Effects of Early Angiotensin-Converting Enzyme Inhibition on Cardiac Gene Expression After Acute Myocardial Infarction

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Background—ACE inhibition after myocardial infarction (MI) has been shown to have beneficial effects on cardiac anatomy and function. The purpose of this study was to examine the effects of ACE inhibition on cardiac gene expression after MI.

Methods and Results—Rats were randomized to receive captopril or no treatment 1 day after MI. Eight weeks later, cardiac function and hemodynamics were measured by use of indwelling catheters and perivascular flow probes. Myocardial gene expression was assessed with DNA microarrays and real-time reverse transcription–polymerase chain reaction. The ratios of heart and left ventricular weights to body weight were significantly increased by MI and normalized by captopril. Cardiac index and stroke volume index were lower in the untreated MI group than in sham controls but were normal in the MI + captopril group. Thirty-seven genes were found to be differentially expressed between the untreated MI group and sham controls; 31 were induced and 6 repressed. Captopril partially or completely inhibited changes in 10 of the genes. The 37 genes clustered into 11 functional groups, and 6 had genes whose expression was modified by ACE inhibition.

Conclusions—ACE inhibition after MI inhibits cardiac hypertrophy, preserves cardiac function, and attenuates changes in myocardial gene expression. Gene expression profiling reveals, however, that some elements of the pathophysiology may be unaffected by the treatment and be targets for new therapies. (Circulation. 2001;103:736-742.)

Key Words: heart failure ▪ molecular biology ▪ cardiomyopathy ▪ genes

Captopril administration after acute myocardial infarction (MI) has been shown to inhibit cardiac remodeling, preserve myocardial function, and prolong survival in experimental heart failure in rats1,2 and in human clinical trials.3-5 Data on the effects of this treatment on myocardial gene expression are lacking, however. The purpose of this study was to determine how captopril treatment affects changes in cardiac gene expression in a rat model of MI.

Rats with moderate-sized infarcts (≈30% of the left ventricle) were used in the study because captopril was shown to have the greatest effect on survival in rats with infarcts in this size range.2 At 8 weeks, ACE inhibition is still effective at normalizing cardiac function and ratio of heart weight to body weight (BW), a measure of cardiac hypertrophy, in MI rats, so this time point was chosen to determine the effects of treatment on gene expression.

DNA microarrays were used to generate a list of candidate genes that were induced/repressed in the myocardium of rats with MI. Then real-time reverse transcription–polymerase chain reaction (RT-PCR) was used to verify the microarray data and to study the effects of captopril on the expression of these genes.

Our findings provide new insights into the pathophysiology of ischemic cardiomyopathy and reveal how early intervention with captopril can attenuate changes in cardiac gene expression induced by MI. Also informative, however, are the genes that are unaffected by captopril. Mortality and morbidity from heart failure remain high, despite optimal ACE inhibitor therapy. Thus, elements of the pathophysiology revealed by gene expression analysis to be resistant to this therapy may provide targets for new therapeutic approaches.

Methods

Animal Model

The animal use protocols were approved by Genentech’s Institutional Animal Care and Use Committee.

MI was produced in male Sprague-Dawley rats by left coronary arterial ligation as described previously.6 Initially, rats with ECG evidence of infarctions were selected for inclusion into the study and randomly assigned to the MI + captopril or untreated MI groups. The dose of captopril used, 2 g/L in the drinking water for 8 weeks, can
almost completely inhibit the activity of ACE. Shim-operated control animals did not receive treatment.

**Measurement of Infarct Size by MRI**

After 7 weeks of treatment, the animals were evaluated at 4.7 T in a 6-cm quadrature volume coil. Cine-style fast, low-angle shot (FLASH) imaging was used. Infant sizes were calculated as the percentage of ventricle that was markedly thinned and hypokinetic. Ultimately, the MI groups were culled on the basis of MRI analysis of infarct size to eliminate rats with small infarcts and to balance the infarct size between the 2 MI cohorts.

**Catheterization**

After 8 weeks of treatment, rats were anesthetized with ketamine hydrochloride (80 mg/kg IP) and xylazine (10 mg/kg IP). A catheter was implanted into the abdominal aorta, through the right femoral artery, for measurement of mean arterial pressure (MAP) and heart rate (HR). An ultrasonic, perivascular flow probe was placed around the ascending aorta for measurement of cardiac output (CO).

**Assessments of Hemodynamics and Cardiac Function**

Two days after catheterization, MAP and HR were measured in conscious rats. The flow-probe cable was connected to a model T 201 flowmeter (Transonic Systems, Inc) to measure CO. Other cardiovascular parameters were calculated as follows: cardiac index (CI)=CO/BW; stroke volume index (SVI)=CO/(HR)(BW); and systemic vascular resistance (SVR)=MAP/CI. Results are expressed as mean±SEM. One-way ANOVA was performed to assess differences in parameters between groups. Significant differences were then subjected to post hoc analysis using the Newman-Keuls method. A value of P<0.05 was considered significant.

**RNA Preparation**

The left ventricular free wall (including the scar in rats with MI) and septum were frozen in liquid nitrogen and stored at −70°C until used. Total RNA was isolated with the RNeasy Maxi Kit (Qiagen).

**Affymetrix GeneChip Probe Array Analyses**

Targets were prepared for microarray hybridization according to previously described protocols. The samples were hybridized for 16 hours at 45°C to GeneChip Test-2 arrays (to assess sample quality), then to Rat Genome arrays (U34A). The arrays were washed, then stained with streptavidin-phycoerythrin (Genome Array Technology). The arrays were scanned with the GeneArray scanner (Agilent Technologies).

To identify differentially expressed transcripts, pairwise comparison analyses were carried out with GeneChip Analysis Suite 3.2. Each of the 6 MI samples were compared with each of the 6 sham samples, resulting in 36 pairwise comparisons. This approach, which is based on the Mann-Whitney pairwise comparison test, allows the ranking of results by concordance, as well as the calculation of significance (P value) of each identified change in gene expression. Genes for which the concordance in the pairwise comparisons exceeded 80.6% were considered to be statistically significant (P<0.05). Genes with concordance between 63.9% and 80.6% were considered to be statistically significant if they were detected with redundant probe sets, was another member of a previously identified functional class, or was found to be highly differentially expressed. This conservative analytical approach was used to limit the number of false-positives.

**Real-Time RT-PCR**

Real-time RT-PCR was performed as described previously with the TaqMan Model 7700 Sequence Detector (ABI-Perkin Elmer). Expression values for each gene were normalized to ribosomal protein L19 (RPL19), which was unaffected by MI or by captopril. Results are expressed as mean±SEM. One-way ANOVA was performed to assess differences between groups.

**Results**

**Effects of MI and Captopril on BW and Heart Weight**

Eight weeks after left coronary artery ligation, BW was significantly decreased in the MI group treated with captopril (Table 1). Absolute heart, ventricular, and left ventricular weights were also lower in the MI+captopril cohort. Infarct size was not different between the 2 MI groups and was ~31% (mean±SEM, 31.2±2.4% and 31.5±3.5% for untreated and captopril-treated rats, respectively, n=6/group), well within the range known to be responsive to captopril treatment. MI induced cardiac hypertrophy, as evidenced by an increase in the ratios of heart, ventricular, and left ventricular weights to BW (Figure 1). Captopril completely inhibited the hypertrophy associated with MI.

**Effects of MI and Captopril on Hemodynamics and Cardiac Function**

Captopril lowered MAP relative to both the sham and MI groups, which were not significantly different (Table 1). HR was not different among the 3 cohorts. MI caused a reduction in CI and SVI compared with sham surgery, but captopril treatment normalized these parameters (Figure 2). SVR was increased by MI, and captopril significantly reduced SVR in rats with MI.

**Effects of MI on Myocardial Gene Expression**

Using DNA microarrays, we identified 37 genes that were most likely differentially expressed between rats with untreated MI and sham controls. Real-time RT-PCR was used to verify these results and to determine the effects of captopril treatment (Tables 2 and 3). Eight weeks after MI, there was induction of genes involved in inflammation, wound healing, and extracellular matrix deposition. Complement appears to be important in this process, with involvement of the alternative pathway indicated by induction of factor B. Transforming growth factor (TGF)-β is also involved. In addition to TGF-β, a latent TGF binding protein-2–like molecule was upregulated by 15.2-fold. Two other highly induced genes in these categories were thrombospondin-4 and lipopolysaccharide binding protein, 10-3- and 10-fold, respectively.

There was induction of genes coding for growth factors and proteins involved in steroid metabolism. Notable was the coor-
dinate upregulation of insulin-like growth factor (IGF)-1 and IGF binding protein 6. The muscle genes smooth muscle LIM protein, SM22, atrial myosin light chain-1, and acidic calponin, as well as the potassium channel lsk protein and protein p9Ka, were also upregulated. Finally, we detected induction of monoamine oxidase A and catechol O-methyltransferase, which are involved in catechol metabolism, and upregulation of atrial and brain natriuretic factors.

Some genes were repressed by MI. Expression of the water channel aquaporin 7 was decreased by 2.3-fold, and cytochrome P450 2E1 and cytosolic epoxide hydrolase, which are involved in arachidonic acid metabolism, were decreased by 4.4- and 2.4-fold, respectively. Three genes involved in fatty acid metabolism were also repressed in MI hearts.

Effects of Captopril on MI-Induced Gene Expression

ACE inhibition partially or completely reversed 10 of the 37 changes in gene expression (27%) detected in this study (Tables 2 and 3). Six of the functional gene clusters contained ≥1 gene affected by the treatment. Captopril normalized the expression of monoamine oxidase, cytochrome P450 2E1, and cytosolic epoxide hydrolase. The expression levels of 2 of the 3 most highly induced genes in MI rats were also significantly reduced, thrombospondin-4 from 10.3- to 4.3-fold and latent TGF binding protein-2–like protein from 15.2- to 8.8-fold. Other genes affected by ACE inhibition were biglycan, IGF binding protein 6, smooth muscle LIM protein, complement C1 inhibitor, and osteoblast-specific factor 2.

Regional Expression of Selected Genes

A limitation of the protocol used in this study was that RNA was prepared from the entire left ventricle, including the scar, in rats with MI. Thus, it was unknown whether there would be sufficient sensitivity to survey genes whose differential expression was confined to only 1 region of the heart, ie, scar or uninvolved myocardium. To address this concern, a separate study was performed in which RNA was prepared from 3 separate regions of the hearts of rats with MI: the scar, the peri-infarct zone, and the uninvolved area. RNA was also prepared from comparably located regions of the hearts of rats with sham surgery. The regional expression of several of the genes identified by the arrays was then determined by use of real-time RT-PCR. The results in Figures 3 to 5 show that the data set reported here is representative not only of genes differentially expressed globally in the heart but also of those with regionally biased expression differences.

Discussion

The genes differentially expressed between rats with MI and sham controls provide interesting insights into the pathology of ischemic cardiomyopathy. We found an
It is clear from the human data that the classic complement pathway is activated. Complement C4 and complement factor B were both upregulated in this study, indicating possible involvement of the classic and alternative pathways in this rat model system.

Inflammation/wound healing

- Complement C4: 1.19 ± 0.19 (Sham), 11.67 ± 1.81$ (MI)
- Complement B2: 3.64 ± 0.38$ (Sham), 3.63 ± 0.15$ (MI)
- Complement C1 inhibitor: 2.34 ± 0.17$ (Sham), 2.33 ± 0.17$ (MI)
- Complement C4: 4.47 ± 0.80$ (Sham), 4.46 ± 0.45$ (MI)

Extracellular matrix

- Collagen I: 0.62 ± 0.32$ (Sham), 0.51 ± 0.26$ (MI)
- Collagen III: 2.99 ± 0.44 (Sham), 3.99 ± 0.44 (MI)
- Lysyl oxidase: 0.21 ± 0.96$ (Sham), 0.19 ± 0.11$ (MI)

Growth factors/inhibitors

- Atrial natriuretic peptide: 0.96 ± 1.64$ (Sham), 13.79 ± 3.79 $ (MI)
- Insulin-like growth factor-1: 1.77 ± 0.12 (Sham), 4.66 ± 0.84$ (MI)
- Tumor-suppressive gene: 1.19 ± 0.11$ (Sham), 1.22 ± 0.11$ (MI)
- Thrombospondin-4: 0.93 ± 0.06 (Sham), 9.61 ± 2.63$ (MI)

Muscle proteins

- Atrial natriuretic peptide: 0.93 ± 0.06 (Sham), 9.61 ± 2.63$ (MI)
- Smooth muscle cell LIM protein: 0.92 ± 0.08 (Sham), 2.64 ± 0.41$ (MI)
- SM22: 2.25 ± 0.35$ (Sham), 2.25 ± 0.35$ (MI)
- Myofibrillar light chain-1: 4.84 ± 0.89$ (Sham), 3.66 ± 0.64#P < 0.01 (MI)
- Myosin light chain: 3.64 ± 0.62$ (Sham), 3.66 ± 0.64#P < 0.01 (MI)

Catechol metabolism

- Monoamine oxidase A: 1.79 ± 0.22 (Sham), 3.88 ± 0.38$ (MI)
- Catechol-O-methyltransferase: 0.92 ± 0.03 (Sham), 1.22 ± 0.11$ (MI)

Ion binding/channels

- 1sk protein: 4.09 ± 2.05 (Sham), 9.78 ± 2.25#P < 0.01 (MI)
- Protein p9Ka: 3.46 ± 0.62$ (Sham), 2.99 ± 0.25$ (MI)

Steroid binding/metabolism

- α2-µ Globulin: 4.43 ± 0.62 (Sham), 13.79 ± 0.96$ (MI)
- Cytochrome P450 1B1: 3.37 ± 0.25$ (Sham), 3.52 ± 0.35$ (MI)

Growth factors/inhibitors

- Insulin-like growth factor-1: 1.95 ± 0.21$ (Sham), 1.99 ± 0.19$ (MI)
- Insulin-like growth factor binding protein 6: 1.41 ± 0.07$ (Sham), 1.41 ± 0.07$ (MI)
- Tumor-suppressive gene: 2.35 ± 0.48$ (Sham), 1.54 ± 0.14#P < 0.01 (MI)

It is clear from the human data that the classic complement pathway is activated. Complement C4 and complement factor B were both upregulated in this study, indicating possible involvement of the classic and alternative pathways in this rat model system.
At an induction of 10-fold, lipopolysaccharide binding protein was one of the most highly induced genes in MI hearts. This finding adds to the increasing body of evidence that factors involved in mediating innate immunity may contribute to the pathophysiology of heart failure. Recent findings that endotoxin levels are increased in heart failure patients during periods of acute edematous exacerbation in the absence of clinical signs of infection. Taken together, these results suggest a mechanism by which immune cytokine production, which is known to have adverse effects on cardiac myocyte function and survival, can be localized to the heart and contribute to deteriorating cardiac function in heart failure patients.

In heart failure, the primary cardiac energy substrate changes from fatty acids to glucose. Our results are consistent with previous findings that show that genes encoding enzymes in the fatty acid oxidation pathway are down-regulated in the failing heart. We found that the inhibition of cardiac hypertrophy and improvement in cardiac function in MI rats treated with captopril were accompanied by a trend toward normalized myocardial gene expression. Ten of the 37 myocardial genes differentially expressed between untreated MI and sham controls were either partially or completely normalized by ACE inhibition. Furthermore, 50% of the functional gene clusters contained ≥1 affected genes. There was also no regional bias toward genes affected by captopril, because

**Table 3. Genes Decreased in Rats With MI Versus Sham Controls**

<table>
<thead>
<tr>
<th>Accession No.</th>
<th>Description</th>
<th>Expression Relative to RPL19</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sham</td>
</tr>
<tr>
<td>AB000507</td>
<td>Aquaporin 7</td>
<td>3.03±0.62</td>
</tr>
<tr>
<td>AF056333</td>
<td>Cytochrome P450 2E1</td>
<td>10.34±1.7</td>
</tr>
<tr>
<td>X60328</td>
<td>Cytosolic epoxide hydrolase</td>
<td>1.64±0.12</td>
</tr>
<tr>
<td>M33648</td>
<td>HMG-CoA synthase</td>
<td>9.33±4.06</td>
</tr>
<tr>
<td>J02791</td>
<td>Acyl coenzyme A dehydrogenase</td>
<td>1.23±0.15</td>
</tr>
<tr>
<td>D00569</td>
<td>2,4-Dienoyl-CoA reductase</td>
<td>1.40±0.09</td>
</tr>
</tbody>
</table>

Data are mean±SEM (n=5 or 6 per group).
*P<0.05 sham vs MI by array.
†P<0.05, †P<0.01 sham by TaqMan.
§P<0.05 MI vs MI + captopril by TaqMan.

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**Figure 3.** Regional expression of atrial natriuretic factor, cytochrome P450 2E1 (CYP 2E1), and cytosolic epoxide hydrolase in infarct scar, peri-infarct zone, and normal or uninvolved regions of MI hearts (solid bars) and in comparably located areas of control hearts (open bars) 6 weeks after left coronary artery ligation or sham surgery. Data are mean±SEM, n=5 or 6 per group. *P<0.05, **P<0.01.
thrombospondin-4 (induced in the infarct scar and peri-infarct zone), monoamine oxidase (induced in the peri-infarct zone and uninvolved myocardium), and CYP2E1 and cytosolic epoxide hydrolase (repressed globally) were all affected by the treatment.

The enzymes monoamine oxidase A and catechol O-methyltransferase were induced in rats with MI. These enzymes metabolize norepinephrine and epinephrine; thus, increased myocardial degradation of catecholamines may in part explain the reduction in β-adrenergic sensitivity observed in heart failure. The expression of monoamine oxidase was normalized by captopril, and this may contribute to the beneficial effects of ACE inhibition on cardiac performance.

The expression of CYP2E1, which metabolizes arachidonic acid, was linked spatially to another enzyme in the arachidonic acid metabolic pathway, cytosolic epoxide hydrolase. The expression of both of these genes was normalized by captopril treatment. It is tempting to speculate that the reduced expression of these genes may be causally related to an element of the pathophysiology that is improved by ACE inhibition.

Angiotensin II has been linked to stimulation of the TGF-β axis in isolated cardiac fibroblasts and myofibroblasts and in models of cardiac repair after myocardial infarction. At 15.3-fold induction, the rat homologue of latent TGF binding protein-2 was the most highly differentially expressed gene we detected between untreated MI hearts and sham controls, and its expression was significantly inhibited by captopril. The induction of TGF-β3, however, was not affected by ACE inhibition. The induction of 2 extracellular matrix proteins, thrombospondin-4 and biglycan, was also significantly reduced by captopril, but the induction of other matrix proteins, including collagen I and III and fibronectin, was unaffected. Captopril also reduced the expression of IGF binding protein 6, but not IGF-1, indicating that the primary effect of the treatment on the IGF axis may be on IGF-2.

Because mortality and morbidity from heart failure after MI remain high despite optimal ACE inhibitor therapy, the genes whose expression was not affected by captopril may provide clues to new treatment strategies. In this regard, the lack of effect of ACE inhibition on the mediators of inflammation is notable. Captopril also did not affect the expression of enzymes involved in cardiac energy substrate utilization or the expression of ion and water channels.

In summary, our results show that early ACE inhibition can inhibit some of the changes in gene expression that are induced by MI. The data also indicate, however, that some aspects of the pathophysiology are apparently unaffected by the treatment, and these areas may be targets for new therapeutic approaches.
Acknowledgments

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References

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