Medroxyprogesterone interferes with ovarian steroid protection against coronary vasospasm

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Cardiovascular disease, the major cause of death in post-menopausal women, can be reduced by replacement of ovarian steroid hormones. To compare medroxyprogesterone with progesterone as the progestin in hormone replacement therapy from the standpoint of coronary artery vasospasm, we treated ovariectomized rhesus monkeys with physiological levels of estradiol-17β in combination with medroxyprogesterone or progesterone for four weeks. Coronary vasospasm in response to pathophysiological stimulation without injury showed that progesterone plus estradiol protected but medroxyprogesterone plus estradiol failed to protect, allowing vasospasm. We conclude that medroxyprogesterone in contrast to progesterone increases the risk of coronary vasospasm.

Cardiovascular disease, including sudden heart death, is the leading cause of death in women[1], and there is growing recognition that replacement of ovarian steroid hormones can significantly reduce this cardiovascular adversity[2]. Hormone replacement therapy for hypoestrogenic women has been designed primarily based on estrogen. Unopposed estrogen can increase the risk of endometrial cancer[1] although the addition of a progestin to the regimen eliminates this increased risk[1,2]. However it is cardiovascular disease that is the major risk factor in hypoestrogenic women, with a fatality rate that is about twice that caused by all forms of cancer combined[1].

Although progestins are generally viewed as equivalent, one prospective study suggested adverse effects of medroxyprogesterone (MPA) on high density lipoprotein (HDL) cholesterol[4]. Furthermore, the initial report from the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial showed that estrogen plus micronized progesterone has cardioprotective effects similar to unopposed estrogen, and greater than estrogen plus MPA from the standpoint of HDL levels[3]. Oral contraceptives present a different situation but also involve prolonged exposure to synthetic progestins. Recent reports of the World Health Organization (WHO) Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception have emphasized the need for research to explain the increased incidence of unexpected death or venous thromboembolism in women taking oral contraceptives[5–8]. These WHO data notably include idiopathic cardiovascular deaths that correlated with specific synthetic progestins[7].

Studies to examine the effects of progestins on coronary reactivity are rare, probably due to the lack of an animal model that has the cyclical and other endocrine complexities found in primates. Nearly all of the research on coronary artery and sudden heart death pathophysiology has been carried out on dogs, pigs, rabbits, pigeons or guinea pigs. Unfortunately, these non-primates lack many of the cardiovascular reflexes and blood vessel characteristics, including vasospasm, found in humans[9]. Although primates have been used in studies of steroids in atherosclerosis[10], there have been few studies of factors that could explain how ovarian steroids protect against the hyperreactivity aspect of coronary vasospasm and myocardial ischemia. We recently reported that coronary vasospasm is induced in non-human primates by increased reactivity to the combination of serotonin and thromboxane A_2 (ref. 11). Reactivity-based coronary vasospasm has characteristics that accurately mimic functional (vasospastic) human coronary vasospasm[11]; although it may not be widely appreciated, increased reactivity may be an important element. The key condition for vasospasm to occur in rhesus monkeys without injury, atherosclerosis or vascular pathology, is ovariectomy[11]. The reactivity-based vasospasm protocol (referred to as the S+U protocol) consists of the pathophysiological combination of serotonin and U46619 (the stable thromboxane A_2 mimetic) injected into a coronary artery (such as the left anterior descending or circumflex) of an ovariectomized macaque, in which, without hormone replacement therapy, vasospasm is reliably induced[11]. This sex steroid dependency for the occurrence of vasospasm in rhesus coronary arteries corresponds closely with that reported in humans[1–3].

We approached the question of which steroids protect against coronary artery vasospasm by treatment with estradiol-17β (E_2) along with either the natural hormone progesterone or the synthetic derivative MPA. MPA is commonly prescribed long term, either as a basis for birth control or as one component of hormone replacement therapy (HRT) for hypoestrogenic women[12]. MPA hormone replacement therapy formulations include slow release depot injections and tablets in combination with conjugated equine estrogens, ostensibly as a progesterone equivalent. The results of this study in non-human primates imply the necessity to reconsider this practice.

Results

Table 1 shows the major finding of this study: that progesterone in combination with E_2 protected against vasospasm while MPA with E_2 did not. All six monkeys that had been exposed to MPA and E_2 for four weeks were sensitive to the S+U protocol, and showed vasospasm before the protocol was complete. E_2 + MPA treated monkeys (MPA group) responded to 1–5 S+U challenges (mean = 2.7 ± 0.7) with vasospasm. In contrast, none of the six E_2 + progesterone-treated monkeys (P group) showed coronary vasospasm, even after six or more S+U doses, the last of which produced cardiogenic shock.

Table 1 Drug-induced coronary vasospasm incidence and steroid levels

Parameter	Progesterone	MPA
Vasospasms	0/6	6/6*
E_2 (pg/ml)	104±10	99±9
P (pg/ml)	6670±676	<100*
MPA (pg/ml)	<100	1737±1129*

All monkeys were treated with estradiol 17β via silastic implants for the entire treatment period. Progesterone or MPA was added during the last two weeks as a second silastic implant, to simulate the luteal phase (P) or hormone replacement therapy (MPA). All steroid implants were left in place, including during angiography. Steroid measurements were made by RIA[15] on serum samples taken just before angiography.
*Significant differences between P and MPA at $P < 0.05$ ($n = 6$ for each group).

Diameters (ϕ) of the proximal left anterior descending (LAD) or left circumflex coronary arteries under control conditions averaged 1.14 ± 0.21 for progesterone and 1.15 ± 0.10 mm for MPA groups. Dilator responses to low dose (0.18 μg) acetylcholine (ACh) were equal in progesterone (1.20 ± 0.25 mm) and MPA (1.21 ± 0.19 mm) groups. However, vasoconstrictor stimulation with the S+U protocol resulted in minimum arterial diameters significantly smaller in the MPA (0.15 ± 0.04 mm) than the P (0.37 ± 0.02 mm) group, representing 87% and 67% reductions from controls, respectively (Table 2).

Angiographic examples at the point of peak responses to intracoronary (IC) vasoconstrictor challenges in the P group are shown in Fig. 1. Coronary arteries had normal dilator function, as shown at the lower dose of serotonin (Fig. 1, *a* and *b*) or with ACh. Even with the potent S+U challenge repeated six or more times, there was no vasospasm in the P group protected arteries (Fig. 1, *c* and *d*). However, there was vasoconstriction and decreased contractile function, as shown by decreasing blood pressure 3–10 minutes after injection and ST segment changes in the electrocardiogram (EKG), indicating myocardial ischemia. Vasoconstrictions to the S+U protocol in the P group never showed the vasospasm hallmark pattern of focal constriction with downstream dilation.

MPA group monkeys were not protected against vasospasm, as shown in Fig. 2 and Tables 1 and 2. Although there was also apparently normal dilator function, as shown by a low dose of ACh (Fig. 2, *a* and *b*), the S+U protocol resulted in segmental occlusion and vasospasm (Fig. 2*c*). This strong (92%) constriction, including at least one hour-glass pattern, was sustained for 15 minutes, even longer than our criterion for vasospasm. After 15 minutes, IC mibefradil was injected to relieve the vasospasm and restore cardiac performance in one animal (Fig. 2*d*). Blood pressure, which reached a nadir of 38/20 mm Hg in this monkey returned to 124/84 mm Hg after mibefradil administration.

Serum levels of E_2 and progesterone were within physiological ranges and MPA was in the low therapeutic range (Table 1). E_2 levels were not significantly different between progesterone and MPA groups and both were near 100 pg/ml. The progesterone levels used were appropriate for the luteal phase range at about 7000 pg/ml and MPA levels were in the in low therapeutic range for women on hormone replacement therapy, averaging about 600 pg/ml. The false indication of MPA observed in one of the P group is explained by cross-reaction of the antiserum with progesterone that was more than 50 times higher. Progesterone levels falsely indicated in three MPA treated monkeys were only slightly above background.

Table 2 Coronary artery diameters, blood pressures and heart rates

Parameter	Progesterone	MPA
Control ϕ in mm	1.14±0.21	1.15±0.10
S+U ϕ_(minimum)	0.37±0.02	0.15±0.04*
Control SBP in mm Hg	101±12	101±13
Control DBP in mm Hg	60±5	61±5
Control Heart rate in bpm	130±24	134±26
S+U stim SBP in mm Hg	51±18	45±11
S+U stim DBP in mm Hg	30±12	26±8
S+U stim Heart rate in bpm	66±34	69±24

The S+U stimulated values represent the minimum diameter (ϕ), blood pressure and heart rate responses, measured at different times. ϕ minimum times ranged from 3–5 min and blood pressure and heart rate from 5–20 min after IC injection of S+U. Image analysis of single frames from angiography recorded on videotape, allowed high resolution measurements of ϕ. *Significant differences between P and MPA at $P < 0.05$ ($n = 6$ for each group).

Discussion

A major unanswered question in hormone replacement therapy for hypoestrogenic women is how to balance estrogen with a progestin. Use of unopposed estrogen has been thought to increase the risk of breast and endometrial cancer and this risk may be lessened by continuous or cyclic addition of a progestin[1–6,12]. Estrogens are widely administered either in a conjugated (Premarin or Ogen) or unconjugated (Estrase) form, to which various forms of progestin can be added in either physiological or low doses[12,13], and thus many alternatives exist, among which is the combination of conjugated estrogens with MPA as a single tablet.

However, in the face of the much discussed cancer risk associated with estrogen administration, an important fact is not being given sufficient attention; death due to cardiac causes is the greater danger. The cardiovascular system is critically important because cardiovascular disease, especially coronary artery disease, sudden heart death and stroke, are by far the most common causes of death in women[1]. Combined annual female cardiovascular fatalities (over 350,000) are more than twice the combined number of deaths for all forms of cancer (160,000) in the United States[1,2] and studies show that hypoestrogenic women are in the highest risk group[1–3,5–8].

The heart and blood vessels respond differently than reproductive tissues to estrogens and progestins and must therefore be considered independently. From the cardiovascular risk standpoint, estrogens are beneficial in maintaining vasodilator capacity, especially of coronary arteries[10]. Furthermore, estrogens decrease LDL-cholesterol particularly oxidized LDL, and thus the tendency toward atherosclerosis[1,12]. These benefits are countered by an increased risk of endometrial carcinoma, but probably only when estrogens are used unopposed by progesterone[1]. Progestins counter the tendency for increased cancer risk factors, but may adversely affect the lipid benefits, depending on whether progesterone (the natural hormone), MPA or one of the 19-nortestosterone steroids is used[1,3,4,13]. The testosterone derivatives have a particularly adverse effect, with respect to HDL-cholesterol[13,14]. MPA is thought to have a less adverse effect, but per-

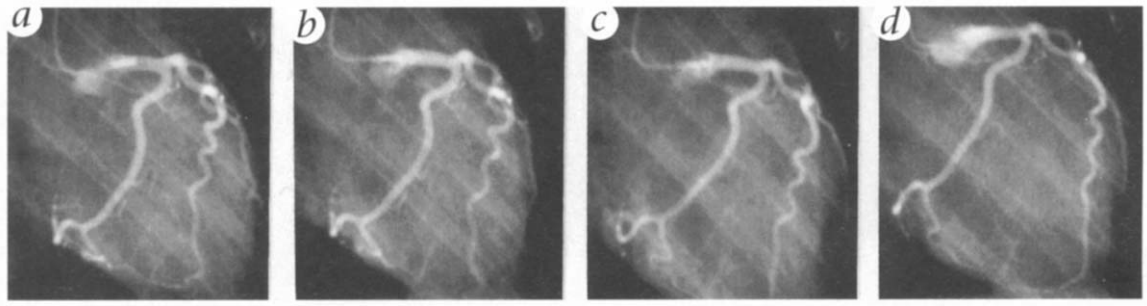

Fig. 1 Coronary angiograms of an E_2 + progesterone treated monkey show the control (*a*), dilation with low dose (100 µm) serotonin (*b*), constriction with 1 µm U46619 (*c*) and further constriction with the third repeat of 100 µm serotonin + 1 µm U46619 (*d*). Although there was strong coronary vasoconstriction that severely diminished cardiac contractility (a fall in systolic/diastolic blood pressure to 45/22 mm Hg; more than 30 s required to clear the radiocontrast media; and ST segment changes in the EKG that suggested extensive left ventricular ischemia) there was no vasospasm.

haps only at lower doses[3,4]. It is a generally held supposition that all progestins (natural and synthetic) can be used without negating the beneficial cardiovascular effects of estrogen. Natural progesterone appears neutral to blood levels of lipids and may therefore be the best choice[1,3].

Vasospasms provide another definitive end-point that reflects regulation of contraction signals. The vasospasms reported in these experiments could be relieved by the Ca^{2+} antagonist, mibefradil, implying that the intracellular Ca^{2+} was an important element[15]. At the level of vasoconstriction or vasodilation of the coronary arteries, estrogens and progestins exert an important but little recognized influence that may involve the Ca^{2+} signal for contraction[11,16]. Studies of coronary reactivity in the cardiac catheterization laboratory suggest that estrogens and progestins may be a dominant factor in the increased reactivity leading to coronary vasospasm[11]. Estrogens favor production of the potent dilators prostacyclin and endothelial dependent relaxant factor, and do not favor the constrictors, thromboxane, endothelin and superoxide ions[13]. Different forms of progestins vary in actions and have been examined only rarely[2,5–8] with respect to these parameters. Testosterone derivatives can have constrictor tendencies, including decreases in prostacyclin[12]. MPA may have similar actions, but has not been thoroughly researched[4,13]. However, recent preliminary reports suggest that MPA decreases insulin sensitivity (Groczykowski, Wong, Stanczyk & Lobo; Society for Gynecologic Investigation, 1996) and counters the beneficial effect of estrogen on endothelin and oxidized LDL levels (Wilcox *et al.*, Society for Gynecologic Investigation, 1996). Natural progesterone has also been inadequately studied with respect to those parameters and it has been reported that higher doses of progesterone can interfere with the relaxant action of estrogen in dog coronary artery[17]. In this report, we have contrasted the actions of MPA with natural progesterone in coronary arteries of monkeys using experimentally controlled physiological estrogen levels[12,14,18–20]. These results demonstrate disparate consequences of MPA and progesterone use that could influence decisions about hormone replacement therapy.

It is important to further explore the basis for differences among progesterone, MPA and other progestins for protection against coronary vasospasm[1,2,4]. Studies on rabbits with high steroid concentrations have shown vasodilation in response to progesterone and estrogen individually[21], but there are no analogous studies with MPA. It is clearly important to explore the differences in progestins systematically and determine which are the most cardioprotective[3–8,22]. The data reported here take the first step by emphasizing the disparity between the abilities of MPA and progesterone to interfere with protection by E_2 against vasospasm. Although MPA is a widely used, highly successful progestin, the cardiovascular consequences of treatment may outweigh the benefits. Serum levels of 350–650 pg/ml were associated with vasospasm, even though this is only about 10% of the peak level occurring in women treated with MPA (Groczykowski, Wong, Stanczyk & Lobo, Society for Gynecologic Investigation, 1996).

Limitations of this report include the duration of E_2 plus progesterone or MPA treatments (four weeks as a combination and six weeks total for E_2). The comparative effects of longer treatment by E_2 plus MPA vs E_2 plus P on vasospasm are unknown. On the other hand, MPA carries at least an increased risk of vasospasm, which could also result from adverse effects on endothelium, blood platelets and coagulation. This should be further investigated as a possible contributor to hyperreactivity.

Our most significant observation is that moderate levels of MPA interfere with the protective effects of E_2 against increased coronary reactivity and vasospasm. As the major cause of death in the large and growing population of hypoestrogenic women, this coronary risk factor must be respected[22]. It is possible that a reduc-

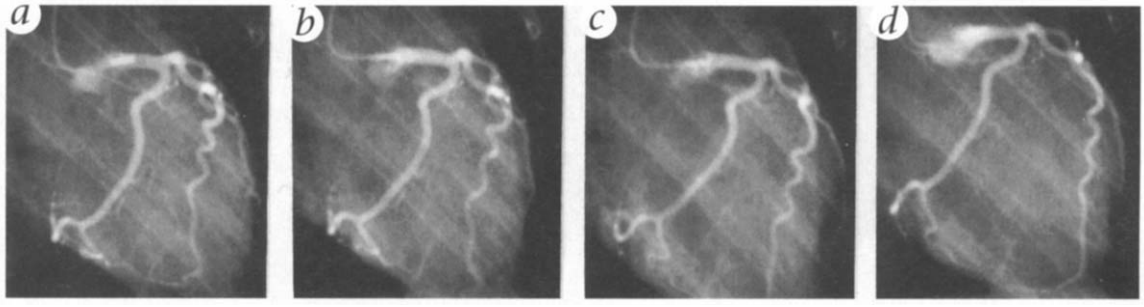

Fig. 2 Coronary angiograms of an E_2 + MPA treated monkey show the control artery diameters (*a*), dilation with low dose (1 µm) ACh (*b*), vasospasm 15 min after the first injection of 100 µm serotonin + 1 µm U46619 (*c*) and vasodilation with mibefradil 15 min after a subsequent vasospasm (*d*). The time elapsed between *c* and *d* is 33 min. Thus, MPA + E_2 eliminated the E_2 protection against vasospasm, as shown in *c* and all MPA treated monkeys (Table 1).

tion in cardiovascular death of up to 50% may be feasible via beneficial effects of HRT[23]. Reasons for choosing MPA over progesterone as components of HRT have been based on familiarity and convenience. Based on the results presented here, formulations of natural progesterone would appear to offer the wiser alternative.

Methods

Animals and steroid administration. Twelve rhesus monkeys (*Macaca mulatta*), aged 8–16 years and with weights of 5–7 kg, were ovariectomized at least three months before the beginning of the hormone study and selected randomly for one of two treatments. After documenting the lack of cardiovascular disease or previous exposure to atherogenic diet and after a normal physical examination (including blood chemistry), monkeys received subdermal silastic implants containing 200 mg of E_2. Two weeks later, silastic implants containing either 400 mg of progesterone or 400 mg of MPA were also implanted. All steroids were purchased from Steraloids (Wilton, NH). Both estrogen and progestin implants, providing sustained release steroids[18], remained in place through the catheterization study six weeks after the beginning of the study. Venous blood samples (5 ml) were collected just before steroid administration and at weekly intervals, for measurements of serum E_2, progesterone and MPA. Target levels of 80–160 pg/ml for E_2, 4000–8000 pg/ml for progesterone, and 400–800 pg/ml for MPA were based on levels that are effective in reproductive system indices in women[12] and monkeys[18]. Levels were measured in blood serum at catheterization by specific radio-immunoassays as described[14,16,19,20].

Angiograms. Angiography was carried out under isoflurane (0.75–1.25%) general anesthesia with 70% O_2 and 30% N_2O and body temperature supported with a heating pad. After ketamine sedation (10 mg/kg) and endotracheal tube placement, both femoral arteries were cannulated for simultaneous measurement of systemic blood pressure and coronary catheterization. Intravenous anticoagulation with 1000 units of heparin and fluid replacement with 75–150 ml of lactated Ringer's solution, and if needed 10–50 ml of dextran, to reach a minimum control diastolic blood pressure of 60 mm Hg. Image analysis (Image Pro from Media Cybernetics, Silver Spring, MD) of single frames acquired via Imagraph (Chelmsford, MA) A-D conversion, from angiography video recordings, allowed high resolution arterial diameter measurements.

S+U protocol. Coronary arteries (left anterior descending or left circumflex) were catheterized and vasoactive agents injected via 3F IC catheter in a 12 step protocol. Hexabrix (kindly provided by Mallinckrodt) was warmed to body temperature and 1–1.5 ml injected by hand as the radio-opaque contrast media. IC injections of 1 μM ACh in step 1, 0.1 or 1 mM serotonin in steps 2 and 3, and 0.1 or 1 μM U46619 (the stable thromboxane A_2 mimetic) in steps 4 and 5 allowed us to test both vasodilator and vasoconstrictor responses (Fig. 1 and 2). Steps 6–8 consisted of the IC combination of 100 μM serotonin and 1 μM U46619 (S+U) in three iterations. One or more of these S+U challenges produced vasospasm in susceptible monkeys, as determined by focal constrictions with downstream dilations (the hourglass pattern). In step 9 (challenge 4), the U46619 was increased to 3 μM, a high dose that usually led to decreased cardiac function and cardiogenic shock. If no vasospasm or cardiogenic shock occurred by step 9, step 10 (challenge 5) added 1 μM angiotensin and step 11 (challenge 6) added 1 nM endothelin, which were repeated until vasospasm or cardiogenic shock occurred (one or the other constituting the end-point). IC mibefradil (1 μM) was used to relieve vasospasm or cardiogenic shock (step 12). Every IC injection of drug was made as 1 ml over a 30 second period. All injections up to step 12 were made into non-constricted coronary arteries and we estimate a ×15 dilution by blood flow during the injection[11]. At the end of the catheterization study, monkeys were killed for gross pathological evaluation with special attention to cardiac or coronary abnormalities. Sections of heart and coronary artery were also studied by histopathology. A more complete account of the protocol is found elsewhere[11].

Vasospasm. Vasospasm has not been rigorously defined to date, and thus has several possible meanings. The limited definition that we use here involves abnormal contractions of large coronary arteries that result in myocardial injury, in contrast to physiological vasoconstriction that is part of the normal autoregulatory response[11,16]. Contraction of coronary arteries occurs with different strengths and durations. The upper range of such contractions are excessive, with focal constrictions reducing control diameters by 76–90% coincident with downstream dilation. These strong, persistent vasoconstrictions result in transmural ischemia, changes of the ST segment of the EKG and ultimately myocardial infarction. We therefore define vasospasm to be contractions of epicardial coronary arteries that include focal or diffuse areas of constriction to less than 25% of control diameter, followed by adjacent downstream dilation, that persist for at least 5 min.

Statistical analyses used the Student's *t*-test (non-paired) or Chi-square, with the $P < 0.05$ representing a significant result.

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