Effects of Continuous Combined Hormone Replacement Therapy on Inflammation in Hypertensive and/or Overweight Postmenopausal Women

Kwang Kon Koh, Jeong Yeal Ahn, Dong Kyu Jin, Byung-Koo Yoon, Hyung Sik Kim, Dae Sung Kim, Mi-Seung Shin, Ji Won Son, In Suck Choi, Eak Kyun Shin

Objective—We observed that estrogen did not show cardioprotective benefits in type 2 diabetic postmenopausal women. We hypothesized that hypertensive and/or overweight women may be less likely to realize cardiovascular benefits from estrogen.

Methods and Results—We administered micronized progesterone (MP) 100 mg or medroxyprogesterone acetate (MPA) 2.5 mg with conjugated equine estrogen (CEE) 0.625 mg daily during 2 months to 35 hypertensive and/or overweight postmenopausal women with a randomized, double-blind, crossover design. With significant changes of lipoproteins, CEE+MP or MPA significantly improved flow-mediated dilation and reduced plasma E-selectin, intercellular adhesion molecule type-1, monocyte chemoattractant protein-1, and tumor necrosis factor-α levels (P<0.001, P<0.001, P=0.021, P<0.001, and P<0.001 by ANOVA, respectively), but not C-reactive protein and fibrinogen levels. Of note, there were no significant differences between each therapy regarding these effects. However, the magnitude of improvement of flow-mediated dilation in these women was less than in healthy postmenopausal women and more than in diabetic postmenopausal women reported by our previous studies. The effects of CEE+MP or MPA on inflammatory markers were comparable to healthy postmenopausal women, but not comparable to diabetic postmenopausal women.

Conclusions—Estrogen combined with synthetic progestin significantly improved flow-mediated brachial artery dilator response and reduced inflammation markers in hypertensive and/or overweight women, comparable to estrogen combined with natural progesterone. (Arterioscler Thromb Vasc Biol. 2002;22:1459-1464.)

Key Words: synthetic progestin ■ inflammation ■ hypertension ■ overweight ■ menopause

Vascular inflammation plays an important role in the pathogenesis of atherosclerosis. The vessel wall in patients with coronary heart disease (CHD) or risk factors for CHD may promote inflammation, which may contribute to development and clinical expression of atherosclerosis including myocardial infarction and stroke.1

Prospective cohort surveys suggest that hormone replacement therapy (HRT) decreases the risk of coronary artery disease in relatively young and healthy postmenopausal women.2,3 In contrast, two recent, randomized studies for secondary prevention, the Heart and Estrogen/progestin Replacement Study4 and Estrogen Replacement and Atherosclerosis trial,5 reported that there were no significant differences between HRT and placebo groups in postmenopausal women with established coronary artery disease. The effects of synthetic progestin, proinflammatory effects of estrogen, increased age, or multiple risk factors of CHD are theorized as the causes of these negative observations.

Estrogen has both anti-inflammatory and pro-inflammatory effects. Estrogen blocks monocyte/macrophage production of tumor necrosis factor (TNF)-α,6 and was found to reduce expression of the cell adhesion molecules in endothelial cells activated by interleukin-1.7 We and others have found that HRT decreases serum levels of the cell adhesion molecules.8,9 However, Cid et al10 reported that estradiol enhanced expression of these same cell adhesion molecules in endothelial cells activated with TNF-α. Further, estrogen increased C-reactive protein (CRP),8 which stimulates the expression of cell adhesion molecules and monocyte chemoattractant protein (MCP)-1 in endothelial cells.11,12

Meanwhile, the concept that the loss of a healthy endothelium may prevent patients from deriving pronounced cardioprotective benefits from HRT is supported by observational and clinical studies. This concept holds that many of the antiatherogenic and other favorable vascular effects of estrogen are receptor mediated and endothelium dependent. Con-
Effects of HRT on Lipids

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>CEE + MP</th>
<th>CEE + MPA</th>
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<tbody>
<tr>
<td>Lipids, mg/dL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>217 ± 6</td>
<td>204 ± 6†</td>
<td>201 ± 5‡</td>
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<tr>
<td>HDL-C</td>
<td>52 ± 2</td>
<td>57 ± 2‡</td>
<td>55 ± 2*</td>
</tr>
<tr>
<td>LDL-C</td>
<td>133 ± 6</td>
<td>110 ± 5‡</td>
<td>110 ± 6</td>
</tr>
<tr>
<td>LDL-C/HDL-C</td>
<td>2.63 ± 0.13</td>
<td>2.03 ± 0.14‡</td>
<td>2.06 ± 0.14†</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>157 ± 13</td>
<td>175 ± 19</td>
<td>158 ± 16</td>
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Data are expressed as mean ± SEM. HDL-C indicates HDL cholesterol; LDL-C, LDL cholesterol.

*P < 0.05; †P < 0.01; ‡P < 0.001 vs Baseline.

sequently, endothelial injury\(^{13}\) or declines in vascular estrogen receptor (ER) populations\(^{14}\) can diminish the anti-inflammatory, anti-thrombotic, and other cardioprotective benefits of this reproductive hormone. In this regard, we observed that HRT increased brachial artery flow-mediated dilator by more than 100% in healthy postmenopausal women (mean body mass index was 22.1 ± 0.3) compared with baseline.\(^{15}\) In contrast, we observed that compared with placebo, estrogen did not significantly improve the percent flow-mediated dilator response to hyperemia in type 2 diabetic postmenopausal women.\(^{16}\) Furthermore, we recently reported that HRT did not significantly decrease plasminogen activator inhibitor type-1 antigen levels and, rather, tended to increase prothrombin fragment 1 + 2 levels from baseline in 20 hypertensive and/or overweight postmenopausal women,\(^{17}\) which is consistent with the Heart and Estrogen/progestin Replacement Study.

Because hypertension and obesity are associated with endothelial dysfunction, these women may be less likely to realize cardiovascular benefits from estrogen. In this regard, two recent articles reported that HRT did not improve endothelial function in postmenopausal women with risk factors, compared with postmenopausal women without risk factors.\(^{18,19}\) In contrast, the Estrogen in the Prevention of Atherosclerosis Trial reported the opposite.\(^{20}\) Thus, the purpose of this study is to determine 1) whether HRT improves NO bioactivity and reduces serological markers of inflammation potentially affected by NO-potentiating properties in hypertensive and/or overweight postmenopausal women, 2) whether HRT-induced reduction in markers of inflammation is mediated by improvement in NO bioactivity or lipoprotein changes, and 3) the mechanism of CRP and other cytokines regulation because CRP induces the synthesis of cell adhesion molecules and MCP-1 in monocytes and endothelial cells.\(^{11,12}\)

**Methods**

**Study Population and Design**

Thirty-five postmenopausal women (mean ± SEM, 58 ± 1 years) participated in this study, all with plasma 17β-estradiol levels <50 pg/mL and cessation of menses for at least 1 year. No subject had taken any cholesterol-lowering agent, estrogen therapy, antioxidant vitamin supplements, or angiotensin-converting enzyme inhibitors during the preceding 2 months. Baseline 17β-estradiol and lipoprotein levels are shown in the Table. We used the National Heart, Lung, and Blood Institute’s definitions\(^{21}\) for overweight and obesity as the cutoff points, body mass index ≥ 25.0 and ≥ 30.0 kg/m², respectively. We used World Health Organization/International Society of Hypertension definitions\(^{22}\) for hypertension, defined as systolic and diastolic blood pressure ≥ 140 or ≥ 90 mm Hg, respectively. Severe hypertension was excluded. Eight, 5, and 22 women were overweight, hypertensive, and both, respectively. Four of 30 were obese. Mean body mass index was 27.3 ± 0.5. The diagnosis of diabetes was based on a history of diabetes or criteria according to the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus.\(^{23}\) None were diabetic, smoked, or had previous angina. This study was randomized, double-blind, crossover in design. Study participants received micronized progesterone (MP) 100 mg or medroxyprogesterone acetate (MPA) 2.5 mg with conjugated equine estrogen (CEE) 0.625 mg daily during 2 months with the second treatment period initiated on completion of the first treatment period. The study was approved by the Gil Hospital Institute Review Board, and all participants gave written, informed consent.

**Laboratory Assays**

Blood samples for laboratory assays and vascular studies were obtained at approximately 8:00 AM after overnight fasting except hormone replacement, at baseline, and at the end of each treatment period, and the samples were immediately coded so that investigators performing laboratory assays were blinded to subject identity or study sequence. Assays for lipids, plasma E-selectin, intercellular adhesion molecule type-1 (ICAM-1), vascular cell adhesion molecule type-1 (VCAM-1), MCP-1, and TNF-α were performed in duplicate by ELISA (R & D Systems) as previously described.\(^{5,15,16,24}\) In all patients, serum was collected for the measurement of CRP levels, which were determined with an immunonephelometry system according to methods described by the manufacturer (Rate Nephelometry, IMMAGE®, Beckman Coulter). The measurement range is 0.1 to 98 mg/dL. All samples from the same patient (batch samples) were measured in blinded pairs on the same ELISA kit to minimize run-to-run variability.

**Vascular Studies**

Imaging studies of the right brachial artery were performed with an ATL HDI 3000 ultrasound machine equipped with a 10-MHz linear array transducer, based on a previously published technique.\(^{15,16,24}\) All images were transmitted to a personal computer via Ethernet with DICOM format (Digital Imaging and Communication in Medicine) and then saved on the hard disk of personal computer as a BMP format. Arterial diameters were measured with Image Tool 2.0 (University of Texas Health Science Center, San Antonio, Tex). Measurements were performed by two independent investigators (D.K.J. and H.S.K.) blinded to the subjects’ identity and medication status. Measurements of maximum diameter and percent flow-mediated dilation were made in 10 studies selected at random. The interobserver and intraobserver variability for repeated measurement of percent flow-mediated dilation were 0.07 ± 0.27% and 0.15 ± 1.24%, respectively.

**Statistical Analysis**

Data are expressed as mean ± SEM or median (range, 25% to 75%). After testing data for normality, we used the Student paired t test or Wilcoxon signed rank test to compare values at baseline and after each therapy, as reported in the Table. We presumed that the second baseline after the washout was not different from the first baseline, because we determined no carryover effect of CEE and progestogen for 6 to 8 weeks from our previous studies\(^{15,16,24,25}\) and thus, we decided 2 months as treatment period without washout and the second baseline. Indeed, we found no carryover effect in this study (see Results). The effects of the two therapies on vascular function and markers of inflammation relative to baseline values were analyzed by one-way repeated measures ANOVA or Friedman’s
Effects of Therapies on Vasomotor Function

Basal brachial artery diameter and forearm blood flows were similar during the two treatment periods as were the peak brachial artery diameters and forearm blood flows during reactive hyperemia and the percent increase in flow during hyperemia (data not shown). Both therapies significantly improved the percent flow-mediated dilator response to hyperemia relative to baseline measurements (P<0.001 by ANOVA) to a similar degree (P=0.674). The brachial artery dilator response to nitroglycerin between each therapy was not significantly changed from baseline measurements (P=0.269 by ANOVA).

Effects of Therapies on Markers of Inflammation

The therapies decreased plasma levels of E-selectin by 11±18% and 14±18% from baseline values (P<0.001 by ANOVA; Figure 2A) and ICAM-1 levels by 8±16% and 12±25% from baseline values (P=0.021 by ANOVA; Figure 2B). There were no significant differences between each therapy in E-selectin and ICAM-1 levels (P=0.342 and P=0.296, respectively). However, both therapies did not significantly decrease VCAM-1 levels from baseline values (P=0.570 by ANOVA).

CEE+MP and CEE+MPA therapies significantly decreased plasma levels of MCP-1 by 12±19% and 16±19%, respectively, from baseline values (P<0.001 by ANOVA; Figure 3A) and TNF-α levels by 19±31% and 25±32%, respectively, from baseline values (P<0.001 by ANOVA; Figure 3B). However, both therapies did not significantly change serum levels of CRP from 0.13 mg/dL (0.10 to 0.43) to 0.23 mg/dL (0.10 to 0.43) or 0.21 mg/dL (0.10 to 0.39) and fibrinogen from 275±11 mg/dL to 267±12 mg/dL or 275±10 mg/dL (P=0.361 and P=0.724 by ANOVA, respectively). There were no significant differences between each

Results

To assess the possibility of a carryover effect from the initial treatment periods to the next treatment period, we compared the percent changes of 1) the first treatment CEE+MP and the first treatment CEE+MPA after 2 months, 2) the cumulative effect of both therapies after 4 months, 3) the first treatment CEE+MP and the second treatment CEE+MP, and 4) the first treatment CEE+MPA and the second treatment CEE+MPA, relative to baseline values. There were no significant differences in age and baseline values, vascular function (diameter and flow), and markers of inflammation between each group. No significant differences were found in above four comparisons. (data not shown) After 2 months of CEE combined with natural or synthetic progesterone, plasma levels of 17β-estradiol significantly increased from baseline values and to a similar degree (P<0.001 by ANOVA, Table.)
therapy in MCP-1, TNF-α, CRP, or fibrinogen levels ($P=0.077$, $P=0.340$, $P=0.419$, and $P=0.554$, respectively).

There were significant inverse correlations between pretreatment MCP-1 levels and the degree of change in those levels ($r=-0.392$, $P=0.002$) and between pretreatment TNF-α levels and the degree of change in those levels ($r=-0.327$, $P=0.011$) after CEE+MP and CEE+MPA.

To identify a mechanism for the regulation of E-selectin, ICAM-1, MCP-1, or TNF-α levels, we assessed correlations between percent changes of lipoprotein levels or flow-mediated dilation and percent changes of E-selectin, ICAM-1, MCP-1, or TNF-α levels on CEE+MP and CEE+MPA. There were significant inverse correlations between the degree of change in flow-mediated dilation and the degree of change in ICAM-1 levels ($r=-0.366$, $P=0.002$; Figure 4) after CEE+MP and CEE+MPA. Further, to identify a mechanism for the regulation of CRP, E-selectin, ICAM-1, VCAM-1, and MCP-1 levels, suggested by experimental studies, we assessed correlations between absolute levels or percent changes of CRP levels and absolute levels or percent changes of E-selectin, ICAM-1, VCAM-1, or MCP-1 levels on CEE+MP and CEE+MPA. There were no significant correlations (all $r\leq0.155$) after CEE+MP and CEE+MPA.

**Discussion**

We observed that estrogen combined with synthetic progestin significantly improved flow-mediated brachial artery dilator response and reduced inflammation markers in hypertensive and/or overweight women, comparable to estrogen combined with natural progesterone. Herrington et al.$^{27}$ reported that hormone therapy with MPA 2.5 mg combined with CEE 0.625 mg daily significantly improved flow-mediated dilation of brachial artery in postmenopausal women. In contrast, Sorensen and coworkers$^{28}$ reported that cyclical estradiol and norethisterone administered for 2.9 years did not improve endothelial function. Gerhard et al.$^{30}$ demonstrated that progesterone added to estradiol therapy did not significantly attenuate the improvement in flow-mediated dilation that was observed with estradiol administered alone. In the present study, we observed that CEE+MPA increased HDL cholesterol levels less than CEE+MP; nonetheless, both HRTs improved brachial artery endothelium-dependent vasodilatation, which was consistent with other groups.$^{27,29}$ Even myocardial blood flows were similar for women on estrogen alone or estrogen plus a progestogen.$^{19}$

The average magnitude of improvement, 51% to 55%, in hypertensive and/or overweight women was less than 105% to 117% in healthy postmenopausal women and more than 17% in diabetic postmenopausal women reported by our previous studies.$^{15,16}$ Most our participants were mildly hypertensive and/or overweight. The Estrogen in the Prevention of Atherosclerosis Trial subjects were similar to ours regarding demographic characteristics including age and weight. That study demonstrated that the average rate of progression of subclinical atherosclerosis was slower in $17\beta$-estradiol users compared with non-users.$^{20}$ Previous work had established a link between endothelial injury or lack of expression of the $ER\alpha$ gene and atherosclerosis.$^{13,14}$ Indeed, a recent ex vivo study conducted by Post and colleagues$^{30}$ demonstrated that the promoter region of the $ER\alpha$ gene exhibited age-related rises in methylation and, hence, inactivation. In addition, endothelial cells explanted from coronary atheromata in patients displayed significant increases in $ER\alpha$ gene methylation as compared with grossly normal segments of the proximal aorta. Of interest, Williams and coworkers$^{31}$ observed that aging inhibited $E_2$-effects on both smooth muscle- and endothelium-mediated vascular reactivity, and furthermore, impaired $E_2$-mediated vascular reactivity may be associated with aging effects on $ER\beta$ function. This observation was confirmed by another preliminary study.$^{32}$ One recent article observed that HRT did not improve endothelium-dependent vasodilatation in women more than 80 years old.$^{18}$

To gain insight as to mechanisms of potential vasculoprotective effects of HRT, we measured markers of inflammation. Both HRTs showed average percent changes of E-selectin ($-11\%$ to $-14\%$), ICAM-1 ($-8\%$ to $-12\%$), and MCP-1 ($-12\%$ to $-16\%$) in hypertensive and/or overweight postmenopausal women and E-selectin ($-13\%$ to $-21\%$), ICAM-1 ($-8\%$ to $-8\%$), and MCP-1 ($-7\%$ to $-13\%$) in healthy postmenopausal women, and E-selectin (+1%), ICAM-1 (+8%), and MCP-1 (+4%) in diabetic postmenopausal women by our previous studies.$^{16,17}$ The changes in E-selectin, ICAM-1, and MCP-1 levels in hypertensive...
and/or overweight postmenopausal women were comparable to healthy postmenopausal women, but not comparable to diabetic postmenopausal women. Of note, there were no significant differences between CEE + MPA and CEE + MP regarding these effects in the present study, comparable to healthy postmenopausal women using the same regimen, dosages, and duration.15

As to the clinical relevance of MCP-1, restenotic patients had statistically significant (P<0.0001) elevated levels of MCP-1 compared with nonrestenotic patients after coronary angioplasty.33 and stable and unstable angina patients had statistically significant (P<0.001) elevated levels of MCP-1 compared with controls, particularly higher levels in unstable angina than in stable angina.34 Ridker et al35 observed that plasma levels of TNF-α were persistently elevated among postmyocardial infarct patients at increased risk for recurrent coronary events.

With regard to proinflammatory effects of estrogen, an epidemiologic study36 and a clinical trial8 reported significantly higher levels of CRP in western women using HRT. The Postmenopausal Estrogen/Progestin Interventions study demonstrated that HRT regimens increased CRP levels with a decrease in E-selectin levels.8 Accordingly, the effect of HRT on serum markers of inflammation in postmenopausal women seems to be divergent. In the present study, we observed that oral HRT showed a trend toward an increase in CRP levels in Asian women.

Increases in CRP with oral estrogen therapy may result in part from a direct stimulatory effect on hepatic CRP synthesis or release during the first pass through the liver estrogen absorbed from the intestines, as transdermal application of estrogen does not increase CRP levels.37 Although likely a first pass effect of orally administered estrogen on the hepatic synthesis of CRP, elevated CRP could have deleterious effects on vascular inflammation. CRP induced the synthesis of chemokines and cell adhesion molecule in endothelial cells.11,12 However, we did not observe any correlations between absolute levels or the degree of change in CRP levels and absolute levels or the degree of change in cell adhesion molecules or MCP-1 after HRT. Walsh et al38 also observed no consistent correlations between cytokines and CRP after HRT. Recently, a cross-sectional study reported that forearm blood flow responses to acetylcholine were inversely correlated with CRP serum levels, suggesting an independent predictor of a blunted endothelial vasodilator capacity in patients with coronary artery disease.39 However, we did not observe any correlations between flow-mediated dilation response of brachial artery to reactive hyperemia and CRP serum levels on CEE+MP and CEE+MPA (-0.047≤r≤0.502).

Plasma levels of inflammatory markers were increased and correlated with the extent of disease in patients with atherosclerosis of the coronary and peripheral arteries.40 Hingorani et al41 demonstrated that acute systemic inflammation with Salmonella typhi vaccine impaired endothelium-dependent dilation in humans. Of interest, Raza et al42 reported flow-mediated dilation was significantly impaired in adults with primary systemic necrotizing vasculitis. Further, suppression of inflammation restored and normalized impaired endothelial function in these patients. We observed significant inverse correlations between the degree of change in ICAM-1 levels and the degree of change in flow-mediated dilation after HRT.

There are several limitations in the present study. We did not have a placebo group and an estrogen-alone group. It is possible that each of the progestagens tested blunted the effects of estrogen alone. We did not directly compare the effects of HRT in mild hypertensive and/or overweight postmenopausal women and type II diabetes, and thus, another prospective study should be conducted to obtain the concrete conclusion.

In summary, we observed that estrogen combined with synthetic progestin significantly improved flow-mediated brachial artery dilator response and reduced inflammation markers in hypertensive and/or overweight women, comparable to estrogen combined with natural progesterone. The potential benefit of HRT, however, should be tested in prospective clinical trials.

Acknowledgments

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