The Cardiac Myosin Inhibitor, CK-3773274, Reduces Contractility in the R403Q Mouse Model of Hypertrophic Cardiomyopathy

Darren T. Hwee, Yangsong Wu, Peadar Cremin, Bradley P. Morgan, Fady I. Malik, Eva R. Chin

Cytokinetics, Inc., South San Francisco, CA

ABSTRACT

Cardiac sarcomere hypercontractility appears to underlie pathological hypertrophy and fibrosis in select genetic hypertrophic cardiomyopathy (HCM) patients. The small molecule CK-3773274 is a novel cardiac myosin inhibitor that decreases contractility in vitro and in healthy animal models. The objective of this study was to evaluate the effect of the novel small molecule CK-3773274 in the genetic R403Q mouse model of hypertrophic cardiomyopathy. A paired oral dose administration of CK-3773274 ranging from 0.5 to 1.5 mg/kg, and fractional shortening (FS) and heart rate were assessed at select time points over a 24-hour period. One hour after dose administration, CK-3773274 significantly reduced FS in a dose-related fashion relative to pre-dose baseline levels. At all dose levels, FS returned to baseline levels 24 hours after dosing. Statistical comparisons by %FS baseline levels are presented in the accompanying table. Values are expressed as mean ± standard error of the mean.

METHODS

To isolate the human R403Q-I185–myosin heavy chain mutation, a mouse model with an R403Q mutation in a myosin heavy chain gene exons using CRISPR-Cas9 technology by the Jackson Laboratory, Bar Harbor, Maine. All mice were treated with CK-3773274 (0.5, 1.25, or 1.5 mg/kg) in a 0.5% polyxylylglutamine (PEG) or 0.5% polylysine vehicle at a stock concentration of 15 mg/mL. A >7× difference between the IC50, unbound* (nM) was identified as a threshold for a statistically significant effect on contractility. Baseline FS was significantly different between wild type (WT) and R403Q (n=9) mice. Posterior wall WT: 0.84 ± 0.04 mm vs. R403Q: 1.22 ± 0.08 mm; posterior wall WT: 0.93 ± 0.03 mm vs. R403Q: 1.09 ± 0.04 mm. The mouse model of hypertrophic cardiomyopathy. At approximately 40 weeks of age, left ventricular wall dimensions, heart rate, and cardiac fractional shortening (FS) and heart rate were assessed at select time points 0, 1, 4, 8, 16, and 24 hours after administration. CK-3773274 significantly reduced FS in a dose-related fashion relative to pre-dose baseline values. 50 of the IC50 in both wild type and R403Q mice in a concentration-dependent manner in the genetic R403Q mouse model of hypertrophic cardiomyopathy. Cardiac myosin inhibition may be a viable approach to reduce underlying hypercontractility of the cardiac sarcomere in hypertrophic cardiomyopathies.

INTRODUCTION

• Hypercontractility of the cardiac sarcomere may be essential for the underlying pathological hypertrophy and fibrosis seen in many hypertrophic cardiomyopathy (HCM) patients. • Direct modulation of the sarcomere is a novel approach to potentially most conditions with maladaptive changes in cardiac contractility. • The objective of this study was to evaluate the effect of the novel small molecule cardiac myosin inhibitor CK-3773274 in the genetic R403Q mouse model of HCM.

RESULTS

Table 1. At 40- to 48-weeks of age, R403Q mice have significantly greater wall thickness than WT mice

<table>
<thead>
<tr>
<th>Body Mass (g)</th>
<th>Heart Mass (mg)</th>
<th>Interventricular Septal Wall Thickness (mm)</th>
<th>Left Ventricular Posterior Wall Thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type (n=10)</td>
<td>31.1 ± 0.04</td>
<td>6.7 ± 0.28</td>
<td>0.93 ± 0.03</td>
</tr>
<tr>
<td>R403Q (n=9)</td>
<td>33.7 ± 1.7</td>
<td>7.4 ± 0.30</td>
<td>1.22 ± 0.08</td>
</tr>
</tbody>
</table>

The objective of this study was to evaluate the effect of CK-3773274 in the genetic R403Q mouse model of hypertrophic cardiomyopathy. At approximately 40 weeks of age, left ventricular wall dimensions, heart rate, and cardiac fractional shortening (FS) and heart rate were assessed at select time points 0, 1, 4, 8, 16, and 24 hours after administration. CK-3773274 significantly reduced FS in a dose-related fashion relative to pre-dose baseline values. At all dose levels, FS returned to baseline values by 24 hours. This plasma concentration of 15% and 50% relative to baseline (IC50, unbound*) was 8.11 and 0.78 µM, respectively. In summary, single oral dose administration of CK-3773274 reduced FS in a dose- and concentration-dependent manner in the genetic R403Q mouse model of hypertrophic cardiomyopathy. Cardiac myosin inhibition may be a viable approach to reduce underlying hypercontractility of the cardiac sarcomere in hypertrophic cardiomyopathies.

SUMMARY

• Single oral dose administration of the cardiac myosin inhibitor CK-3773274 reduced fractional shortening in a dose- and concentration-dependent manner in the genetic R403Q mouse model of hypertrophic cardiomyopathy. CK-3773274 achieved a 10% (IC50) in contractility at unbound nanomolar concentrations. At ≥7× difference between the IC50, unbound* in wild type and R403Q mice may translate into a measured, gradual-on-target effect. Cardiac myosin inhibition may be a viable approach to reduce underlying hypercontractility of the cardiac sarcomere in hypertrophic cardiomyopathies.

REFERENCES


DISCLOSURES

This study was funded by Cytokinetics, Inc. All authors were employees of Cytokinetics at the time of the study and were compensated financially for their work.

Figure 1. Single doses of CK-3773274 do not affect heart rate in either WT (A) or R403Q (B) mice

Figure 2. CK-3773274 decreased fractional shortening in a dose-related manner in WT (A) and R403Q (B) mice

Figure 3. CK-3773274 decreased fractional shortening to a similar extent in WT and R403Q mice in a dose-related manner

Figure 4. CF-3773274 decreased fractional shortening in a dose-related manner in WT (A) and R403Q (B) mice

Figure 5. Single doses of CK-3773274 do not affect heart rate in either WT (A) or R403Q (B) mice

Figure 6. CF-3773274 decreased fractional shortening in a dose-related manner in WT (A) and R403Q (B) mice

Figure 7. Single doses of CK-3773274 do not affect heart rate in either WT (A) or R403Q (B) mice