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 Lmna^H222P/H222P mice that develop dilated cardiomyopathy. We recently showed that partial inhibition of ERK and JNK signaling before the onset of cardiomyopathy in Lmna^H222P/H222P mice prevented the development of left ventricle 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 Lmna^H222P/H222P mice after they developed left ventricular dilatation and decreased ejection fraction. Lmna^H222P/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 IB,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.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 Lmna^H222P knock-in mice, a model of the human disease. Male Lmna^H222P/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

Received June 4, 2010; accepted October 25, 2010.

From the Department of Medicine (W.W., A.M., H.J.W.), Department of Pathology and Cell Biology (W.W., A.M., H.J.W.), Department of Physiology and Cellular Biophysics (J.S.), and Clyde and Helen Wu Center for Molecular Cardiology (J.S.), College of Physicians and Surgeons, Columbia University, New York, NY; and INSERM, U974 (G.B.), Université Pierre et Marie Curie-Paris, UMR-S974, CNRS, UMR-7215, Institut de Myologie, IFR14 (G.B.), and AP-HP, Groupe Hospitalier Pitié-Salpêtrière, U.F. Cardiogénétique et Myogénétique, Service de Biochimie Métabolique (G.B.), Paris, France.

*Drs Wu and Muchir contributed equally to this article.

The online-only Data Supplement is available with this article at http://circ.ahajournals.org/cgi/content/full/CIRCULATIONAHA.110.970673/DC1.

Circulation is available at http://circ.ahajournals.org

DOI: 10.1161/CIRCULATIONAHA.110.970673
also shown that lamin A variants that cause cardiomyopathy activate ERK and JNK when expressed in cultured cells.\({\textsuperscript{14}}\) 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 \(\text{Lmna}\,^{\text{H222P/H222P}}\) 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.\({\textsuperscript{15,16}}\)

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 prophyactic treatment in asymptomatic humans with \(\text{LMNA}\) 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 \(\text{Lmna}\,^{\text{H222P/H222P}}\) 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. \(\text{Lmna}\,^{\text{H222P/H222P}}\) mice were generated and genotyped with polymerase chain reaction primers as described.\({\textsuperscript{13}}\) Drugs dissolved in dimethyl sulfoxide (DMSO) were delivered into the peritoneal cavity by injection at 3 mg \(\cdot\) kg\(^{-1}\) \cdot d\(^{-1}\) 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 \(\times 10\) 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 \(\text{Lmna}\,^{\text{H222P/H222P}}\) 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 \(\text{Lmna}\,^{\text{H222P/H222P}}\) 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 \(\text{Lmna}\,^{\text{H222P/H222P}}\) 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 \(\text{Lmna}\,^{\text{H222P/H222P}}\) mice are known to have markedly increased LV end-diastolic diameter (LVEDD) and LV end-systolic diameter (LVESD) compared with \(\text{Lmna}\,^{\text{+/+}}\) mice.\({\textsuperscript{13,15,16}}\) \(\text{Lmna}\,^{\text{H222P/H222P}}\) mice also have depressed cardiac contractility, with fractional shortening decreased by 20% to 40% compared with \(\text{Lmna}\,^{\text{+/+}}\) mice.\({\textsuperscript{13,15}}\) Myocardial fibrosis occurs in \(\text{Lmna}\,^{\text{H222P/H222P}}\) mice at 16 weeks of age.\({\textsuperscript{16}}\) At 20 weeks, LVEDD and LVESD increase further in \(\text{Lmna}\,^{\text{H222P/H222P}}\) mice, and cardiac contractility also progressively deteriorates.\({\textsuperscript{15,16}}\) 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 \(\text{Lmna}\,^{\text{H222P/H222P}}\) 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 \(\cdot\) kg\(^{-1}\) \cdot d\(^{-1}\), PD98059 was highly selective for blocking ERK signaling because phosphorylation of JNK was not significantly inhibited (Figure 1A). At 3 mg \(\cdot\) kg\(^{-1}\) \cdot d\(^{-1}\), SP600125 was specific of the JNK signaling because phosphorylation of ERK1/2 was not significantly inhibited (Figure 1B).

#### Effect of the 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.\({\textsuperscript{17,18}}\) Upregulation of genes involved in sarcomere organization also occurs.\({\textsuperscript{19,20}}\) We therefore assessed the expression of \(\text{Mlc-2a}\) messenger RNA (mRNA), encoding a cardiac isoform of myosin light chain, and \(\text{NppA}\) and \(\text{NppB}\) mRNAs, encoding natriuretic peptides precursors, in hearts from \(\text{Lmna}\,^{\text{+/+}}\) mice, DMSO-treated \(\text{Lmna}\,^{\text{H222P/H222P}}\) mice, and inhibitor-treated \(\text{Lmna}\,^{\text{H222P/H222P}}\) mice (Figure 2). In hearts from DMSO-treated \(\text{Lmna}\,^{\text{H222P/H222P}}\) mice, expression of \(\text{Mlc-2a}\) mRNA was significantly increased \(\approx 30\)-fold compared with hearts of \(\text{Lmna}\,^{\text{+/+}}\) mice (Figure 2). Similarly, in hearts from \(\text{Lmna}\,^{\text{H222P/H222P}}\) mice, \(\text{NppA}\) and \(\text{NppB}\) mRNA levels showed significant 36-fold and 17-fold increases in expression compared with hearts of \(\text{Lmna}\,^{\text{+/+}}\) mice (Figure 2). Treatment of \(\text{Lmna}\,^{\text{H222P/H222P}}\) mice with PD98059 or SP600125 significantly decreased the expression of \(\text{Mlc-2a}\), \(\text{NppA}\), and \(\text{NppB}\) mRNAs at 20 weeks of age (Figure 2). Hence, pharmacological inhibition of ERK or JNK signaling reversed the molecular compensatory processes that occur in \(\text{Lmna}\,^{\text{H222P/H222P}}\) mice with cardiomyopathy.

#### Effect of PD98059 and SP600125 on LV Dilatation and Contractility in \(\text{Lmna}\,^{\text{H222P/H222P}}\) Mice

After 4 weeks of treatment with DMSO, PD98059, or SP600125, \(\text{Lmna}\,^{\text{H222P/H222P}}\) 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 LVEDD and LVESD. The EF of DMSO-treated Lmna<sup>+/+</sup> mice, Lmna<sup>H222P/H222P</sup> mice treated with PD98059 or SP600125 had a lower degree of cardiac fibrosis than DMSO-treated mice (Figure 3).

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 LVEDD compared with mice treated with DMSO; however, LVESD 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 (Fn1 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 Colla1, a 4-fold increase in Colla2, and a 4-fold increase in Fn1 mRNAs compared with hearts from Lmna<sup>+/+</sup> mice (Figure 5). Treatment with PD98059 and SP600125 significantly lowered the expression of Colla1, Colla2, and Fn1 (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 of 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 of 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 Lmna<sup>H222P/H222P</sup> 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. Although other abnormalities in nuclear morphology have been observed in hearts of Lmna<sup>H222P/H222P</sup> mice when cardiac tissue is examined by electron microscopy, we could not assess these ultrastructural alterations with the light microscopic methods we used.

**Pilot Study of PD98059 and SP600125 to Treat More Advanced Heart Disease in Lmna<sup>H222P/H222P</sup> Mice**

In a pilot study, we assessed treatment of Lmna<sup>H222P/H222P</sup> mice with PD98059 and SP600125 at a more advanced stage of disease and for a longer time: We assigned 19-week-old male Lmna<sup>H222P/H222P</sup> 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 Lmna<sup>H222P/H222P</sup> mice partially blocked phosphorylation of ERK1/2 and JNK in hearts from 24-week-old mice (Figure IA in the online-only Data Supplement). At 24 weeks, Lmna<sup>H222P/H222P</sup> mice treated with PD98059 had decreased LV dilatation and increased fractional shortening compared with DMSO-treated mice (Figure IB in the online-only Data Supplement). There was also a trend toward decreased LV dilatation and increased fractional shortening in the Lmna<sup>H222P/H222P</sup> mice treated with SP600125 (Figure IB in the online-only Data Supplement).

Cardiac expression of Mic-2a, NppA, NppB, Col1a1, and Col1a2 mRNAs was also significantly reduced in the inhibitor-treated Lmna<sup>H222P/H222P</sup> mice at 24 weeks, except for NppB in those treated with SP600125 (Figure IC 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
H222P/H222P mice.\textsuperscript{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
H222P/H222P 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
H222P/H222P 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 agent\textsuperscript{25} or a β-blocker\textsuperscript{24} 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,\textsuperscript{26} alter cardiac performance over the long term. Part of such remodeling includes fibrosis, which results in exaggerated mechanical stiffness and causes systolic dysfunction.\textsuperscript{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,\textsuperscript{28–30} angiotensin receptor blockers,\textsuperscript{31,32} diuretics,\textsuperscript{33} and aldosterone antagonists.\textsuperscript{34–36} Treatment of Lmna
H222P/H222P mice with MEK or JNK inhibitors had a profound beneficial effect on myocardial fibrosis, a characteristic of later-stage cardiomyopathy caused by LMNA mutations.\textsuperscript{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.\textsuperscript{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
H222P/H222P 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,\textsuperscript{38} both of which were the case in our study. Furthermore, Lmna
H222P/H222P 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{39,40} A study by Heywood et al\textsuperscript{41} 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
H222P/H222P 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
H222P/H222P mice up to 24 weeks of age, when they have a mortality rate of ≈25%,\textsuperscript{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.\textsuperscript{42–44} Both PD98059 and SP600125, which we used in this study to inhibit ERK and JNK

<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+/+ (DMSO)</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>
</tbody>
</table>
| Lmna
H222P/H222P (DMSO)      | 22| 372     | 3.87±0.11* | 3.00±0.13† | 53.87±2.58‡ | 27.86±1.54† |
| Lmna
H222P/H222P (PD98059)    | 19| 350     | 3.55±0.11 | 2.41±0.11† | 65.46±2.64§ | 35.91±1.88§ |
| Lmna
H222P/H222P (SP600125)  | 26| 363     | 3.73±0.08 | 2.67±0.10† | 61.88±1.66§ | 33.11±1.16§ |

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

Comparison between DMSO-treated Lmna
H222P/H222P and Lmna+/+ mice: *P<0.05, †P<0.005. Comparison between SP600125-treated, PD98059-treated, and DMSO-treated Lmna
H222P/H222P 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.

Figure 4. Sirius red (A) and Gomori trichrome (B) staining of cross sections of hearts from LmnaH222PH222P mice treated with PD98059, SP600125, or DMSO. A cross section of a heart from an Lmna−/− 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−/− mice and LmnaH222PH222P 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. Superior JNK inhibitors are also in preclinical development for use in humans. Hence, our results in Lmna<sup>H222P/H222P</sup> 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.

Sources of Funding
This work was supported by grants from the National Institutes of Health (AR048997) and the Muscular Dystrophy Association (MDA4287).

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.
Mitogen-Activated Protein Kinase Inhibitors Improve Heart Function and Prevent Fibrosis in Cardiomyopathy Caused by Mutation in Lamin A/C Gene
Wei Wu, Antoine Muchir, Jian Shan, Gisèle Bonne and Howard J. Worman

Circulation, published online December 20, 2010;
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2010 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/early/2010/12/20/CIRCULATIONAHA.110.970673

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2010/11/18/CIRCULATIONAHA.110.970673.DC1
http://circ.ahajournals.org/content/suppl/2012/01/02/CIRCULATIONAHA.110.970673.DC2

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/
Lamin A/C 유전체 돌연변이에 의한 선천성 심근병증에서 MAPK 억제제는 심근의 섬유화를 방지하고 심근기능을 호전시킬 수 있다.

최동주 교수

Summary

배경
Lamin A/C 유전자의 LMNA의 돌연변이는 확장성 심근병증을 유발할 수 있다. 본 연구는 돌연변이 Lmna<sup>1202P</sup>생쥐에서 ERK와 JNK 단백 kinase의 신호전달체계의 변화가 심근병증을 유발한다고 알려져 있다.

결론
ERK와 JNK 억제제는 Lmna 돌연변이에 의한 심근병증 치료제로 사용될 수 있다.

방법 및 결과
ERK와 JNK 신호전달체계를 억제하는 약물이 LMNA 돌연변이에 의한 심근병증 치료체로 임상적 유효성있는지를 알아보고 위하여, Lmna<sup>1202P</sup>생쥐에서 심기능 저하와 좌심실 확장이 발생한 이후 억제제를 투여하였다. 지표연구로 생후 16-20주 혹은 생후 19-24주의 Lmna<sup>1202P</sup>생쥐에 ERK와 JNK 억제제를 투여하였고, 억제제에 의하여 natriuretic peptide 전구물질 RNA의 발현과 수축기 피로는 구조 단백 발현이 억제되었고, 좌심실 확장을 예방하였고, 구축률(ejection fraction)을 증가시켰으며, 좌심실 섬유화를 억제하였다.
일반적으로 유전자 돌연변이에 의한 실험변수 약제 처리에 의한 효과를 고려하기 어렵습니다. 그러나 본 연구에서는 MAPK의 일종인 ERK와 JNK를 억제하는 약물이 LMMNA 돌연변이 실험변증의 효과를 가질 수 있음을 보고 있습니다.

세포의 핵을 구성하는 A-type nuclear lamin은 LMMNA 유전자 돌연변이 실험변증의 요소로, 이 연구에서는 실험변증의 사례를 제시한 세 가지 이상의 실험변증 실험변증을 포함합니다. 즉, 근이형증을 동반하는 실험변증으로, Emery-Driffus 근이형증, 제1형 수지대형 근이형증 혹은 제2형 실험변증이 유발합니다. 이러한 LMMNA 돌연변이는 가족성 확장성 실험변증의 8%를 차지합니다. LMMNA 실험변증의 증상은 매우 다양하며, 출생 후 60대까지 두 발생할 수 있으나, 30대에서의 발현이 가장 흔하다. 이는 다른 가족형 확장성 실험변증에 비해 이상적이며, 일반에 나타나는 특이적 발생에 의한 돌연변이의 발현이 기여한 실험변증으로 진행하여 실타이식을 필요로 한다.

본 연구에서는 LMMNA 실험변증의 치료 가능성을 검증하기 위하여 본 질병의 동물 모델인 Lmna H222P 유전자 변이형쥐를 사용하였습니다. Lmna H222P 변장주는 출생 후 3-4주에 빠른 성장 속도가 감소하기 시작하며, 16주에는 과소실 확장과 기능 저하가 확장성 실험변증과 같은 상태에 진행된다. 이 연구에서 Lmna H222P 유전자 변이형쥐에서 확장성 실험변증이 발생할 때 ERK(extracellular signal-regulated kinase)와 JNK(c-Jun N-terminal kinase) 등의 MAPK(mitogen-activated protein kinase)들이 비정상적으로 활성화됨을 밝혔다. 즉, LMMNA 돌연변이가 ERK와 JNK를 활성화시킨다. 또한, ERK와 JNK 억제제는 확장성 실험변증 발현 중인 Lmna H222P 유전자 변이형쥐 투여하였을 때 실험변증 발생을 예방하였다. 이에 대해 실험시험에서는 Lmna 돌연변이에 의한 실험변증의 발생과 억제에 관한 연구를 수행하는데, 실험변증의 이상을 일으키는 약제의 효과가 지속되며, 실험변증의 억제 효과가 있음을 보고하였다.