Estrogen-Induced Vasoprotection Is Estrogen Receptor Dependent
Evidence From the Balloon-Injured Rat Carotid Artery Model

Stephen Bakir, MD; Tatsuhiko Mori, MD; Joan Durand, BS; Yiu-Fai Chen, PhD; J. Anthony Thompson, PhD; Suzanne Oparil, MD

Background—Previous studies have shown that estrogen (E2) is vasoprotective in multiple animal models of vascular injury, including mice with homologous disruptions of either the α or β isoforms of the estrogen receptor (ER) gene, calling into question the ER dependency of the vasoprotective effect. This study used ICI 182,780, a nonselective ER antagonist, to test the hypothesis that the vasoprotective effect of E2 in the rat carotid injury model is ER mediated.

Methods and Results—Intact female Sprague-Dawley rats were divided into 4 groups and treated with the nonselective ER antagonist ICI 182,780 (ICI; 0.5, 1.5, or 5 mg·kg⁻¹·d⁻¹, subcutaneously [S.C.]) or vehicle, beginning before balloon injury of the right common carotid artery and continuing for 14 days afterward. Four groups of ovariectomized rats (OVX) were treated with 17β estradiol (E2) (20 µg·kg⁻¹·d⁻¹, S.C.) alone or combined with ICI 5 mg·kg⁻¹·d⁻¹, S.C.; with ICI 5 mg·kg⁻¹·d⁻¹ alone; or with vehicle according to a similar protocol. Two weeks after injury, rats were killed, and the carotid arteries were evaluated for neointima formation using morphometric analysis. ICI 182,780 blunted the E2-related protective effect and increased neointima formation in injured carotid arteries of intact female rats in a dose-dependent fashion. ICI had no effect on neointima formation in OVX, but addition of ICI to E2 in OVX blocked the inhibitory effect of exogenous E2 on neointima formation.

Conclusions—These results indicate that the vasoprotective effect of E2 in the balloon-injured rat carotid artery model is mediated by ER. (Circulation. 2000;101:2342-2344.)

Key Words: restenosis ■ hormones ■ carotid arteries ■ estrogen receptors

Previous work from our laboratory has demonstrated a sexual dimorphism in the response to balloon injury of the rat carotid artery, with intact male rats developing a more robust neointimal response to injury than intact females.1 Gonadectomy of female but not male rats resulted in increased neointima formation, and treatment with E2 but not testosterone inhibited neointima formation in gonadectomized rats of both sexes, indicating that the observed sexual dimorphism was E2-dependent. Studies using a mouse carotid injury model showed that E2 provided vascular protection in wild-type mice and in mice with homologous disruptions of either the α or β isoform of the ER.2,3 This suggests that expression of either of the 2 functionally distinct ERs is sufficient to protect against pathological responses to vascular injury, that a third, uncharacterised ER may be responsible for the vascular protective effects of E2, or that a nongenomic, nonreceptor mediated signaling mechanism is involved.

In this study, we used ICI 182,780, a nonselective ER antagonist, alone in intact female rats (INT) or alone or in combination with 17β-estradiol in ovariectomized (OVX) rats to test the hypothesis that E2-dependent vasoprotection in this model is ER-mediated.

Methods

Ten-week-old female Sprague-Dawley rats obtained from Charles River Breeding Laboratories (Wilmington, MA) were randomized to undergo bilateral ovariectomy under ether anesthesia or to remain intact. Three days later, INT were divided into 4 groups and were treated with either the nonselective ER antagonist ICI 182,780 (0.5, 1.5, or 5 mg·kg⁻¹·d⁻¹, subcutaneously [S.C.]) or vehicle (V) initiated 3 days before balloon injury of the right common carotid artery and continuing for 14 days afterward. Four groups of OVX rats were treated with either 17β estradiol 20 µg·kg⁻¹·d⁻¹, S.C. (OVX+E2); ICI 5 mg·kg⁻¹·d⁻¹ (OVX+ICI 5.0), combined E2 and ICI 5 mg·kg⁻¹·d⁻¹ (OVX+E2+ICI 5.0); or vehicle (OVX+V) according to a similar protocol.

Rats were anesthetized by intraperitoneal injection of ketamine (80 mg/kg) and xylazine (5 mg/kg). The right carotid artery was isolated by a middle cervical incision, suspended on ties, exposed, and injured with an inflated 2F Fogarty balloon catheter (Baxter V. Mueller), as previously described.1 Two weeks after injury, rats were killed with an overdose of sodium pentobarbital (75 mg/kg) and perfused with 10% formalin at a pressure of 120 mm Hg. The uterus was removed, blotted dry, and weighed to assess E2 effects. The carotid arteries were fixed and processed for morphometric examination as previously described.1 Uteri were processed similarly for histologic examination. Morphometric analysis was performed with a Bioquant II Morphometric

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system by a single examiner, who was blinded with respect to the experimental group to which each sample belonged. Neointima formation in the injured artery was expressed as the absolute area of intima and the ratio of the intimal area to the medial area. All protocols were approved by the Institutional Animal Care and Use Committee at the University of Alabama at Birmingham and were consistent with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication No. 85-23, revised 1985).

At the time of sacrifice, a 1 mL blood sample was removed from the abdominal aorta. Serum 17β-estradiol levels were determined by commercially available radioimmunoassay kits (Diagnostic Products Corp) with an 8 pg/mL sensitivity.

Results were expressed as mean±SEM, and data were analyzed with StatView 4.0 software. Statistical comparisons of body weight, uterine weight, serum estradiol level, neointimal area, medial area, and ratio of the intimal area to the medial area among experimental groups were performed with a 1-way ANOVA. When significant differences were identified, the Scéffé’s multiple range test was applied to determine the level of significance. *P<0.05 was considered to be significant.

Results

Neointima formation in INT rats was greatly attenuated compared with OVX rats (Figures 1D and 2). Treatment of INT rats with ICI increased neointima formation in a dose-dependent manner, and the neointimal area in the INT+ICI 5.0 group was not significantly different from that of the OVX+V rats (Figures 1E and 2). Neointima formation was robust in OVX rats (Figures 1A and 2) and was not altered by ICI administration. Treatment with E2 inhibited neointima production by 61% (Figures 1B and 2). Addition of ICI 5.0 to OVX rats treated with E2 completely blocked the E2-mediated reduction in neointima formation (Figures 1C and 2).

Morphometric analysis showed that the neointimal area of carotid arteries from INT+V rats was 0.072±0.010 mm², a 48.9% reduction from the OVX+V group (0.141±0.010 mm²). Administration of ICI to INT rats dose dependently increased neointima formation (Table), and in the highest ICI dose group, neointimal area (0.122±0.015 mm²) was similar to that seen in OVX+V rats. Neointimal area in OVX+E2 rats was 0.051±0.004 mm², a 61% reduction compared with OVX+V rats (Table). Treatment with ICI 5.0 did not alter neointimal area in OVX rats but did abolish the protective effect of E2 (neointima 0.124±0.017 mm²) in these animals. In this model of vascular injury, the medial area was not altered, so intima/media ratios (Figure 2) closely reflected absolute neointimal area.

Serum 17β-estradiol levels were similar in OVX+E2 and INT+V rats (Table); ICI treatment in the INT rats increased estradiol levels dose dependently. In the highest ICI dose group, 17β estradiol levels were 2-fold higher than in INT+V. In OVX+E2 rats, ICI produced a nonsignificant increase in serum 17β-estradiol levels compared with OVX+E2 (P=0.95, Table). Serum 17β-estradiol concentration in OVX+V rats was at the level of sensitivity of the assay.

In INT rats, ICI treatment decreased uterine weight in a dose-dependent fashion (Table). Uterine weights in OVX rats were significantly increased by E2 treatment to values similar to those in INT; addition of ICI completely blocked the trophic effect of E2 on the uterus, as reflected in both uterine weights and the histologic appearance of uterine epithelial (endometrial) cells (data not shown).
Physiologic Indices 14 Days After Balloon Injury in INT and OVX Rats Treated with V, E2, or Varying Concentrations of ICI

<table>
<thead>
<tr>
<th></th>
<th>Int+V</th>
<th>Int+ICI 0.5</th>
<th>Int+ICI 1.5</th>
<th>Int+ICI 5.0</th>
<th>OVX+V</th>
<th>OVX+ICI 5.0</th>
<th>OVX+E2</th>
<th>OVX+ICI 5.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>6</td>
<td>5</td>
<td>7</td>
<td>7</td>
<td>4</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>Intima/media ratio</td>
<td>0.68±0.072</td>
<td>0.99±0.09</td>
<td>1.20±0.07</td>
<td>1.29±0.11</td>
<td>1.31±0.08</td>
<td>1.022±0.08</td>
<td>0.50±0.05</td>
<td>1.07±0.05</td>
</tr>
<tr>
<td>Intimal area, mm²</td>
<td>0.07±0.01</td>
<td>0.11±0.01</td>
<td>0.12±0.02</td>
<td>0.12±0.02</td>
<td>0.15±0.01</td>
<td>0.11±0.01</td>
<td>0.05±0.00</td>
<td>0.12±0.00</td>
</tr>
<tr>
<td>Medial area, mm²</td>
<td>0.11±0.01</td>
<td>0.11±0.01</td>
<td>0.10±0.01</td>
<td>0.09±0.00</td>
<td>0.12±0.01</td>
<td>0.11±0.01</td>
<td>0.10±0.00</td>
<td>0.12±0.01</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>257±7</td>
<td>253±8</td>
<td>257±12</td>
<td>255±8</td>
<td>288±7</td>
<td>308±11</td>
<td>239±5</td>
<td>243±7</td>
</tr>
<tr>
<td>Uterine weight, g</td>
<td>0.35±0.04</td>
<td>0.21±0.01</td>
<td>0.14±0.01</td>
<td>0.15±0.01</td>
<td>0.11±0.01</td>
<td>0.08±0.01</td>
<td>0.48±0.04</td>
<td>0.20±0.01</td>
</tr>
<tr>
<td>Serum E2, pg/mL</td>
<td>25±6.9</td>
<td>27±4.2</td>
<td>46.6±5.5</td>
<td>57.9±9.3</td>
<td>10.5±3.0</td>
<td>10.5±3.0</td>
<td>32.0±4.8</td>
<td>31.3±5.6</td>
</tr>
<tr>
<td>Uterine epithelial thickness, mm</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.01±0.001</td>
<td>0.01±0.001</td>
<td>0.04±0.003</td>
<td>0.02±0.001</td>
</tr>
</tbody>
</table>

**Discussion**

Our results demonstrated the following: (1) ICI blocked the vasoprotective effects of endogenous E2 on neointima formation in INT rats in a dose-dependent fashion. (2) When ICI was combined with exogenous E2 in a dose that resulted in physiological levels of circulating E2, vasoprotective effects of E2 were abolished in OVX rats. (3) ICI administration did not alter neointima formation in OVX rats. (4) ICI administration did not alter endogenous estrogen levels in OVX rats. (5) ICI administration did not alter the vasoprotective effects of endogenous E2 on neointima formation in INT rats in a dose-dependent fashion. (6) When ICI was combined with exogenous E2 in a dose that resulted in physiological levels of circulating E2, vasoprotective effects of E2 were abolished in OVX rats. (7) ICI administration did not alter endogenous estrogen levels in OVX rats. (8) ICI administration did not alter the vasoprotective effects of endogenous E2 on neointima formation in INT rats in a dose-dependent fashion. (9) ICI administration did not alter endogenous estrogen levels in OVX rats. (10) ICI administration did not alter the vasoprotective effects of endogenous E2 on neointima formation in INT rats in a dose-dependent fashion.

This study provided the first direct evidence that the vasoprotective effects of E2 in the rat carotid injury model are mediated through ERs. Together with the previous observations of Iafriati et al.² and Karas et al.³, these findings suggest that expression of either the ERα or ERβ subtype in injured blood vessels is sufficient to activate the signaling mechanism(s) responsible for E2-mediated vasoprotection or that a novel, not yet discovered, mechanism exists that facilitates the signaling pathway involved. A clearer understanding of these E2-related effects may facilitate translation of the benefits seen in animal models into effective preventative or therapeutic strategies for cardiovascular disease in humans.

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**References**

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