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

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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-
vitamin supplements, or angiotensin-converting enzyme inhibitors

substantially, endothelial injury or declines in vascular estrogen receptor (ER) populations can diminish the anti-inflammatory, antithrombotic, 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. 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. 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, 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. In contrast, the Estrogen in the Prevention of Atherosclerosis Trial reported the opposite. 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.

Methods

Study Population and Design

Twenty hypertensive and/or overweight postmenopausal women, 17 of whom had hypertension, 17 had diabetes mellitus, 14 had hypertension and diabetes, and 13 had the combination of hypertension, diabetes, and obesity. Two of 20 women 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. 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. 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® for Windows version 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 maximum diameter were 0.004 ± 0.039 mm and 0.005 ± 0.089 mm, respectively. The interobserver and intraobserver variability for repeated measurement of percent flow-mediated dilation were 0.07 ± 1.27% 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 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
repeated ANOVA on ranks. After demonstration of significant differences among therapies by ANOVA, post hoc comparisons between treatment pairs were made by use of the Student-Newman-Keuls multiple comparison procedures. Pearson correlation coefficient analysis was used to assess associations between measured parameters. We calculated that 30 subjects would provide 80% power for detecting difference of absolute increase, \( \geq 2.1\% \) flow-mediated dilation of the brachial artery between CEE+MP and CEE+MPA, with \( \alpha=0.05 \) based on our previous studies\(^{15,24} \) and others.\(^26\) The comparison of endothelium-dependent dilation among the two treatment schemes was prospectively designated as the primary end point. All other comparisons were considered secondary. Therefore, probability values less than the Bonferroni-adjusted \( \alpha \) of 0.05/7=0.007 were deemed as statistically significant for the secondary end points.

**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 progestogen, plasma levels of 17β-estradiol significantly increased from baseline values and to a similar degree (\( P<0.001 \) by ANOVA, Table.)

**Effects of Therapies on Lipids**

The effects of therapies on lipids are shown in the Table. CEE+MP and CEE+MPA therapies significantly decreased total cholesterol by 5\( \pm 1\% \) and 7\( \pm 1\% \), respectively from baseline values (\( P<0.001 \) by ANOVA) and LDL cholesterol levels by 15\( \pm 20\% \) and 16\( \pm 20\% \), respectively from baseline values (\( P<0.001 \) by ANOVA). CEE+MP and CEE+MPA therapies significantly increased HDL cholesterol levels by 10\( \pm 15\% \) and 8\( \pm 18\% \), respectively from baseline values (\( P=0.002 \) by ANOVA). Thus, both therapies significantly decreased the ratio of LDL to HDL cholesterol levels by 22\( \pm 20\% \) and 20\( \pm 22\% \), respectively from baseline values (\( P<0.001 \) by ANOVA). However, there were no significant differences in lipoprotein levels between each therapy.

**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, Figure 1) by 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).

**Figure 1.** Flow-mediated dilatation before therapy (Baseline) and after administration of CEE+MP or CEE+MPA. 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. SEM is identified by the bars.

**Effects of Therapies on Markers of Inflammation**

The therapies decreased plasma levels of E-selectin by 11\( \pm 18\% \) and 14\( \pm 18\% \) from baseline values (\( P<0.001 \) by ANOVA; Figure 2A) and ICAM-1 levels by 8\( \pm 16\% \) and 12\( \pm 25\% \) 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\( \pm 19\% \) and 16\( \pm 19\% \), respectively, from baseline values (\( P<0.001 \) by ANOVA; Figure 3A) and TNF-α levels by 19\( \pm 31\% \) and 25\( \pm 32\% \), 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\( \pm 11\) mg/dL to 267\( \pm 12\) mg/dL or 275\( \pm 10\) mg/dL (\( P=0.361 \) and \( P=0.724 \) by ANOVA, respectively). There were no significant differences between each therapy.
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.\(^\text{27}\) 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\(^\text{28}\) reported that cyclic estradiol and norethisterone administered for 2.9 years did not improve endothelial function. Gerhard et al.\(^\text{29}\) demonstrated that progestosterone 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.\(^\text{27,29}\)

Even myocardial blood flows were similar for women on estrogen alone or estrogen plus a progestogen.\(^\text{19}\)

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.\(^\text{15,16}\) 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 17β-estradiol users compared with non-users.\(^\text{20}\) Previous work had established a link between endothelial injury or lack of expression of the ER gene and atherosclerosis.\(^\text{13,14}\) Indeed, a recent ex vivo study conducted by Post and colleagues\(^\text{30}\) 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 atheromatous 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\(^\text{31}\) observed that aging inhibited E2 effects on both smooth muscle- and endothelium-mediated vascular reactivity, and furthermore, impaired E2-mediated vascular reactivity may be associated with aging effects on ER-\(\beta\) function. This observation was confirmed by another preliminary study.\(^\text{32}\) One recent article observed that HRT did not improve endothelium-dependent vasodilation in women more than 80 years old.\(^\text{18}\)

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.\(^\text{16,17}\) 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.9 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.

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