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ORIGINAL ARTICLE

Medroxyprogesterone Acetate and Progesterone, Used Short Term, Do not Adversely Affect Forearm Reactive Hyperemia in Postmenopausal Women on Estradiol Therapy

Cho MM[a], Hodis HN[b], Mack WJ[b], Roy S[c], Paulson RJ[c], Li Y[c], Stanczyk FZ[c]

[a]Department of Obstetrics, Gynecology, and Women's Health, UMDNJ-New Jersey Medical School, Newark, NJ; [b]Departments of Medicine and Preventive Medicine, Atherosclerosis Research Unit; [c]Department of Obstetrics and Gynecology, USC Keck School of Medicine, Los Angeles, CA , USA

This study aimed at investigating whet-her oral medroxyprogesterone acetate (MPA) or micronized progesterone (P) combined with micronized estradiol (E2) adversely affect endothelial function in postmenopausal women.
Materials and Methods: Randomized, double-blind, crossover trial of MPA or micronized P with oral E2 treatment in menopausal women.
Result: Flow-mediated, endothelium-dependent vasodilation of the brachial artery was not significantly affected by short-term administration of E2 alone or E2 combined with cyclic MPA, or E2 combined with cyclic P.
Conclusion: Short-term oral E2 treatment of postmenopausal women alone or with cyclic oral MPA or oral P did not affect flow-mediated endothelium-dependent vasodilation.

Key Words: Medroxyprogesterone acetate, progesterone, estradiol, forearm reactive hyperemia.

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Introduction

Cardiovascular disease is the leading cause of death in postmenopausal women.[1] The cardioprotective effect of estrogen is controversial. Among the mechanisms thought to be responsible for the cardioprotective effects of estrogen are those directly involving vascular tissue. These include the effects of estrogen on endothelin-derived relaxing factor (nitric oxide), endothelin production, prostacyclin, and thromboxane.[2] An important concern of using unopposed estrogen is the development of endometrial cancer. The addition of a progestogen provides the needed balance to eliminate the unopposed estrogen therapy-associated endometrial cancer.

In general, the natural progestogen, proges-terone (P), and synthetic progestogens are used interchangeably in hormone therapy (HT). However, the biologic effects of these progestogens do not appear to be identical. Estrogen has beneficial effects on blood flow but there is some question as to the negative effect that a progestogen may have on the blood flow. Estrogen plus micronized P results in increases of high-density lipoprotein (HDL) similar to that of unopposed estrogen, but greater than that of estrogen plus medroxyprogesterone acetate (MPA).[3] Studies with non-human primates show that estrogen increases endothelium-dependent

coronary dilation,[4] but MPA diminishes it.[5] In addition, MPA, but not P, increases the risk of coronary vasospasm in rhesus monkeys.[6,7]

In postmenopausal women, arterial production of prostacyclin is significantly decreased in uterine arteries.[8] Administration of estrogen in healthy postmenopausal women selectively potentiates endothelium-dependent vasodilation. In postmenopausal women with risk factors for atherosclerosis and evidence of impaired vascular function, estrogen infusion potentiates both endotheliumdependent and endothelium-independent forearm vasodilation response to acetylcholine.[9] Ethinyl estradiol infusion also decreases basal coronary vasomotor tone (as manifested by increased coronary flow, decreased resistance, and increased epicardial cross-sectional area), and attenuates an abnormal coronary vasomotor response to acetylcholine in postmenopausal women.[10] When postmenopausal women are placed on extended estrogen therapy, the metabolism of prostacyclin and thromboxane A2 is altered, favoring prostacyclin.[11] Limited data exist on the effect of progestogens on vascular reactivity in women.

This study was performed to compare the effects of micronized P and MPA administration on vascular reactivity in estrogen-treated postmenopausal women.

Materials and Methods

Subjects

Twenty postmenopausal women (48-53 years old), who had had their last menstrual period at least 1 year previously and had serum follicle-stimulating hormone (FSH) levels greater than 25 mIU/mL, were studied. They had taken no medications (including non-steroidal anti-inflammatory drugs) for at least the 3 previous months. The study was approved by the University of Southern California Institutional Review Board. All subjects gave written informed consent.

Study Design

During the 3 months-per-subject study period, all women ingested 1 mg micronized 17β-estradiol (Estrace, Bristol Myers Squibb, Princeton, New Jersey) daily for 30 days at bedtime during the first and third months of the study, with an intervening medication-free month to allow for washout. Each subject was then randomized to receive either 10 mg of MPA (Provera, Pfizer, New York, New York) or 200 mg of micronized P (Prometrium, Solvay Pharmaceuticals, Inc., Marietta, Georgia) daily at bedtime during the last 12 days of the month while on daily E2 treatment. After the washout in month 2, each subject started back on 1 mg of micronized E2 daily in month 3 and crossed over to the other progestogen during the last 12 days of the month. Subjects were randomized to the order of administration of MPA vs. P using a list of random digits.

Assays

A venous blood sample and a forearm reactive hyperemia test were obtained at baseline, before initiation of progestogen treatment (while on E2 treatment only), and at the end of each progestogen treatment (while on both E2 and progestogen treatment).

Androstenedione, testosterone, estrone (E1) and E2 were quantified by radioimmunoassay (RIA) following their extraction with ethyl acetate:hexane (3:2) and separation by Celite column partition chromatography.[12-14] Estrone sulfate was measured following an initial extraction step with ethyl acetate:hexane (3:2) to remove the unconjugated estrogens, hydrolysis of the sulfate group by use of an aryl sulfatase, an additional extraction step with ethyl acetate:hexane (3:2), and then RIA of E1.[15] P and MPA were quantified by RIA following their extraction with hexane:ethyl acetate (9:1) and ethyl acetate:hexane (3:2), respectively.[16,17] Sex Hormone Binding Globulin (SHBG) and FSH were measured by direct immunochemiluminometric assay using the Immulite analyzer (Diagnostic Products Corporation, Inglewood, California). Intra-assay and interassay coefficients of variation

ranged from 4 to 8% and 8 to 13%, respectively. Serum levels of lipids and lipoproteins were determined on the Vitros analyzer using standardized methods. LDL cholesterol was calculated by use of the following equation: LDL=total cholesterol – HDL cholesterol – triglycerides/5.

Brachial Artery Flow-mediated Dilation

All studies were conducted in a temperature-controlled (22°C) research laboratory with subjects in the supine position. The right brachial artery was imaged at predefined areas along the brachial artery, first transversely then longitudinally, using a high-resolution ultrasound machine. Forearm blood flow was obstructed by inflating a blood pressure cuff over the upper arm to suprasystolic pressure (50 mm Hg above systolic blood pressure) for 3 minutes. The occlusion phase was verified by Doppler flow. After the 3-minute period, the blood pressure cuff was released, and the brachial artery imaged for an additional 5 minutes during the reactive hyperemia phase. Endothelium-dependent vasodilation was measured as the percentage change from the baseline diameter of the brachial artery to the diameter during the reactive hyperemia phase. Subsequent to the flow-mediated vasodilation, 0.4 mg nitroglycerin (NTG) was administered sublingually and the brachial artery imaged for an additional 5 minutes to assess endothelium-independent vasodilation. The peak diameter changes at approximately 1 minute after cuff deflation and 3 minutes after NTG administration were used to measure the percentage change from the baseline diameter.

Statistical Analysis

Data are expressed as mean±standard deviation. The paired t test and the Wilcoxon signed-rank test were used to compare within-subject values at baseline and after each therapy.

Results

Baseline hormone levels confirmed the menopausal status of the participants. Of the twenty subjects enrolled, one subject withdrew prior to initiation of progestogen treatment, another withdrew secondary to intolerance of the blood pressure cuff inflation and five subjects were excluded secondary to non-compliance to the study protocol. Thirteen subjects completed the study.

Brachial artery flow-mediated dilation was similar at all treatment points (Fig. 1).

No statistically significant differences were observed in dilation between baseline, treatment with estrogen only, estrogen with P, or estrogen with MPA.

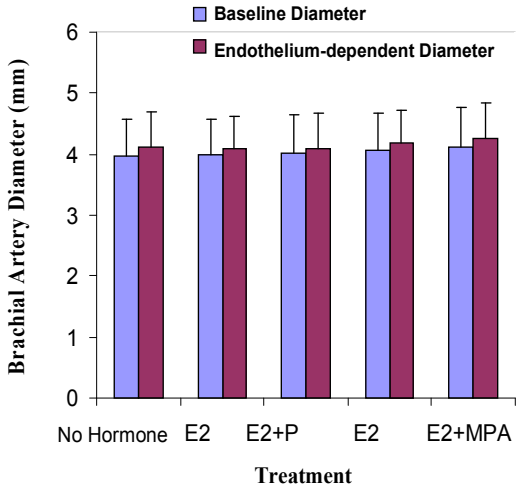

Fig. 1. Effects of hormone treatment on brachial artery endothelium-dependent dilation

There were also no differences in the forearm brachial artery endothelium-independent dilation after administration of NTG at all treatment points (Fig. 2). No statistically significant differences were observed in dilation between baseline, treatment with estrogen only, estrogen with P, or estrogen with MPA.

The baseline serum concentration of E2 in all subjects remained within the postmenopausal range prior to initiation of treatment and during the wash-out period between treatments. As shown in Table 1, estrogen treatment raised levels of E2, E1, and estrone sulfate, as well as SHBG levels. Levels of testosterone and androstenedione were not affected by the treatment of estrogen alone or estrogen in combination with either MPA or P. P levels increased compared to baseline levels when subjects received micronized P (Table 1), and were not different from baseline levels when subjects received MPA. MPA levels were measurable when subjects received MPA and not detectable when subjects did not receive MPA.

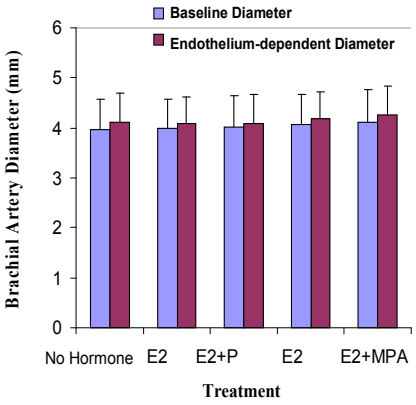

Fig. 2. Effects of hormone treatment on brachial artery endothelium-independent dilation after administration of NTG

E2 alone or in combination with MPA or P reduced total- and LDL-cholesterol concentrations, but the reduction was not significant. E2 did not significantly affect the serum concentration of HDL-cholesterol as compared with baseline values. There were also no effects observed on HDL-cholesterol during the combined phase of E2 and P or MPA. Also, neither E2 alone, E2 in combination with P, nor E2 in combination with MPA significantly altered the serum concentration of triglycerides (Table 2).

Table 1. Serum hormone and sex hormone binding globulin levels

	Treatment					
	Baseline	E2	E2 + P	Baseline	E2	E2+MPA
Estradiol (pg/mL)	15.0±9.0*	74.8±41.9†	46.5±21.6†	15.9±5.93	84.3±48.8†	63.3±59.3†
Estrone (pg/mL)	48.7±38.3	379±225†	221±144†	44.9±22.0	451±273†	278±200†
Estrone Sulfate (ng/mL)	1.44±1.13	15.2±9.33†	13.9±13.6†	1.03±0.41	20.8±13.7†	12.5±12.1†
Testosterone (ng/dL)	18.8±7.62	22.4±13.6	19.9±7.71	19.2±5.32	22.3±8.40	18.3±6.62
Androstenedione (ng/mL)	0.66±0.26	0.70±0.29	0.68±0.24	0.62±0.18	0.66±0.22	0.60±0.25
Progesterone (ng/mL)	0.26±0.18	0.22±0.09	2.24±1.94†	0.22±0.08	0.20±0.08	0.26±0.21
Medroxyprogesterone Acetate (ng/mL)	ND	ND	ND	ND	ND	480±308.1
Sex Hormone-Binding Globulin (nmol/L)	29.5±12.6	39.7±13.2†	40.2±12.1†	25.4±9.97†	43.0±13.7†	36.4±12.3†

* (Mean±Standard deviation); ND = non-detectable; † $p<0.05$; from paired t-test comparing within subject hormone value on treatment to no treatment (baseline);

Table 2. Lipid profiles in various treatment groups

		Treatment				
	Baseline	**E2**	**E2 + P**	**Baseline**	**E2**	**E2+MPA**
Total Cholesterol (mg/dL)	228.0±44.0*	222.0±37.4	217.0±33.1	221.0±39.7	214.0±30.1†	213.0±34.0
Triglycerides (mg/dL)	135.0±70.2	139.0±48.0	159.0±62.96	139.0±49.1	128.0± 58.7	135.0±60.5
HDL-C (mg/dL)	57.0±8.28	57.6±7.62	56.9±6.56	58.0±8.29	53.5±6.73	56.9±11.4
LDL-C (mg/dL)	144.0±36.5	137.0±31.5	128.0±29.7	135.0±33.6	135.0±21.1	129.0±23.5

*(Mean±Standard deviation); † $P<0.05$, from paired t-test comparing within subject lipid values on treatment to no treatment (baseline)

Discussion

In the present study, we found that estrogen per se or in combination with P or MPA did not affect brachial artery endothe-liumdependent vasodilation in healthy postmenopausal women. There were also no treatment effects on brachial artery endothelium-independent vasodilation in response to NTG.

The best way to assess endothelial function in the forearm is the intra-arterial infusion of a vasoactive agent. Measurement of brachial artery diameter during reactive hyperemia provides a noninvasive method for assessing endothelial function.[18] Reactive hyperemia is mediated largely by the release of nitric oxide (NO). Studies have shown that estrogen directly upregulates expression of endothelial NO synthase messenger ribonucleic acid and protein, resulting in increased endothelial NO synthase activity. This estrogen-dependent increase in endogenous NO production could improve vascular function after estrogen therapy in postmenopausal women. Lieberman et al.[19] found that 8 weeks of oral treatment with either 1 or 2 mg doses of E2 per day increased endothelium-dependent, flow-mediated vasodilation. Al-Khalili et al.[20] noted that flow-mediated dilation increased in a dose-dependent fashion after intravenous infusion of 2.5 mg or 5 mg of conjugated equine estrogen (CEE). The present study found no effect on endothelium-dependent flow-mediated vaso-dilation after 17 days of daily oral treatment with 1 mg of E2 (Fig. 1). Additionally, there were no statistically significant reductions in total cholesterol and LDL-cholesterol with estrogen treatment. Lack of an effect of estrogen on flow-mediated vasodilation and lipid metabolism may be due to the duration of treatment, as this was a short-term study.

Studies in animal models have examined the effects of MPA on endothelial function. Miyagawa et al.[6] reported that the intracoronary administration of serotonin and a thromboxane mimetic induce coronary vasospasm in monkeys treated with E2 and MPA but not in monkeys treated with E2 and P. Kawano et al.[21] reported that short-term administration of MPA plus transdermal E2 diminished the improvement in endothelium-dependent vasodilation of the brachial artery compared with E2 therapy alone in postmenopausal women. However, Sanada et al.[22] found no change in blood flow after treatment with either CEE (0.625 mg daily) alone for 3 months or concurrently with MPA (2 mg daily). The investigators also found no significant changes associated with hormone therapy in forearm blood flow induced by sublingual administration of NTG. In the present study, the addition of P or MPA did not change the brachial artery diameter over the response to oral E2 alone (Fig. 1). There was no significant increase in HDL-cholesterol or significant decrease in LDL-cholesterol concentration with E2 treatment alone or when combined with P or

MPA, probably due to the small sample size (lack of power) and short duration of treatment.

Several of our subjects had hyperlipidemia (fasting total cholesterol greater than 220 mg/dL), even though there was no past medical history at the time of entry into our study. Sanada et al.[23] reported that there is an augmentation of blood flow in hypercholesterolemic subjects compared to normocholesterolemic subjects treated with a 6-month course of concurrent 0.625 mg CEE and 2.5 mg MPA. When we confined our analyses to subjects with hyperlipidemia, no differences in results could be discerned from that obtained in the total sample. But, the sample size was very small.

It has been reported that the addition of micronized P does not attenuate the favorable effect of E2 on endothelium-dependent vasod-ilation and has been suggested that the vascular effects of progestogens combined with estrogen might be different for synthetic and natural progestogens.[24] Koh et al. reported that after 2 months of cyclic treatment, CEE and P and CEE and MPA similarly improve brachial artery endothelium-dependent vasodilation and have similar effects on markers of inflammation, hemostasis, and fibrinolysis in healthy postmenopausal women.[25]

This study has several limitations. The use of agonists to stimulate NO release, such as acetycholine, as well as NO antagonists would have allowed us to draw more specific conclusions concerning the role of basal and stimulated NO production mediated by HT in forearm circulation. Measurement of NO production and assay of ENOS activity would have been definitely more informative; hence reactive hyperemia is only a surrogate of NO production. The number of participants in our study was relatively small and out of those twenty participants, seven withdrew.

The duration of our study may have been too short to fully evaluate the effect of the addition of P and MPA on endothelial function.

In conclusion, this study suggests that the cyclic addition of MPA or P to short-term daily oral E2 does not affect endothelial function of the brachial artery relative to oral E2 alone. Short-term E2 alone showed no effect on brachial artery endothelial function.

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