Effect of Transdermal Estradiol and Oral Conjugated Estrogen on C-Reactive Protein in Retinoid-Placebo Trial in Healthy Women

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Background—The increase in C-reactive protein (CRP) during oral conjugated equine estrogen (CEE) may explain the initial excess of cardiovascular disease observed in clinical studies. Because the effect of transdermal estradiol (E2) on CRP is unclear, we compared CRP changes after 6 and 12 months of transdermal E2 and oral CEE in a randomized 2×2 retinoid-placebo trial.

Methods and Results—A total of 189 postmenopausal women were randomized to 50 μg/d transdermal E2 and 100 mg BID of the retinoid fenretinide (n=45), 50 μg/d transdermal E2 and placebo (n=49), 0.625 mg/d oral CEE and 100 mg BID fenretinide (n=46), or 0.625 mg/d oral CEE and placebo (n=49) for 1 year. Sequential medroxyprogesterone acetate was added in each group. Relative to baseline, CRP increased by 10% (95% CI −9% to 33%) and by 48% (95% CI 22% to 78%) after 6 months of transdermal E2 and oral CEE, respectively. The corresponding figures at 12 months were 3% (95% CI −14% to 23%) for transdermal E2 and 64% (95% CI 38% to 96%) for oral CEE. Fenretinide did not change CRP levels at 6 and 12 months relative to placebo. Relative to oral CEE, the mean change in CRP after 12 months of transdermal E2 was −48% (95% CI −85% to −7%, P=0.012), whereas fenretinide was associated with a mean change of −1% (95% CI −34% to 40%, P=0.79) compared with placebo.

Conclusions—In contrast to oral CEE, transdermal E2 does not elevate CRP levels up to 12 months of treatment. The implications for early risk of coronary heart disease require further studies. (Circulation. 2002;106:1224-1228.)

Key Words: hormones ▪ inflammation ▪ coronary disease ▪ prevention ▪ risk factors

Despite significant epidemiological evidence of a beneficial effect of postmenopausal hormones on coronary heart disease (CHD), recent results from the Heart and Estrogen/Progestin Replacement Study (HERS), a secondary prevention trial, showed no overall benefit from hormone replacement therapy (HRT) but did show a significant time trend, with an excess of cardiovascular events in the first year and a lower risk in the following years in the oral conjugated equine estrogen (CEE)/progestin group compared with the placebo group. This pattern of events has been confirmed in other reports, including a preliminary announcement by the Women’s Health Initiative (WHI) HRT Study, a large trial of oral CEE for the primary prevention of CHD. An explanation for these unexpected findings has been suggested by the observation that oral CEE increases, by =80%, the levels of ultrasensitive C-reactive protein (CRP), an index of low-grade inflammation associated with endothelial dysfunction, which is an independent risk factor for CHD in healthy men and women. Therefore, the dual effect of oral CEE on CHD could be due to an initial alteration of endothelial activation, which is then followed by an inhibition of atherosclerosis through different mechanisms.

In contrast to oral CEE, little is known of the long-term effects of transdermal 17β-estradiol (E2) on women’s health, including CHD and breast cancer risk. In the present study, we compared the effects of oral CEE, transdermal E2, fenretinide, and placebo on the 6-month and 12-month changes in CRP levels in a biomarker trial of breast cancer and CHD risk.

Methods

Protocol

The aims of the trial were (1) to determine the effect of fenretinide, a retinoic acid derivative, and (2) to compare the effect of oral CEE and transdermal E2 at biologically comparable doses on biomarkers of breast cancer and cardiovascular risk in healthy women...
undergoing HRT. The rationale for the present study was provided by the results of a secondary prevention trial, in which fenretinide decreased second breast malignancies in premenopausal women but not in postmenopausal women, thus suggesting a permissive effect of steroidal hormones. A similar interaction had previously been noted on the 12-month change in circulating insulin-like growth factor-I (IGF-I). The study biomarkers include the 12-month changes in plasma IGF-I (primary end point), the ratio of IGF-I to IGF-binding protein-3, mammographic density, atypia in breast fine-needle aspirate, lipoproteins, CRP, and homocysteine. Although the study is still under way, the results of the HERS study and the WHI study prompted us to study the 6-month changes in CRP levels for safety reasons. A 12-month point measurement was also included to determine whether changes in CRP were transient or sustained. The Data Safety and Monitoring Committee deemed it appropriate to prepare the present report. The accrual started on September 1, 1998, and randomization was closed on October 1, 2000, with a total of 226 subjects (114 on transdermal E2 and 112 on oral CEE). As of December 31, 2001, 33 subjects had dropped out because of adverse events (5 on E2 and 6 on CEE) or voluntary withdrawal (10 on E2 and 12 on CEE) and 4 subjects had insufficient serum available, thus leaving a total of 189 subjects suitable for the present study. The post hoc powers to detect a 50% to 80% increase in CRP after 6 months of oral CEE compared with no change with transdermal E2 ranged from 75% to 97%, according to a pooled 2-sample t test on the log[CRP] values. Percentage increases in CRP with oral CEE of 80% have been reported in previous studies, but no effects of transdermal estrogen replacement therapy (ERT) on CRP have been reported in healthy women, and we anticipated no change by fenretinide because of a different route.

Participants
Study participants were postmenopausal de novo ERT users with 6- to 60-month amenorrhea and follicle-stimulating hormone levels >40 U/L. Women meeting any of the following criteria were excluded: prior HRT; hysterectomy; previous malignancy; first-degree relative with breast cancer aged <50 years; endometrial proliferative disorders; alterations of metabolic, liver, renal, and/or cardiac function; retinoid hypersensitivity; photodermatitis; retinal diseases or glaucoma; venous thromboembolic disease; infections; severe depression; porphyria; and otosclerosis.

Women were assessed at baseline and at 2, 6, 12, and 18 months. Blood samples were drawn every 6 months during the combined phase to adjust for the progestin effect. Assessment by pill count showed a >90% compliance in ~80% of the subjects.

Assignment
Subjects were randomized by a 2×2 factorial design to transdermal E2 (50 µg/d) released by a weekly patch (Climara, Schering SpA) and fenretinide (R.W. Johnson Pharmaceutical Research Institute) at 100 mg BID by oral capsules (n=49), transdermal E2 and placebo capsules (n=49), 0.625 mg/d oral CEE (Premarin, Wyeth-Lederle) and fenretinide (n=46), or oral CEE and placebo (n=49) for 1 year. Assignment to the ERT route was unblinded because the comparison in symptom relief was not a study end point. Sequential medroxyprogesterone acetate (Farlutal, Pharmacia) at 10 mg/d PO for the first 12 days of each month was added to continuous ERT. A 3-day rest period from the retinoid capsules was prescribed monthly to increase plasma retinol levels, thus allowing sufficient uptake for normal night vision. Randomization was centrally performed by telephone with the use of permuted blocks of 4 and was stratified for the 4 participating centers.

Outcome Measure
Serum levels of CRP were measured in 94 women allocated to transdermal E2 and 95 women allocated to oral CEE who had both baseline and 6-month blood measurements. This time interval was considered appropriate because earlier results from the HERS study showed the highest risk of CHD events to be in the first 4 to 8 months of oral CEE therapy. A 12-month measurement was also included to determine whether changes in CRP were transient. Our hypothesis was that there would be an 80% increase in CRP from baseline to 6 and 12 months among women using oral CEE, no effect of transdermal E2, no effect of fenretinide compared with placebo, and no different effect of fenretinide in the 2 ERT groups given the unknown anti-inflammatory properties of this compound.

Assay Methods
Serum concentrations of CRP were determined by a high-sensitivity assay using a 2-site chemiluminescent enzyme immunoassay (Diagnostic Products Corp) for the IMMULITE automated analyzer. The sensitivity of the test is 0.01 mg/dL. The intra-assay and interassay coefficients of variation were 3.6% and 3.9%, respectively.

Statistical Analyses
Because the distributions of CRP at baseline and at 6 and 12 months are, as previously observed, very skewed, with long tails toward higher values, the analysis was carried out by using the logarithmic transformation of CRP. A linear regression model was developed with baseline information used only to investigate the relationship between baseline log[CRP] and the covariates measured at baseline. These were age, months since last period, fibrinogen, leukocyte count, total cholesterol, HDL cholesterol, LDL cholesterol, total cholesterol–to–HDL cholesterol ratio, body mass index (BMI), waist-to-hip ratio, and smoking status. All variables were entered into the model initially, and a backward selection process was adopted.

The main analysis to investigate the effects of the route of HRT and fenretinide on CRP was carried out in 2 ways. First, the 6-month and 12-month CRP values were used as the main end points and were adjusted for any baseline imbalance in the 4 groups of women through a repeated-measures ANCOVA using a mixed-effect model. This is the appropriate method of correcting for baseline values. Second, we analyzed the percentage change in CRP at 6 and 12 months because this is a clinically relevant end point and because previous studies have reported data regarding this change. The Kruskal-Wallis test was used to assess differences, if any, occurring in the median percentage change. Bias-corrected bootstrap CIs for the median percentage change were based on 5000 bootstrap samples. A robust regression analysis of the percentage change in CRP was also conducted to minimize the effect of the outliers in the percentage changes associated with low baseline values of CRP. This is based on a linear regression after omitting 10% of the most extreme percentage changes. It is robust because it is unaffected by these potential outliers. All statistical analyses were carried out with the use of Splus 2000 (MathSoft Inc).

Results
Baseline Variables
The distributions of baseline variables are shown in Table 1. All variables were evenly distributed among groups. Baseline levels of CRP, shown in Table 2, were comparable to levels reported in recent studies involving healthy subjects in their age range. There was a slight nonsignificant imbalance between the 2 ERT routes regarding baseline CRP levels. Women who were randomized to oral CEE showed nonsignificantly higher CRP values at baseline compared with women randomized to transdermal E2 (P=0.18). At baseline, only BMI and fibrinogen were significantly and positively associated with CRP levels (the correlations between log[CRP] and BMI and fibrinogen are 0.53 and 0.52, respectively; both P<0.001), and 40% of the variation in log[CRP] was explained by BMI and fibrinogen.
Repete-Measures Analysis of CRP at 6 and 12 Months
The mean levels of CRP at baseline and at 6 and 12 months in the 4 treatment arms are reported in Table 2. Relative to baseline, CRP increased at 6 and 12 months among women taking oral CEE but not among those taking transdermal E2.

Repeated-measures ANCOVA models showed that the use of oral CEE was associated with higher CRP at 6 and 12 months compared with transdermal E2 (P=0.0007; Figure). There was no change in CRP from 6 to 12 months (P=0.87). Fenretinide was associated with no significant change in CRP at 6 and 12 months relative to placebo (P=0.34). For a woman with median CRP (0.075 mg/dL) and median BMI (24.1 kg/m²), the model predicted a 10% increase (95% CI 22% to 78%) if she had been given transdermal E2 and a 48% increase (95% CI 38% to 96%) on oral CEE (Figure). Both graphs imply a regression to the mean, in that women with high baseline CRP values tend to have slightly lower 6- and 12-month values, and women with low baseline CRP values tend to have slightly higher endpoint values. Moreover, there was a strong positive association between CRP at 6 and 12 months and CRP at baseline (P<0.01 for both), in that heavier women, who exhibit higher baseline CRP values than lean women, also had higher end-point values over and above the effect of ERT. However, the effects of the ERT route on CRP levels at 6 and 12 months were not significantly modified by baseline CRP and BMI, possibly because of a low power to test such interactions (P=0.14 and P=0.30, respectively, for the interactions).

The levels of CRP at 6 months were slightly lower among women receiving fenretinide compared with those receiving placebo, but this difference was not statistically significant (P=0.18, adjusting for ERT route, BMI, and CRP). In addition, there was no evidence of a different effect of fenretinide depending on the route of ERT administration (P=0.76 for the interaction).

Percentage Change in CRP From Baseline
Table 3 shows the differences over the 4 treatment groups regarding the median percentage change in raw CRP levels from baseline to 6 months (P=0.01, Kruskal-Wallis test) and from baseline to 12 months (P=0.002). The results of robust analysis indicate that transdermal E2 induced a mean percentage change in CRP at 6 months of −38% (95% CI −86% to −12%) relative to oral CEE (P=0.004), whereas fenretinide was associated with a mean percentage change in CRP of −29% (95% CI −60% to 12%) relative to placebo (P=0.18). At 12 months, the mean percentage change associated with transdermal E2 was −48% (95% CI −85% to −7%) relative to oral CEE (P=0.012), whereas fenretinide was associated with a mean percentage change of −1% (95% CI −34% to 40%) relative to placebo (P=0.79).

Because no subject had any form of cardiovascular disease during the study (2 women experienced superficial phlebitis 3

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**TABLE 1. Main Subject Characteristics at Baseline**

<table>
<thead>
<tr>
<th></th>
<th>E2 + Fenretinide (n=45)</th>
<th>E2 + Placebo (n=49)</th>
<th>CEE + Fenretinide (n=46)</th>
<th>CEE + Placebo (n=49)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>52.5±3.1</td>
<td>52.1±3.1</td>
<td>52.1±3.4</td>
<td>51.4±2.9</td>
</tr>
<tr>
<td>Duration of menopause, mo</td>
<td>23.2±16.5</td>
<td>20.6±12.7</td>
<td>18.9±13.8</td>
<td>21.7±16.7</td>
</tr>
<tr>
<td>Fibrinogen, mg/dL</td>
<td>314±58</td>
<td>311±67</td>
<td>301±62</td>
<td>310±66</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.8±3.5</td>
<td>25.3±5.0</td>
<td>23.7±3.6</td>
<td>24.6±3.6</td>
</tr>
<tr>
<td>CRP, mg/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>0.07</td>
<td>0.06</td>
<td>0.10</td>
<td>0.11</td>
</tr>
<tr>
<td>Range</td>
<td>0.01−0.74</td>
<td>0.00−1.63</td>
<td>0.02−1.21</td>
<td>0.01−1.50</td>
</tr>
<tr>
<td>Leukocytes (×10³/mm³), n</td>
<td>5.6±1.2</td>
<td>5.5±1.4</td>
<td>5.7±1.6</td>
<td>5.2±1.2</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.83±0.10</td>
<td>0.83±0.10</td>
<td>0.82±0.07</td>
<td>0.82±0.06</td>
</tr>
<tr>
<td>Total cholesterol/HDL-C ratio</td>
<td>3.7±1.3</td>
<td>3.6±1.2</td>
<td>3.4±1.0</td>
<td>3.5±0.9</td>
</tr>
<tr>
<td>Smoking habit (never/current/former), n</td>
<td>19/17/9</td>
<td>33/11/5</td>
<td>29/13/4</td>
<td>33/10/6</td>
</tr>
</tbody>
</table>

HDL-C indicates HDL cholesterol. Values are mean±SD.

**TABLE 2. Geometric Mean and 95% CIs for CRP (mg/dL) During ERT**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>6 mo</th>
<th>12 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>LCL</td>
<td>UCL</td>
</tr>
<tr>
<td>E2 + fenretinide (n=45)</td>
<td>0.076</td>
<td>0.055</td>
<td>0.106</td>
</tr>
<tr>
<td>E2 + placebo (n=49)</td>
<td>0.072</td>
<td>0.051</td>
<td>0.102</td>
</tr>
<tr>
<td>CEE + fenretinide (n=46)</td>
<td>0.087</td>
<td>0.064</td>
<td>0.118</td>
</tr>
<tr>
<td>CEE + placebo (n=49)</td>
<td>0.097</td>
<td>0.070</td>
<td>0.135</td>
</tr>
</tbody>
</table>

LCL indicates lower confidence limit; UCL, upper confidence limit. Geometric mean is calculated because distribution of CRP is skewed with long tail to high values. It is calculated as exponential of the mean of log [CRP].
and 11 months after randomization, respectively), no association between CRP increase and clinical events could be assessed.

Discussion
Our results indicate no significant changes in CRP levels for up to 12 months of treatment with transdermal E2, whereas 6 and 12 months of oral CEE increased CRP by 48% (95% CI 22% to 78%) and 64% (95% CI 38% to 96%), respectively, relative to baseline. The difference in the effect of ERT cannot be attributed to slightly higher baseline CRP levels in the oral CEE arms, inasmuch as women with elevated CRP levels at randomization showed a tendency to slightly regress to the mean for both ERT routes. Because increased CRP level is among the strongest of the risk factors for CHD in healthy women and because its normalization is associated with a significant improvement in endothelium-dependent vascular reactivity, our findings suggest that transdermal E2 may be associated with a safer effect on CHD during the initial 12 months of ERT. However, because the causal association between the changes in CRP induced by oral CEE and an increased risk of CHD events is still unproved, the clinical effects of transdermal E2 on early and late CHD events remain to be determined.

Our observation adds further support to the notion that transdermal E2 and oral CEE exert a remarkably different pattern of hormonal and metabolic effects, presumably as a consequence of the elevated concentration of orally administered estrogens at first-pass hepatic level. In contrast to oral CEE, transdermal E2 has only marginally favorable effects on serum lipids, lipoproteins, and fibrinolysis. CRP, a member of the pentraxin family of proteins involved with pattern recognition in innate immunity, is the principal downstream mediator of the acute-phase response and is primarily derived via interleukin-6-dependent hepatic biosynthesis. Therefore, the different pattern of protein expression induced by the ERT route includes biomarkers of subclinical systemic inflammation, such as CRP and, possibly, its precursor, interleukin-6. Although it is unclear whether the increase in CRP during oral CEE really reflects an increase in vascular inflammation and atherogenesis or is simply the result of a metabolic aberration due to high liver synthesis, recent data indicate that elevated CRP not only is a marker of vascular inflammation but also has a direct effect on its amplification regardless of the reason for this elevation. Given the absence of cardiovascular events, the present study cannot provide further insight into this important issue.

As already noted, BMI showed a positive association with CRP. Moreover, BMI had a positive effect on the CRP increase over and above the effect of oral CEE, although the interaction term was not statistically significant, possibly because of a low power to test it. This observation may offer clues regarding the results of the HERS trial, in which an initial excess of CHD during oral CEE occurred in a predominantly obese population. Therefore, women with prior CHD and high BMI would be at further increased risk of an early CHD recurrence compared with lean women as a result of their persistently elevated CRP levels during oral CEE therapy. In contrast, most epidemiological evidence of a

TABLE 3. Median Percentage Change and 95% Bootstrap CIs in CRP From Baseline to 6 and 12 Mo

<table>
<thead>
<tr>
<th></th>
<th>6 mo</th>
<th>12 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>LCL</td>
</tr>
<tr>
<td>E2 + fenretinide (n=45)</td>
<td>−18.6</td>
<td>−32.8</td>
</tr>
<tr>
<td>E2 + placebo (n=49)</td>
<td>18.7</td>
<td>0.0</td>
</tr>
<tr>
<td>CEE + fenretinide (n=46)</td>
<td>39.1</td>
<td>−16.0</td>
</tr>
<tr>
<td>CEE + placebo (n=49)</td>
<td>79.0</td>
<td>42.9</td>
</tr>
</tbody>
</table>
benefit of oral CEE is based on studies in healthier and leaner women, who tend to use HRT more frequently and to benefit from its use in terms of CHD risk. If confirmed in larger clinical trials, our observation supports the notion that transdermal E2 should be the preferred form of ERT to control menopausal symptoms in heavier women with high CRP values or other cardiovascular risk factors.

Our findings of a different effect of ERT route on CRP are consistent with those recently reported by Vehkavaara et al for a small series of 27 women, in whom there was no change in CRP after 12 weeks of transdermal E2, but in whom an increase with oral E2 was noted. At variance, Sattar et al showed that transdermal E2 (80 μg/d) and continuous oral norethisterone given for 6 months significantly reduced CRP in 33 women with type-2 diabetes. However, women with diabetes had much higher baseline levels, which is in line with the involvement of CRP in type-2 diabetes pathogenesis. Although the different type of progestin and the higher diabetes had much higher baseline levels, which is in line in 33 women with type-2 diabetes. However, women with norethisterone given for 6 months significantly reduced CRP values or other cardiovascular risk factors.

As expected, fenretinide was associated with no significant changes of CRP levels relative to placebo. Although previous studies have shown that retinoids possess a moderate anti-inflammatory activity through inhibition of interleukin-6 production, fenretinide had no appreciable modulation of high CRP levels in women taking oral CEE. Interestingly, statins can significantly lower CRP levels and may reduce venous thromboembolic formation in HRT users. A trial specifically testing the effect of statins on oral CEE-associated CRP increase is warranted.

In conclusion, we demonstrated that transdermal E2, in contrast to oral CEE, does not increase CRP in healthy women. Heavier women with high BMI showed high end-point CRP values over and above the effect of oral CEE. Fenretinide had no significant effect on CRP. Although the causal relationship between CRP changes and CHD risk remains to be established, our results suggest that the neutral effect of transdermal E2 on CRP might be associated with a safer profile on early CHD risk. Further studies are necessary to address this issue.

Acknowledgments

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References

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