Hz. To determine contractility, myocytes were imaged through a 40x objective and the images were digitized at a sampling speed of 240 Hz.
Frame grabber, myopacer, acquisition, and analysis software were obtained from IonOptix (Milton, MA). After an initial 1 min basal contractility period, compounds were perfused for 5 minutes before a 2 min washout period.
Calcium transient analysis: Myocytes were loaded with 2 uM fura-2 AM and simultaneous contractility and fura-2 ratios determined using an IonOptix system modified for fluorescence analysis.
Cell Analysis: For each cell, ten or more contractility and calcium ratio transients at basal and after compound addition, were averaged and compared. Contractility average transients were analyzed using the IonWizard analysis program to determine changes in diastolic length, and fractional shortening (% decrease in the diastolic length; FS). For FS, data are normalized to basal values (basal equals 100%) and expressed as FS (% of basal). The averaged calcium ratio transients were analyzed to determine changes in fura-2 diastolic and systolic ratios and the 75% time to baseline (T_{75}).
***In vivo* experiments:** Adult male SD rats were anesthetized with isoflurane and infused with 0.25 - 1.2 mg/kg/hr (1.5 ml/kg/hr) CK-1827452. Terminal plasmas were taken.
Echocardiography: Using an Aplio ultrasound system (Toshiba), a 10 Hz probe was placed at the level of the papillary muscles and 2-D M-mode images of the left ventricle were taken. Diastolic and systolic wall thickness and dimensions, and fractional shortening (FS) were determined before and after infusion.
Heart failure: Myocardial infarctions (MI) were induced by ligation of the left coronary artery (LCA). Only animals with fractional shortening 3 SD lower than the average from the Sham animals (surgery without tying off LCA) were utilized in experiments. Experiments were performed 12 weeks post-surgery.
Reagents: CK-1827452 a diarylurea, provided by the Cytokinetics Chemistry department. Fura-2 from Molecular Probes and all other reagents from Sigma.
Statistics: Data are mean +/- SEM. Statistics were performed using the Students *t* –test or ANOVA and post-hoc Newman-Keuls as appropriate. P< 0.05 was considered significant.

Results (continued)

CK-1827452 increases myocyte contractility without increasing the calcium transient



Individual isolated adult rat cardiac myocyte tracings (above) and group data (below) showing contractility and calcium transients after CK-1827452 treatment. CK-1827452 treatment elicits a significant increase in contractility with no significant change in the fura-2 calcium ratio.

Treatment	N	FS (% of basal)	Diastolic Fura-2 Ratio	Systolic Fura-2 Ratio	T_{75} (seconds)
Basal	8	100	0.82 +/- 0.01	1.26 +/- 0.01	0.32 +/- .01
0.2 μM CK1827452		133.1 +/- 6.8*	0.84 +/- 0.01	1.23 +/- 0.01	0.31 +/- .02

* p =< 0.05

CK-1827452 overcomes BDM inhibition without increasing calcium transient parameters

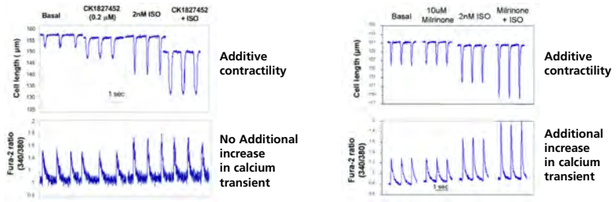
Treatment	N	FS (% of basal)	Diastolic Fura-2 Ratio	Systolic Fura-2 Ratio	T_{75} (seconds)
Basal	4	100	0.67 ± 0.02	1.23 ± 0.01	0.37 ± 0.02
10 mM BDM		4.2 ± 2.8	0.67 ± 0.01	1.16 ± 0.03	0.42 ± 0.03
BDM + 10 μM CK-1827452		28.0 ± 11.2*	0.86 ± 0.02	1.14 ± 0.02	0.41 ± 0.03

To determine possible effects on the calcium transient at high concentrations of CK-1827452 experiments were conducted in the presence of the myosin inhibitor BDM. BDM inhibits the myosin cross-bridges but doesn't alter the calcium cycling. A large concentration of CK-1827452 (10 μM) overcomes BDM inhibition but does not alter the calcium transient (panel above). In contrast, the β-adrenergic agonist Isoproterenol (ISO) alters the calcium transient parameters (below).

* p =< 0.05

Treatment	N	FS (% of basal)	Diastolic Fura-2 Ratio	Systolic Fura-2 Ratio	T_{75} (seconds)
Basal	5	100	0.96 ± 0.02	1.25 ± 0.01	0.24 ± 0.01
10 mM BDM		5.8 ± 1.7	0.97 ± 0.02	1.24 ± 0.01	0.26 ± 0.01
BDM + 5 nM ISO		47.4 ± 14.7*	1.02 ± 0.03	1.45 ± 0.03*	0.14 ± 0.01*

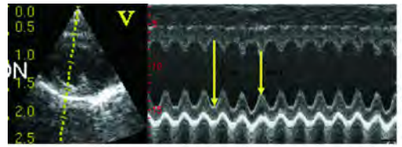
CK-1827452 lacks PDE activity



Individual myocyte tracings showing contractility and calcium transient changes after treatment alone or in combination with CK-1827452, ISO or the PDE inhibitor milrinone. Combination treatment with CK-1827452 and ISO results in additive contractility but no additional increase in the calcium transient (no PDE inhibitory activity). In contrast, the PDE inhibitor milrinone in combination with ISO increases contractility and the calcium transient.

In vitro summary: In biochemical studies, CK-1827452 selectively activates cardiac myosin. In isolated cardiac myocytes, treatment with CK-1827452 results in increased fractional shortening without increasing the calcium transient or inhibiting the PDE pathway.

CK-1827452 increases fractional shortening *in vivo*…



Cardiac function was determined using M-mode echocardiography.

Fractional Shortening (F.S.) : Measure of cardiac contractility
F.S. = (EDd – ESd)/EDd x 100
EDd = End Diastolic Diameter ESd = End Systolic Diameter

… in Sprague Dawley rats

CK-1827452 (mg/kg/hr)	N	Plasma (μM)	FS (% of baseline)	Diastolic Diameter (% of baseline)	Systolic Diameter (% of baseline)
0	6	< 0.01	108.1 +/- 2.3	99.3 +/- 1.4	92.3 +/- 2.5
0.25	5	0.21 +/- 0.02	104.4 +/- 2.1	101.3 +/- 3.1	96.5 +/- 3.7
0.7	6	0.43 +/- 0.06	119.3 +/- 3.5*	96.5 +/- 2.6	76.8 +/- 1.6*
1.2	6	0.72 +/- 0.04	120.4 +/- 4.3*	95.4 +/- 2.2	75.0 +/- 4.8*

Echocardiography parameters after 30 min infusion of 0.25 – 1.2 mg/kg/hr CK-1827452. A dose response increase in fractional shortening (FS) is observed.

… and in animals with heart failure

Group	N	Body Weight (kg)	Heart Weight (g)	BWt/HWt Ratio	Fractional Shortening
SHAM	15	0.503 ± 0.042	1.629 ± 0.153	3.261 ± 0.423	49.6 ± 0.9
MI	28	0.514 ± 0.057	1.946 ± 0.361*	3.816 ± 0.762*	21.5 ± 0.7*

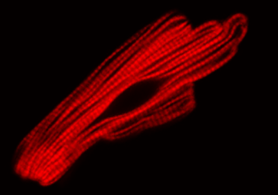
Rats with myocardial infarction (MI) had similar body weights to sham controls but had increased heart wts, HW/BW ratios and decreased FS consistent with heart failure.

SHAM (mg/kg/hr)	N	Plasma (μM)	FS (% of Baseline)	Diastolic Diameter (% of Baseline)	Systolic Diameter (% of Baseline)
0	4	ND	107.8 ± 4.6	96.8 ± 3.6	90.3 ± 5.6
0.25	4	0.26 ± 0.02	108.9 ± 4.7	94.9 ± 3.9	87.3 ± 6.1
0.7	4	0.57 ± 0.06	117.2 ± 3.6*	92.8 ± 3.6	76.9 ± 7.4*
1.2	4	1.17 ± 0.14	120.6 ± 6.3*	90.3 ± 4.3*	70.9 ± 7.7*
MI (mg/kg/hr)	**N**	**Plasma (μM)**	**FS (% of Baseline)**	**Diastolic Diameter (% of Baseline)**	**Systolic Diameter (% of Baseline)**
0	7	ND	95.4 ± 8.0	92.5 ± 8.8	101.6 ± 2.9
0.25	7	0.24 ± 0.03	108.5 ± 4.6	96.5 ± 5.5	94.1 ± 1.6*
0.7	7	0.47 ± 0.03	118.2 ± 3.7*	93.2 ± 9.5	94.8 ± 2.1
1.2	7	0.98 ± 0.13	114.4 ± 6.3*	95.4 ± 1.6	92.0 ± 2.6*

Echocardiography parameters after 30 min infusion of 0.25 – 1.2 mg/kg/hr CK-1827452. A dose response increase in fractional shortening (FS) is observed in both groups demonstrating *in vivo* efficacy of CK-1827452 in sham as well as heart failure animals.

* p =< 0.05

***In vivo* summary:** Infusion of the myosin activator CK-1827452 results in increased fractional shortening in SD rats, sham animals and in rats with heart failure. In SD rats, infusion with CK-1827452 significantly increases fractional shortening starting at 0.4 μM plasma concentrations. In sham and heart failure rats, infusion of CK-1827452 results in similar and significant increases in fractional shortening fraction starting at plasma concentrations of 0.4-0.6 μM.



Conclusions

The cardiac myosin activator CK-1827452:

- Selectively activates cardiac myosin
- Increases contractility in cardiac myocytes without increasing calcium transient
- Significantly increases cardiac contractility in SD rats and animals with defined heart failure
- These data suggest that CK-1827452 may be a useful therapeutic in the treatment of human heart failure

References

(1) Cohn JN, Goldstein SO, Greenberg BH, Lorell BH, Bourge RC, Jaski BE, Gottlieb SO, McGrew F 3rd, DeMets DL, White BG. A dose-dependent increase in mortality with vesnarinone among patients with severe heart failure. Vesnarinone Trial Investigators. *N Engl J Med.* 1998 Dec 17;339(25):1810-6.

(2) Packer M, Carver JR, Rodeheffer RJ, et al. Effect of oral milrinone on mortality in severe chronic heart failure. *N. Engl. J. Med.* 1991;325:1468-1475.

(3) Rodriguez H, Sylvester S, Qian X, Morgan B, Morgans Jr. D, Malik F, Sakowicz R. Activation of Cardiac Sarcomere ATPase by CK-1122534, a Small Molecule Agent that Specifically Targets Cardiac Myosin. *American Society of Cell Biology*, December 2004.

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