Mitogen-Activated Protein Kinase Inhibitors Improve Heart Function and Prevent Fibrosis in Cardiomyopathy Caused by Mutation in Lamin A/C Gene

Wei Wu, MD*; Antoine Muchir, PhD*; Jian Shan, MD; Gisèle Bonne, PhD; Howard J. Worman, MD

Background—Mutations in the lamin A/C gene, LMNA, can cause dilated cardiomyopathy. We have shown abnormal activation of the extracellular signal-regulated kinase (ERK) and the c-jun N-terminal kinase (JNK) branches of the mitogen-activated protein kinase signaling cascade in hearts from LmnaH222P/H222P mice that develop dilated cardiomyopathy. We recently showed that partial inhibition of ERK and JNK signaling before the onset of cardiomyopathy in LmnaH222P/H222P mice prevented the development of left ventricular dilatation and decreased cardiac ejection fraction at a time when they occurred in untreated mice.

Methods and Results—To determine whether pharmacological inhibitors of ERK and JNK signaling could be clinically useful to treat cardiomyopathy caused by LMNA mutation, we administered them to LmnaH222P/H222P mice after they developed left ventricular dilatation and decreased ejection fraction. LmnaH222P/H222P mice were treated with ERK and JNK signaling inhibitors from 16 to 20 or, in pilot experiments, 19 to 24 weeks of age. The inhibitors blocked increased expression of RNAs encoding natriuretic peptide precursors and proteins involved in sarcomere architecture that occurred in placebo-treated mice. Echocardiography and histological analysis demonstrated that treatment prevented left ventricular end-systolic dilatation, increased ejection fraction, and decreased myocardial fibrosis.

Conclusion—Inhibitors of ERK and JNK signaling could potentially be used to treat humans with cardiomyopathy caused by LMNA mutations. (Circulation. 2011;123:53-61.)

Key Words: cardiomyopathy ■ LMNA ■ mitogen-activated protein kinases ■ pharmacology

Dilated cardiomyopathy is characterized by ventricular dilatation and impaired systolic function with 20% to 48% of cases familial.1 Mutations in LMNA encoding A-type nuclear lamins have been shown to cause several human diseases2 with at least 3 having dilated cardiomyopathy as a predominant feature: autosomal Emery-Dreifuss muscular dystrophy,3 limb girdle muscular dystrophy type 1B,4 and dilated cardiomyopathy type 1A.5 Given the phenotypic overlap of these disorders, they can be described as LMNA dilated cardiomyopathy with variable skeletal muscle involvement.6 LMNA mutations appear to be responsible for ≈8% of familial cardiomyopathies.7–10 The onset of symptoms in LMNA cardiomyopathy is variable, ranging from the first to sixth decade of life and occurring most frequently in the third decade.7–11 Its natural history is more aggressive than most other familial cardiomyopathies, with high rates of arrhythmias leading to sudden death and advanced heart failure necessitating cardiac transplantation.7,11,12

Clinical Perspective on p 61

To identify potential targets to treat cardiomyopathy caused by LMNA mutation, we have been examining cellular signaling pathways in hearts of LmnaH222P knock-in mice, a model of the human disease. Male LmnaH222P/H222P mice develop left ventricular (LV) dilatation and depressed contractile function starting at ≈8 to 10 weeks of age and invariably develop LV dilatation and decreased cardiac contractility at 16 weeks.13 We have shown abnormal activation of the extracellular signal-regulated kinase (ERK) and the c-Jun N-terminal kinase (JNK) branches of the mitogen-activated protein kinase (MAPK) signaling cascade in hearts of Lmna H222P knock-in mice before the onset of clinically detectable cardiomyopathy.14 We have

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also shown that lamin A variants that cause cardiomyopathy activate ERK and JNK when expressed in cultured cells. From these results, we hypothesized that activation of ERK and JNK plays a primary pathogenic role in the development of cardiomyopathy. Our recent work has shown that small-molecule inhibitors of ERK and JNK signaling administered to male Lmna<sup>H222P/H222P</sup> mice before the onset of detectable cardiomyopathy prevented LV dilatation and decreases in cardiac ejection fraction (EF) at an age when placebo-treated mice had significant abnormalities in these parameters.15,16

A critical question relevant to potential treatment of human subjects with ERK and JNK inhibitors regards their effectiveness after the onset of cardiac dysfunction. It would be impractical to use such drugs as prophylactic treatment in asymptomatic humans with LMNA mutations, especially given the variable age of onset, usually adulthood. To help answer this question, we initiated the present study to determine whether inhibitors of ERK and JNK signaling would be beneficial in Lmna<sup>H222P/H222P</sup> mice after LV dilatation and decreased cardiac EF have already occurred.

Methods

An expanded Materials and Methods section is available in the online-only Data Supplement. Lmna<sup>H222P/H222P</sup> mice were generated and genotyped with polymerase chain reaction primers as described.13 Drugs dissolved in dimethyl sulfoxide (DMSO) were delivered into the peritoneal cavity by injection at 3 mg·kg<sup>−1</sup>·d<sup>−1</sup> for 5 days a week. Equal volumes of DMSO were administered as placebo. Cardiac structure and contractility were assessed by echocardiography. Representative stained cardiac sections were photographed with a Microphot SA (Nikon) light microscope attached to a Spot RT Slide camera (Diagnostic Instruments) with a ×10 objective. Images were processed with Adobe Photoshop CS (Adobe Systems). RNA transcripts measured with real-time reverse-transcription polymerase chain reaction were quantified with iQ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA). Statistical comparisons were made with an unpaired Student t test or a 1-way ANOVA with the Tukey posthoc test to evaluate the significance of differences between means.

Results

Rationale for Treatment of Lmna<sup>H222P/H222P</sup> Mice

Our hypothesis was that treatment with a MAPK/ERK kinase (MEK) 1/2 inhibitor, which inhibits activation of ERK, or a JNK inhibitor would improve cardiac structure and function in Lmna<sup>H222P/H222P</sup> mice when the compounds are administered after these parameters are significantly abnormal. Because the animal care facility at Columbia University Medical Center prohibits removal and reentry of mice from its barrier facility, we could not obtain echocardiograms on individual subjects before and after treatment. To test our hypothesis, we therefore assigned 16-week-old male Lmna<sup>H222P/H222P</sup> mice to 3 different treatment arms (placebo DMSO, n = 28; MEK1/2 inhibitor PD98059, n = 22; JNK inhibitor SP600125, n = 29) and examined parameters of cardiac structure and function at 20 weeks of age, after 4 weeks of treatment. At 16 weeks, male Lmna<sup>H222P/H222P</sup> mice are known to have markedly increased LV end-diastolic diameter (LVEDD) and LV end-systolic diameter (LVESD) compared with Lmna<sup>+/+</sup> mice.13,15,16 Lmna<sup>H222P/H222P</sup> mice also have depressed cardiac contractility, with fractional shortening decreased by 20% to 40% compared with Lmna<sup>+/+</sup> mice.13,15 Myocardial fibrosis occurs in Lmna<sup>H222P/H222P</sup> mice at 16 weeks of age.16 At 20 weeks, LVEDD and LVESD increase further in Lmna<sup>H222P/H222P</sup> mice, and cardiac contractility also progressively deteriorates.16 During the 4-week treatment protocol, 6 mice in the DMSO group, 3 in the PD98059 group, and 3 in the SP600125 group died before reaching 20 weeks of age for evaluation.

Effect of PD98059 and SP600125 on ERK and JNK Signaling

Systemic administration of the MEK1/2 inhibitor PD98059 and the JNK inhibitor SP600125 to Lmna<sup>H222P/H222P</sup> mice from 16 to 20 weeks of age partially blocked the phosphorylation of ERK1/2 (Figure 1A) and JNK (Figure 1B), respectively, in hearts. At 3 mg·kg<sup>−1</sup>·d<sup>−1</sup>, PD98059 was highly selective for blocking ERK signaling because phosphorylation of JNK was not significantly inhibited (Figure 1A). At 3 mg·kg<sup>−1</sup>·d<sup>−1</sup>, SP600125 was specific of the JNK signaling because phosphorylation of ERK1/2 was not significantly inhibited (Figure 1B).

Effect of PD98059 and SP600125 on Cardiac Expression of Natriuretic Peptides and Myosin Light Chain

One of the features of dilated cardiomyopathy is the upregulation of cardiac hormones such as natriuretic peptides as a compensatory mechanism to maintain cardiac output.17,18 Upregulation of genes involved in sarcomere organization also occurs.19,20 We therefore assayed the expression of Mlc-2a messenger RNA (mRNA), encoding a cardiac isoform of myosin light chain, and NppA and NppB mRNAs, encoding natriuretic peptides precursors, in hearts from Lmna<sup>+/+</sup> mice, DMSO-treated Lmna<sup>H222P/H222P</sup> mice, and inhibitor-treated Lmna<sup>H222P/H222P</sup> mice (Figure 2). In hearts from DMSO-treated Lmna<sup>H222P/H222P</sup> mice, expression of Mlc-2a mRNA was significantly increased ∼30-fold compared with hearts of Lmna<sup>+/+</sup> mice (Figure 2). Similarly, in hearts from Lmna<sup>H222P/H222P</sup> mice, NppA and NppB mRNA levels showed significant 36-fold and 17-fold increases in expression compared with hearts of Lmna<sup>+/+</sup> mice (Figure 2). Treatment of Lmna<sup>H222P/H222P</sup> mice with PD98059 or SP600125 significantly decreased the expression of Mlc-2a, NppA, and NppB mRNAs at 20 weeks of age (Figure 2). Hence, pharmacological inhibition of ERK or JNK signaling reversed the molecular compensatory processes that occur in Lmna<sup>H222P/H222P</sup> mice with cardiomyopathy.

Effect of PD98059 and SP600125 on LV Dilatation and Contractility in Lmna<sup>H222P/H222P</sup> Mice

After 4 weeks of treatment with DMSO, PD98059, or SP600125, Lmna<sup>H222P/H222P</sup> mice were anesthetized, and cardiac dimensions and function were measured by echocardiography. M-mode transthoracic echocardiography showed
increased LVEDD and LVESD in Lmna<sup>H222P/H222P</sup> mice treated with DMSO compared with Lmna<sup>+/+</sup> mice (Figure 3). Lmna<sup>H222P/H222P</sup> mice treated with PD98059 and SP600125 had significantly smaller LVESD compared with the DMSO-treated mice (Figure 3). Fractional shortening and EF were reduced in Lmna<sup>H222P/H222P</sup> mice compared with Lmna<sup>+/+</sup> mice but increased in the Lmna<sup>H222P/H222P</sup> mice treated with PD98059 or SP600125.

The Table shows the composite echocardiographic data for the 3 treatment arms for Lmna<sup>H222P/H222P</sup> mice and Lmna<sup>+/+</sup> mice for comparison. Compared with Lmna<sup>+/+</sup> mice, Lmna<sup>H222P/H222P</sup> mice treated with DMSO had significantly increased LVEDD and LVESD. The EF of DMSO-treated male Lmna<sup>H222P/H222P</sup> mice at 20 weeks was 53.87 ± 2.58%, which was decreased by 28% compared with Lmna<sup>+/+</sup> mice. Lmna<sup>H222P/H222P</sup> mice treated with PD98059 or SP600125 had a statistically significant reduction in the LVESD compared with mice treated with DMSO; however, LVEDD was not significantly different. Lmna<sup>H222P/H222P</sup> mice treated with PD98059 had an EF of 65.46 ± 2.64%, an increase of ≈22% (P < 0.005) compared with the DMSO-treated group. The EF of Lmna<sup>H222P/H222P</sup> mice treated with SP600125 was 61.88 ± 1.66%, an increase of ≈15% (P < 0.005) compared with the DMSO-treated group. Overall, these results showed that PD98059 and SP600125 have positive effects on cardiac contractility when administered after cardiac dysfunction occurs in Lmna<sup>H222P/H222P</sup> mice.

**Effect of PD98059 and SP600125 on Myocardial Fibrosis in Lmna<sup>H222P/H222P</sup> Mice**

Later-stage cardiomyopathy caused by LMNA mutations is characterized by myocardial fibrosis. As shown by Sirius Red and Gomori trichrome staining, hearts from Lmna<sup>H222P/H222P</sup> mice 20 weeks of age treated with DMSO had a significant increase in fibrosis compared with hearts from Lmna<sup>+/+</sup> mice (Figure 4A and 4B). In contrast, Lmna<sup>H222P/H222P</sup> mice treated with PD98059 or SP600125 had a lower degree of cardiac fibrosis than DMSO-treated mice (Figure 4A and 4B).

We quantified the myocardial fibrotic area of each animal by determining the ratio of fibrotic tissue (stained blue with Gomori trichrome) to the total tissue area in each micrograph (Figure 4C). Hearts from DMSO-treated Lmna<sup>H222P/H222P</sup> mice had 15.01 ± 0.9% fibrotic tissue per total surface examined (Figure 4D). Systemic treatment with PD98059 or SP600125 significantly lowered the area of fibrotic tissue to 4.48 ± 1% (P < 0.0005) and 5.86 ± 0.4% (P < 0.0005), respectively (Figure 4D).

Excessive extracellular matrix, predominantly collagen proteins, defines fibrotic tissue. We therefore determined the expression of genes encoding a protein of the extracellular matrix (Fln1 encoding fibronectin) and genes encoding type I collagen (Col1a1 and Col1a2) using real-time reverse-transcription polymerase chain reaction. At 20 weeks of age, hearts from Lmna<sup>H222P/H222P</sup> mice treated with DMSO had a 5-fold increase in Col1a1, a 4-fold increase in Col1a2, and a 4-fold increase in Fln1 mRNAs compared with hearts from Lmna<sup>+/+</sup> mice (Figure 5). Treatment with PD98059 and SP600125 significantly lowered the expression of Col1a1, Col1a2, and Fln1 (Figure 5). These results demonstrated that Lmna<sup>H222P/H222P</sup> mice treated with either MEK1/2 or JNK inhibitors had decreased progression of myocardial fibrosis.

**Effect of PD98059 and SP600125 on Nuclear Shape in Cardiomyocytes in Lmna<sup>H222P/H222P</sup> Mice**

We have reported abnormal elongation of nuclei in cardiomyocytes of Lmna<sup>H222P/H222P</sup> mice. Nuclei in cardiomyocytes in hearts from Lmna<sup>H222P/H222P</sup> mice treated with DMSO were elongated compared with those in Lmna<sup>+/+</sup> mice (Figure 6A). Nuclei of cardiomyocytes in hearts from Lmna<sup>H222P/H222P</sup> mice treated with PD98059 or SP600125 had an overall shape that was more “rounded” than those in hearts of mice treated with DMSO (Figure 6A). Mean length of cardiomyocyte nuclei in hearts from Lmna<sup>H222P/H222P</sup> mice treated with DMSO was significantly longer than in hearts from Lmna<sup>+/+</sup> mice (P < 0.0005; Figure 6B). The mean lengths of nuclei in cardiomyocytes in
hearts from LmnaH222P/H222P mice treated with PD98059 or SP600125 were significantly shorter than in the hearts of mice in the DMSO-treated group (P < 0.0005; Figure 6B). Similar nuclear elongation has also been reported in Lmna knockout mice, suggesting a role of lamins in determining nuclear shape in cardiomyocytes.23,24 Although other abnormalities in nuclear morphology have been observed in hearts of LmnaH222P/H222P mice when cardiac tissue is examined by electron microscopy,13 we could not assess these ultrastructural alterations with the light microscopic methods we used.

Figure 2. Effect of PD98059 and SP600125 on cardiac expression of natriuretic peptides and myosin light chain in LmnaH222P/H222P mice. Dot diagrams indicate the expression levels of Mlc-2a mRNA encoding the cardiac isoform of myosin light chain, Nppa mRNA encoding the atrial natriuretic factor and Nppb encoding the brain natriuretic peptide in hearts from Lmna+/+ mice and LmnaH222P/H222P mice treated with PD98059 (n=5), SP600125 (n=6) or DMSO (n=8). Values were obtained using the ΔCT method using Gapdh as the housekeeping gene (see the Materials and Methods section in the online-only Data Supplement). *P < 0.05, **P < 0.005, #P < 0.05, ##P < 0.005.

Pilot Study of PD98059 and SP600125 to Treat More Advanced Heart Disease in LmnaH222P/H222P Mice
In a pilot study, we assessed treatment of LmnaH222P/H222P mice with PD98059 and SP600125 at a more advanced stage of disease and for a longer time. We assigned 19-week-old male LmnaH222P/H222P mice to 3 different treatment arms (placebo DMSO, n=4; MEK1/2 inhibitor PD98059, n=3; JNK inhibitor SP600125, n=3) and examined parameters of cardiac structure and function. Systemic administration of PD98059 and SP600125 to LmnaH222P/H222P mice partially blocked phosphorylation of ERK1/2 and JNK in hearts from 24-week-old mice (Figure 1A in the online-only Data Supplement). At 24 weeks, LmnaH222P/H222P mice treated with PD98059 had decreased LV dilatation and increased fractional shortening compared with DMSO-treated mice (Figure 1B in the online-only Data Supplement). There was also a trend toward decreased LV dilatation and increased fractional shortening in the LmnaH222P/H222P mice treated with SP600125 (Figure 1B in the online-only Data Supplement). Cardiac expression of Mlc-2a, NppA, NppB, Colla1, and Colla2 mRNAs was also significantly reduced in the inhibitor-treated LmnaH222P/H222P mice at 24 weeks, except for NppB in those treated with SP600125 (Figure 1C in the online-only Data Supplement).

Discussion
Our previous work has documented the effectiveness of inhibiting ERK and JNK signaling in preventing or delaying
the onset of cardiomyopathy in Lmna<sup>−/−</sup> mice.15,16 In those studies, MEK and JNK inhibitors were administered before the onset of any detectable structural or functional cardiac abnormalities. A critical remaining question was whether MEK and JNK inhibitors would be effective in improving heart function in Lmna<sup>H222P/H222P</sup> mice when initiated after the onset of cardiac disease, which would be more analogous to potential treatment in human patients. In this study, we therefore tested the extent to which a treatment course starting after the onset of cardiac disease in Lmna<sup>H222P/H222P</sup> mice would be beneficial. Our results showed that pharmacological inhibitors of ERK and JNK signaling blocked increased expression of RNAs encoding natriuretic peptide precursors and proteins involved in sarcomere architecture, prevented LV end-systolic dilatation, increased cardiac EF, and decreased myocardial fibrosis. Two recent studies showed that either a calcium-sensitizing agent25 or a β-blocker24 also improved cardiac function in mouse models of Lmna-associated cardiomyopathy. Our work provides support for the possibility that MEK or JNK inhibitors could overcome the lack of definitive treatments for human patients suffering from cardiac disease caused by LMNA mutations.

Changes in myocardial structure and function in response to injury and proliferation of the nonmyocyte cell populations of the heart, referred to as myocardial remodeling,26 alter cardiac performance over the long term. Part of such remodeling includes fibrosis, which results in exaggerated mechanical stiffness and causes systolic dysfunction.27 Established therapies for heart failure may also derive a significant part of their benefit from actions on cardiac fibroblasts. A beneficial effect on cardiac fibrosis has been reported for angiotensin-converting enzyme inhibitors,28–30 angiotensin receptor blockers,31,32 diuretics,33 and aldosterone antagonists.34–36 Treatment of Lmna<sup>H222P/H222P</sup> mice with MEK or JNK inhibitors had a profound beneficial effect on myocardial fibrosis, a characteristic of later-stage cardiomyopathy caused by LMNA mutations.21,22 Activation of ERK and JNK signaling pathways by various stimuli has been correlated to several cellular processes such as cell proliferation and remodeling of extracellular matrix.37 Inhibition of ERK and JNK signaling pathways could therefore have a beneficial effect on cardiac function by also acting directly to decrease the proliferation of myocardial fibroblasts. Such a hypothesis needs to be tested. It also remains to be determined whether simultaneous inhibition of both ERK and JNK signaling has additive effects in cardiomyopathy caused by Lmna mutation.

Our study in Lmna<sup>H222P/H222P</sup> mice was designed similar to a human clinical trial. It assessed primary end points (LV dilatation, EF) and “surrogate” secondary end points (expression of natriuretic peptide precursors) that are used in many human clinical heart failure trials. Although mortality is a reasonable end point in a phase III clinical trial for advanced heart failure, it is rarely, if ever, used in the initial drug assessment phase or in treatment of subjects with heart disease that is not end stage,38 both of which were the case in our study. Furthermore, Lmna<sup>H222P/H222P</sup> mice have diaphragmatic muscle involvement (not reported in humans with LMNA mutations) and significant skeletal muscle pathology as they age, which may be noncardiac causes of mortality.13 Nonetheless, the measurements of LV function we used correlate with prognosis in many human clinical trials, and their behavior parallels changes in mortality with treatment.38 For example, LV end-systolic volume, which is determined by measuring LVESD, is the major determinant of survival in human subjects after recovery from myocardial infarction and after coronary artery bypass grafting for impaired LV function.39,40 A study by Heywood et al41 also showed in human subjects with an EF <40% treated with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers that an increase of >15% in EF resulted in mortality of only ~2%/y. In our study, PD98059 and SP600125 improved the EF of Lmna<sup>H222P/H222P</sup> mice ~22% and 15%, respectively, compared with placebo. Taking into account the fact that EF improvement is an important predictor for survival in human subjects with systolic dysfunction, we speculate that small-molecule inhibitors of the ERK and JNK signaling pathways could have a positive effect on survival of patients with LMNA mutations. Although not an end point in our study, during the 4-week treatment protocol starting at 16 weeks of age, 6 mice in the DMSO group, 3 in the PD98059 group, and 3 in the SP600125 group died before reaching 20 weeks of age, suggesting that treatment with MEK1/2 or JNK inhibitors trended toward improved survival. Furthermore, our pilot study treating Lmna<sup>H222P/H222P</sup> mice up to 24 weeks of age, when they have a mortality rate of ~25%,13 showed improvements in echocardiographic and cardiac biochemical parameters.

The choice of therapeutic agents in clinical trials is predicated, at least in part, on the efficacy of drugs studied in murine models of disease.42–44 Both PD98059 and SP600125, which we used in this study to inhibit ERK and JNK

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**Table. Echocardiographic Data at 20 Weeks of Age for Lmna<sup>+/+</sup> Mice and Lmna<sup>H222P/H222P</sup> Mice Treated With DMSO Placebo or Treated With SP600125 or PD98059**

<table>
<thead>
<tr>
<th>Genotype (Treatment Group)</th>
<th>n</th>
<th>HR, bpm</th>
<th>LVEDD, mm</th>
<th>LVESD, mm</th>
<th>EF, %</th>
<th>FS, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lmna&lt;sup&gt;+/+&lt;/sup&gt;</td>
<td>12</td>
<td>400</td>
<td>3.50±0.06</td>
<td>2.07±0.08</td>
<td>73.21±1.17</td>
<td>41.71±1.01</td>
</tr>
<tr>
<td>Lmna&lt;sup&gt;H222P/H222P&lt;/sup&gt; (DMSO)</td>
<td>22</td>
<td>372</td>
<td>3.87±0.11*</td>
<td>3.00±0.13†</td>
<td>53.87±2.58‡</td>
<td>27.86±1.54‡</td>
</tr>
<tr>
<td>Lmna&lt;sup&gt;H222P/H222P&lt;/sup&gt; (PD98059)</td>
<td>19</td>
<td>350</td>
<td>3.55±0.11</td>
<td>2.41±0.11</td>
<td>65.46±2.64§</td>
<td>35.91±1.88§</td>
</tr>
<tr>
<td>Lmna&lt;sup&gt;H222P/H222P&lt;/sup&gt; (SP600125)</td>
<td>26</td>
<td>363</td>
<td>3.73±0.08</td>
<td>2.67±0.10†</td>
<td>61.88±1.66§</td>
<td>33.11±1.16§</td>
</tr>
</tbody>
</table>

HR indicates heart rate; FS, fractional shortening. Values are mean±SEM.

Comparison between DMSO-treated Lmna<sup>H222P/H222P</sup> and Lmna<sup>+/+</sup> mice: *P<0.05, †P<0.0005. Comparison between SP600125-treated, PD98059-treated, and DMSO-treated Lmna<sup>H222P/H222P</sup> mice: ‡P<0.05, §P<0.005, ††P<0.0005.
signaling, respectively, are tool compounds and are not suitable for use in humans secondary to problems with bioavailability and toxicity. Therefore, any future clinical trial of MEK or JNK inhibitor in human subjects with cardiomyopathy caused by LMNA mutations would require the use of superior drugs, including possibly those that have already entered the pipeline of pharmaceutical companies for other indications. For example, a second-generation oral MEK inhibitor, PD0325901 (Pfizer), has markedly improved properties, including better potency against MEK, better bioavailability, increased metabolic stability, and a longer MEK suppression. PD0325901 has been administered to humans and has entered a phase II clinical trial to treat advanced non–small-cell lung cancer. Similarly,

**Figure 4.** Sirius red (A) and Gomori trichrome (B) staining of cross sections of hearts from Lmna<sup>H222P/H222P</sup> mice treated with PD98059, SP600125, or DMSO. A cross section of a heart from an Lmna<sup>+/+</sup> mouse is shown for comparison. Scale bar: 50 μm. C, Quantification of fibrotic area in hearts from mice. n=3 in each group. Y axis corresponds to the area (pixels); X axis represents the color spectrum (red corresponds to the muscle tissue, blue corresponds to the connective tissue). D, Bars indicate the percentage of fibrosis per surface area of myocardium examined in hearts from Lmna<sup>+/+</sup> mice and Lmna<sup>H222P/H222P</sup> mice treated with PD98059, SP600125, or DMSO. n=3 in each group. ***P<0.0005, ###P<0.0005.
AZD6244/ARRY-142886 (AstraZeneca/Array Biopharma) is in phase II clinical trials for patients with cancers.49 Superior JNK inhibitors are also in preclinical development for use in humans.50 Hence, our results in LmnaH222P/H222P mice with cardiac dysfunction could lay the foundation for clinical trials of MEK and JNK inhibitors that are currently being developed for cancer and inflammatory conditions in human subjects with cardiomyopathy caused by LMNA mutations.

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Disclosures
Drs Worman and Muchir are inventors on a pending PCT patent application on methods for treating and/or preventing cardiomyopathies by ERK and JNK inhibition filed by the trustees of Columbia University in New York, NY. The other authors report no conflicts.

References


Heart failure is responsible for considerable morbidity and mortality, and dilated cardiomyopathy (DCM) is a major cause. Molecular genetic studies have revealed mutations in various genes in patients with familial DCM, but the precise mechanisms of how they lead to heart muscle damage remain largely unknown. Mutations in LMNA encoding A-type nuclear lamins appear to be responsible for ~8% of cases of familial DCM, and patients with LMNA mutations have a poorer prognosis than those with DCM caused by mutations in most other genes. We have previously shown an abnormal activation of the extracellular signal-regulated kinase (ERK) and the c-jun N-terminal kinase (JNK) branches of the mitogen-activated protein kinase signaling cascade in hearts of mice with DCM caused by a mutation in Lmna. We now establish that treating these mice with chemical inhibitors of ERK and JNK after the onset of left ventricular dilatation and decreased cardiac ejection fraction, a time when human patients would be considered for therapy, improves cardiac function and significantly decreases myocardial fibrosis. These results provide proof of concept that pharmacological inhibitors of ERK and JNK signaling, some of which are currently in clinical development for other indications, could be studied in human clinical trials of patients with DCM caused by LMNA mutations.
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Summary

배경
Lamin A/C 유전자 LMNA의 돌연변이 유전자 돌연변이로 인해 심근병증을 유발할 수 있다. 본 유전자 돌연변이 Lmna<sup>1029del</sup>
<sup>1029</sup> 생쥐에서 ERK와 JNK 단백 kinase의 신호전달체제
의 변화가 심근병증을 유발한다고 알려져 있다.

방법 및 결과
ERK와 JNK 신호전달체제를 억제하는 약물이 LMNA 돌
연변이에 의한 심근병증 치료제로서 임상적 유용성이
있는지를 알아보기 위하여, Lmna<sup>1029del</sup> 생쥐에서 심
기능 저하와 좌심실 확장이 발생한 이후 억제제를 투
여하였다. 지표연구로 생후 16-20주 혹은 생후 19-24주
의 Lmna<sup>1029del</sup> 생쥐에서 ERK와 JNK 억제제를 투여하였
다. 억제제에 의하여 natriuretic peptide 신규물질 RNA
의 발현과 수축에 관여하는 구조 단백 발현이 억제되었
다. 심초음파와 조직검사에서 억제제 치료는 수축기말
좌심실 확장은 억제하였고, 구출률(ejection fraction)을
증가시켰으며, 좌심실 섬유화를 억제하였다.
일반적으로 유전체 돌연변이에 의한 실험체는 약제 치료에 의한 호전을 기대하기 어렵다. 그러나 본 연구에서 MAPK의 일종인 ERK와 JNK를 억제하는 약물이 LMNA 돌연변이 실험체의 호전을 가져올 수 있음을 보였다.

세포의 핵을 구성하는 A-type nucleolar lamin은 LMNA 유전자에서 전사하는데, 이 유전자의 돌연변이로 사망에 의한 가장 이상의 확장성 실험체들을 유발한다. 주로 근이음증을 동반하는 실험체로, Emery-Dreifuss 근이음증, 제1형 근수지형 근이음증 혹은 제1형 실험체로 유발된다. 이러한 LMNA 돌연변이는 가족성 확장성 실험체들의 8%가량을 차지한다. LMNA 실험체는 중증은 매우 다양하며 출생 후 60대까지 두 부 발생할 수 있으나, 특히 30대에서의 발병이 가장 흔하다. 이는 다른 가족성 확장성 실험체로 비교하여 유전성이 설립하여 주로가 나타난다. 특히 부정맥 발생이 의한 돌연사와 밀가 심부전으로 진행하여 실험체로 필요하다.

본 연구에서는 LMNA 실험체의 지표 가능성을 모이기 위하여 본 실험이 노출된 Lmna H222P 유전자 변이형쥐를 사용하였다. LmnaKO/H222P 쥐는 출생 후 0-14주에 자식충식기와 적지하기 시작하며, 16주후에 자식충식 확장성 지표의 가장 지표가 확장성 실험체들과 같은 상태가 된다. 이는 연구에서는 Lmna H222P 유전자 변이형 쥐에서 확장성 실험체들이 발생하기 전에 ERK/Extracellular signal-regulated kinase(N-Jnk(N-terminal kinase) 등의 MAPK/mitogen-activated protein kinase)들이 비정상적으로 활성화됨을 보았다. 즉, LMNA 돌연변이와 ERK의 JNK를 활성화시킨다. 또한, ERK와 JNK 억제제는 확장성 실험체들 발생 전에 Lmna H222P 유전자 변이형 쥐에서 투여하였을 때 실험체들의 염두를 예방하였다. 그러나 실험 자료에서의 실험체들이 발생하기 위해 이들 약제들의 예방적 투여는 바람직하지 못하다. 따라서 이에 확장성 실험체들의 발생된 이후에도 이들 약제의 효과가 지속되다고 의문이다.

본 연구에서는 Lmna H222P 유전자 변이형 쥐에서 확장성 실험체들과 심방자 발현이 미루어 발생한 이후의 지표가 확장성 실험체들의 예방적 예방이 필요하며 예방적 예방이 필요하다. 약제의 투여는 새로운 약제의 예방적 예방이 필요하며 예방적 예방이 필요하다. 약제의 투여는 새로운 약제의 예방적 예방이 필요하다.