Differential Regulation of Mitogen-Activated Protein Kinases in the Failing Human Heart in Response to Mechanical Unloading

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Background—Mechanical unloading of the heart with a left ventricular assist device (LVAD) leads to favorable changes in the biology of the failing cardiac myocyte. To determine a potential mechanism for these improvements, we examined the regulation of mitogen-activated protein kinases (MAPKs) in the failing heart in the presence and absence of LVAD support.

Methods and Results—We examined the degree of activation (ie, phosphorylation) of p44/42 extracellularly regulated kinase, p38 kinase, and c-Jun N-terminal kinase (JNK1/2), and the corresponding activity levels of these MAPKs in myocardial samples obtained from 11 patients with LVAD support and in 11 patients without LVAD support. MAPK activity was also examined in an additional 6 patients from whom paired samples were obtained before and after LVAD support. The activity of p44/42 and JNK1/2 were reduced significantly, whereas p38 activity levels were significantly increased after LVAD support. We examined functional parameters that are linked to MAPK activation, namely cardiac myocyte hypertrophy and apoptosis. Both cardiac myocyte cell size and the incidence of cardiac myocyte apoptosis were significantly reduced after LVAD support.

Conclusions—Mechanical unloading of the failing heart leads to differential regulation of MAPKs. These changes in MAPK activity are associated with changes in myocyte hypertrophy and viability, suggesting a potential mechanistic basis for some of the observed salutary changes after LVAD support. (Circulation. 2001;104:2273-2276.)

Key Words: cardiomyopathy ■ heart-assist device ■ heart failure ■ myocyte ■ signal transduction

Mechanical unloading of the failing heart with a left ventricular (LV) assist device (LVAD) results in regression of myocyte hypertrophy, as well as improvement in cardiac myocyte function. The cellular and molecular changes that are responsible for these salutary changes in myocyte structure and function after mechanical unloading, however, are unknown. Relevant to this discussion is the observation that several cell signal–transduction pathways are regulated in the heart in direct response to changes in mechanical loading conditions. In this regard, one of the best-characterized signal-transduction pathways is the family of mitogen-activated protein kinases (MAPKs), including p44/42 extracellularly regulated kinase, p38 kinase, and c-Jun N-terminal kinase (JNK1/2). All 3 kinases are involved in the regulation of cell growth and programmed cell death in the heart, and all 3 are upregulated in patients with ischemic and dilated cardiomyopathy. Accordingly, in the present study we sought to determine whether LVAD support was associated with changes in the regulation of MAPKs in the failing human heart.

Methods

Tissue Samples
LV tissue was harvested at the time of orthotopic heart transplantation in 22 patients with dilated cardiomyopathy (New York Heart Association class IV). Of these, 11 patients had LVAD support and 11 patients were not supported with LVAD before transplant. In an additional cohort of 6 patients, we examined paired LV tissue samples before and after LVAD explantation (see Data Supplement, available at http://www.circulationaha.org).

Activation of Mitogen-Activated Protein Kinases
We determined total MAPK levels using antibodies to p44/42, p38, or JNK1/2 and the degree of MAPK phosphorylation (ie, activation) using phosphospecific antibodies to p44/42, p38, or JNK1/2. MAPK activity was determined by measuring the degree of phosphorylation of specific transcription factors downstream from p44/42, p38 and JNK1/2, respectively—namely, Elk-1, ATF-2, or c-Jun (Data Supplement).

Functional Changes After LVAD Support
Previous studies showed that p44/42 activation is linked to cardiac myocyte hypertrophy, whereas the stress-activated kinases, p38 and...
JNK1/2, are linked to cardiac myocyte apoptosis. Accordingly, we examined myocyte size and the incidence of myocyte apoptosis after LVAD support (Data Supplement).

**Statistics**

Data are expressed as mean±SEM. A Student’s t test was used to compare mean differences in levels of total MAPK, MAPK phosphorylation, and the degree of phosphorylation of MAPK substrates. *P*<0.05 was regarded as statistically significant.

**Results**

**Patient Demographics**

The Table shows that there were no significant differences in patient age or cardiac index between the patients with LVAD support (3.2±0.6 months) and those without LVAD support. There were, however, statistically significant differences (*P*<0.05) in the ratio of female/male patients, systolic blood pressure, and ejection fraction between the 2 groups. To corroborate the results obtained with unpaired myocardial samples, we also obtained paired myocardial samples from an additional 6 patients (mean age, 57±3 years) who had undergone LVAD support for 53±19 days. Patients with and without LVAD support were treated with conventional therapy for heart failure; none of the LVAD-supported patients received inotropes, whereas all of the non-LVAD–supported patients received inotropes (2 patients received intravenous milrinone; 6, intravenous dobutamine; and 3, intravenous milrinone and dobutamine).

**Activation and Activity of MAPKs**

Figure 1 shows 3 important findings with respect to the regulation of MAPKs in LVAD-supported hearts. First, as shown by the Western blot in Figure 1A, the intensity of the bands corresponding to phosphorylated p44/42 was decreased in LVAD-supported hearts. There was no change in the intensity of the bands corresponding to total p44/42. Analysis of group data showed that there was a trend toward a significant decrease (*P*=0.056) in the ratio of phospho-p44/42:total p44/42 after LVAD support (Figure 1B), whereas there was a significant (*P*<0.05) decrease in p44/42 activity, as demonstrated by the decrease in phosphorylation of Elk-1 in the LVAD-supported patients (Figure 1C). Importantly, analysis of p44/42 activity in paired myocardial samples taken from the same patient before and after LVAD support also showed a significant (*P*<0.05) decrease in Elk-1 phosphorylation after LVAD support (263±57 versus 56±49 [arbitrary units]).

Second, there was an increase in the intensity of the bands corresponding to phosphorylated p38 (Figure 1D) in LVAD-supported hearts. The group data summarized in Figure 1E show that there was a significant (*P*<0.05) increase in the ratio of phosphorylated p38:total p38 and a significant increase (*P*<0.05) in the level of phosphorylation of ATF-2, a p38 substrate (Figure 1F). To confirm these results we showed that p38 activity was also increased in paired myocardial samples from the same patient after LVAD support (pre-LVAD, 84±43 versus post-LVAD, 193±49 [arbitrary units]; *P*<0.05).

Third, there was a significant decrease in total JNK1/2 levels (Figures 1G, H) and JNK1/2 activity (Figure 1I) in the myocardial samples from the patients who underwent LVAD support. However, we were unable to detect phospho-JNK1/2 in these failing hearts (Figure 1G), despite using 3 different phosphospecific antibodies (Cellular Signaling, Pro-Mega, SantaCruz). There were insufficient paired myocardial samples to determine JNK1/2 activity.

**Demographics and Clinical Data**

<table>
<thead>
<tr>
<th></th>
<th>Without LVAD (n=11)</th>
<th>With LVAD (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>53±4</td>
<td>50±2</td>
</tr>
<tr>
<td>Female/male</td>
<td>5/6</td>
<td>1/10</td>
</tr>
<tr>
<td>Heart failure duration, mo</td>
<td>54±18</td>
<td>46±8*</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>108±6</td>
<td>136±9†</td>
</tr>
<tr>
<td>Diastolic</td>
<td>62±3</td>
<td>68±5†</td>
</tr>
<tr>
<td>Cardiac index</td>
<td>2.5±0.3</td>
<td>2.9±0.2†</td>
</tr>
<tr>
<td>Ejection fraction</td>
<td>10.0±0.8</td>
<td>13.1±0.7†</td>
</tr>
</tbody>
</table>

*P*<0.05 compared to group without LVAD.
†Measurements obtained with LVAD support.

**Figure 1.** MAPK regulation in the presence and absence of LVAD support. A, D, and G show, respectively, representative Western blots for p44/42, p38, and JNK1/2 activation (upper lane), total p44/42, p38, and JNK1/2 (middle lane), and p44/42, p38, and JNK1/2 activity (lower lane). The results for group data for MAPK activation (phosphorylation), are expressed as the ratio of the intensity of the bands corresponding to phosphorylated p44/42 and p38 divided by the intensity of the bands corresponding to total p44/42 (B) and p38 (E). Panel H shows the results for group data for total JNK1/2 before and after LVAD. Panels C, F, and I show the results for group data for p44/42, p38 and JNK1/2 activity levels, respectively. + indicates LVAD; –, no LVAD; and C, HeLa cells treated with 200 U/mL TNF for 15 minutes (positive control). *P*<0.05 compared with no LVAD.
Functional Changes After LVAD Support

Myocyte Cell Volume

Figure 2B shows that the average myocyte volume from hearts without LVAD support was significantly (>0.01) greater than the average myocyte volume from hearts with LVAD support.

Cardiac Myocyte Apoptosis

Apoptotic cardiac myocyte nuclei were detected in 6 of 10 hearts examined before LVAD support and in 1 of 10 samples examined after mechanical unloading. Figure 2D shows that there was an overall significant (>0.05) decrease in the incidence of apoptotic cardiac myocytes after LVAD support.

Discussion

The major new finding of the present study is that mechanical unloading of the failing human heart results in a differential regulation of MAPK activity, with a significant decrease in p44/42 and JNK1/2 activity and a corresponding increase in p38 activity. The decrease in p44/42 activity is likely due to a decreased phosphorylation (activation) of p44/42 rather than to changes in total p44/42 protein levels, insofar as p44/42 protein levels were not different in the hearts of patients with and without LVAD support (Figure 1A, G). The mechanism for the decrease in JNK1/2 activity, however, is less clear. Although there was a decrease in total JNK1/2 protein levels (Figure 1G, I) in LVAD-supported hearts, we were unable to detect phosphorylated JNK1/2. Thus, the decrease in JNK1/2 activity could be secondary to a decrease in total JNK1/2 levels or to a combination of decreased JNK1/2 phosphorylation and decreased JNK1/2 levels. Lastly, the increase in p38 activity is likely due to increased phosphorylation of p38 (Figure 1E) rather than to an increase in the level of p38 protein, which was not different in the presence and absence of LVAD support (Figure 1D).

The observed changes in p44/42 and JNK1/2 activity in human tissue are consistent with the known effects of mechanical unloading on increased MAPK phosphorylation in cardiac myocytes. However, the increase in p38 activity and activation was an unexpected finding, insofar as p38 was originally identified as a kinase in yeast (Hog-1) that was sensitive to increases in cell osmolarity. Nonetheless, the results of the present study are consistent with the experimental literature, which shows that p38 is activated under hyper- and hypo-osmotic conditions, thus suggesting that p38 may be activated in response to both increased and decreased hemodynamic loading conditions. It is important to emphasize that the antibody used in the present study does not distinguish between the 4 different p38 isoforms (α, β, γ, δ); accordingly, we cannot comment on the effect of mechanical unloading on the activity of these different p38 isoforms. Finally, although it is possible that medication use may have been different in the presence and absence of LVAD support and that such differences may have contributed to the observed differences in MAPK activity, our data show a remarkable consistency in terms of decreased p44/42 and JNK1/2 activity and increased p38 activity in 9 of 11 patients examined in the unpaired samples and in 5 of 6 patients examined in the paired samples. Thus, we consider it unlikely that differences in medication use alone were responsible for the changes in MAPK activity.

Although it was not possible to determine the exact functional significance of differential MAPK activation in this clinical study, we did examine surrogate markers that are downstream from p44/42 and p38 activation (ie, myocyte hypertrophy) as well as p38 and JNK1/2 activation (ie, cardiac myocyte apoptosis). As shown in Figure 2A and B, there was a significant decrease in myocyte cell volume after LVAD support, consistent with the role of p44/42 in regulating cell growth. Moreover, Figure 2C shows that there was a decrease in the incidence of apoptosis after LVAD support, consistent with reports that have shown that decreased JNK1/2 activity and increased p38 activity are linked to decreased myocyte apoptosis. It is important to recognize, however, that these studies do not establish a clear-cut cause-and-effect relationship between changes in MAPK activity and changes in myocyte structure and viability. Indeed, it is likely that a variety of mechanisms contribute to the observed changes in myocyte phenotype after LVAD support. Nonetheless, these studies do raise the intriguing possibility that selective modulation of selective signal-transduction pathways may be used to modulate myocyte structure or viability in the failing human heart.

Acknowledgments

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References

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