Attenuation of Endothelin-1 Induced Vasoconstriction by 17β Estradiol Is Not Sustained During Long-Term Therapy in Postmenopausal Women With Coronary Heart Disease

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OBJECTIVES The goal of this study was to determine the long-term effects of estrogen replacement therapy on the response to endothelin-1 (ET-1) in postmenopausal women with coronary heart disease.

BACKGROUND It is thought that the vasoconstrictor ET-1 is involved in the development and progression of atherosclerosis. Estrogen replacement may slow the development of atherosclerosis in postmenopausal women.

METHODS Nineteen of 20 postmenopausal women randomized to either three months of 2 mg oral estradiol or placebo completed the double-blind placebo-controlled protocol. Change in forearm blood flow (FBF) in response to a 60 min brachial arterial infusion of ET-1 (5 pmol/min) was measured before randomization, after one month of randomized therapy and after three months of therapy using venous occlusion plethysmography.

RESULTS Estrogen treatment had no effect on baseline FBF. Systolic and diastolic blood pressure and heart rate did not change in response to estrogen therapy or ET-1. Before randomization, in response to ET-1, FBF was reduced by 21.9% (mean response over 60 min) in the placebo group and 19.0% in the estradiol group (p = 0.67). After one month of therapy, the response was attenuated in the estrogen group, 10.0%, compared with the placebo group, 23.6 (difference in means 13.6%, 95% confidence interval [0.7%, 26.6%), p = 0.041). After three months of therapy, there was no difference in response between the placebo group, 27.0%, and estrogen group, 30.2% (p = 0.65).

CONCLUSIONS In postmenopausal women with coronary heart disease, estrogen therapy inhibits the vasoconstrictor response to ET-1 after one month of therapy. This effect is lost after three months of therapy, suggesting that tachyphylaxis to one potentially beneficial action of estradiol develops during chronic treatment. (J Am Coll Cardiol 2001;37:1367–73) © 2001 by the American College of Cardiology

It is widely believed that estrogen protects against the development of atherosclerosis in women because natural ovarian failure and ovariectomy are both associated with an enhanced risk of coronary heart disease (1). Similarly, a number of experimental and observational human studies suggest that estrogen replacement therapy can delay or prevent the onset of atherosclerosis (2), though the recent Heart and Estrogen/progestin Replacement Study (HERS) (3) does not seem to support a role for estrogen in secondary prevention.

The mechanisms of this putative antiatherosclerotic effect of estrogen are not fully understood. Beneficial serum lipid changes (4), inhibition of atherosclerotic plaque forming cells (5), reduction in low-density lipoprotein oxidation (6) and a decrease in oxidative stress (7) may play a part but are unlikely to be the whole explanation (8). Interactions with other vascular mediators are also potentially important. The synthesis, release or action of potentially beneficial mediators, such as nitric oxide and prostacyclin, may be promoted (8,9). Conversely the actions of potentially adverse mediators may be diminished by estrogen. One such adverse mediator is endothelin-1 (ET-1), the most potent vasoconstrictor known and a powerful mitogen (10). Plasma concentrations of ET-1 are increased in hypercholesterolemic subjects (11) and patients with established vascular disease (12). Furthermore, ET-1 immunoreactivity is enhanced in the walls of atherosclerotic human vessels (12,13). Increased expression and activity of endothelin-converting enzyme (ECE) has also been demonstrated in smooth muscle cells and macrophages in human atherosclerotic plaques (13,14). Hence, higher ECE activity has been associated with progression of atherosclerosis and inhibition of ECE by phosphoramidon has been shown to retard vascular injury and atherosclerosis in experimental animals (15). Estrogen might, therefore, exert its putative antiatherosclerotic effect...
by inhibiting the synthesis and actions of ET-1. There is, indeed, some evidence that estrogen decreases ET-1 secretion in healthy postmenopausal women (16). There is also some experimental evidence that estrogen decreases the vascular actions of ET-1. Estradiol has been shown to reduce the vasoconstrictor effect of endothelin in coronary arteries from rabbits, dogs and pigs (17–19). Conversely, ovariectomy has been reported to increase the vasoconstrictive action of ET-1 in rabbit small cerebral arteries (20). Lastly, the mitogenic effect of ET-1 is attenuated by estrogen in human umbilical vein cultured smooth muscle cells (21).

However, the long-term in vivo actions of estrogen on the vascular response to ET-1 in humans are not known. In this study, we examined the effects on the vascular response to ET-1 of three months of estrogen replacement therapy in postmenopausal women with coronary heart disease.

**METHODS**

The study was performed with the approval of the Local Ethics Committee of the West Glasgow Hospitals University NHS Trust. All patients gave written informed consent. **Patients and estrogen therapy.** Twenty postmenopausal women with known coronary heart disease took part in the study. All patients had either a previously documented myocardial infarction or angiographically proven coronary artery disease. They were randomized to three months of treatment with 2 mg of oral estradiol (Progynova, Schering Health, Burgess Hill, United Kingdom) or matching placebo. Treatment could be decreased to 1 mg if 2 mg was not tolerated. Plasma luteinizing hormone (LH) and follicle stimulating hormone (FSH) concentrations were measured at baseline to confirm menopausal status, and plasma estradiol concentrations were measured during the study to monitor treatment adherence.

**Forearm blood flow (FBF) protocols.** Each patient was studied on three occasions: at baseline before randomization, after one month of randomized therapy and after three months of randomized treatment. Patients discontinued treatment with vasoactive medications for 48 h before each study day, and aspirin was discontinued for 14 days before each study day. All other therapies were taken as normal. Patients abstained from alcohol for 24 h and from food, caffeine-containing drinks and cigarettes for at least 6 h before each study. On the study day baseline, FBF measurements were obtained after 45 min of rest, including 30 min of brachial arterial saline infusion, before ET-1 was infused intra-arterially.

**ET-1 infusion.** Pharmaceutical grade ET-1 (Clinalfa AG, Nottingham, United Kingdom) was infused into the nondominant brachial artery at a rate of 5 pmol/min for 60 min. This dose has previously been shown to cause slow onset vasoconstriction in human forearm resistance vessels (22).

**FBF measurements.** Studies were performed with patients lying supine in a quiet clinical laboratory maintained at a constant temperature between 23°C and 25°C (22–24). After local anesthesia with 1% lidocaine (Astra Pharmaceuticals, Kings Langley, United Kingdom), a 27-gauge steel needle (Terumo Medical Corporation, Leuven, Belgium) was inserted into the nondominant brachial artery and connected to a constant rate infusion pump (IVAC P1000, Alaris Medical Systems, San Diego, California) via a 16-gauge epidural catheter (Portex Ltd., Hythe, United Kingdom). Physiological saline solution (0.9%, Baxter Healthcare Ltd., Deerfield, Illinois) was infused at 1 ml/min for at least 20 min before infusion of the locally acting dose of ET-1 (see the previous text).

Forearm blood flow was measured simultaneously in the infused and noninfused arms by venous occlusion plethysmography using indium-in-gallium Silastic strain gauges applied to the widest aspect of each forearm (25). To obtain blood flow measurements, the hand circulation was excluded by inflation of wrist cuffs to 220 mm Hg, and upper-arm cuffs were inflated to 40 mm Hg to obstruct venous outflow for 12 of every 16 s (22–24). Cuffs were inflated using rapid cuff inflators (Model E-20; D.E. Hokanson Inc., Bellevue, Washington). Voltage output from plethysmographs (D.E. Hokanson Inc.) was transferred via a MacLab 4c analog-to-digital converter (AD Instruments, Hastings, United Kingdom) to a Macintosh personal computer (PowerMac, Apple Computer Inc., Cupertino, California) for analysis using Chart software (version 3.2.8, AD Instruments). Plethysmographic recordings were made for a period of 2 1/2 minutes at 15 min intervals during saline infusion, with the final measurement being made 2 1/2 min immediately before the start of the ET-1 infusion. During the drug infusion, measurements were made at 10-min intervals. The last five measurements from each 2 1/2 min recording period were transferred from Chart to Excel software (version 7.0, Microsoft Corp., Seattle, Washington) averaged, and the mean percentage change from baseline in the ratio of flow between the infused and noninfused arms was calculated. This uses the noninfused arm as a contemporaneous control and distinguishes the effects of drug infusion from any other external or environmental factors (26,27).

Blood pressure and heart rate were manually recorded in the noninfused arm at 15-min intervals throughout each study and at the end of the study period.

**Statistical methods.** All hemodynamic data were recorded blind to treatment allocation. The study design gave repeated measurements at baseline (defined as the time point
just before ET-1 infusion was commenced) and at 10, 20, 30, 40, 50 and 60 min after infusion, at three periods (study days): before randomization and at one and three months after randomization. The statistical model fitted to the percent change over baseline FBF was a covariance pattern mixed model assuming a compound symmetry correlation pattern over the six postinfusion time points (following Brown et al. [28]). Three separate models were fitted, one for each time period, with treatment, time and the interaction of treatment and time included as fixed effects. When no interaction was found, a main effects model was used. Statistical significance was taken as \( p < 0.05 \). All blood flow results are expressed as mean values with 95% confidence intervals in the text and mean values \( \pm 1 \) SEM in the figures. All other data are tabulated as mean \( \pm \) SEM.

### RESULTS

**Patients.** Ten women were randomized to each treatment group. Nineteen patients completed the study protocol, one woman withdrawing from the placebo group after the baseline study because of a urinary tract infection. Details of these patients are given in Table 1. One woman randomized to estrogen had to reduce the dose from 2 mg to 1 mg/day because of breast tenderness (at study day 2, i.e., after one month of treatment).

Local infusion of ET-1 caused no adverse local or systemic effects, and patients did not report any discomfort. Heart rate, blood pressure and FBF in the noninfused forearm did not change significantly on any of the study days, confirming that the drug had only local actions on the forearm vasculature of the infused arm and had no systemic hemodynamic effects. There were no significant differences in baseline heart rate and blood pressure between any of the different study days.

**Plasma FSH and LH concentrations.** Before randomization, mean \( \pm \) SEM FSH concentration was 61.2 \( \pm \) 8 U/L in the placebo group and 70 \( \pm \) 13 U/L in the estrogen group (normal postmenopausal concentrations >30 IU/L) (Table 2). Before randomization, mean \( \pm \) SEM plasma LH concentration was 29.5 \( \pm \) 4 IU/L in the placebo group and 36.7 \( \pm \) 4 IU/L in the estrogen group (normal postmenopausal concentrations >30 IU/L) (Table 2).

**Plasma 17\( \beta \) estradiol concentrations.** Before randomization, mean \( \pm \) SEM plasma estradiol concentration was 60 \( \pm \) 10 pmol/l in the placebo group and 53 \( \pm \) 3 pmol/l in the active therapy group. Plasma concentrations after one month and three months of treatment were 55 \( \pm \) 23 and <50 pmol/l in the placebo group and 381 \( \pm \) 125 and 340 \( \pm \) 93 pmol/l in the estrogen group (Table 2).

**Effect of 17\( \beta \) estradiol on baseline FBF.** Baseline FBF before randomization, after one month and after three months of treatment was 2.50 \( \pm \) 0.21, 2.72 \( \pm \) 0.40, 2.20 \( \pm \) 0.17 ml/min/100 ml forearm volume in the estrogen treatment group (no significant between day differences).

**Effect of 17\( \beta \) estradiol on blood pressure.** Mean \( \pm \) SEM systolic/diastolic blood pressure before randomization, after one month of treatment and after three months of treatment was: 144 \( \pm \) 8/80 \( \pm \) 3, 143 \( \pm \) 8/79 \( \pm \) 3 and 142 \( \pm \) 8/78 \( \pm \) 3 mm Hg in the placebo group, while in the estrogen therapy group, the blood pressures were 151 \( \pm \) 7/82 \( \pm \) 3, 148 \( \pm \) 8/80 \( \pm \) 3 and 148 \( \pm \) 6/80 \( \pm \) 3 mm Hg. There were no significant differences between the groups or study days.

**Effect of 17\( \beta \) estradiol on the vasoconstrictor response to ET-1.** As expected, ET-1 reduced FBF in the infused arm compared with the noninfused arm, and the average peak reduction of all 19 patients at the prerandomization visit was \(-35.96\%\) with a 95% confidence interval of \(-42.8\%\) to \(-29.2\%). This was similar to that reported previously using similar doses [22]. There was little evidence of an interaction between

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### Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>17( \beta ) Estradiol (n = 10)</th>
<th>Placebo (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs (mean ( \pm ) SD)</td>
<td>65.5 ( \pm ) 7.9</td>
<td>66.4 ( \pm ) 6.6</td>
</tr>
<tr>
<td>Hysterecomy, n</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Myocardial infarction, n</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>CAD by angiography (no MI), n</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Hypertension, n</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Current smokers, n</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Ex-smokers, n</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Type II disease, n</td>
<td>Diet only</td>
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</tr>
<tr>
<td>Drug therapy, n</td>
<td>Aspirin</td>
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</tr>
<tr>
<td>ACE inhibitor</td>
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<td></td>
</tr>
<tr>
<td>Beta-blockade</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Nitrates</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Diuretics</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Serum total cholesterol concentration at baseline (mmol/L)</td>
<td>5.97 ( \pm ) 0.33</td>
<td>5.87 ( \pm ) 0.42</td>
</tr>
</tbody>
</table>

ACE = angiotensin-converting enzyme; CAD = coronary artery disease; MI = myocardial infarction.

### Table 2. Plasma Hormone Concentrations (mean \( \pm \) SEM)

<table>
<thead>
<tr>
<th>Study Day</th>
<th>17( \beta ) Estradiol (n = 10)</th>
<th>Placebo (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH (IU/L)</td>
<td>Prerandomization</td>
<td>36.7 ( \pm ) 9.0</td>
</tr>
<tr>
<td>After 1 month of therapy</td>
<td>40.0 ( \pm ) 15.2</td>
<td>30.6 ( \pm ) 6.2</td>
</tr>
<tr>
<td>After 3 months of therapy</td>
<td>36.9 ( \pm ) 13.9</td>
<td>27.4 ( \pm ) 3.5</td>
</tr>
<tr>
<td>FSH (IU/L)</td>
<td>Prerandomization</td>
<td>70.3 ( \pm ) 12.9</td>
</tr>
<tr>
<td>After 1 month of therapy</td>
<td>56.6 ( \pm ) 16.8</td>
<td>60.9 ( \pm ) 7.6</td>
</tr>
<tr>
<td>After 3 months of therapy</td>
<td>48.6 ( \pm ) 14.3</td>
<td>62.6 ( \pm ) 7.6</td>
</tr>
<tr>
<td>Estradiol (pmol/L)</td>
<td>Prerandomization</td>
<td>53.1 ( \pm ) 3.1</td>
</tr>
<tr>
<td>After 1 month of therapy</td>
<td>381 ( \pm ) 125</td>
<td>55.2 ( \pm ) 22.5</td>
</tr>
<tr>
<td>After 3 months of therapy</td>
<td>340 ( \pm ) 93</td>
<td>&lt;50</td>
</tr>
</tbody>
</table>

FSH = follicle stimulating hormone; LH = luteinizing hormone.
treatment and time for any of the periods (study days) \( p = 0.26, p = 0.80, p = 0.16 \) for pre-, one month and three months after randomization, respectively.

Before randomization, there was a similar mean reduction in FBF in response to ET-1 in the two treatment groups, \(-21.9\%\) in the placebo group and \(-19.0\%\) in the estradiol group (difference in means \( 2.9\%, 95\% \) confidence interval \([-11.1\%, 16.9\%]\), \( p = 0.67 \)) (Fig. 1). After one month of therapy, the vasoconstrictor response to ET-1 was significantly attenuated in the estrogen treatment group compared with the placebo treatment group (Fig. 2). The mean percentage reductions in the ratio of FBF were \(-23.6\%\) in the placebo group and \(-10.0\%\) in the estradiol group (difference in means \( 13.6\%\) \( [0.7\%, 26.6\%] \), \( p = 0.041 \)). After three months of therapy, there was no difference in the vasoconstrictor response to ET-1 in the two treatment groups, \(-27.0\%\) in the placebo group and \(-30.2\%\) in the estradiol group (difference in means \(-3.2\%\) \( [-17.6\%, 11.2\%] \), \( p = 0.65 \)) (Fig. 3).

**DISCUSSION**

We found that ET-1 mediated arterial constriction was reduced after one month of treatment with estradiol. After three months of therapy with estrogen, this effect was lost even though circulation levels of estrogen remained similar to those recorded after one month of treatment with estrogen.

**Short-term effects of estrogen.** Our findings are in keeping with earlier studies in experimental animals. Jiang et al. (17) found that \( 17\beta \) estradiol at concentrations of between 1 and 30 \( \mu \)mol/L shifted ET-1, calcium and BAY K8644 concentration-dependent contraction curves to the right in endothelium-denuded coronary arteries taken from male and nonpregnant female rabbits. Both \( 17\beta \) estradiol and verapamil also induced dose-dependent relaxation in endothelium intact and endothelium-denuded coronary arteries submaximally precontracted by ET-1. Lampling et al. (18) subsequently reported that estradiol at a concentration of 1 \( \mu \)mol/L significantly reduced the contractile response to ET-1 in isolated pressurized coronary microvessels taken from the left ventricle of male and female dogs. Estradiol also produced dose-dependent relaxation of these vessels after preconstriction with ET-1. This relaxation was significantly blunted, by about two-thirds, by the combination of indomethacin and NG-nitro-L-arginine. Lastly, Sudhir and colleagues (19) found that intracoronary estradiol (1 nmol/L) reduced the vasoconstrictor response to intracoronary ET-1 (1 to 10 pmol/L), in vivo, in pigs. Interestingly, the effects of sarafotoxin, an agonist more selective for the vascular smooth muscle endothelin type B receptor, were not reduced by estradiol.

Collectively, these studies in several species of experimental animals suggest that estradiol modulates the arterial vasoconstrictor response through endothelium-dependent mechanisms.
pathways, possibly involving vasodilator prostaglandins and nitric oxide. At very high concentrations, an endothelium-independent effect of estradiol may also be demonstrated. The mechanism of action of this counterregulatory effect of estradiol seems to directly or indirectly involve vascular smooth muscle endothelin type A receptor mediated vasoconstriction. Our one-month data are consistent with these animal data but show that the inhibitory effect of estradiol is apparent with much lower concentrations of this hormone (in the 300 to 400 pmol/L range). Despite these lower concentrations of estradiol, the vasconstrictor effect of a similar concentration of ET-1 to that used by Sudhir et al. (19) was reduced by approximately half.

Long-term estrogen treatment. What makes our study unique, however, is that we continued estradiol therapy for three months and showed that, with chronic dosing, the inhibitory effect of this hormone on ET-1 mediated vasoconstriction is completely lost. This observation clearly demands explanation. The most obvious question to ask is whether the women in our study continued to take their hormone treatment. Reported adherence was complete, and this is substantiated by the persistently high plasma concentrations of estradiol in the patients randomized to this treatment.

A more intriguing question is whether or not tachyphylaxis to the effects of estradiol may have occurred with longer term treatment. Relatively little is known about the long-term vascular effects of estrogen therapy. Though there are very many acute studies (29–32), there are very few reports of estrogen therapy given for more than one month (33–35). In two well-controlled studies, Lieberman et al. (33) and Gerhard et al. (34) studied the effect of long-term estradiol on brachial artery diameter changes, measured using high-resolution ultrasound, in response to reactive hyperemia. Lieberman et al. (33) administered estradiol, 1 or 2 mg per day or placebo for nine weeks. In a placebo-controlled crossover trial, Gerhard et al. (34) gave 0.2 mg of transdermal estradiol with or without vaginal micronized progesterone for 14 weeks. In the first study, plasma estradiol concentrations were not reported, but in the second study they were approximately 530 pmol/L. In both studies flow mediated, endothelium-dependent, conduit artery dilation was enhanced by estradiol therapy. However, though brachial artery diameter increased, peak reactive hyperemic flow did not. In a more recent but less well controlled study, Cagnacci et al. (35) used similar methods to examine brachial artery diameter changes in response to reactive hyperemia before and after two months treatment with 50 mg of transdermal estradiol daily (achieving plasma concentrations of approximately 140 pmol/L). In contrast with the two previous groups, these authors did not demonstrate any effect of estradiol on flow mediated vasodilation. Though two of these three studies suggest that tolerance does not develop to at least some of the vascular actions of estradiol, there is evidence from experimental animals that prolonged high concentration exposure does
lead to tachyphylaxis (36). If we accept that flow mediated vasodilation is persistently enhanced during long-term estradiol therapy and that this is related to nitric oxide release, it would seem that the nitric oxide pathway is not the mechanism through which estradiol inhibits the forearm vasoconstrictor response to ET-1 because the latter inhibition is lost during chronic therapy.

That at least one of the vascular actions of estradiol is lost during chronic therapy may have important therapeutic implications. Though there are many possible explanations for the results of HERS, the sort of tachyphylaxis we have observed could well be relevant (3).

Study limitations. In this study we did not determine whether the effect of estrogen was due to alterations in endothelin receptor density or affinity. A previous study in porcine arterial endothelial cells suggests that these changes do not play a role in the effect of estrogen (37).

Summary. We have reported that one month of treatment with 2 mg oral estradiol substantially reduced the forearm vasoconstrictor response to ET-1. This effect was lost by three months. It appears that at least one of the potentially beneficial vascular actions of estradiol is subject to tachyphylaxis.

References


11. Bath PMW, Martin JF. Serum platelet-derived growth factor and...


