Conjugated Estrogens Acutely Abolish Abnormal Cold-Induced Coronary Vasoconstriction in Male Cardiac Allografts

Steven E. Reis, MD; Vishwajeth Bhoopalam, MD; Kathleen A. Zell, BSN; Peter J. Counihan, MD; A.J. Conrad Smith, MD; Si Pham, MD; Srinivas Murali, MD

Background—Transplant-associated coronary arteriopathy is manifested in its early stages by paradoxical coronary artery constriction in response to endothelium-dependent vasodilator stimuli such as the cold pressor test (CPT) and is a major cause of death or retransplantation. Estrogen has vasoactive properties that abolish coronary artery endothelial dysfunction in native hearts. We hypothesized that estrogen attenuates inappropriate coronary artery constriction in cardiac allografts.

Methods and Results—Coronary artery diameter and systemic hemodynamic responses to a 90-second CPT were measured before and 15 minutes after double-blind, randomized administration of intravenous conjugated estrogens (1.25 mg) or placebo in men with male cardiac allografts. Before estrogen, 9 men exhibited an abnormal 15.1 ± 3.0% CPT-induced decrease in coronary artery diameter. However, repeat CPT did not induce significant coronary artery constriction when performed 15 minutes after estrogen. CPT responses before and after estrogen were significantly different (P = .02). Placebo did not influence coronary artery responses to CPT in 6 men. Systemic hemodynamic responses to CPT were not influenced by estrogen or placebo. Estrogen was the only significant determinant of changes in coronary artery responses to CPT.

Conclusions—Conjugated estrogens acutely abolish abnormal CPT-induced coronary artery constriction in male cardiac allografts. This favorable vasomotor effect suggests that estrogen may prevent inappropriate coronary artery constriction in men with cardiac transplants. (Circulation. 1998;97:23-25.)

Key Words: transplantation • hormones • endothelium • coronary disease • atherosclerosis

Allograft coronary arteriopathy is a major cause of death or retransplantation in cardiac transplant recipients. Early transplant-associated arteriopathy is manifested by coronary artery endothelial dysfunction, which precedes the development of angiographically documented disease. This physiological abnormality may be detected in cardiac allografts by measurement of coronary artery responses to endothelium-dependent vasodilator stimuli, such as the cold pressor test (CPT), that induce dilatation in coronary arteries with intact endothelium and paradoxical constriction in those with endothelial dysfunction.

Estrogen has vasomotor properties that prevent in vitro arterial constriction and inappropriate coronary artery constriction in native human hearts. Estrogen-induced attenuation of abnormal coronary vasoreactivity may provide a clinical cardioprotective effect because inappropriate coronary artery constriction has been implicated in the pathogenesis of acute cardiac syndromes. We hypothesized that estrogen attenuates inappropriate coronary artery constriction in cardiac allografts.

Methods
Cardiac transplant recipients undergoing routine posttransplant coronary angiography were eligible to participate. All subjects gave written informed consent approved by the University of Pittsburgh Biomedical Institutional Review Board.

Assessment of Coronary Hemodynamics Vasodilator drugs were discontinued for at least 24 hours. Routine coronary angiography was performed by standard techniques, and a “normal” coronary artery was selected for assessment. Heart rate and systemic blood pressure were recorded, and coronary artery diameter was measured at a fixed anatomic location relative to a branch point by quantitative angiography (AWOS, Siemens Medical Systems) performed by an investigator (V.B.) blinded to treatment group. The reliability (ratio of the inherent variance of the measurement between patients to the variance from all sources) is 0.94, as assessed by analysis of the variance components.

Effects of Estrogen on Coronary Artery Responses to the CPT After baseline hemodynamics and coronary artery diameter were measured, a CPT was performed with the subject’s hand immersed in ice water for 90 seconds. At the conclusion of the CPT, systemic hemodynamics and coronary artery diameter were reassessed. Intravenous conjugated estrogens (Premarin 1.25 mg, Wyeth-Ayerst) or placebo was administered in a double-blind, randomized fashion. Fifteen minutes later, hemodynamics and coronary artery diameter were reassessed at baseline and after a repeat CPT. Intrinsic vasodilator...
There were no statistically significant differences between groups.

difference between coronary artery diameter were then quantified by calculation of the coronary artery diameter (from 3.3 \pm 0.3 to 2.8 \pm 0.3 mm, P<.01) in response to the initial CPT but no significant diameter change in response to repeat CPT performed 15 minutes after estrogen (3.1 \pm 0.3 to 3.2 \pm 0.2 mm, P=NS). Coronary artery constriction elicited by the first CPT was significantly different from the coronary artery response elicited by the postestrogen CPT (P=.02).

Placebo did not influence coronary artery responses to CPT. Before placebo, the studied men exhibited an insignificant 9.0 \pm 3.8% decrease in coronary artery diameter (from 2.7 \pm 0.4 to 2.5 \pm 0.4 mm, P=.07) in response to CPT. Fifteen minutes after placebo, their coronary artery diameters also did not change significantly in response to repeat CPT (2.5 \pm 0.4 to 2.5 \pm 0.4 mm, P=NS). Coronary artery responses to the CPT did not differ before and after placebo.

Multivariate analysis demonstrated that estrogen was the only significant determinant of changes in coronary artery diameter responses to CPT. Other variables, including age, baseline coronary artery diameter, systemic hemodynamic response to CPT, intrinsic coronary artery vasodilator capacity, and degree of rejection did not influence coronary artery responses to CPT.

### Effects of Estrogen on CPT-Induced Changes in Systemic Hemodynamics

CPT-induced endothelium-dependent vasodilatation requires a cold-induced increase in catecholamines, which may be assessed indirectly by measurement of changes in blood pressure. In subjects assigned to estrogen, baseline CPT induced a mean 11.3 \pm 2.2% increase in mean blood pressure (from 113.4 \pm 5.9 to 126.2 \pm 6.9 mm Hg, P<.01), which was not significantly different from the 12.5 \pm 3.1% increase (from 118.0 \pm 5.4 to 132.9 \pm 7.2 mm Hg, P<.01) induced by repeat CPT performed 15 minutes after estrogen. In placebo subjects, baseline CPT induced a 17.1 \pm 3.0% increase in mean blood pressure (from 111.5 \pm 5.0 to 130.0 \pm 4.3 mm Hg, P<.01), which did not differ from the 23.4 \pm 7.3% increase (from 107.7 \pm 5.2 to 131.5 \pm 4.9 mm Hg, P=.01) induced by repeat CPT performed after placebo. These blood pressure responses to CPT are similar to those observed in patients who did not receive transplants.\(^\text{10,11}\)

Although our transplant patients exhibited a normal blood pressure response to CPT, cardiac allografts are denervated and may not manifest an appropriate chronotropic response to adrenergic stimulation. In the men assigned to estrogen, baseline CPT induced a 2.7 \pm 0.7% increase in heart rate (from 78.8 \pm 3.0 to 80.9 \pm 3.1 bpm, P<.01), and postestrogen CPT induced a similar 3.2 \pm 1.3% increase in heart rate (from 77.1 \pm 3.0 to 79.4 \pm 2.8 bpm, P=.04). Placebo patients increased their heart rate by 5.8 \pm 0.8% (from 76.0 \pm 5.7 to 80.3 \pm 5.9 bpm, P<.01) in response to placebo CPT and did not exhibit a significant change in heart rate (from 79.3 \pm 6.0 to 80.0 \pm 6.3 bpm, P=NS) in response to postplacebo CPT. Overall, the study population had smaller CPT-induced increases in heart rate than a nontransplant population.\(^\text{10,11}\) However, heart rate is not a determinant of baseline coronary artery diameter or of coronary artery responses to CPT.

### Statistical Analysis

The data are expressed as mean \pm SEM. Values of P <.05 are considered significant. Only subjects who exhibited no change or a paradoxical decrease in coronary artery diameter in response to CPT were selected for analysis, because we previously demonstrated that estrogen does not improve vasoreactivity in coronary arteries that dilate normally in response to an endothelium-dependent stimulus. Baseline characteristics were compared between groups by \(\chi^2\) analysis and Fisher’s exact test and the independent Student’s t test. Within each group, the influence of CPT on hemodynamics and coronary artery diameter was assessed by the paired Student’s t test.

To assess the influence of pharmacological intervention (estrogen or placebo) on coronary artery responses to CPT, we first calculated the difference between coronary artery diameter after CPT and at baseline (\(\Delta D=\text{diameter}_{\text{CPT}}-\text{diameter}_{\text{baseline}}\)). The effects of intervention on coronary artery diameter were then quantified by calculation of the difference between \(\Delta D\) before and after intervention \(\Delta(D(D)=\Delta D_{\text{after intervention}}-\Delta D_{\text{before intervention}})\) followed by linear regression analysis.

### Results

Twenty-seven patients were enrolled and randomized to estrogen or placebo. Fifteen exhibited abnormal CPT-induced coronary artery constriction and were included in the analysis. All subjects were men, because no postmenopausal female cardiac transplant recipients underwent coronary angiography during the study. All allografts were from male donors. Nine of the analyzed subjects received estrogen, and 6 received placebo. The Table demonstrates no significant differences in baseline characteristics between groups.

### Table 1. Demographic Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Estrogen (n=9)</th>
<th>Placebo (n=6)</th>
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<tbody>
<tr>
<td>Age, y</td>
<td>54.0 \pm 2.8</td>
<td>53.7 \pm 3.9</td>
</tr>
<tr>
<td>Time since transplantation, y</td>
<td>4.6 \pm 0.8</td>
<td>4.8 \pm 0.8</td>
</tr>
<tr>
<td>Hyperlipidemia, %</td>
<td>33.3</td>
<td>20.0</td>
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<tr>
<td>Diabetes, %</td>
<td>11.1</td>
<td>16.7</td>
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<tr>
<td>Hypertension, %</td>
<td>100.0</td>
<td>66.7</td>
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<tr>
<td>Rejection, %</td>
<td>33.3</td>
<td>20.0</td>
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<tr>
<td>Baseline hemodynamics</td>
<td></td>
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<tr>
<td>Coronary diameter, mm</td>
<td>3.3 \pm 0.3</td>
<td>2.7 \pm 0.4</td>
</tr>
<tr>
<td>Mean blood pressure, mm Hg</td>
<td>113.4 \pm 5.9</td>
<td>111.5 \pm 5.0</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>78.0 \pm 3.0</td>
<td>76.0 \pm 5.7</td>
</tr>
<tr>
<td>Coronary diameter response to NTG, %</td>
<td>15.1 \pm 6.3</td>
<td>13.4 \pm 4.4</td>
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Initial CPT-induced changes

<table>
<thead>
<tr>
<th></th>
<th>Estrogen (n=9)</th>
<th>Placebo (n=6)</th>
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<tbody>
<tr>
<td>Coronary diameter, mm</td>
<td>15.1 \pm 3.0</td>
<td>9.0 \pm 3.8</td>
</tr>
<tr>
<td>Mean blood pressure, mm Hg</td>
<td>113.2 \pm 2.2</td>
<td>17.1 \pm 3.0</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>2.7 \pm 0.7</td>
<td>5.8 \pm 0.8</td>
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</table>

NTG indicates Intracoronary nitroglycerin (200 \(\mu g\)). CPT, cold pressor test. There were no statistically significant differences between groups.
Discussion

This study demonstrates that estrogen attenuates abnormal coronary artery vasoreactivity in male cardiac allografts. Intravenous conjugated estrogens acutely abolished the abnormal 15.1% decrease in coronary artery diameter induced by the CPT in men with cardiac transplants. This finding was independent of cold-induced changes in systemic hemodynamics and degree of rejection. In contrast, placebo did not influence coronary artery responses to exogenous cold exposure.

Previous studies of transplant recipients have described abnormal coronary artery constriction in response to exogenous cold exposure that has been attributed to immune system–mediated graft endothelial dysfunction. The present study confirms this finding and demonstrates that it may be acutely abolished by intravenous conjugated estrogens. Although we used conjugated estrogens because they contain a potent vasoactive compound (17α-dihydroequilenin), it is possible that multiple components of this preparation influenced coronary artery responses to CPT. The favorable vaso-motor effects of estrogen in cardiac allografts may be related to estrogen-induced inhibition of endothelin-1 and calcium-mediated vasoconstriction, an increase in arterial endothelial nitric oxide, and/or alteration of arterial myocyte ATP-sensitive potassium channels.

Another possible explanation for our findings is that estrogen may lessen the degree of cold-induced sympathetic stimulation of the coronary arteries. Although we did not measure catecholamine responses to each of the two CPTs, we demonstrated that estrogen does not affect CPT-induced changes in blood pressure, which is a physiological surrogate marker of catecholamines. Therefore, it is unlikely that inhibition of cold-induced sympathetic stimulation explains the observed favorable effect of estrogen.

Our study is limited by our use of the CPT to assess coronary vasoreactivity. Cardiac allografts are denervated, as manifested in this study by their attenuated chronotropic response to CPT. However, α- and β-adrenergic receptors on graft coronary arteries regulate vasomotor tone and respond to circulating catecholamines in an endothelium–dependent manner independent of cardiac denervation. In addition, our findings are consistent with those reported in coronary arteries of native hearts.

Estrogen modulates the immune system and inhibits in vivo allograft rejection. Lou and colleagues demonstrated that chronic estradiol treatment abolishes MHC class II antigen expression, decreases cellular infiltration of the arterial wall, and inhibits myointimal proliferation in coronary arteries of New Zealand White rabbits that underwent cardiac transplantation. Local administration of estrogen has also been shown to inhibit insulin-like growth factor I–induced atherosclerosis in rat orthotopic abdominal aortic allografts. These findings suggest that chronic estrogen therapy may be a novel approach to inhibiting the progression of coronary atherosclerosis in human cardiac allografts. Our results demonstrate that estrogen also acutely prevents inappropriate coronary artery constriction. Although it is not known whether these results may be extrapolated to chronic estrogen therapy, estrogen-induced improvement in coronary artery vasomotor tone may also provide a cardiovascular benefit in cardiac transplant patients. Other studies have demonstrated that nonfeminizing phytoestrogens have favorable vascular properties, suggesting their potential utility in men. Therefore, estrogen should be evaluated as a novel antiatherosclerotic therapy in male and female transplant recipients.

References

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