Effect of *Medroxyprogesterone Acetate* Plus *Estradiol* on Endothelium-Dependent Vasodilation in Postmenopausal Women

Hiroaki Kawano, MD, Takeshi Motoyama, MD, Nobutaka Hirai, MD, Toshihiro Yoshimura, MD, Kiyotaka Kugiyama, MD, Hisao Ogawa, MD, Hitoshi Okamura, MD, and Hirofumi Yasue, MD

Estrogen replacement therapy reduces risk of cardiovascular events[1–5] and is required by a great number of postmenopausal women. Because the addition of progestins had been demonstrated to remove the risk of endometrial cancer induced by long-term use of estrogen,[3,6] estrogen plus progestin treatment has become the most utilized therapy today. The effect of progestins, however, still remains controversial. Some investigators have reported that progestin does not affect the risk of coronary artery disease,[1,3–5] whereas others have reported that it increases the risk.[3,7–9] Recently, noninvasive examination of endothelial function has become possible by measuring flow-mediated dilation of the brachial artery during reactive hyperemia after transient occlusion.[10–14] The objective of the present study was to determine the effects of medroxyprogesterone acetate (MPA), a progestin widely used and believed to be highly successful when added to estrogen replacement therapy, on endothelial function in postmenopausal women.

• • •

The present study included 25 postmenopausal women with mild hypercholesterolemia (mean age 54 ± 1 years). Menopause was confirmed by follicle-stimulating hormone (FSH) levels >40 IU/L and absence of menstruation for at least 1 year. All subjects were asymptomatic, normotensive, nondiabetic, and nonsmokers. None had coronary artery disease, congestive heart failure, or any other serious disease. All subjects gave written informed consent before the study, and the study was conducted in accordance with the guidelines approved by the ethics committee at our institution.

All subjects were enrolled in a randomized, double-blind, placebo-controlled study to evaluate the effect of estradiol with and without MPA on endothelial function. Thirteen women were randomized to receive 8 weeks of hormone replacement, which comprised 4 consecutive treatment phases; 2 weeks of 17-β estradiol (2 mg/2 days) (Estraderm TTS, Novartis, Basel, Switzerland) plus placebo corresponding to MPA, 2 weeks of 17-β estradiol plus MPA (2.5 mg/day) (Provera, Pharmacia & Upjohn, Peapack, New Jersey), 2 weeks of 17-β estradiol plus placebo, and 2 weeks of placebo corresponding to 17-β estradiol plus placebo corresponding to MPA. The remaining 12 women received the same treatment in the opposite order. All subjects were studied during fasting state in the morning (8:00 A.M.) before the study began and at the end of each supplementation, as shown in Figure 1.

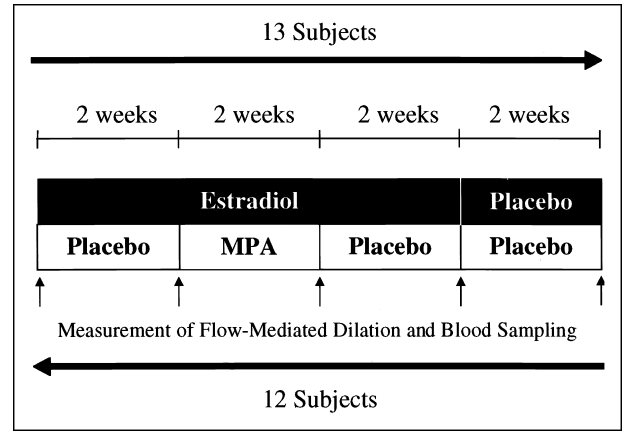

FIGURE 1. Study protocol schematic of the experimental protocol.

Flow-mediated dilation of the brachial artery after transient occlusion was measured by 2 ultrasound technicians. The validity of this method has been confirmed in our previous studies and by investigators.[10–14] Briefly, the diameter of the brachial artery was measured from B-mode ultrasound images using a 7.5-MHz linear array transducer (SSH-160A, Toshiba, Tokyo, Japan). Flow velocity in the brachial artery was measured using a pulsed Doppler signal. The brachial artery was scanned in the antecubital fossa in a longitudinal fashion. Depth and gain settings were optimized at the beginning of the study and were kept constant throughout the recording period. When a satisfactory transducer position was found, the surface of the skin was marked, and the arm position was maintained throughout the study.

Each subject rested for 10 minutes before the first scan. After baseline measurements of the diameter and the flow velocity in the brachial artery, a blood pressure cuff was placed around the forearm and inflated to a pressure of 250 to 300 mm Hg; the cuff was released after 5 minutes. Measurements of the arterial diameter and the flow velocity were continuously performed during cuff inflation and after cuff deflation.

From the Departments of Cardiovascular Medicine, and Obstetrics and Gynecology, Kumamoto University School of Medicine, Kumamoto, Japan. This study was supported in part by Grant-in-Aid for Scientific Research A 12770346 from the Ministry of Education, Science, Sports and Culture, Tokyo, Japan. Dr. Kawano's address is: Department of Cardiovascular Medicine, Kumamoto University School of Medicine, 1-1-1 Honjo, Kumamoto 860-8556, Japan. E-mail: koumei@gpo.kumamoto-u.ac.jp. Manuscript received May 1, 2000; revised manuscript received and accepted July 17, 2000.

0002-9149/01/$–see front matter
PII S0002-9149(00)01329-1

TABLE 1 Hemodynamic and Blood Sampling Data

	Baseline	Estradiol + Placebo	Estradiol + MPA	Estradiol + Placebo	Placebo + Placebo
Heart rate (beats/min)	65 ± 3	67 ± 3	69 ± 3	71 ± 2	63 ± 4
Mean blood pressure (mm/Hg)	104 ± 4	102 ± 2	102 ± 3	102 ± 2	100 ± 3
Baseline diameter (mm)	3 ± 0.15	3 ± 0.09	3 ± 0.10	3 ± 0.15	3 ± 0.13
Baseline flow (ml/min)	161 ± 19	163 ± 16	165 ± 19	169 ± 22	159 ± 15
Increase in flow during reactive hyperemia (%)	271 ± 32	256 ± 25	249 ± 30	260 ± 36	266 ± 24
Increase in diameter after nitroglycerin (%)	15 ± 2	16 ± 2	16 ± 1	15 ± 2	15 ± 2
Total cholesterol (mg/dl)	223 ± 13	218 ± 11	220 ± 11	221 ± 13	221 ± 12
LDL cholesterol (mg/dl)	132 ± 12	128 ± 12	130 ± 12	128 ± 11	130 ± 12
HDL cholesterol (mg/dl)	63 ± 7	63 ± 6	62 ± 6	64 ± 5	62 ± 6
Triglyceride (mg/dl)	126 ± 16	123 ± 16	127 ± 15	125 ± 17	125 ± 16
Estradiol (pg/ml)	15 ± 9	56 ± 13*	52 ± 15*	59 ± 16	15 ± 8
Progesterone (ng/ml)	0.38 ± 0.6	0.34 ± 0.5	0.39 ± 0.6	0.38 ± 0.7	0.37 ± 0.2
FSH (pg/ml)	87 ± 9	51 ± 5†	50 ± 6†	54 ± 5†	90 ± 10
LH (pg/ml)	39 ± 10	38 ± 12	35 ± 11	40 ± 11	39 ± 11

*p <0.01 versus baseline and placebo + placebo; †p <0.01 versus baseline and placebo + placebo.
HDL = high density lipoprotein; LDL = low density lipoprotein; LH = luteinizing hormone.

Thereafter, the subjects rested for 15 minutes. After confirming that the arterial diameter and the flow velocity returned to baseline levels, sublingual nitroglycerin (0.3 mg) was administrated, and 3 to 4 minutes later the last measurements were performed.[10–14]

The ultrasound images were recorded on a super VHS videocassette recorder (BR-S601 M, Victor, Tokyo, Japan), and arterial diameter was measured at a fixed distance from an anatomic marker with ultrasonic calipers by 2 independent observers, who were blinded as to which phase of the therapy the images had been acquired. Measurements were taken from the anterior to the posterior interface between the media and adventitia ("m" line) at the end-diastole, incident with the R wave on a continuously recorded electrocardiogram.[10–13,15] The diameters at 4 cardiac cycles were analyzed for each scan, and the measurements were averaged. Diameter measurements for the reactive hyperemia were taken 45 to 90 seconds after the cuff deflation to measure peak diameter. Blood flow was calculated by multiplying the velocity-time integral of the Doppler flow signal by heart rate and the vessel cross-sectional area. Increase in blood flow was calculated by dividing the maximum flow within the first 15 seconds after the cuff deflation by the flow at baseline.[10–14]

In our study, the kappa coefficients of interobserver and intraobserver variability for the repeated measurements of resting arterial diameter were 0.85 ± 0.09 and 0.95 ± 0.04, respectively. In a preliminary study, when these procedures were performed at the same time on 2 separate days in 20 volunteers, the average intrasubject test-retest difference for the measurements of the arterial diameter during the reactive hyperemia was 0.05 ± 0.04 mm.[10,13]

Blood samples were obtained from all study subjects on the study day while they were in the fasting state. Serum levels of estradiol, progesterone, FSH, and luteinizing hormone were measured by a specific immunoradiometric assay.[10,12]

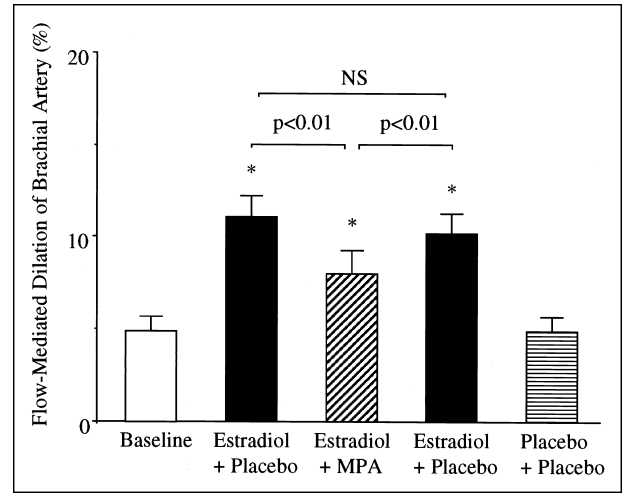

FIGURE 2. Effects of hormone replacement on the flow-mediated dilation of the brachial artery. *p <0.01 versus baseline and placebo + placebo. See text for details. Data are expressed as mean ± SE.

The data were analyzed by analysis of variance. When statistically significant effects were found, the Newman-Keuls test was used to isolate the differences between groups. Statistical significance was defined as $p < 0.05$. All data are expressed as mean ± SEM.

All subjects well tolerated the study. Heart rate, mean blood pressure, lipid profiles, increase in blood flow during the reactive hyperemia, and the nitroglycerin-induced arterial dilation (endothelium-independent vasodilation) did not change throughout the study (Table 1). The active treatments (hormone supplementations) increased estradiol levels and decreased FSH levels.

Flow-mediated vasodilation was increased by the initial estradiol supplementation, as shown in Figure 2 (10.72 ± 0.18%, $p < 0.01$ vs 4.92 ± 0.19% baseline and 4.88 ± 0.18% placebo + placebo). The estradiol plus MPA supplementation also increased the flow-

mediated vasodilation (7.97 ± 0.14%, p <0.01 vs baseline and placebo + placebo). However, the enhancement with estradiol plus MPA was less pronounced than that with estradiol alone (10.72 ± 0.18% vs 7.97 ± 0.14%, p <0.01). After the secondary estradiol supplementation performed in succession to estradiol plus MPA, the flow-mediated vasodilation recovered to the degree of the initial estradiol supplementation (10.50 ± 0.21%, p <0.01 vs baseline, estradiol + MPA and placebo + placebo).

• • •

Many observational studies have demonstrated that estrogen replacement therapy has an effect on lipid profiles and risk of cardiovascular events.[1–5,16] Not only the effect on coronary risk factors, such as lipid profiles, but also the direct effect of estrogen replacement therapy on the vascular endothelium, plays an important role in the prevention of coronary artery disease.[16] Oral estrogen supplementation decreases low density lipoprotein levels and increases high density lipoprotein[1–5,7,16]; however, because the supplementation we performed was of short duration and transdermal, lipid profiles did not change during the therapy. The effect of estradiol therapy, with or without MPA, on vascular reactivity in the present study appears independent of its effect on lipid profiles. In the present study, short-term administration of MPA degraded the improvement of endothelial function of the brachial artery as induced by estrogen replacement. The endothelium-dependent relaxation of the brachial artery correlates with that of coronary artery.[13,14] The results of the present study indicate that the hormone replacement therapy with MPA plus estradiol reduces cardiovascular events or angina in postmenopausal women, which is similar to the results of estradiol alone. However, the effect may be less produced with MPA plus estradiol than estradiol alone.

Some recent studies that questioned the effect of estrogen replacement therapy, most of which utilized MPA, reported that estrogen replacement therapy does not necessarily reduce the prevalence of coronary heart disease.[17,18] Unfortunately, we only examined the short-term effect of estradiol with and without MPA on endothelial function, and the effect of MPA was not strong enough to completely suppress the beneficial effect of estradiol. There may be a difference between the effect of a continuous MPA regimen and that of a cyclical MPA regimen on the endothelial function during estrogen replacement therapy. Further studies are needed to clarify the long-term effect of MPA on the endothelial function in women.

Serum levels of natural progesterone did not change throughout the study, and low doses of MPA have been reported not to elicit any changes in serum natural progesterone concentration in monkeys.[19] Thus, the low dose of MPA (2.5 mg/day) may not affect natural progesterone concentration in women.

In conclusion, MPA, when added to estradiol treatment, diminishes the improvement in endothelium-dependent dilation of the brachial artery that is achieved with estrogen therapy.

1. Witteman JCM, Grobbee DE, Kok FJ, Hofman A, Valkenburg HA. Increased risk of atherosclerosis in women after the menopause. *BMJ* 1989;298:642–644.
2. Samaan SA, Crawford MH. Estrogen and cardiovascular function after menopause. *J Am Coll Cardiol* 1995;26:1403–1410.
3. Belchetz PE. Hormone treatment of postmenopausal women. *N Engl J Med* 1994;330:1062–1071.
4. Stampfer MJ, Colditz GA, Willett WC, Manson JE, Rosner B, Speizer FE, Hennekens CH. Postmenopausal estrogen therapy and cardiovascular disease. *N Engl J Med* 1991;325:756–762.
5. Grodstein F, Stampfer MJ, Manson JE, Colditz GA, Willett WC, Rosner B, Speizer FE, Hennekens CH. Postmenopausal estrogen and progestin use and the risk of cardiovascular disease. *N Engl J Med* 1996;335:435–461.
6. Grady D, Rubin SM, Petitti DB, Fox CS. Hormone therapy to prevent disease and prolong life in postmenopausal women. *Ann Intern Med* 1992;117:1016–1037.
7. Hulley S, Grady D, Bush T, Furberg C, Herrington D, Riggs B, Vittinghoff E. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. *JAMA* 1998;280:605–613.
8. Miller VT, Muesing RA, LaRosa JC, Stoy DB, Phillips EA, Stillman RJ. Effects of conjugated equine estrogen with and without ferent progesterones on lipoproteins, high-density lipoprotein subfractions, and apolipoprotein A-I. *Obstet Gynecol* 1991;77:235–240.
9. Barrett-Connor E, Bush TL. Estrogen and coronary heart disease in women. *JAMA* 1991;265:1861–1867.
10. Kawano H, Motoyama T, Kugiyama K, Hirashima O, Ohgushi M, Fujii H, Ogawa H, Yasue H. Gender difference in improvement of endothelium-dependent vasodilation after estrogen supplementation. *J Am Coll Cardiol* 1997;30:914–919.
11. Gerhard M, Walsh BW, Tawakol A, Haley EA, Creager SJ, Seely EW, Ganz P, Creager MA. Estradiol therapy combined with progesterone and endothelium-dependent vasodilation in postmenopausal women. *Circulation* 1998;98:1158–1163.
12. Kawano H, Motoyama T, Kugiyama K, Hirashima O, Ohgushi M, Yoshimura M, Ogawa H, Okumura K, Yasue H. Menstrual cyclic variation of endothelium-dependent vasodilation of the brachial artery. *Proc Assoc Am Physicians* 1996;108:473–480.
13. Motoyama T, Kawano H, Kugiyama K, Hirashima O, Ohgushi M, Tsunoda R, Moriyama Y, Miyao Y, Yoshimura M, Ogawa H, Yasue H. Vitamin E administration improves impairment of endothelium-dependent vasodilation in patients with coronary spastic angina. *J Am Coll Cardiol* 1998;32:1672–1679.
14. Anderson TJ, Uehata A, Gerhard MD, Meredith IT, Knab S, Delagrange D, Lieberman EH, Ganz P, Creager MA, Yeung AC, Selwyn AP. Close relation of endothelial function in human coronary and peripheral circulations. *J Am Coll Cardiol* 1995;26:1235–1241.
15. Pignoli P, Tremoli E, Poli A, Oreste P, Paoletti R. Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. *Circulation* 1986;74:1399–1406.
16. Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. *N Engl J Med* 1999;340:1801–1811.
17. Barrett-Connor E, Struenkel C. Hormone and heart disease in women. *J Clin Endocrinol Metab* 1999;84:1848–1853.
18. Blumenthal RS, Zacur HA, Reis SE, Post WS. Beyond the null hypothesis—do the HERS results disprove the estrogen/coronary heart disease hypothesis? *Am J Cardiol* 2000;85:1015–1017.
19. Miyagawa K, Rosch J, Stanczyk F, Hermsmeyer K. Medroxyprogesterone interferes with ovarian steroid protection against coronary vasospasm. *Nat Med* 1997;3:324–327.