Acute and mid-term combined hormone replacement therapy improves endothelial function in post-menopausal women with angina and angiographically normal coronary arteries

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Aims and background Coronary endothelial dysfunction improves after acute oestradiol treatment in women with angina and normal coronary angiograms. We sought to analyse whether this effect is also seen in the peripheral circulation and whether it is sustained after a mid-term period of treatment.

Methods We studied 20 women with angina, signs suggestive of myocardial ischaemia and normal coronary angiograms. In five of them, coronary and peripheral endothelial functions were studied at baseline. Brachial artery flow-mediated dilation was reanalysed after 24 h of transdermal oestradiol treatment. In the other 15 women, brachial artery vasoreactivity was studied at baseline and after a 6-week period of treatment with transdermal oestradiol and medroxyprogesterone (HRT) or placebo in a double-blinded crossover fashion.

Results An abnormal coronary artery response to acetylcholine was observed in all women as well as impaired brachial flow-mediated dilation. Brachial flow-mediated dilation significantly increased after 24 h of oestradiol treatment (4.8 ± 0.8% vs 0.06 ± 0.6%, P<0.001). Peripheral flow-mediated dilation also increased after a 6-week period of HRT compared with baseline (4.1 ± 3% vs 0.4 ± 1%, P<0.01) and placebo treatment (4.1 ± 3% vs 0.6 ± 1.7%, P<0.01).

Conclusion Impaired endothelium-dependent vasodilation exists both at the coronary and peripheral circulation in post-menopausal women with angina and normal coronary angiograms. Flow-mediated dilation improves in these women after short and mid-term therapy with transdermal oestradiol irrespective of concomitant progesterone use.

Key Words: Endothelial function, oestradiol, hormone replacement therapy, syndrome X.

See page 2051, doi:10.1053/euhr.2001.2771 for the Editorial comment on this article

Introduction

Cardiovascular disease continues to be the leading cause of morbidity and mortality among women in developed countries. Risk of death from heart disease is tenfold higher than risk of death from cancer in a 50-year-old woman[1]. Moreover, as life expectancy increases, more than half of the life of a woman will take place during menopause. Therefore, menopause and the consequent risk of vascular disease will be an increasingly important health care problem in the ensuing years.

Chest pain with normal coronary angiograms is a common clinical presentation in female patients, especially among the post-menopausal population. A possible link between oestrogen deficiency and this syndrome has been postulated[2].
There is a growing body of evidence concerning the benefits of oestrogens on vascular biology and many studies have demonstrated their beneficial effect on the lipid profile and their protection against atherosclerosis in different experimental models and in humans. Oestradiol has also been shown to improve peripheral vascular reactivity in healthy post-menopausal women and in women with hyperlipidaemia. In addition, variations in brachial artery vasomotion have been reported during the menstrual cycle that correlate with oestradiol plasma levels.

We have previously reported that short-term (24-h) treatment with transdermal oestradiol improves the endothelium-dependent coronary vasomotion in women with angiography and angiographically normal coronary arteries. The aims of the present study were (1) to find out whether acute transdermal oestradiol improves on peripheral vascular reactivity, as has been demonstrated in coronary arteries and (2) to evaluate whether the effect is maintained through a mid-term period of treatment.

Methods

Patients

Post-menopausal women admitted to hospital with prolonged or progressive angina were screened. Patients included in the study had typical angina plus acute electrocardiographic changes during pain or a positive exercise test, and normal coronary angiograms. Menopause was defined as follicle stimulating hormone (FSH) plasma levels >40 UI.L⁻¹ and oestradiol plasma levels <30 pg.ml⁻¹, determined at least 1 year after the last menstrual bleeding. Angina was defined as typical constrictive chest pain radiating to the arms, jaw or back and relieved by rest or sublingual nitrates. Myocardial ischaemia was suggested by the presence of ST-segment depression (≥ 1 mm) or T wave inversion on at least two consecutive leads of the ECG, or by the existence of a positive stress test or a perfusion defect on cardiac scintigraphy. Exclusion criteria were the following: (a) age >70 years, as hormonal replacement therapy is infrequently prescribed in this group of patients, (b) diabetes mellitus, per se related to endothelial dysfunction, (c) prior myocardial infarction or concomitant valvular heart disease, (d) significant left ventricular hypertrophy (septum or posterior wall thickness >12 mm), and (e) significant high blood pressure (systolic blood pressure >160 mmHg and/or diastolic blood pressure >100 mmHg). The study was approved by our Institution Ethics Committee and all patients gave written consent.

Study design

The study was structured into two phases and, accordingly, patients were divided into two groups. The first group of patients underwent endothelial function studies at baseline and after 24 h of transdermal oestradiol treatment to evaluate the acute effects of oestradiol on coronary and peripheral vasoreactivity (first phase of the study). The second group of patients underwent only peripheral endothelial studies at baseline, after a 6-week period of hormone replacement therapy (HRT) and after placebo to test the mid-term effect of HRT on brachial artery reactivity (second phase of the study).

Acute oestradiol treatment study protocol: coronary and brachial vasoreactivity assessment

Coronary artery endothelial function was analysed by means of intracoronary acetylcholine infusion as previously described. Endothelium-independent coronary vasodilation was evaluated in response to intracoronary nitroglycerin. Quantitative coronary angiography was used to measure the changes in lumen diameter in response to those drugs. An abnormal response to acetylcholine (10⁻⁷ to 10⁻⁵ μM) was defined as the absence of coronary vasodilation after its administration. Treatment with vasodilator drugs and beta-blockers was withdrawn for at least 24 h before the coronary vasoreactivity studies.

An ultrasound vascular study of the brachial artery to assess endothelium-dependent and endothelium-independent vasodilation was also performed on the same day. After both studies, a patch rated to deliver 100 μg · day⁻¹ of oestradiol was applied to each patient on her left buttock. Twenty-four hours after patch application, patients underwent a second brachial artery ultrasound study. Oestradiol plasma levels were determined at baseline and 24 h after oestradiol treatment.

Peripheral endothelial function study

Peripheral vascular studies were performed on the brachial artery using a previously validated ultrasound technique at baseline and after oestradiol treatment. These studies were carried out in a quiet, temperature-controlled room (24 °C) with patients in the supine position, using a high resolution 5.5–7.5 MHz vascular probe connected to a conventional ultrasound machine (Sonos 5500, Agilent Technologies, Andover, MA, U.S.A.). All peripheral vascular studies were performed at the same time of day (afternoon) with the same ultrasound machine and by the same operator. All patients rested for at least 10 min before the study. Blood pressure, heart rate and ECG were continuously monitored throughout the procedure. A longitudinal section of a non-tortuous segment of the right brachial artery 2–5 cm above the elbow was scanned. The centre of the artery was identified by obtaining the clearest image of the anterior and posterior arterial wall layers. Depth and gain settings were optimized and remained

independent vasodilation, a baseline scan was obtained. To assess endothelium-dependent vasodilation and was calculated as the percentage change in brachial artery diameter after nitroglycerin administration from the second baseline scan.

Using this methodology and a nested-analysis of variance, inter-observer and intra-observer variance for brachial artery diameter measurement in our laboratory are 0.0002 (0.04% of total variability) and 0.001 (0.22% of total variability), respectively.

**Mid-term hormone replacement study protocol**

The second phase of the study was designed to evaluate whether the effect of oestradiol on peripheral vascular reactivity persisted with time. All patients underwent a gynaecological examination, Papanicolau’s smear test and a mammogram prior to inclusion in the study to rule out any contraindication to oestrogen treatment. Subjects were randomized in a double-blind, crossover fashion to receive transdermal oestradiol patches rated to deliver 50 μg.day⁻¹ (Evopad®, Jansen-Cilag) or placebo during two consecutive phases of 6 weeks, each one separated from the other by a 2-week wash-out period. Women with an intact uterus also received 2.5 mg.day⁻¹ of medroxyprogesterone acetate or placebo during 3 of the 6 weeks throughout each treatment phase. Both types of patches and pills were identical in size and appearance.

Patients underwent vascular brachial artery studies at baseline and on the last day of each treatment phase. Therefore, every woman was her own study control. Patients were also asked to write down the number of episodes of chest pain and the number of sublingual nitroglycerin tablets taken during each phase of the study.

**Blood tests**

The following blood determinations were performed at baseline and on the last day of each treatment phase: oestradiol, nitrites/nitrates, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides and lipoprotein(a). Follicle stimulating hormone and luteinic hormone were also determined at the beginning of the study. Oestradiol, follicle stimulating hormone and luteinic hormone levels were determined using a chemo-luminescence immunoenzymatic assay (IMMULITE DPC Diagnostic Products). Nitrites/nitrates plasma concentrations were measured using the Griess reactive method. Lipid profile was determined using a routine enzymatic method and LDL-cholesterol was calculated using the Friedewald formula[15].

**Platelet function analysis**

The haemostatic performance of platelets was assessed in a Platelet Function Analyzer (PFA-100) device (Dade

unchanged during the test. In order to achieve a steady image throughout the whole study, the probe was fixed with a mechanical clamp, and the sample volume of the pulsed wave Doppler was placed in the middle of the artery lumen as a reference marker. Hard copies of the images were also obtained and the position of the probe and the arm was registered to ensure accurate reproduction of the same arterial segment for repeated studies. Each scan consisted of a longitudinal image of the brachial artery and a pulsed wave Doppler spectral display of the brachial artery flow. All scans were recorded on superVHS videotape and a magneto-optical disk. After a clear image was obtained, a baseline scan was recorded. Endothelium-dependent vasodilation was assessed by analysis of the brachial artery diameter changes in response to an increase in flow. Reactive hyperaemia was achieved by the rapid release of a pneumatic pressure cuff placed around the forearm, distal to the arterial segment scanned, which was inflated up to 300 mmHg during 4-5 min. A pulsed wave Doppler signal of the brachial artery flow and bidimensional images were recorded 55–65 s after cuff release. After a 10–15 min rest to allow vessel recovery, a second baseline scan was obtained. To assess endothelium-independent vasodilation, 400 μg of sublingual nitroglycerin were administered and a fourth scan was obtained 3 min later.

**Image analysis**

Images were analysed by two independent observers blinded for treatment allocation. The arterial diameter was measured from the images recorded on optical disks (digital imaging) using the callipers from the ultrasound machine. All measurements were made at end-diastole at the peak of the R wave of the ECG, with callipers placed from the trailing edge of the anterior wall interface to the leading edge of the posterior wall interface. Each observer measured four to eight different cardiac cycles and the mean arterial diameter was obtained. If measurements differed by more than 3%, a third blinded observer again measured the artery diameter and if differences still persisted, the study was disregarded. Flow-mediated vasodilatation, which is nitric oxide dependent[13], was used as an index of endothelium-dependent vasodilation and was calculated as the percentage change in brachial artery mean diameter after reactive hyperaemia over that obtained at baseline. Endothelium-dependent vasodilation was considered abnormal when flow-mediated vasodilation was <5%. Reactive hyperaemia was calculated as the relative ratio of maximal flow after cuff release to that measured at baseline. Flow was estimated from the velocity–time integral of the pulse wave Doppler signal and heart rate. Although flow absolute values are not accurate using this method because of the incident angle of the Doppler ultrasound beam, relative values have been previously validated[19]. Vasodilation induced by sublingual nitroglycerin was used as an index of endothelium-dependent vasodilation and was calculated as the percentage change in brachial artery diameter after nitroglycerin administration from the second baseline scan.

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**Platelet function analysis**

The haemostatic performance of platelets was assessed in a Platelet Function Analyzer (PFA-100) device (Dade
Diagnosing) using collagen–ADP and collagen–epinephrine cartridges. This is a new in vitro system for the evaluation of platelet function which determines platelet aggregation on a collagen–epinephrine or a collagen–ADP coated membrane, as whole blood is aspirated under controlled flow conditions through a microscopic aperture cut into a membrane[16]. The time required by the platelet plug to occlude the aperture (closure time) was registered at baseline and after each of the study treatments.

Statistical analysis

Discrete variables are presented as percentages and analysed using the chi-squared test. Continuous variables are expressed as mean value ± SD and compared using a paired two-tailed Student’s t-test. Sample size for the double-blind, crossover study to analyse the effect of mid-term transdermal oestradiol on vascular reactivity was calculated following the method proposed by Sorensen et al.[17]. According to this, 15 patients were necessary to show an absolute increase of 2% in flow-mediated dilation after HRT with a statistical power of 80% and an a error of 0.5. A P value <0.05 was considered statistically significant.

Results

Baseline characteristics

Twenty post-menopausal women with angina and normal coronary artery angiograms were included in the study. Baseline characteristics are shown in Table 1. All patients had at least one sign suggestive of myocardial ischaemia: seven (35%) patients had T wave inversion ≥1 mm or ST down-sloping ≥1 mm on at least two consecutive leads, nine (45%) had a positive stress test and four (20%) showed an abnormal myocardial perfusion on a cardiac scintigraphy. None of them had ever received HRT.

Coronary and brachial vascular studies: acute effect of transdermal oestradiol

Five women underwent coronary and brachial artery endothelial function studies on the same day. All of them showed an impaired response to intracoronary acetylcholine with a maximum decrease in mean coronary artery diameter of −15 ± 6%, while normal vasodilation was seen after intracoronary nitroglycerin (increase in mean coronary artery diameter 29 ± 17%; Fig. 1). Similarly, all of them showed an impaired flow-mediated dilation of the brachial artery (0.06 ± 0.6%) and a normal response to sublingual nitroglycerin (16 ± 7%). Both coronary angiography with intracoronary acetylcholine infusion and ultrasound brachial artery studies demonstrated endothelial dysfunction in each of these five women (Table 2).

Twenty-four hours after transdermal oestradiol treatment, plasma levels of oestradiol were 87 ± 24 pg.dl⁻¹ compared with <10 pg.dl⁻¹ at baseline. Vascular studies performed before and after oestradiol showed no change either in baseline brachial artery diameter (3.91 ± 0.3 vs 3.98 ± 0.3 mm, respectively, P=ns), or in reactive hyperaemia (349 ± 147% vs 307 ± 78%, respectively, P=ns). However, brachial flow-mediated dilation increased to 4.8 ± 0.8% after oestradiol therapy (P<0.001 compared to baseline flow-mediated dilation), while no change was seen in endothelium-independent vasodilation (17 ± 7%, P=ns compared to baseline nitroglycerin-induced vasodilation) (Fig. 2).

Effect of mid-term hormone replacement therapy on peripheral vascular reactivity

Fifteen women were randomized to receive hormone replacement therapy or placebo during two periods of 6 weeks each in a double-blinded crossover fashion. Eleven women received oestrogen plus medroxyprogesterone acetate, and the remaining four women received oestrogen alone. No significant differences were observed in all parameters analysed between the two treatment groups and, therefore, results represent pooled data from all patients.

Vascular ultrasound studies were performed at baseline and on the last day of each study period. Baseline mean brachial artery diameter did not change in any of the three vascular examinations (beginning of the study 4 ± 0.7 mm; post-HRT 4 ± 0.8 mm; post-placebo 4 ± 0.6 mm, P=ns). Endothelium-dependent vasodilation assessed by means of flow-mediated dilation was impaired at baseline in all 15 women (mean flow-mediated dilation 0±4 ± 1%). However, after 6 weeks of

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**Table 1 Baseline characteristics**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>61 ± 7</td>
</tr>
<tr>
<td>Years from menopause</td>
<td>12 ± 9</td>
</tr>
<tr>
<td>Oestradiol (pg.dl⁻¹)</td>
<td>12 ± 5</td>
</tr>
<tr>
<td>FSH (U.l⁻¹)</td>
<td>78 ± 32</td>
</tr>
<tr>
<td>LH (U.l⁻¹)</td>
<td>42 ± 18</td>
</tr>
<tr>
<td>Mild hypertension</td>
<td>6 (30%)</td>
</tr>
<tr>
<td>Total cholesterol (mg.dl⁻¹)</td>
<td>208 ± 32</td>
</tr>
<tr>
<td>HDL-cholesterol (mg.dl⁻¹)</td>
<td>65 ± 15</td>
</tr>
<tr>
<td>LDL-cholesterol (mg.dl⁻¹)</td>
<td>124 ± 29</td>
</tr>
<tr>
<td>Triglycerides (mg.dl⁻¹)</td>
<td>96 ± 35</td>
</tr>
<tr>
<td>Lipoprotein(a) (mg.dl⁻¹)</td>
<td>28 ± 24</td>
</tr>
<tr>
<td>Baseline brachial artery diameter (mm)</td>
<td>4 ± 0.7</td>
</tr>
<tr>
<td>Brachial artery FMD (%)</td>
<td>0.45 ± 1.2</td>
</tr>
<tr>
<td>Reactive hyperaemia (%)</td>
<td>488 ± 275</td>
</tr>
<tr>
<td>Brachial artery NTG-dilation (%)</td>
<td>18 ± 8</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD and as number of patients (%). MILD hypertension was defined as a systolic blood pressure of 140–160 mmHg or diastolic blood pressure of 90–100 mmHg. FMD=flow mediated dilatation; FSH=follicle stimulating hormone; LH=luteinic hormone; NTG-dilation=nitroglycerin-mediated vasodilation.

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flow-mediated dilation was significantly improved compared with baseline (4.1 ± 3% vs 0.4 ± 1%, respectively, \(P<0.01\)) and placebo (4.1 ± 3% vs 0.6 ± 1.7%, respectively, \(P<0.01\)). There were no differences in nitroglycerin-induced vasodilation between the three examinations (Fig. 3). Reactive hyperaemia response and mean heart rate and blood pressure were comparable at baseline and after any of the two treatments (HRT or placebo).

The number of episodes of chest pain and tablets of sublingual nitroglycerin taken were not different while on HRT or placebo (4 ± 4 vs 5 ± 5, \(P=\text{ns}\), and 3 ± 5 vs 3 ± 5, \(P=\text{ns}\), respectively). Eight (54%) patients referred to improvement of symptoms with HRT compared with five (33%) women who did not experience any change in their symptomatology \(P=\text{ns}\); the remaining two (13%) patients felt better with placebo.

**Blood tests analysis**

Oestradiol plasma levels determined on the last day of treatment (72 h after last patch application) increased from \(13 \pm 5 \text{pg.dl}^{-1}\) at baseline to \(26 \pm 14 \text{pg.dl}^{-1}\) \((P<0.01)\). Nitrite/nitrate levels remained unchanged at baseline and after oestradiol or placebo. After HRT, total plasma cholesterol, LDL-cholesterol, HDL-cholesterol, lipoprotein(a) and triglycerides were not significantly different from baseline or after placebo. Finally, a shorter time to clot formation was observed while women were on HRT, although the difference did not reach statistical significance when it was compared with baseline or treatment with placebo (Table 3).

**Discussion**

We have shown that impaired endothelium-dependent vasodilation exists both in the coronary and peripheral circulation in post-menopausal women with angina and normal coronary angiograms. Abnormal endothelium-dependent vasodilation improves in these women after short and mid-term therapy with transdermal oestradiol and despite the concomitant use of progesterone. Moreover, this effect is observed regardless of the potential benefit on lipid profile, which was not observed during this study period. However, endothelium-independent vasodilation induced by nitroglycerin remains normal in these women and does not change after HRT.
We have studied a selected group of patients with syndrome X, all of whom had some sign that suggested myocardial ischaemia. The exact mechanisms that underlie this clinical presentation are unclear, but microvascular angina and endothelial dysfunction have been proposed\[18–20\]. Endothelial dysfunction is thought to be an early phenomenon in the pathogenesis of atherosclerosis\[21\]. It has been recently reported that endothelial dysfunction in patients with mild coronary artery disease is associated with increased cardiac events during long-term follow-up\[22\]. Consequently, reversal of abnormal endothelium-dependent vasodilation may lead to an improvement in survival.

**Syndrome X and endothelial dysfunction**

Flow-mediated dilation, which is mediated by nitric oxide, is considered as a measure of endothelial function\[13\]. A close relationship between endothelium-dependent vasomotor responses in the brachial and

**Figure 2** Brachial artery vasoreactivity and short-term transdermal oestradiol treatment. Endothelium-dependent flow-mediated dilation (FMD) was significantly better after transdermal oestradiol [□] compared with baseline [■] (P<0·001, left graph). No significant differences were observed between either group regarding nitroglycerin-induced, endothelium-independent vasodilation (NTG) (P=ns, right graph).

**Figure 3** Brachial artery vasoreactivity and mid-term HRT treatment. Endothelium-dependent, flow-mediated dilation (FMD) was significantly better after HRT [●] compared with baseline [■] (P<0·01) and placebo [□] (P<0·01) (left graph). However, there were no significant differences in nitroglycerin-induced, endothelium-independent vasodilation (NTG) (P=ns) (right graph). *P<0·01 as compared to baseline and placebo.

**Table 3 Results of blood sample assays**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>HRT</th>
<th>Placebo</th>
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</thead>
<tbody>
<tr>
<td>Oestradiol (pg.ml⁻¹)</td>
<td>13 ± 5</td>
<td>26 ± 14*</td>
<td>14 ± 7</td>
</tr>
<tr>
<td>Progesterone (ng.ml⁻¹)</td>
<td>0·60 ± 0·3</td>
<td>0·50 ± 0·3</td>
<td>0·53 ± 0·3</td>
</tr>
<tr>
<td>Total cholesterol (mg.dl⁻¹)</td>
<td>211 ± 34</td>
<td>203 ± 31</td>
<td>203 ± 32</td>
</tr>
<tr>
<td>HDL-cholesterol (mg.dl⁻¹)</td>
<td>66 ± 14</td>
<td>63 ± 14</td>
<td>63 ± 13</td>
</tr>
<tr>
<td>LDL-cholesterol (mg.dl⁻¹)</td>
<td>126 ± 30</td>
<td>124 ± 25</td>
<td>126 ± 26</td>
</tr>
<tr>
<td>Triglycerides (mg.dl⁻¹)</td>
<td>95 ± 37</td>
<td>104 ± 50</td>
<td>93 ± 39</td>
</tr>
<tr>
<td>Lipoprotein(a) (mg.dl⁻¹)</td>
<td>27 ± 27</td>
<td>25 ± 22</td>
<td>28 ± 22</td>
</tr>
<tr>
<td>Nitrites/Nitrates (nmol.ml⁻¹)</td>
<td>36 ± 9</td>
<td>56 ± 53</td>
<td>54 ± 54</td>
</tr>
<tr>
<td>aPTT (s)</td>
<td>27 ± 2</td>
<td>27 ± 1</td>
<td>28 ± 3</td>
</tr>
<tr>
<td>Time to clot formation (s)†</td>
<td>153 ± 55</td>
<td>132 ± 27</td>
<td>150 ± 43</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD. aPTT=activated partial thromboplastin time. †Time to clot formation was assessed by means of the PFA-100 device (see text).

*P<0·01 vs baseline and placebo.
coronary arteries has been reported in women with coronary artery disease\cite{23}. Accordingly, we found that all the studied women with an abnormal vascular response to intracoronary acetylcholine, also had impaired flow-mediated dilation of the brachial artery.

**Oestrogens and endothelial function**

Previous studies using oral\cite{24}, intravenous\cite{25} or even intracoronary oestradiol\cite{26-28} to assess coronary artery response to acetylcholine, showed that acute administration of oestradiol improves coronary endothelium-dependent vasodilation. The beneficial effect of oestrogens on vascular reactivity has also been documented in the peripheral circulation using various forms of oral, intravenous and intra-arterial oestrogens\cite{29-32}. In contrast, it has been shown that withdrawal of oestrogen therapy leads to deterioration in endothelial function in post-menopausal women with coronary artery disease\cite{33}. However, all these previous studies were carried out in heterogeneous groups of women with coronary artery disease or coronary risk factors.

In this regard, we have previously reported an improvement in coronary vasoreactivity after short-term transdermal oestradiol therapy in post-menopausal women with syndrome X, angina, signs of myocardial ischaemia and normal coronary angiogram\cite{31}. The present study confirms the beneficial effect of short-term transdermal oestradiol on endothelial function in these patients and shows that this effect is maintained through a mid-term period of treatment. Of interest is that this beneficial effect was observed with modest oestradiol plasma levels. This observation may be clinically relevant since replacement therapy at low oestrogen doses can be as effective as with higher oestradiol concentrations.

**Progesterone and endothelial function**

Chronic oestrogen therapy, combined with progesterone in order to decrease the associated risk of endometrial cancer in women with an intact uterus, has not reduced the cardioprotective effects of oestrogens in large epidemiological series\cite{34}. Although acute vaginal administration of natural progesterone decreased forearm blood flow in 12 post-menopausal women\cite{35}, it has not been established that combined therapy has a negative effect on peripheral vasoreactivity. Improvement in endothelial function has been reported despite the use of progestins in combination with transdermal and oral oestradiol\cite{7,9}. Nevertheless, variable vascular responses can be seen with different types of progestins. In this regard, Rosano et al. reported an increase in brachial artery flow-mediated dilation in post-menopausal women taking oral conjugated oestrogens and medroxyprogesterone acetate, while it was reduced in those taking transdermal oestradiol and norethisterone acetate as compared with baseline\cite{36}. Use of norethisterone and oral conjugated oestrogen in a larger study among healthy post-menopausal women conferred no benefit on endothelium-dependent vasomotor function in women who took HRT compared with those who did not\cite{37}.

**Blood tests results**

In the present study, an improvement in endothelium-dependent dilation was observed after HRT despite the lipid profile remaining unchanged; therefore, an enhancement of endothelial function was observed regardless of the effect on lipids, supporting the hypothesis that oestrogens have a direct effect on the vascular wall, probably through increased bioavailability of nitric oxide\cite{38}. Unfortunately, we could not demonstrate a significant increase in the nitrite/nitrate plasma concentration as a marker of increased nitric oxide synthesis; however, it is known that this concentration may be affected by dietary variations and other concomitant diseases, such as infections, which were not controlled in the present study\cite{39}.

Even though plasma levels of oestradiol after HRT were low in our study, they were in accordance with data previously reported when using patches that deliver 50 or 100 µg.day\(^{-1}\)\cite{40}. Although these levels were lower than those observed in pre-menopause, endothelial function improved after HRT. Moreover, transdermal oestrogen administration is associated with higher rates of treatment compliance, which is an essential issue for chronic therapies such as HRT that may last up to 30 years\cite{40}.

Importantly, we found no significant increase in platelet haemostatic performance in this small sample of women under HRT, although a trend towards it was apparent. This study was conducted in women with angina and normal coronary arteriograms and, therefore, any extrapolation of these results to other trials performed among women with coronary artery disease must be made with extreme caution. Nevertheless, the initial prothrombic risk suggested by the results of the HERS study\cite{41} may be related to the clot formation process and this certainly deserves further investigation.

**Study limitations**

The present study was designed to evaluate the effect of HRT on vascular reactivity and therefore it had insufficient power to evaluate changes in the lipid profile. Consequently, a failure to show a decrease in total cholesterol and LDL-cholesterol may not be interpreted as being in conflict with the results of other previous studies specifically designed for that purpose\cite{4,9}. Moreover, the effects observed on plasma cholesterol in studies using transdermal oestradiol have been controversial; several studies specifically designed to
evaluate that have failed to show a beneficial effect on the plasma lipid profile, probably related to the lack of an hepatic first pass. Furthermore, we used medroxyprogesterone acetate instead of micronized progestosterone, which is known to have less androgenic properties and a better cardiovascular risk profile. However, a benefit to endothelial performance was observed despite the use of medroxyprogesterone. Finally, although a trend towards a decrease in symptoms was noted, no significant difference was seen while on HRT or placebo in these women who are usually refractory to antianginal therapy. Larger and longer follow-up studies are required to show whether improvement in endothelial function will translate into net clinical benefit.

Conclusions

In this study, HRT (transdermal oestradiol plus medroxyprogesterone) improved flow-mediated, endothelium-dependent vasodilation both in coronary and brachial arteries of post-menopausal women with angina and normal coronary angiograms, regardless of the effect on lipid plasma levels and despite the combination with progesterone. Vasodilation in response to endothelium-independent stimuli such as nitroglycerin remained normal and unchanged after HRT. Further investigations on the mechanisms by which oestrogens potentiate endothelial function will enable the development of specific drugs that improve flow-mediated, nitric oxide-dependent vasodilation.

We are indebted to Dolors Viles, Alfredo Cuppoletti and Manel Morales for their help in data collection.

References


