Effect of Medroxyprogesterone Acetate on Vascular Inflammatory Markers in Postmenopausal Women Receiving Estrogen

Akihiko Wakatsuki, MD; Yuji Okatani, MD; Nobuo Ikenoue, MD; Takao Fukaya, MD

**Background**—Estrogen increases C-reactive protein (CRP) in postmenopausal women. Estrogen also decreases cell adhesion molecules, whereas elevated CRP stimulates the expression of cell adhesion molecules. Because androgens have antiinflammatory effects, androgenic progestins such as medroxyprogesterone acetate (MPA) may inhibit proinflammatory effects of estrogen. We investigated the effects of MPA on estrogen-induced changes in acute inflammatory proteins and cell adhesion molecules in postmenopausal women.

**Methods and Results**—Postmenopausal women were treated daily with conjugated equine estrogen (CEE, 0.625 mg), CEE plus MPA 2.5 mg, or CEE plus MPA 5.0 mg for 3 months. CEE significantly increased CRP concentrations by 320.1 ± 210.2% (P < 0.05). The addition of MPA to CEE, however, inhibited the increase in CRP in a concentration-dependent manner (MPA 2.5 mg, 169.8 ± 66.9%, P < 0.05; MPA 5 mg, 55.0 ± 30.4%, not significant). Similarly, CEE increased amyloid A protein concentrations, whereas MPA reversed this effect. Interleukin-6 concentration did not change significantly in any treatment group. CEE alone significantly decreased the concentration of E-selectin, but the concentrations of intercellular adhesion molecule and vascular cellular adhesion molecule did not change significantly. The addition of MPA tended to decrease the levels of cell adhesion molecules, and use of 5.0 mg MPA showed significant decreases in all cell-adhesion molecule concentrations.

**Conclusions**—Concurrent MPA administration may attenuate estrogen’s proinflammatory effect. Because MPA in combination with CEE decreased cell adhesion molecule concentrations, the anti-inflammatory effect of MPA may actually be responsible for the favorable effect of estrogen-progestogen combinations on cell adhesion molecules in postmenopausal women. (Circulation. 2002;105:1436-1439.)

**Key Words:** cell adhesion molecules ■ hormones ■ inflammation ■ lipoproteins ■ women

Vascular inflammation has been considered an important part of the pathogenesis of atherosclerosis. Increased C-reactive protein (CRP), a circulating marker of inflammation, is an independent risk factor for cardiovascular disease in healthy postmenopausal women.1 Myocardial events and ischemic stroke can be predicted by elevated CRP.2 Postmenopausal estrogen replacement therapy (ERT) has beneficial effects on plasma lipids, low-density lipoprotein (LDL) oxidation, and hemostatic factors. In addition, estrogen favorably affects endothelial function by increasing expression of endothelial NO synthase,3 leading to increased endothelium-dependent vasodilation. Long-term postmenopausal ERT significantly reduced mortality from congestive heart disease (CHD) and other cardiovascular disease.4 In contrast, the Heart and Estrogen/Progestin Replacement Study (HERS) demonstrated that estrogen and progestin therapy did not reduce the overall rate of coronary events in postmenopausal women with established coronary disease.5 Estrogens has been reported to elevate plasma concentrations of CRP.6,7 Because elevated CRP may be associated with plaque destabilization and rupture, a proinflammatory effect of estrogen might explain the increased number of cardiovascular events demonstrated in women with existing cardiovascular disease during the first year of the HERS trial. ERT has been reported to decrease the concentrations of cell adhesion molecules.8 However, a recent study has demonstrated that elevated CRP induces expression of human endothelial cell–derived adhesion molecules, such as vascular cell adhesion molecule (VCAM)-1, intercellular adhesion molecule (ICAM)-1, and E-selectin.9 Accordingly, it is likely to be possible that estrogen-induced increase in CRP may offset the favorable effect of estrogen on cell adhesion molecules.

Medroxyprogesterone acetate (MPA) is commonly used as a progestin combined with estrogen to reduce the risks for endometrial hyperplasia and carcinoma in postmenopausal women who have not undergone hysterectomy10 and was the hormone combination used in the HERS trial. Parkar et al11 have demonstrated that androgens have anti-inflammatory

Received November 29, 2001; revision received January 16, 2002; accepted January 18, 2002.
From the Department of Obstetrics and Gynecology, Kochi Medical School, Kochi, Japan.
Correspondence to Akihiko Wakatsuki, MD, Department of Obstetrics and Gynecology, Kochi Medical School, Oko cho, Nankoku, Kochi, Japan, 783-8505, E-mail wakatuki@kochi-ms.ac.jp
© 2002 American Heart Association, Inc.
Circulation is available at http://www.circulationaha.org DOI: 10.1161/hc1202.105945

1436
effects. Because synthetic progestins such as MPA also have androgenic effects, concurrent MPA administration may inhibit the proinflammatory effect of estrogen.

In the present study, we investigated the effects of clinically used doses of MPA combined with estrogen on vascular inflammatory markers and cell adhesion molecules in postmenopausal women.

Methods

Subjects

We studied 45 naturally postmenopausal Japanese women with the following characteristics: mean age, 52 years (range, 40 to 60); mean body mass index (BMI), 21.8 kg/m² (range, 17.7 to 23.5); and mean menopausal interval, 3.8 years (range, 2 to 7). No subject had undergone ovariectomy, and none of the subjects had menstruated for at least 1 year. None of the subjects smoked, used caffeine or alcohol, or had a history of hypertension, thyroid disease, liver disease, diabetes mellitus, or cardiovascular disease, and none were presently taking any medication known to influence lipoprotein metabolism. None of the subjects received ERT before the study. No subject underwent exercise or dietary therapy before the study. Written informed consent was obtained from each subject before participation. The ethics committee of Kochi Medical School approved the study design. Patients were randomly assigned in open, parallel-group fashion to 3 treatment groups. For 3 months, subjects in the conjugated equine estrogen (CEE) group received 0.625 mg of oral CEE daily (n=15), whereas those in the CEE+MPA 2.5 group received CEE 0.625 mg plus MPA 2.5 mg daily (n=15) and those in the CEE+MPA 5.0 group received CEE 0.625 mg plus MPA 5.0 mg daily (n=15). Endometrial biopsies were performed before and after treatment in all subjects.

Laboratory Assays

Before and at completion of treatments, venous blood samples were obtained between 8:00 and 10:00 AM after a 12-hour fast. The concentrations of total cholesterol and triglyceride were measured by enzymatic methods as previously described. The concentration of high-density lipoprotein (HDL) cholesterol was determined by similar methods after apolipoprotein B–containing lipoproteins had been precipitated with sodium phosphotungstate in the presence of magnesium chloride. Using ultracentrifugation according to the method of Havel et al., LDL (density, 1.019 to 1.063) was fractionated from plasma samples (<24 hours). Concentrations of LDL cholesterol were assayed enzymatically. The concentrations of estrone (E1), estradiol (E2), and progesterone were measured using radioimmunoassays.

The concentrations of high sensitive (hs) CRP was analyzed using the Behring Latex-Enhanced CRP assay on the Behring NephoMeter Analyzer System (Dade Behring). Serum amyloid A protein (SAA) concentrations were determined by a latex agglutination turbidimetric immunoassay. Assays for ICAM-1, VCAM-1, and E-selectin were analyzed with an ELISA kit. The concentrations of interleukin-6 (IL-6) were measured by chemiluminescent enzyme immunoassay.

Statistical Analysis

Data are expressed as mean±SEM. Differences between the 3 groups in baseline subject characteristics, lipid and hormone concentrations, inflammatory markers, and cell adhesion molecules were analyzed by one-way ANOVA. Treatment-induced changes in these parameters were analyzed by Student’s paired t test. A value of P<0.05 was accepted as statistically significant.

Results

General Physiological Characteristics

Histological analysis of endometrial biopsy specimens did not demonstrate hyperplasia in any patient either before or after treatment. ANOVA between the 3 groups showed no significant differences in age, BMI, baseline concentrations of lipids, hormone concentrations, inflammatory markers, and cell adhesion molecules. BMI did not change significantly during the study period in the 3 treatment groups.

Lipid and Hormone Concentrations

The total and LDL cholesterol concentrations were significantly reduced after treatment in all 3 groups. The HDL cholesterol and triglyceride concentrations were significantly increased in the CEE and CEE+MPA 2.5 groups compared with baseline. These concentrations, however, did not increase significantly in the CEE+MPA 5.0 group. The concentrations of E1 and E2 were significantly increased by all treatments, whereas progesterone concentration did not change significantly (Table 1).

Inflammatory Markers and Cell Adhesion Molecules

Concentrations of hs-CRP and SAA were significantly increased in both CEE and CEE+MPA 2.5 groups, whereas no significant change was observed in the CEE+MPA 5.0 group. The concentration of IL-6 did not change in any treatment group (Table 2). Concentration of E-selectin was significantly decreased in the CEE group, but concentrations of VCAM-1 and ICAM-1 did not change significantly. Concentrations of ICAM-1 and E-selectin, but not VCAM-1, were significantly reduced in the CEE+MPA 2.5 group. In the CEE+MPA 5.0 group, concentrations of ICAM-1, VCAM-1, and E-selectin were all significantly reduced (Table 3).

Discussion

Lipids

CEE alone or in combination with MPA reduced total and LDL cholesterol concentrations to a similar degree, indicating
that MPA may not alter estrogen-induced reduction in LDL cholesterol. The addition of MPA, however, significantly reduced CEE-induced increases in the concentrations of plasma HDL cholesterol in a concentration-dependent manner. These results indicate that MPA may offset estrogen’s favorable effects on HDL cholesterol, consistent with findings of the Postmenopausal Estrogen Progesterone Intervention trial. In contrast, MPA reduced estrogen-induced increase in plasma triglycerides. Progestin has been reported to decrease the plasma concentrations of triglyceride and triacylglycerol-rich lipoproteins in subjects with hypertriglyceridemia.

Markers of Inflammation

The present study demonstrated that CEE increased acute inflammatory markers such as CRP and SAA. Because elevated CRP is a risk factor for future cardiovascular events, estrogen’s proinflammatory effect may increase plaque vulnerability and may lead to the increased cardiovascular events. Adding MPA, however, blunted the CEE-induced increase in these proteins in a concentration-dependent manner. These findings indicate that MPA may inhibit a proinflammatory effect of estrogen. Most studies have demonstrated that HRT elevates plasma concentrations of CRP. However, in one clinical trial where transdermal estradiol was combined with norethisterone, a 19-nortestosterone progestogen with androgenic properties, CRP estradiol was combined with norethisterone, a 19-

Table 2: Plasma Concentrations of Inflammatory Markers

<table>
<thead>
<tr>
<th></th>
<th>CEE</th>
<th>CEE + MPA 2.5</th>
<th>CEE + MPA 5.0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>hs-CRP, ng/mL</td>
<td>579.8 ± 136.6</td>
<td>1238.4 ± 328.9*</td>
<td>532.9 ± 165.6</td>
</tr>
<tr>
<td>SAA, µg/mL</td>
<td>5.80 ± 1.02</td>
<td>6.85 ± 0.90*</td>
<td>5.10 ± 0.76</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>1.52 ± 0.29</td>
<td>1.35 ± 0.23</td>
<td>1.26 ± 0.22</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. *P < 0.05 vs pretreatment.

Also have androgenic effects, MPA may reduce CRP concentration in a similar manner.

Inflammatory stimuli induce IL-6 production that in turn stimulates hepatic secretion of CRP. In the present study, plasma levels of IL-6 did not change in any treatment group. Therefore, it is unlikely that IL-6 has a principal role in estrogen-induced increases in CRP. Because estrogen directly passes hepatic circulation when estrogen is administered orally, estrogen’s hepatic stimulation may result in an increased production of CRP. Although smoking and obesity are factors known to influence CRP levels, none of the subjects smoked, and BMI did not change during the study period in any treatment group. Additional studies are needed to clarify the mechanism behind estrogen-induced increases in CRP.

Although CRP and SAA are markers of the acute inflammatory response, CRP may have a direct role in the pathogenesis in the development of atherosclerosis. Specially, CRP activates the complement cascade and has been colocalized with complement components in atherosclerotic lesions of human coronary arteries. CRP stimulates the release of inflammatory cytokines and induces tissue factor expression from human monocytes. In addition, a recent study has demonstrated that CRP induces the expression of cell adhesion molecules.

Cell Adhesion Molecules

Cell adhesion molecules, once expressed on the surfaces of endothelial cell or leukocytes after cytokine stimulation, are shed from the surface within 24 hours. Plasma levels of cell adhesion molecules are associated with the extent of atherosclerosis and the occurrence of coronary events. Concentrations of cell adhesion molecules increase after menopause, whereas HRT has been reported to decrease plasma levels of cell adhesion molecules, which may lead to the reduction in the risk of CHD in postmenopausal women. In the present study, however, CEE alone did not decrease ICAM-1 and VCAM-1 concentrations. Because CRP induces adhesion molecule expression, favorable effects of estrogen on cell adhesion molecules could be offset by estrogen-induced increases in CRP. Addition of MPA tended to decrease the

Table 3: Concentrations of Cell Adhesion Molecules

<table>
<thead>
<tr>
<th></th>
<th>CEE</th>
<th>CEE + MPA 2.5</th>
<th>CEE + MPA 5.0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>VCAM-1, ng/mL</td>
<td>530.4 ± 24.8</td>
<td>521.1 ± 26.7</td>
<td>511.4 ± 20.8</td>
</tr>
<tr>
<td>ICAM-1, ng/mL</td>
<td>216.8 ± 16.5</td>
<td>213.0 ± 14.5</td>
<td>224.5 ± 19.5</td>
</tr>
<tr>
<td>E-selectin, ng/mL</td>
<td>49.2 ± 4.9</td>
<td>44.9 ± 4.0*</td>
<td>47.3 ± 9.6</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. *P < 0.05, †P < 0.01 vs pretreatment.
concentrations of cell adhesion molecule. MPA 5.0 mg combined with CEE significantly reduced all concentrations of ICAM-1, VCAM-1, and E-selectin. Thus, MPA-induced reduction in CRP seems to preserve estrogen’s favorable effect on cell adhesion molecules. According to Otsuki et al., progestin but not MPA inhibits VCAM-1 expression in human vascular endothelial cells. This indicates that MPA’s anti-inflammatory effect, but not its direct effect, may decrease cell adhesion molecule concentrations.

Study Limitations
We investigated changes in the plasma concentrations of cell adhesion molecules in the present study. Although the clinical relevance of cell adhesion molecules has been supported by several studies, biological function of cell adhesion molecules in sera remains unclear.

Plasma concentrations of progesterone did not change significantly in each group, because serum concentrations of physiologically progestrone may not be affected by low doses of MPA. Plasma concentrations of 400 to 800 pg/mL of MPA are reported to show effects in the reproductive system in women as well as in monkeys. Because plasma concentrations of MPA were not measured in the present study, we could not determine a cutoff level where MPA begins to offset the proinflammatory effect of estrogen.

Conclusions
The present study demonstrates that although estrogen increased CRP and SAA concentrations, MPA attenuates the proinflammatory effect of estrogen. Anti-inflammatory effects of MPA may inhibit plaque rupture in women with CHD and also preserve estrogen’s favorable effect on cell adhesion molecules. However, addition of MPA 2.5 mg, as in the HERS trial, did not prevent estrogen-induced increases in CRP and SAA in postmenopausal women, indicating that the standard dose of MPA used for continuous combined therapy may not have an anti-inflammatory effect. Addition of increased doses of MPA may be needed to act as an anti-inflammatory agent, but caution should be taken because MPA also has atherogenic effects by impairing endothelial function and reducing HDL cholesterol. Additional studies are needed to investigate whether long-term use of androgenic progestins combined with estrogen can prevent early increases in coronary events in women with established coronary disease, as demonstrated in the HERS trial.

References
Effect of Medroxyprogesterone Acetate on Vascular Inflammatory Markers in Postmenopausal Women Receiving Estrogen
Akihiko Wakatsuki, Yuji Okatani, Nobuo Ikenoue and Takao Fukaya

Circulation. 2002;105:1436-1439: originally published online March 4, 2002;
doi: 10.1161/hc1202.105945
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/105/12/1436

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/