Improvement of Endocardial and Vascular Endothelial Function on Myocardial Performance by Captopril Treatment in Postinfarct Rat Hearts

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Background—Endocardial (EE) and myocardial capillary vascular endothelial (myocap VE) cells have been shown to modulate the contractile characteristics of myocardium in a calcium-dependent manner. We evaluated the endothelial-myocardial interaction in the rat postinfarction myocardial infarction (MI) model and the effects of captopril.

Methods and Results—Wistar rats were divided into 4 groups treated for 4 weeks: (1) control; (2) infarcted controls (left anterior coronary artery ligation); (3) infarcted+captopril 2 g/L in drinking water; and (4) infarct+captopril+triton intracoronary injection. Coronary VE function was evaluated by infusion of serotonin in Langendorff preparations (n=31), and the myocardial contractile characteristics were investigated by use of isolated papillary muscles (n=44). Cardiac mRNA for endothelial constitutive nitric oxide synthase (ecNOS) was measured, and its cellular location was evaluated by immunohistochemistry. Serotonin-induced increase in coronary flow was decreased in infarct controls compared with controls (4.6% versus 53.4%, P<0.01) but not in the 2 infarct+captopril groups. Intracoronary triton injection decreased serotonin-induced coronary flow in the infarct+captopril+triton group. All MI groups had decreased total tension in isolated papillary muscles. EE removal by triton immersion decreased total tension in all groups except for infarct controls (3.3 versus 3.2 g/mm²). Cardiac ecNOS mRNA decreased in the control infarct group but remained normal in the infarct+captopril group.

Conclusions—Chronic postinfarction endothelium-induced coronary vasodilatation is impaired, and both EE and myocap VE dysfunction contribute to myocardial depression. Captopril use prevents these abnormalities and the reduction of cardiac ecNOS mRNA. (Circulation. 1999;100:1338-1345.)

Key Words: myocardial infarction ▪ endothelium ▪ contractility ▪ heart failure

Postinfarction cardiac dysfunction is characterized by loss of myocardium, adverse ventricular remodeling, loss of the intrinsic contractility of the remaining myocardium, and a decreased ability of this myocardium to respond to stimuli that increase contractility, such as β-adrenergic agonists. In addition, the development of coronary vascular endothelial (VE) dysfunction is associated with a reduction in endothelial constitutive nitric oxide synthase (ecNOS) activity.1-4 The one study that investigated the effects of therapeutic interventions on congestive heart failure (CHF)–associated endothelial dysfunction found a correlation between clinical improvement and recovery of endothelium-derived vasodilatation in the peripheral vasculature, suggesting that VE dysfunction may play a pathophysiological role in CHF.5

In 1988, Brutsaert et al6 and later others7 demonstrated that endocardial endothelium (EE) and myocardial capillary vascular endothelium (myocap VE) had modulatory effects on the contractile characteristics of the subjacent myocardium. The myocardial contractile effects of EE and myocap VE can take many forms, much as has been shown for the effects of VE on vascular smooth muscle.4,8-11 In disease, some of the myocardial contractile effects of endothelium have been shown to be abnormal and to contribute to the pathophysiological process itself.12,13 These abnormalities include a loss of nitric oxide release, excessive production of endothelin-1, and a loss of the modulatory effects of endothelium on the contractile effects of other inotropic substances.1-4,8 Yet, whether and to what extent the direct modulatory effects of EE and myocap VE on myocardial contractility are affected in cardiac dysfunction needs further study.

ACE inhibitors (ACEIs) have been shown to improve morbidity and mortality in addition to preserving ventricular function in patients after large myocardial infarction (MI). ACEIs have also been shown to improve endothelium-
dependent vasodilatation in patients with CHF, and a correlation between ACEI-induced endothelium-dependent vasodilatation and clinical improvement has been documented. What effect ACEIs may have on abnormalities in coronary endothelium-dependent vasodilatation, however, is less clear. It is also not known to what extent reversal of the loss of endothelium-derived, ie, from EE or myocap VE, positive inotropic effects on myocardium contributes to the beneficial effects of ACEIs on ventricular function in this setting.

In this study, we evaluated whether in the rat postinfarction model, vascular receptor-mediated NO–induced coronary vasodilatation and the direct effects of EE and myocap VE on myocardial contractility were modified. We also evaluated the changes in morphological characteristics of the endothelium and the expression of ecNOS in VE and myocardium. We then assessed whether an ACEI could modify these changes.

Methods

Wistar rats weighing 200 to 250 g were used and prepared in accordance with the guidelines of the Canadian Council on Animal Care. The MI was produced by ligating the anterior coronary artery as described by Pfeffer et al. Rats were then randomized into infarct/no-therapy and infarct + captopril. Two g/L in the drinking water (Bristol-Myers Squibb). Treatments were initiated 4 hours after the operation by an injection of the ACEI captopril (0.25 mg/kg IP). Treatment with captopril was continued until the time of euthanasia, 4 weeks after infarction. Age-matched Wistar rats and operated rats with no visible scar served as controls.

Endothelium-Derived Coronary Vasodilatation

Four weeks after infarction, rats were anesthetized with a mixture of ketamine 87 mg/kg and xylazine 13 mg/kg and anticoagulated by injection of heparin 1000 U/kg IP. The hearts were excised and mounted within 45 seconds on a Langendorff preparation with a perfusion pressure of 80 cm H2O. The hearts were then perfused with Krebs-Henseleit solution containing (in mmol/L) NaCl 118, KCl 3.5, MgSO4 2.43, CaCl2 1.25, KH2PO4 1.2, NaHCO3 24.9, and dextrose 15.0. The solution was kept at 37°C and bubbled with 95% O2–5% CO2 at pH 7.4. The hearts were paced at 280 to 300 bpm by a stimulator (Grass S6) at 1 V and permitted to stabilize for 15 minutes, at which time the coronary perfusion rate was assessed by accumulating perfusate over a 2-minute period and then divided by 2. A serotonin (Sigma Chemical Co) infusion was then delivered just above the aortic valve at a rate calculated to deliver a final concentration of 10^-3 mol/L to the coronary arteries for 4 minutes. Before and after serotonin delivery, the coronary flow was calculated by collection of coronary effluent.

The effect of serotonin was allowed to wash out (8 to 10 minutes). In control hearts with and without an MI (groups 1 and 2) and in half of the captopril-treated hearts with an MI (group 3), continuous perfusion with Krebs-Henseleit was maintained for another 50 minutes. Then a bolus injection of saline at 1% was given over a 1-second period just above the aortic valve. After 15 minutes, serotonin was again delivered at a final concentration of 10^-3 mol/L to the coronary arteries for 4 minutes, and the coronary flow was measured.

In the other half of the captopril-treated hearts with an MI (group 4), a continuous perfusion of Krebs-Henseleit was maintained for another 50 minutes. Then a bolus injection of triton X-100 (Scientillar Mallinckrodt Inc) at 1:400 concentration was given over a 1-second period at 1% of the coronary perfusion rate per minute. This was injected into the aorta just above the coronary arteries. After 15 minutes, serotonin was delivered and the coronary flow measured.

Before and after saline and triton were injected into the coronaries, ~5 mL of the coronary effluents was taken to determine damage to myocardium by measurement of creatine kinase (CK).

Evaluation of Vascular Smooth Muscle Vasodilatory Capacity

At the end of the experiments, with the hearts still on the Langendorff perfusion, 3 hearts in each group had sodium nitroprusside (SNP) delivered by an IVAC pump just above the aortic valve at a rate calculated to a final concentration of 10^-3 mol/L to the coronaries. Coronary flow was measured before and during SNP infusion.

**TABLE 1. Morphology**

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight, g</th>
<th>LV Weight, g</th>
<th>RV Weight, g</th>
<th>Scar Weight, g</th>
<th>Scar Area, cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=9)</td>
<td>404±4</td>
<td>0.66±0.01†</td>
<td>0.19±0.001†</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Infarct/no-therapy (n=8)</td>
<td>399±13</td>
<td>0.84±0.07*</td>
<td>0.45±0.05*</td>
<td>0.14±0.02</td>
<td>1.32±0.15</td>
</tr>
<tr>
<td>Infarct + captopril (n=14)</td>
<td>379±8</td>
<td>0.72±0.03†</td>
<td>0.36±0.03*</td>
<td>0.14±0.01</td>
<td>1.27±0.13</td>
</tr>
<tr>
<td>P, ANOVA</td>
<td>0.097</td>
<td>0.021</td>
<td>&lt;0.001</td>
<td>0.923</td>
<td>0.787</td>
</tr>
</tbody>
</table>

LV indicates left ventricle, and RV, right ventricle.

*P<0.05 vs control; †P<0.05 vs infarct/no-therapy.

**TABLE 2. CK in Coronary Effluent**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Baseline,*</th>
<th>After Triton or Saline Injection,</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U/L</td>
<td>U/L</td>
</tr>
<tr>
<td>Control (n=4)</td>
<td>13±8</td>
<td>8±4</td>
</tr>
<tr>
<td>Infarct/no-therapy (n=3)</td>
<td>12±5</td>
<td>7±4</td>
</tr>
<tr>
<td>Infarct + captopril (n=3)</td>
<td>14±0.3</td>
<td>9±1</td>
</tr>
<tr>
<td>Infarct + captopril + triton</td>
<td>11±2</td>
<td>10±1</td>
</tr>
<tr>
<td>P, ANOVA</td>
<td>0.97</td>
<td>0.87</td>
</tr>
</tbody>
</table>

*Baseline indicates after 25 minutes on Langendorff perfusion.

**Figure 1.** Effects of MI and intracoronary injection of triton X-100 on serotonin-induced coronary flow.
Evaluation of Endothelium-Induced Myocardial Contractile Effects in the Isolated Papillary Muscle

One or 2 left ventricular papillary muscles were quickly excised and mounted in a bath with Krebs-Henseleit solution containing (in mmol/L) NaCl 118, KCl 3.5, MgSO4 2.43, CaCl2 0.7, KH2PO4 1.2, NaHCO3 24.9, and dextrose 5.0. The solution was kept at 29°C (in mmol/L) NaCl 118, KCl 3.5, MgSO4 2.43, CaCl2 0.7, KH2PO4 1.2, NaHCO3 24.9, and dextrose 5.0. The solution was kept at 29°C (in mmol/L) NaCl 118, KCl 3.5, MgSO4 2.43, CaCl2 0.7, KH2PO4 1.2, NaHCO3 24.9, and dextrose 5.0. The solution was kept at 29°C (in mmol/L) NaCl 118, KCl 3.5, MgSO4 2.43, CaCl2 0.7, KH2PO4 1.2, NaHCO3 24.9, and dextrose 5.0. The solution was kept at 29°C (in mmol/L) NaCl 118, KCl 3.5, MgSO4 2.43, CaCl2 0.7, KH2PO4 1.2, NaHCO3 24.9, and dextrose 5.0. The solution was kept at 29°C.

The muscles were stimulated at 10% above threshold at 6 stimuli per minute with a model S-88 stimulator (Grass Instrument Co). The preload was adjusted so that the muscle length was the length at which maximal tension was developed (Lmax), and twitch characteristics were recorded as previously described. The muscles were stimulated at 10% above threshold at 6 stimuli per minute with a model S-88 stimulator (Grass Instrument Co). The preload was adjusted so that the muscle length was the length at which maximal tension was developed (Lmax), and twitch characteristics were recorded as previously described. The muscles were stimulated at 10% above threshold at 6 stimuli per minute with a model S-88 stimulator (Grass Instrument Co). The preload was adjusted so that the muscle length was the length at which maximal tension was developed (Lmax), and twitch characteristics were recorded as previously described.

In previous studies, we and others have demonstrated that because extracellular calcium concentration was increased to 7 mmol/L, and the EE was removed by immersion of the papillary muscles for 90 minutes, and baseline isometric, isotonic, and unloaded (zero load for Vmax) twitch contractions were recorded. Extracellular calcium concentration was then increased stepwise to 7 mmol/L, and repeat contractions at each calcium concentration were recorded. Muscles were restabilized at 0.7 mmol/L extracellular calcium, and the EE was removed by immersion of the papillary muscles for 1 second in 0.5% triton X-100 (Mallinckrodt Inc), followed by vigorous washing to remove the remaining triton X-100. This technique has been shown to destroy the endocardial layer of the endocardium without damaging myocardial cells. Muscles were then permitted to restabilize for 1 hour at 0.7 mmol/L calcium, and isometric, isotonic, and unloaded twitch contractions were recorded. Extracellular calcium concentration was increased to 7 mmol/L, and repeat isometric, isotonic, and unloaded twitch contractions were recorded.

### TABLE 3. Papillary Muscle Contractile Characteristics: Effects of Endothelium Removal

<table>
<thead>
<tr>
<th>Calcium Concentration</th>
<th>TT, g/mm²</th>
<th>+dT/dt, g · mm⁻² · s⁻¹</th>
<th>TTPT, ms</th>
<th>RT₁/₂, ms</th>
<th>Vmax, Lmax/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7 mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.65 ± 0.24†</td>
<td>42.4 ± 2.4†</td>
<td>127 ± 3</td>
<td>113 ± 4†</td>
<td>1.84 ± 0.17‡</td>
</tr>
<tr>
<td>EE off (n=11)</td>
<td>4.11 ± 0.20‡</td>
<td>38.2 ± 1.9‡</td>
<td>113 ± 3†</td>
<td>95 ± 3*</td>
<td>1.82 ± 0.19†</td>
</tr>
<tr>
<td>Infarct/no-therapy</td>
<td>3.30 ± 0.30†</td>
<td>22.9 ± 2.4†</td>
<td>139 ± 4</td>
<td>87 ± 3†</td>
<td>1.09 ± 0.25†</td>
</tr>
<tr>
<td>EE off (n=10)</td>
<td>3.21 ± 0.27†</td>
<td>22.5 ± 2.1†</td>
<td>136 ± 4†</td>
<td>90 ± 3</td>
<td>1.03 ± 0.25†</td>
</tr>
<tr>
<td>Infarct+captopril</td>
<td>4.17 ± 0.23</td>
<td>31.1 ± 3.1†</td>
<td>144 ± 4†</td>
<td>100 ± 8</td>
<td>1.52 ± 0.21</td>
</tr>
<tr>
<td>EE off (n=12)</td>
<td>3.75 ± 0.23*</td>
<td>27.5 ± 3.2†</td>
<td>133 ± 4†</td>
<td>104 ± 2†</td>
<td>1.31 ± 0.16</td>
</tr>
<tr>
<td>Infarct+captopril+triton</td>
<td>3.35 ± 0.39†</td>
<td>27.5 ± 5.0†</td>
<td>141 ± 6†</td>
<td>99 ± 4</td>
<td>1.76 ± 0.19†</td>
</tr>
<tr>
<td>(n=11)</td>
<td>3.08 ± 0.35†</td>
<td>23.9 ± 4.1†</td>
<td>133 ± 3†</td>
<td>102 ± 6</td>
<td>1.66 ± 0.18†</td>
</tr>
<tr>
<td>7 mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.73 ± 0.32†</td>
<td>60.1 ± 4.2†</td>
<td>116 ± 3†</td>
<td>124 ± 5†</td>
<td>3.05 ± 0.34‡</td>
</tr>
<tr>
<td>EE off (n=11)</td>
<td>5.80 ± 0.31‡</td>
<td>60.4 ± 3.9†</td>
<td>116 ± 3†</td>
<td>125 ± 5‡</td>
<td>3.11 ± 0.34‡</td>
</tr>
<tr>
<td>Infarct/no-therapy</td>
<td>4.56 ± 0.41†</td>
<td>39.5 ± 4.2†</td>
<td>138 ± 4†</td>
<td>94 ± 3‡</td>
<td>1.86 ± 0.50†</td>
</tr>
<tr>
<td>EE off (n=10)</td>
<td>4.55 ± 0.41†</td>
<td>39.5 ± 4.0†</td>
<td>136 ± 3†</td>
<td>96 ± 3†</td>
<td>1.83 ± 0.51†</td>
</tr>
<tr>
<td>Infarct+captopril</td>
<td>4.85 ± 0.22</td>
<td>42.0 ± 3.1†</td>
<td>136 ± 4†</td>
<td>116 ± 4‡</td>
<td>1.76 ± 0.22‡</td>
</tr>
<tr>
<td>EE off (n=12)</td>
<td>4.83 ± 0.20</td>
<td>42.4 ± 3.2†</td>
<td>133 ± 3†</td>
<td>115 ± 3</td>
<td>1.64 ± 0.15‡</td>
</tr>
<tr>
<td>Infarct+captopril+triton</td>
<td>4.37 ± 0.45†</td>
<td>41.2 ± 5.8†</td>
<td>134 ± 2†</td>
<td>111 ± 7‡</td>
<td>2.09 ± 0.19†</td>
</tr>
<tr>
<td>(n=11)</td>
<td>4.36 ± 0.42†</td>
<td>41.6 ± 5.5†</td>
<td>132 ± 2†</td>
<td>115 ± 5‡</td>
<td>2.07 ± 0.20‡</td>
</tr>
</tbody>
</table>

TTPT indicates time to peak tension, and RT₁/₂, time from peak tension to half-tension decline.

*P<0.05 vs EE on.
†P<0.05 vs control.
‡P<0.05 vs infarct/no-therapy.

**Figure 2.** Effect of MI, captopril therapy, intracoronary triton X-100, and EE removal on contractile indices TT and +dT/dt.
Morphological Studies
The viability of endothelial cells and myocytes was determined as previously described.7 Immunohistochemical staining for eNOS was performed as follows: Slides were deparaffinized in xylene and rehydrated in a series of graded ethanol, followed by distilled water, and were then washed with PBS (0.01 mol/L, pH 7.4) and immersed in pepsin (0.05% in 0.01 mol/L HCl) at 37°C for 30 minutes. Endogenous peroxide activity was blocked by 0.3% methanolic peroxide. Normal goat serum was applied to reduce nonspecific binding, and primary antiserum for eNOS (Transduction Laboratories) was diluted in PBS (1/150) and incubated overnight at 4°C in a humidity chamber. Sections were soaked with biotinylated antibody (1800 in PBS) for 30 minutes at room temperature and then washed with Tris-HCl 0.1 mol/L–NaCl 0.15 mol/L–0.05% Tween 20 (TNT), and blocked with Tris-HCl 0.1 mol/L with 0.5% DuPont blocking reagent (TNT) for 30 minutes at room temperature. Slides were then treated with streptavidin–horseradish peroxidase (1/50 dilution) in TNT buffer and washed with TNT. Biotinylated tyramide was added (1/50) for enhancement of the reaction, and then the streptavidin–horseradish peroxidase step was repeated exactly as before, followed by washing in TNT and then in Tris-saline. Slides were then treated with diaminobenzidine 0.06% for 5 minutes, and after washing, they were counterstained with Harris hematoxylin for 2 minutes. Destaining was performed in 1% acid alcohol, then slides were dehydrated with ethanol, cleared with xylene, and mounted with coverslips and Permount.

Ribonuclease Protection Assay
eNOS mRNA levels in the viable portion of the left ventricle were measured with a ribonuclease protection assay as previously described.16

Statistics
All data in this study are expressed as mean±SEM. One-way ANOVA was used to assess the effects of the multiple comparisons and followed by a Dunnett comparison test. The differences for the effects of an intervention on the same muscles were determined by a paired t-test. A value of P<0.05 was considered statistically significant.

Results
The weight of the left ventricles was significantly increased only in the infarct/no-therapy group compared with the control group (Table 1). The size and weight of the scar were similar in the infarct/no-therapy and infarct+captopril groups.

Only small amounts of CK were lost in the effluent during the Langendorff experiments (Table 2). Baseline values of CK were similar in the 4 groups and did not increase as a result of the intracoronary injection of triton.

Endothelium-Derived Coronary Vasodilatation
After the 15-minute stabilization period, the intracoronary injection of serotonin induced significant and similar coronary vasodilatation in the control and the infarct+captopril groups (Figure 1A). In the infarct/no-therapy group, serotonin had no effect on coronary flow (54% in the control group versus 4% in the infarct/no-therapy group, P<0.01), consistent with marked VE dysfunction. After the intracoronary injection of triton X-100 in half of the infarct+captopril hearts (group 4), serotonin-induced vasodilatation was abolished (35.0% to −2.0%, P<0.01, Figure 1B), suggesting that intracoronary triton X-100 resulted in VE dysfunction.

At the end of the Langendorff portion of the study, an infusion of SNP induced a similar marked increase in coronary flow (range, 77% to 122%) in all 4 groups, indicating that vascular smooth muscle responsiveness was still present in all 4 groups of hearts.

Effects of Endothelium on the Contractile Characteristics of its Subjacent Myocardium

VE Dysfunction
Muscles from infarct/no-therapy hearts had a significantly lower total tension (TT), maximum rate of tension development (+dT/dt), and Vmax than muscles from control hearts (Figure 2, Table 3). The decrease in TT and Vmax in the infarct+captopril group (without triton X-100), however, was not significant compared with control muscles. The infarct+captopril+triton X-100 group had a decrease in TT and in +dT/dt similar to that of the infarct/no-therapy group but had no decrease in Vmax. Increasing extracellular calcium to 7 mmol/L eliminated all differences among the 3 infarct groups such that all 3 infarct groups had a decrease in contractility similar to that of controls (Table 3).

EE Dysfunction
EE removal with triton X-100 reduced the indices of contractility TT and +dT/dt and abbreviated twitch duration but did not decrease Vmax (Table 3). However, removing EE from papillary muscles from infarct/no-therapy hearts had no effect on any contractile characteristics, which suggests that EE had lost at least some of its effects on subjacent myocardium. Removing EE from papillary muscles of infarct+captopril hearts had effects on contractile characteristics similar to those in controls, suggesting that therapy with captopril helped preserve the contractile effects of EE on subjacent myocardium. That is, removing EE decreased TT and +dT/dt and abbreviated twitch duration (Table 3, Figure 3). This was true whether the infarct+captopril hearts had received intracoronary injection of triton X-100 or not. As occurred with myocap VE dysfunction, increasing extracellular calcium concentration to minimize differences in myofibrillar calcium sensitivity related to the contractile effects of endothelium eliminated all differences in contractile characteristics between the 3 infarct groups.
Morphological Studies of VE and EE

In control hearts with and without intracoronary triton X-100, there were no areas of either myocardial or endothelial (VE or EE) damage or visible abnormalities (Figure 4). In hearts with an infarct, there were large areas of scarring and visible ventricular dilatation. In all areas of the heart, both VE and EE were intact and there was no staining with propidium iodide, which indicated good viability of these cells (Figure 5). In many areas, however, particularly in the areas of the scar, the high number of stress fibers in the EE and VE (increase in F-actin labeling) indicated some morphological changes in the endothelium after infarction (Figure 5). These changes were present in infarcted hearts and were similar whether triton X-100 was injected or not.

Immunohistochemical studies demonstrated the presence of ecNOS in the VE and EE layers (Figure 6). After infarction, endothelial staining of ecNOS was clearly reduced and appeared to be increased by ACEI to levels comparable

![Figure 4](image1.png)
![Figure 5](image2.png)
to those in noninfarcted hearts. This was confirmed by measurement of ecNOS mRNA by a highly quantitative ribonuclease protection assay (Figure 7). Expression of ecNOS, normalized for β-actin, was not different in the sham-operated animals in the presence and absence of captopril.

Hearts from the infarct/no-therapy animals demonstrated a significant decline in ecNOS mRNA expression, which was normalized by treatment with captopril.

Discussion

This study suggests that the abnormality in endothelial function that accompanies postinfarction myocardial dysfunction extends beyond that of a defect in endothelium-mediated coronary vasodilatation to one that includes a loss of the direct contractile effects of EE and myocap VE on subjacent myocardium. This loss of endothelial function is accompanied by a loss of vascular staining and mRNA expression for ecNOS and only very minor histological changes. The use of the ACEI captopril attenuates the development of endothelial dysfunction, both VE and EE, preventing the loss of serotonin-induced coronary vasodilatation and the loss of the direct myocardial contractile effects of both EE and myocap VE. The use of captopril also improves endothelial staining and cardiac expression of ecNOS. This study indicates that at least some of the beneficial effects of ACEIs on the preservation of intrinsic myocardial contractility in this postinfarction model are the result of beneficial effects on endothelial function and that EE and myocap VE dysfunction plays a more important role in the development of left ventricular dysfunction after infarction than has previously been thought.

With the development of ventricular dysfunction, there is a decrease in endothelium-derived peripheral arterial vasodilatation that can be improved by the long-term use of an ACEI. The mechanism by which endothelium-dependent vasodilatation becomes impaired in this condition is thought to be largely the result of a decrease in ecNOS production of nitric oxide. Consistent with this, we found a decreased mRNA expression and endothelial immunostaining for ecNOS in untreated infarcted hearts but few histological changes.
Endothelium can exert direct contractile effects via a putative myofibrillar calcium sensitizing process, by the release of positive and negative inotropic substances such as endothelin-1 and nitric oxide, and by modulating the contractile effects of other inotropic substances such as activated platelets, phenylephrine, angiotensin II, and β-adrenergic agonists. Although the exact mechanism by which endothelium exerts its direct myocardial effects has yet to be determined, there is good experimental evidence that it exerts its effects at the cellular level by increasing myofibrillar calcium affinity. This characteristic has been used to indirectly evaluate the direct effects of endothelium on subjacent myocardium. Indeed, numerous studies have found that increasing extracellular calcium has been effective in compensating for the loss of the myocardial contractile effects that accompany the removal of EE and myocap VE, because this intervention minimizes the influence of changes in myofibrillar calcium affinity by increasing intracellular calcium concentrations. In this study, we used this indirect approach to evaluate the contractile effects of endothelium on subjacent myocardium and found that removal of both EE and VE had no effect on the contractile characteristics of papillary muscles from untreated postinfarct hearts, which supports the concept that endothelium loses its direct contractile effects in this situation. Also, increasing extracellular calcium concentrations eliminated some of the differences in contractile characteristics between muscles from infarcted and noninfarcted hearts, consistent with a loss of the direct endothelial effects on the contractile characteristics of its adjacent myocardium.

In addition, we used a second, more direct approach. We used brief immersion of papillary muscles in triton X-100 to chemically remove EE and used intracoronary injection of triton X-100 to render coronary VE and myocap VE dysfunctional. Lack of damage to myocytes in this study is supported by the reversibility of the loss of contractile changes that accompanied removal of the endocardium by increasing extracellular calcium concentration. We also found that intracoronary injection of triton X-100 did not result in significant morphological damage to myocytes and did not result in a rise in CK levels. As was the case with papillary muscle immersion in triton X-100, contractile abnormalities caused by intracoronary injection of triton X-100 could be completely reversed by increasing extracellular calcium concentration, which suggests that this intervention did not result in functional damage to the myocardium itself.

EE and myocap VE dysfunction appears to have contributed to the decrease in contractility found in our isolated papillary muscle studies and may contribute to the ventricular dysfunction known to occur in this model. Indeed, the fact that an endothelium-derived component to cardiac dysfunction should occur in this model helps reconcile isolated myocyte studies and suggests that isolated myocyte contractility is not very depressed in this model despite a significant decrease in papillary muscle function. However, because removal of the endothelium did not completely eliminate differences between the contractile characteristics of infarcted and noninfarcted papillary muscles, it would appear that it was only partially responsible for the decrease in contractility and that an endothelium-independent decrease in intrinsic myocardial contractility also contributes. What the net result of these changes in EE and myocap VE function would be in vivo is uncertain, because some of the changes would tend to increase and others would tend to decrease contractility. However, it seems clear from this study that the loss of the direct contractile effects of endothelium contributes to the decrease in contractility associated with this condition.

After infarction, ACEIs have been shown to attenuate ventricular remodeling, to preserve ventricular function, and to improve survival. In the present study, we found that ACEIs prevented much of the loss of the direct myocardial contractile effects of both EE and myocap VE related to this postinfarction model of myocardial dysfunction. The mechanism of this beneficial effect may be multifactorial. ACEIs have direct effects on endothelium by reducing the production of angiotensin II and the metabolism of bradykinin. This results in a reduction of local production of oxygen-derived free radicals, which are toxic to the endothelium, and an increase in production of NO and prostaglandins, which, in addition to their vasoactive properties, can have direct myocardial effects. ACEIs have also been shown to preserve ventricular function and to attenuate neurohumoral activation.
after infarction, thus changing the milieu in which the endothelium functions, which may in turn be expected to alter the changes in endothelial function generally associated with this condition. Finally, because captopril normalizes ecNOS expression in hearts with an MI, it is possible that ACEIs may exert part of their actions by directly upregulating ecNOS.

Acknowledgment

This study was supported by the Medical Research Council of Canada.

References

Improve Of Endocardial and Vascular Endothelial Function on Myocardial Performance by Captopril Treatment in Postinfarct Rat Hearts


_Circulation_. 1999;100:1338-1345
doi: 10.1161/01.CIR.100.12.1338

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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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:
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