Statin Attenuates Increase in C-Reactive Protein During Estrogen Replacement Therapy in Postmenopausal Women

Kwang Kon Koh, MD, PhD; William H. Schenke, BA; Myron A. Waclawiw, PhD; Gyorgy Csako, MD; Richard O. Cannon III, MD

Background—HMG-CoA reductase inhibitor (statin) therapy reduces cardiovascular risk, mechanisms of which may include diminished arterial inflammation, as suggested by reduction in levels of C-reactive protein (CRP). Because oral estrogens increase CRP in postmenopausal women, with potential inflammatory and thrombotic consequences that could compromise any benefit to cardiovascular risk, we determined whether the coadministration of a statin might modify the estrogenic effect on CRP.

Methods and Results—In a double-blind, 3-period crossover study, 28 postmenopausal women (average LDL cholesterol 163±36 mg/dL) were randomly assigned to daily conjugated equine estrogens (CEEs) 0.625 mg, simvastatin 10 mg, or their combination for 6 weeks, with each treatment period separated by 6 weeks. CEEs increased median CRP levels from 0.27 to 0.46 mg/dL, simvastatin decreased CRP from 0.29 to 0.28 mg/dL, and the therapies combined increased CRP from 0.28 to 0.36 mg/dL (all P≤0.02 versus respective baseline values). Post hoc testing showed that the 29% increase in CRP on the combination of CEEs with simvastatin was significantly less than the 70% increase in CRP on CEEs alone (P<0.05). The effect of combination therapy on CRP levels did not correlate with baseline CRP or with baseline or treatment-induced changes in levels of interleukin-6, lipoproteins, or flow-mediated dilation of the brachial artery as a measure of nitric oxide bioactivity.

Conclusions—The combination of statin with estrogen may attenuate the potential harmful effects of estrogen therapy in postmenopausal women and maximize any benefit to cardiovascular risk. (Circulation. 2002;105:1531-1533.)

Key Words: hormones ■ women ■ inflammation ■ endothelium ■ hypercholesterolemia

Considerable pathological and experimental evidence indicates that inflammation within large arteries contributes importantly to the development, progression, and clinical expression of atherosclerosis.1 In cohort studies of healthy men and women, serum levels of C-reactive protein (CRP), a marker of inflammation, were positively correlated with cardiovascular risk.2–4 CRP may be released from the liver on stimulation by cytokines such as interleukin-6 (IL-6)5 and from macrophages and smooth muscle cells of atherosclerotic plaques.6 In experimental preparations, CRP induces the synthesis of cytokines, cell adhesion molecules, and tissue factor in monocytes and endothelial cells7,8 and may contribute further to atherogenesis by facilitating uptake of LDL by macrophages, thus accelerating foam-cell formation.9

Prospective cohort surveys suggest that hormone replacement therapy decreases the risk of coronary artery disease in postmenopausal women.10 with potential benefit supported by multiple reports of favorable biological effects of estrogen on lipoproteins, fibrinolysis, and vascular function.11 Several clinical trials, however, have reported an increased incidence of cardiovascular events after initiation of hormone therapy.12–14 A mechanism for the adverse effects of estrogen may be potentiation of vascular inflammation and thrombosis by increased CRP in serum associated with oral hormone therapy in postmenopausal women.15 In contrast, HMG-CoA reductase inhibitor (statin) therapy reduces cardiovascular risk, the mechanism of which may include diminished arterial inflammation, as suggested by a reduction in levels of CRP in serum that appear to be independent of a reduction in LDL cholesterol levels.16,17 Because hormone therapy increases CRP in postmenopausal women, which could compromise any benefit to cardiovascular risk, we determined whether the addition of statin might modify the estrogenic effect on CRP.

Methods

Study Population and Design
Twenty-eight healthy postmenopausal women (age 57±1 years) with mild to moderate elevation of LDL cholesterol (average 163±7 mg/dL) participated in a randomized, double-blind, 3-period crossover trial. Data on the primary end point of this study (brachial artery...
endothelium-dependent reactivity) as well as levels of hormones, lipoproteins, and cell adhesion molecules in blood have been reported previously.10–13 The study participants received conjugated equine estrogens (CEE) 0.625 mg each morning and placebo each night, placebo each morning and simvastatin 10 mg each night, or a combination of the 2 daily therapies for each of three 6-week treatment periods, with 6 weeks of washout between treatment periods. The National Heart, Lung, and Blood Institute Review Board approved the study, and all participants gave written, informed consent.

**Laboratory Assays**

CRP and IL-6 assays were performed on serum samples from this study obtained at the beginning and end of each treatment period, coded to maintain blinding, and frozen at −70°C. Samples were processed as 1 batch for CRP using a high-sensitivity (0.01 mg/dL), 2-site chemiluminescent enzyme immunometric assay (Immulite 2000, DPC). The interassay coefficients of variation were 5.1% and 5.7% at mean values of 12.14 and 0.86 mg/dL, respectively, and the intra-assay coefficients of variation were 2.2%, 5.2%, and 8.1% at mean values of 0.66, 0.24, and 0.04 mg/dL, respectively. IL-6 was assayed in triplicate by ELISA (R&D Systems), with an intra-assay coefficient of variation <4%.

**Statistical Analysis**

Because CRP and IL-6 levels were skewed in our patient population, the Wilcoxon signed-rank test was used to compare median values before and after each treatment and the relative changes in values in response to treatment. The effects of CEEs, simvastatin, and CEEs combined with simvastatin on CRP and on IL-6 were analyzed by a nonparametric repeated-measures ANOVA (Friedman test). Post hoc comparisons between different treatment pairs were made with the Wilcoxon signed-rank test. Spearman’s correlation coefficient was used to determine correlations between data sets when either or both sets showed a skewed distribution.

**Results**

After 6 weeks of treatment, CEEs increased median CRP levels by 70%, simvastatin decreased CRP by 4%, and the therapies combined increased CRP by 29% (Figure 1), with significant differences in the changes in CRP levels among these therapies (P < 0.001 by ANOVA). Post hoc testing showed that the increase in CRP on the combination of CEEs with simvastatin was significantly less than the increase in CRP on CEEs alone (P < 0.05). The reduction in CRP levels with simvastatin correlated inversely with the respective baseline CRP values (r = −0.539, P = 0.003). The effects of CEEs on CRP did not correlate with respective baseline CRP values, whether administered alone (r = −0.017, P = 0.932) or combined with simvastatin (r = 0.327, P = 0.089). Treatment-induced changes in CRP with CEEs, simvastatin, or CEEs combined with simvastatin did not correlate with changes in LDL cholesterol or HDL cholesterol (all r values between −0.250 and 0.274) or with changes in flow-mediated dilation of the brachial artery as a measure of nitric oxide bioactivity (all r values between 0.046 and 0.272) from respective pretreatment baseline values.

Modest to weak correlations were present between CRP and IL-6 at baseline before treatment with CEEs (r = 0.435, P = 0.020), simvastatin (r = 0.221, P = 0.258), and CEEs combined with simvastatin (r = 0.490, P = 0.008). Despite increasing CRP, CEEs did not change median levels of IL-6 (from 1.76 pg/mL at baseline to 1.67 pg/mL, P = 0.601). Simvastatin decreased median levels of IL-6 from 1.91 to 1.82 pg/mL (P = 0.028). The combination of CEEs and simvastatin reduced median levels of IL-6 from 1.81 to 1.58 pg/mL, although this did not achieve statistical significance (P = 0.046). There was no correlation between treatment-induced changes in CRP and changes in IL-6 (all r values between −0.197 and 0.165).

**Discussion**

Consistent with data from the Postmenopausal Estrogen/progestin Interventions (PEPI) trial,15 we found that oral estrogen increases levels of CRP in serum. Although IL-6 has been proposed to be a major cytokine stimulus for CRP synthesis by the liver,5,19 we found no correlation between changes in CRP and IL-6 levels in our study cohort. Walsh and coworkers20 similarly reported that the increase in CRP with oral estrogen may be independent of changes in IL-6. In this regard, Herrington et al21 found that oral CEEs significantly increased IL-6 levels only in obese women, whereas CRP was significantly increased in both obese and lean women. Instead, 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, because transdermal application of estrogen does not increase CRP levels.22 Like other studies,16,17 ours found that a statin preparation reduced CRP levels in postmenopausal women independently of changes in lipoprotein levels. The novel finding in our study is that the addition of statin to estrogen administered to postmenopausal women significantly attenuated the increase in CRP levels observed when estrogen was administered alone. This effect of statin therapy was independent of changes in lipoprotein levels or improvement in brachial artery endothelium-dependent reactivity.

Our findings may have significant implications in the use of hormone therapy in postmenopausal women by reducing the potentially adverse effects on inflammation and thrombosis that may result from increasing CRP to the magnitude observed with estrogen alone. It is possible that if a higher dose of simvastatin or a more potent statin had been used in our study, an even greater attenuation of the estrogenic effect on CRP might have been achieved. Statins are reported to
have anti-inflammatory effects in experimental preparations independent of cholesterol lowering\textsuperscript{23–25} and thus may be useful as an adjunct to hormone replacement therapy even when cholesterol levels fall within National Cholesterol Education Program guidelines. The potential benefit of this combination therapy, however, should be tested in prospective clinical trials.

References

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