The Cardiac Myosin Inhibitor, CK-3772271, Attenuates Cardiac Fibrosis and Diastolic Dysfunction in the Dahl/Salt Sensitive Rat Model of Heart Failure with Preserved Ejection Fraction

Darren T Hwee, James J Hartman, Jingying Wang, Yangsong Wu, Claire McHugh, Xihui Xu, Eva R Chin, Fady I Malik, Bradley P Morgan

Research and Non-Clinical Development, Cytokinetics, Incorporated, South San Francisco, CA, USA

ABSTRACT

Introduction

Heart failure with preserved ejection fraction (HFpEF) is characterized by abnormal contractility and progressive myocardial fibrosis and stiffness. CK-3772271 (CK-271) is a novel small molecule cardiac myosin inhibitor that reduces cardiac myosin ATPase activity and reduces cardiac contractility in unloaded isolated cardiomyocytes in vitro and in healthy rats and dogs in vivo. The effect of chronic CK-271 treatment on cardiac function and morphology was evaluated in the Dahl/Salt Sensitive (DSS) rat hypertension model of HFpEF.

Methods

DSS male rats were fed either a control low salt (LS, 0.3% NaCl) or high salt (HS, 4% NaCl) diet to induce a hypertension-driven HFpEF disease phenotype. Six weeks after HS diet treatment, DSS rats were randomized into two subgroups: continued HS diet or a HS diet formulated with CK-271 (100 ppm) for an additional 6 weeks. Body mass, systolic blood pressure, and cardiac function were measured longitudinally. After 12 weeks of HS treatment hearts were collected to assess cardiac fibrosis.

Results

HS diet treatment increased systolic blood pressure (LS: 132.2 ± 4.7 mmHg vs HS: 163.5 ± 4.0 mmHg, mean ± standard error of the mean, $p < 0.001$) and caused cardiac hypercontractility as evidenced by an increase in the end-systolic pressure-volume relationship (LS: 0.8 ± 0.17 vs HS: 2.1 ± 0.46 µL/mmHg). HS diet also increased isovolumic relaxation time (IVRT) (LS: 16.8 ± 0.6 vs HS: 22.8 ± 0.6 ms, $p < 0.0001$), left atrial area (LS: 30.3 ± 0.8 vs HS: 42.5 ± 2.2 mm^2, $p < 0.0001$), and cardiac fibrosis (LS: 3.4 ± 0.4 vs HS: 5.0 ± 0.6%). Six weeks of CK-271 treatment reduced fractional shortening (HS: 53.8 ± 1.4 vs HS + CK-271: 42.7 ± 1.0%, $p < 0.0001$) and reduced HS diet-induced diastolic dysfunction, including IVRT (HS: 22.8 ± 0.6 vs HS + CK-271: 19.5 ± 0.5 ms, $p < 0.0001$) and left atrial area (HS: 42.5 ± 2.2 vs HS + CK-271: 35.4 ± 0.8 mm^2, $p < 0.0001$). Furthermore, CK-271 reduced the development of cardiac fibrosis (HS: 5.0 ± 0.6 vs HS + CK-271: 3.5 ± 0.3%, $p < 0.05$) induced by HS diet.

Conclusion

The small molecule cardiac myosin inhibitor, CK-3772271, attenuated the development of diastolic dysfunction and fibrosis by normalizing cardiac contractility in the DSS rat model of HFpEF. Cardiac myosin inhibition may be a novel approach to mitigating the development of HFpEF.

INTRODUCTION

- Heart failure with preserved ejection fraction (HFpEF) is by the clinical syndrome of heart failure in the setting of a normal or near normal LVEF. The underlying pathology is varied, but includes abnormal contractility and progressive myocardial fibrosis and stiffness
- CK-3772271 (CK-271) is a novel small molecule cardiac myosin inhibitor that reduces cardiac myosin ATPase activity and reduces cardiac contractility in unloaded isolated cardiomyocytes in vitro and in healthy rats and dogs in vivo
- The effect of chronic CK-271 treatment on cardiac function and morphology was evaluated in the Dahl/Salt Sensitive (DSS) rat hypertension model of HFpEF

METHODS

Preparation of reagents

- Myofibrils were prepared from flash-frozen bovine cardiac, bovine masseter, and rabbit psoas tissue as described in Hwee et al. (2015)[1]; bovine cardiac myosin subfragment-1 was prepared as described in Malik et al. (2011)[2]

ATPase assays

- Steady-state ATPase activity was measured using a pyruvate kinase and lactate dehydrogenase-coupled enzyme system as described in Hwee et al. (2015)[1] and Malik et al. (2011)[2]. Nonmyosin ATPase activity was subtracted from cardiac and slow skeletal myofibril assays (where indicated) by subtracting the ATPase activity in the presence of a saturating concentration of the nonselective myosin II inhibitor blebbistatin

Measure of cardiomyocyte contractility and calcium transients

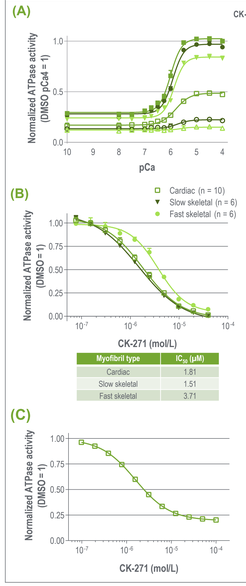
- Adult rat ventricular cardiomyocytes were isolated and loaded with fura-2 as described in Malik et al. (2011)[2]. Cardiomyocyte contractility and calcium transients were measured by edge-detection video microscopy and fluorescence photometry (IonOptix, Milton, MA) as described in Malik et al. (2011)[2]

Evaluation of CK-271 treatment in Dahl/Salt Sensitive (DSS) rats

- 10–11-week-old, DSS male rats were fed either a control low salt (LS, 0.3% NaCl) or high salt (HS, 4% NaCl) diet to induce a hypertension-driven disease phenotype
- After 6 weeks of HS diet treatment, DSS rats were randomized based on body weight and systolic blood pressure into two subgroups: continued HS diet or a HS diet formulated with CK-271 (100 ppm) for an additional 6 weeks
- Systolic blood pressure was measured by tail cuff and cardiac function was assessed by echocardiography and invasive hemodynamics
- After 12 weeks of HS treatment hearts were collected to assess cardiac fibrosis by Masson's trichrome stain

RESULTS

Figure 1. CK-271 inhibits the ATPase activity of bovine cardiac myofibrils and purified bovine cardiac subfragment-1



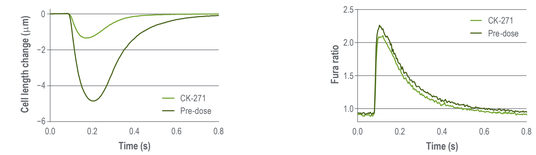
Myofibril type	IC_{50} (µM)
Cardiac	1.81
Slow skeletal	1.51
Fast skeletal	3.71

(A) CK-271 inhibits the ATPase activity of bovine cardiac myofibrils. The ATPase activity of Triton X-100-skinned bovine cardiac myofibrils was measured using a pyruvate kinase/lactate dehydrogenase-coupled assay as described in Hwee et al. (2015). Data shown are mean values ± standard deviation (SD) (n = 4).

(B) CK-271 selectively inhibits the ATPase activity of cardiac and slow skeletal myofibrils compared with fast skeletal myofibrils. Dose-response analysis was performed with cardiac (bovine, n = 10), slow skeletal (bovine, n = 6), and fast skeletal (rabbit, n = 6) detergent-skinned myofibrils. Data shown are mean values ± SD. Calcium concentrations were fixed at approximately the pCa_{75} for each myofibril type. Raw ATPase rates were normalized to reactions containing an equivalent concentration of dimethyl sulfoxide (DMSO). For cardiac and slow skeletal reactions, ATPase rates in the presence of the nonselective myosin inhibitor blebbistatin were subtracted to eliminate the effects of nonmyosin ATPases. Data were fitted using a four-parameter dose-response equation.

(C) CK-271 inhibits the ATPase activity of bovine cardiac myosin subfragment-1. ATPase activity was normalized to control reactions containing 2% DMSO. Data were fitted using a four-parameter dose-response equation (95% confidence interval): top = 1.0 (0.99 to 1.00), bottom = 0.19 (0.19 to 0.20), IC_{50} = 1.58 µM (1.54 to 1.61), Hill Slope = −1.11 (−1.14 to −1.09).

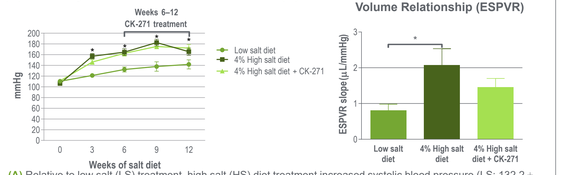
Figure 2. CK-271 decreases shortening of isolated adult rat ventricular myocytes without altering Ca^{2+} transients



Treatment	N	FS (% of basal)	Diastolic fura ratio	Systolic fura ratio	T_{75} (s)
Basal	11	100 ± 4.7	1.02 ± 0.04	1.98 ± 0.11	0.226 ± 0.018
5 µM CK-271	11	27.2 ± 6.3	1.04 ± 0.04	1.99 ± 0.11	0.229 ± 0.023

In the charts are shown an average of 20 cell shortening and calcium transients for a representative adult rat cardiomyocyte before and after exposure to 5 µM CK-271. Pooled data are shown in the table. Basal reference values are: diastolic cell length = 123.5 ± 3.8 µm, fractional shortening (FS) = 5.24 ± 0.63 µm, contraction velocity (CV) = 121.5 ± 16.9 µm/s, relaxation velocity (RV) = 63.4 ± 12.7 µm/s, time to peak = 0.124 ± 0.008 s, and time to baseline (T_{50}) = 0.221 ± 0.018 s. CK-271 (5 µM) significantly decreases fractional shortening ($p < 0.05$) without increasing any calcium transient parameter.
Data are presented as mean ± standard error of the mean

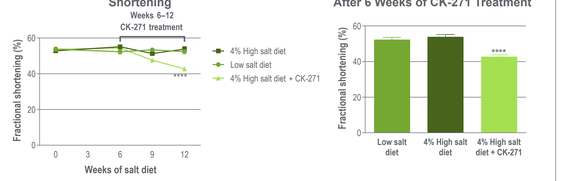
Figure 3. A 4% high salt (HS) diet increased systolic blood pressure and the end systolic pressure volume relationship in DSS rats



(A) Relative to low salt (LS) treatment, high salt (HS) diet treatment increased systolic blood pressure (LS: 132.2 ± 4.7 mmHg vs HS: 163.5 ± 4.0 mmHg, $*p < 0.001$). Cardiac myosin inhibitor CK-271 had no effect on systolic blood pressure.

(B) After 12 weeks of salt diet, the ESPVR slope increased only in the 4% HS group (LS: 0.8 ± 0.17 vs HS: 2.1 ± 0.46 µL/mmHg, $*p < 0.05$ LS vs HS diet).
All data are expressed as mean ± standard error of the mean

Figure 4. Six weeks of CK-271 treatment reduced fractional shortening in high salt diet-fed DSS rats

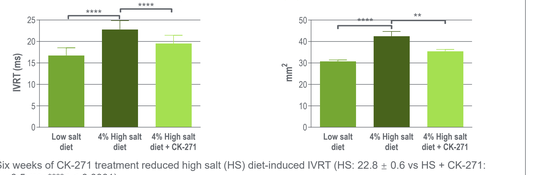


(A) Longitudinal cardiac fractional shortening measurements in DSS rats through 12 weeks of low salt (LS) or high salt (HS) diet.

(B) Six weeks of CK-271 treatment reduced fractional shortening in HS diet-fed DSS rats (HS: 53.8 ± 1.4 vs HS + CK-271: 42.7 ± 1.0%, $****p < 0.0001$ vs LS and HS diet groups).

All data are expressed as mean ± standard error of the mean

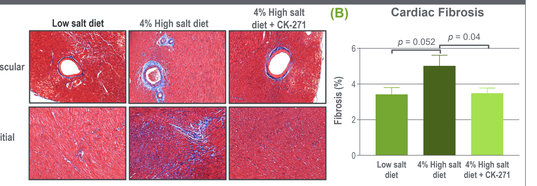
Figure 5. Six weeks of CK-271 treatment reduced measures of high salt diet-induced diastolic dysfunction



(A) Six weeks of CK-271 treatment reduced high salt (HS) diet-induced IVRT (HS: 22.8 ± 0.6 vs HS + CK-271: 19.5 ± 0.5 ms, $****p < 0.0001$).

(B) Six weeks of CK-271 treatment reduced LAA (HS: 42.5 ± 2.2 vs HS + CK-271: 35.4 ± 0.8 mm^2, $**p < 0.01$; $****p < 0.0001$). All data are expressed as mean ± standard error of the mean

Figure 6. Six weeks of CK-271 treatment reduced the development of cardiac fibrosis in DSS rats



(A) Representative Masson's trichrome stains of collagen deposition in the perivascular and interstitial regions of DSS rat hearts.

(B) Cardiac fibrosis increased with 4% salt diet treatment. CK-271 treatment prevented the development of fibrosis induced by high salt (HS) diet (HS: 5.0 ± 0.6 vs HS + CK-271: 3.5 ± 0.3%, $p < 0.05$).
All data are expressed as mean ± standard error of the mean

CONCLUSIONS

- CK-3772271 is a novel small molecule that selectively inhibits cardiac myosin ATPase activity and contractility in vitro
- CK-3772271 attenuated the development of cardiac hypercontractility, diastolic dysfunction, and fibrosis in the DSS rat model of HFpEF
- Cardiac myosin inhibition may be a viable approach to address hypercontractility and associated hemodynamic and subsequent cardiac structural abnormalities associated with HF

References

1. Hwee et al. (2015). *J Pharmacol Exp Ther.* 353(1):159–168.
2. Malik et al. (2011). *Science.* 331(6023):1439–1443.

Acknowledgments

This study was funded by Cytokinetics, Incorporated.

Editorial support was provided by Richard Fay, PhD, CMPP on behalf of Evidence Scientific Solutions, Philadelphia, PA, and was funded by Cytokinetics, Incorporated.

Disclosures

All authors are current or former employees of Cytokinetics, Incorporated and were financially compensated for their work.

Presented at the American Heart Association Scientific Sessions | Virtual Congress | November 13–17, 2020

Cytokinetics