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Effects of estradiol with oral or intravaginal progesterone on risk markers for breast cancer in a postmenopausal monkey model

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Abstract

Objective: To evaluate the effects of oral estradiol given with either oral or intravaginal micronized progesterone (P_4) on risk biomarkers for breast cancer in a postmenopausal monkey model.

Design: This experiment was a two-way crossover study in which 20 ovariectomized adult female cynomolgus macaques were treated (in equivalent doses for women) with oral estradiol (1 mg/d) + oral micronized P_4 (200 mg/d) or intravaginal P_4 delivered by Silastic rings (6- to 10-mg/d release rate). Hormone treatments lasted 2 months and were separated by a 1-month washout period. The primary outcome measure was breast epithelial proliferation.

Results: Serum P_4 concentrations were significantly greater in subjects receiving oral P_4 (10.9 ng/mL) compared with intravaginal P_4 (3.8 ng/mL) at 2 to 3 hours after oral dosing ($P < 0.0001$) but not at 24 to 28 hours after oral dosing (2.9 ng/mL for oral P_4 vs 3.2 ng/mL for intravaginal P_4 at 2 months, $P = 0.19$). Serum estradiol concentrations were significantly lower after oral P_4 than after intravaginal P_4 ($P < 0.05$ for all time points). Oral P_4 resulted in significantly decreased body weight (−2.5%) compared with intravaginal P_4 (+3.6%) ($P = 0.0001$). Markers of breast proliferation, sex steroid receptor expression, and endometrial area did not differ significantly between oral P_4 and intravaginal P_4 treatments ($P > 0.1$ for all).

Conclusions: Despite different pharmacodynamic profiles, oral and intravaginal P_4 had similar effects on biomarkers in the postmenopausal breast.

Key Words: Hormone therapy – Estrogen – Progesterone – Breast cancer – *Macaca*.

Progestogen administration prevents endometrial hyperplasia and cancer resulting from unopposed estrogen therapy in postmenopausal women.[1,2] A variety of different synthetic progestogens (progestins) have been approved for this purpose, with medroxyprogesterone acetate (MPA) being the most widely used progestin in the United States.[3,4] Recent evidence from clinical trials[5,6] and observational studies[7-9] indicates that the addition of oral MPA to an estrogen may increase breast cancer risk in postmenopausal women by at least 30% in long-term users and possibly contribute to other adverse health outcomes.[5] These findings have raised widespread concern about progestin use and increased interest in alternative progestogen types and routes of administration.[4,10,11]

One such alternative is micronized progesterone (P_4), which has been characterized as a more physiologic and potentially safer "bioidentical" progestogen.[12-14] Compared with MPA, micronized P_4 provides a more favorable lipid profile,[15] less endometrial bleeding,[16] and similar protection of the endometrium against estrogen-induced hyperplasia.[17] We have also reported recently that oral P_4, when given with estradiol (E_2), results in less epithelial cell proliferation than E_2 + MPA.[18] Serum P_4 concentrations resulting from daily oral administration are highly variable and poorly sustained, however. After ingestion, oral micronized P_4 is rapidly converted in the intestines and liver to a variety of metabolites,[19,20] some of which may produce unwanted sedative-like effects[21] and potentially contribute to breast epithelial cell growth.[22] This evidence has led to increased interest in parenteral administration of P_4,[4,23-25] which may reduce the overall P_4 metabolite load (given the lack of enteric and hepatic first-pass metabolism) and provide more stable serum P_4 concentrations.

Among parenteral formulations, intravaginal P_4 has been used to effectively target the endometrium while limiting systemic exposure.[26] This concept is based on the "first uterine pass effect," which proposes that intravaginal hormones are preferentially absorbed by the uterus via a portal-like system.[27-29] Intravaginal P_4 has been shown to

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concentrate in uterine tissues[28,29] and to protect the endometrium from estrogen stimulation[23,25,28] while producing substantially lower amounts of systemic metabolites than oral P_4.[19,20] Intravaginal rings in particular have the advantage of continually releasing P_4 over time; thus, high peak serum concentrations and potential compliance issues associated with daily oral dosing are avoided. However, no data currently exist on the comparative effects of intravaginal ring P_4 and oral P_4 on the breast. The purpose of this experiment was to evaluate breast proliferation and other hormonal markers after treatment with E_2 plus either oral or intravaginal P_4. We hypothesized that E_2 + intravaginal P_4 would result in less breast proliferation than E_2 + oral P_4.

METHODS

Animal subjects

In this study we used 25 adult female, surgically menopausal cynomolgus monkeys (*Macaca fascicularis*) with an average age of 8.1 ± 0.3 years. All animals had been ovariectomized for 1.6 years and housed since this time in stable social groups of three to five animals each. All procedures in this study were conducted in compliance with state and federal laws, standards of the U.S. Department of Health and Human Services, and guidelines established by the Wake Forest University Animal Care and Use Committee. The facilities and laboratory animal program of Wake Forest University are fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care.

Study design and treatments

The animals received no hormone treatment for 6 weeks prior to the start of the current experiment. One social group of animals (n = 5) was random1y selected to receive placebo treatment as a reference control group. For the remaining animals (n = 20) the study followed a two-way Latin-square crossover design in which all monkeys received each of the following treatments: (1) oral micronized 17β-estradiol (1 mg/d) (Estrace, Bristol-Myers Squibb, New York, NY) + oral micronized progesterone (oral P_4, 200 mg/d) (Prometrium, Solvay Pharmaceuticals, Marietta, GA) or (2) oral E_2 + intravaginal micronized progesterone, delivered via a Silastic ring implant (ring P_4, 6- to 10-mg/d release). Half of the 20 treatment animals received E_2 + oral P_4 in phase 1 and E_2 + intravaginal P_4 in phase 2, whereas the other half received hormone treatments in the reverse order. Daily doses are expressed in human equivalents; absolute daily doses (in mg/kg body weight) were 0.05 for E_2, 11.1 for oral P_4, and an estimated 0.09 to 0.15 for ring P_4. Hormone doses were designed to represent clinically used regimens approved for postmenopausal women. Treatment phases lasted 2 months and were separated by a 1-month washout period, during which all animals were given placebo. Breast biopsies and other measures were performed at the end of each treatment period. Throughout the experiment, the animals were fed a standard control casein/lactalbumin-based diet.

The animals were treated each morning between 8:00 and 10:00 AM. E_2 was administered within a fruit punch (Crystal Lite) vehicle, whereas oral micronized P_4 was injected into a small marshmallow or piece of fruit (banana or tangerine). Micronized P_4 is formulated in a peanut oil–based vehicle, and the marshmallow or fruit dosing of P_4 was used to avoid parenteral absorption. Control animals received a placebo fruit punch, and all nonoral P_4 animals were given a placebo marshmallow or fruit piece. For dosing, all animals were trained to enter a catch cage, drink the fruit punch from a syringe, and then eat the marshmallow or fruit. Individual oral drug doses were calculated on the basis of body weight at the start of each dosing phase.

Intravaginal P_4 rings

Intravaginal P_4 was administered using vaginal rings similar to those prescribed for women.[30] Rings were custom designed by the Population Council's Center for Biomedical Research (New York, NY) using established procedures.[30,31] The rings were made by mixing micronized progesterone USP (Berlex Laboratories, Wayne, NJ) with silicone elastomers, which were polymerized, covered with a silicone membrane, and fused to form a soft, flexible ring. Release rates for rings containing different amounts of P_4 were tested initially in a series of in vitro experiments. For these assays, an individual ring was incubated for 24 hours in a 37°C water bath with constant shaking. Water aliquots were collected daily for P_4 analysis, followed by changing of the water in each bath. Released P_4 was measured using a Waters Alliance high-performance liquid chromatography system with Phenomenex C_{18} column (Waters, Milford, MA). All in vitro assays were run in triplicate. To evaluate systemic release in vivo, rings were placed intravaginally in small groups of study animals (n = 2-4 per group), and serum P_4 concentrations were determined by radioimmunoassay after 2 weeks. The latter pilot study was conducted during the acclimation period after ovariectomy.

Target serum P_4 concentrations were 3 to 5 ng/mL (9.5-15.9 pmol/L), representing the lower end of the reported range in studies of postmenopausal women using different intravaginal P_4 formulations.[26,32-37] The ring dose providing this target range contained 28 mg of micronized P_4 and had an in vitro release rate of 0.4 to 0.7 mg P_4 per day (Fig. 1A). Rings measured 1.3 cm in diameter and 2.4 mm in thickness. Placebo and oral P_4 animals received control silicone rings containing no P_4. All rings were replaced 1 month into each treatment period and removed at the time of biopsy.

Vaginal swabbing

To confirm a physiologic effect of P_4 on the endometrium, daily vaginal swabs were taken from each animal for 2 weeks after each treatment period of the primary study.

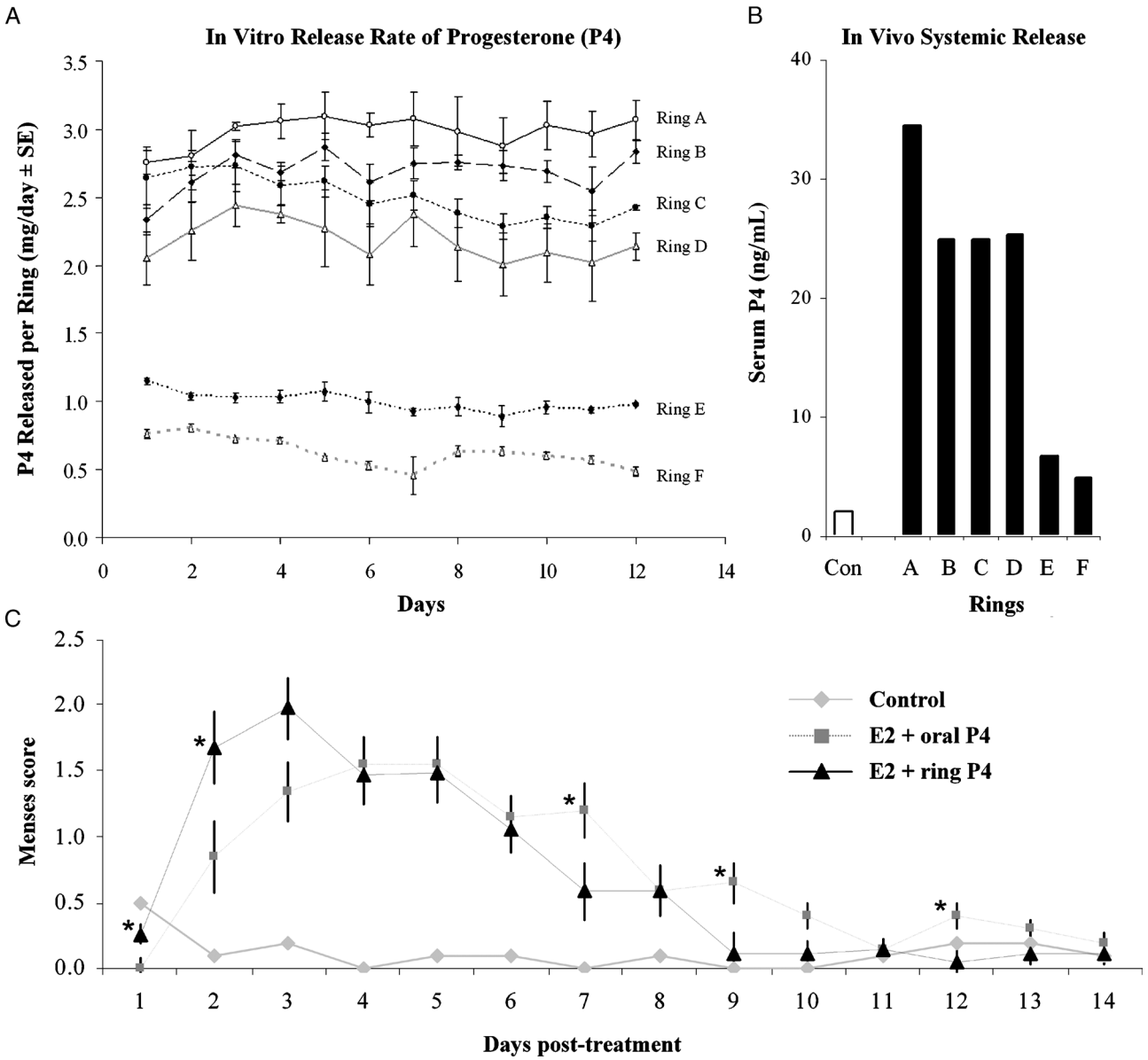

FIG. 1. Dose calibration of intravaginal progesterone (P_4) rings. **A:** In vitro release rates for the six different trial rings (A-F) containing the following amounts of P_4: 580 mg (A); 451 mg (B); 282 mg (C); 217 mg (D); 56 mg (E); and 28 mg (F). **B:** Serum P_4 concentrations in postmenopausal female macaques (n = 2-4 per group) after 2 weeks of ring P_4 administration. **C:** Menses pattern in the 2 weeks after withdrawal of the 28-mg intravaginal P_4 ring (ring F) and oral P_4 given with estradiol (E_2), confirming a physiologic endometrial effect of both intravaginal and oral doses. Asterisks indicate a significant difference between E_2 + oral P_4 and E_2 + ring P_4 groups on a given day ($P < 0.05$). Study treatment groups were placebo (Con), oral estradiol + oral progesterone (E_2 + oral P_4), and oral estradiol + progesterone administered via an intravaginal ring (E_2 + ring P_4). Vertical lines indicate standard error.

Withdrawal bleeding (menses) was subjectively scored as 0, 1+, 2+, or 3+.

Endometrial area

Endometrial area was determined by transabdominal ultrasound using a Sonosite 180 portable ultrasound machine with a 5.0-MHz linear transducer (Sonosite, Bothell, WA). Maximal transverse cross-sectional area was measured on static representative digital images using public domain software (National Institutes of Health ImageJ 1.33j, available at http://rsbweb.nih.gov/ij/).

Vaginal cytology

To confirm the effects of oral E_2, vaginal keratinocytes were collected with a cotton swab, rolled onto a glass slide, fixed, and stained using a modified Papanicolaou method. Maturation value was calculated as follows: (0.2 × % parabasal cells) + (0.6 × % intermediate cells) + (% superficial cells).

Serum estrogens and progesterone

Serum E_2, estrone, and P_4 concentrations were measured from samples collected 2 to 3 hours and 24 to 28 hours

after dosing. The 2- to 3-hour postdosing interval corresponds to the estimated t_{max} range for oral P_4 administration (200 mg) in women.[13] Blood was collected by femoral venipuncture after sedation with ketamine, and serum concentrations were quantitated by radioimmunoassay as described previously.[18] For E_2, serum was extracted with ethyl ether using standard procedures. Intra- and interassay coefficients of variation were less than 10% for all serum assays.

Breast biopsies

At the beginning and end of each treatment period, the animals were anesthetized with ketamine and buprenorphine for breast biopsy. For this procedure, a 1.5-cm incision was made in a preselected breast quadrant, and a small (0.4 g) sample of mammary gland was removed. Biopsies were performed by an experienced veterinary surgeon (C.J.L.). The incision was sutured, and the animals were monitored and given analgesia during recovery following clinical procedures approved by the Animal Care and Use Committee. Half of each biopsy was frozen; the other half was fixed at 4°C in 4% paraformaldehyde for 24 hours, transferred to 70% ethanol, and then processed for histologic analysis using standard procedures.

Immunohistochemistry

Immunostaining procedures were performed on fixed, paraffin-embedded mammary gland tissues using commercially available primary monoclonal antibodies for the proliferation marker Ki67 (Ki67/MIB1, 1:50 dilution, Dako, Carpinteria, CA) and progesterone receptor (PGR) (NCL-PGR, 1:100 dilution, Novocastra, Newcastle-upon-Tyne, UK). Staining methods included antigen retrieval with citrate buffer (pH 6.0), biotinylated rabbit anti-mouse F_c antibody as a linking reagent, alkaline phosphatase–conjugated streptavidin as the label, and Vector Red (Vector Laboratories, Burlingame, CA) as the chromogen. Cell staining was quantified by a computer-assisted counting technique using a grid filter to select cells for counting and our modified procedure of cell selection.[38] Numbers of positively stained cells were measured as a percentage of the total number examined (100 cells). All measurements were made with researchers blinded to treatment group.

Intramammary gene expression

Expression levels of mRNA transcripts for genes associated with breast proliferation (Ki67 and MCM5) and estrogen/progesterone action (estrogen receptor α [ER-α] and PGR) were determined using quantitative real-time reverse transcriptase–polymerase chain reaction. Breast RNA was extracted, purified, quantitated, qualitatively evaluated for intactness, and reverse transcribed using techniques described previously.[39] Macaque-specific primer-probe sets for internal control genes (GAPDH and β-Actin) and ER were generated through the TaqMan Assay-by-Design service (Applied Biosystems, Foster City, CA), whereas human TaqMan assays were used for Ki67, MCM5, and PGR. All probes were designed to span an exon-exon junction. Reactions were performed on an Applied Biosystems Prism 7000 using standard reagents and thermocycling protocol.[39] Relative expression levels were determined using the ΔΔCt method described in Applied Biosciences User Bulletin No. 2 (available online at http://docs.appliedbiosystems.com/pebiodocs/04303859.pdf.). The Ct values for the control genes GAPDH and β-Actin were averaged for use in internal calibration, whereas reference breast tissue RNA was run in parallel as an external calibrator.

Statistics

A mixed general linear model with repeated measures was used to determine means and to test for differences between the two hormone treatments (E_2 + oral P_4 and E_2 + ring P_4). The study was not designed to compare treatment and control groups, and placebo group values are provided for reference only. Phase was incorporated as a fixed-effect covariate for all tissue endpoints, and all variables were screened for phase-by-treatment interactions. No significant phase-by-treatment interactions were detected ($P > 0.1$ for all measures). Variables were also screened for potential carryover effects from the final phase of the previous experiment[18] (before the 6-week washout period); for this analysis, previous treatment was included in the mixed model as a fixed-effect covariate. A significant carryover effect was found for PGR immunolabeling ($P > 0.05$), and this measure was excluded from further analysis; no such carryover effects were found for other measures ($P > 0.1$ for all). A general linear model was used to evaluate group differences in body weight and Ki67 immunolabeling at baseline. Menses scores were analyzed using a nonparametric Wilcoxon rank-sum test. All variables were evaluated for their distribution and equality of variances between diets and transformed when appropriate to improve homogeneity of variance. Data are reported either as mean ± SE for untransformed data or mean (90% CI) for retransformed data. One P_4 ring was lost during treatment, and this animal was excluded from analysis. Other missing data points included a subset of mammary gland samples lacking either lobuloalveolar (n = 1-2 per treatment) or ductal (n = 2-3 per treatment) epithelium on sectioning, three unmeasurable endometrial ultrasound images (n = 1-2 per treatment), and one quantitative polymerase chain reaction sample with poor RNA quality. Data were analyzed using the SAS statistical package (version 8, SAS Institute, Cary, NC). A two-tailed significance level of 0.05 was chosen for all comparisons.

RESULTS

Intravaginal P_4 calibration

To evaluate the systemic delivery of intravaginal P_4, we conducted a series of preliminary studies using rings with different P_4 release rates. Rings with an in vitro release rate greater than 2.0 mg P_4 per day (rings A-D) consistently resulted in supraphysiologic serum P_4 concentrations greater

TABLE 1. *Treatment effects on body weight and reproductive tract measures*[a]

	Control	E_2 + oral P_4	E_2 + ring P_4	P^b
Body weight, kg				
Baseline	4.82 ± 0.35	4.36 ± 0.15	4.17 ± 0.15	0.002
Posttreatment	4.86 ± 0.34	4.26 ± 0.15	4.32 ± 0.15	0.22
Change	0.04 ± 0.06	−0.11 ± 0.04	0.15 ± 0.04	0.0001
Endometrial area, cm^2	0.15 ± 0.07	0.24 ± 0.04	0.26 ± 0.04	0.63
Vaginal maturation index	50.4 ± 4.8	81.4 ± 2.2	66.0 ± 2.7	<0.0001

Values represent mean ± SE.
[a]Control = placebo; E_2 = 17β-estradiol administered orally; oral P_4 = micronized progesterone administered orally; ring P_4 = micronized progesterone administered via an intravaginal ring.
[b]Oral P_4 and ring P_4 groups were compared using a mixed general linear model; control values are provided for reference only. *P* values indicate differences between E_2 + oral P_4 and E_2 + ring P_4 groups.

than 20 ng/mL, whereas rings with lower release rates (1.0 mg P_4 per day for ring E and 0.6 mg P_4 per day for ring F) provided serum P_4 less than 10 ng/mL (Fig. 1A, B). Ring F, containing 28 mg of P_4, resulted in P_4 concentrations in the target range of 3 to 5 ng/mL and was selected for use in the subsequent study.

Withdrawal menses

To confirm a physiologic effect of oral and ring P_4 doses on the endometrium, menses were monitored for 2 weeks after withdrawal of hormone treatments. Vaginal bleeding occurred in both oral P_4 and ring P_4 treatment groups within 2 days after treatment was stopped (Fig. 1C). Menses scores after P_4 ring removal were significantly higher on days 1 and 2 and significantly lower on days 7, 9, and 12 than observed for oral P_4 treatment ($P < 0.05$ for all), suggesting a slightly more rapid onset of menses after withdrawal of intravaginal P_4.

Body weight and reproductive tract measures

Body weight decreased by 2.5% during oral P_4 treatment but increased by 3.6% during intravaginal P_4 ($P = 0.0001$) (Table 1). Endometrial area was not significantly different between treatment groups ($P = 0.63$), whereas vaginal maturation index was significantly greater after oral P_4 ($P < 0.0001$) (Table 1).

Serum estrogens and progesterone

Intravaginal P_4 rings provided more stable systemic concentrations of P_4 than oral administration. The 28-mg ring dose resulted in mean serum P_4 concentrations of 3.2 to 4.8 ng/mL (10.1-15.2 nmol/L) (Table 2), representative of the lower end of the reported range for postmenopausal women taking P_4 via intravaginal ring, gel, or suppository (2.5-20 ng/mL).[26,32-37] In acute serum samples (taken 2-3 hours after oral dosing), serum P_4 concentrations were significantly greater for oral P_4 treatment (10.9 ng/mL) than intravaginal P_4 (3.8 ng/mL) ($P < 0.0001$). In lag serum samples (taken 24-28 hours after oral dosing), serum P_4 concentrations were significantly lower after oral P_4 treatment at the 2-week time point ($P < 0.0001$) but not at the 2-month time point ($P = 0.19$), suggesting a modest decline in ring P_4 delivery toward the end of the treatment period. Serum E_2 was significantly lower after oral P_4 treatment than after ring P_4 at 2 weeks (acute, $P = 0.03$; lag, $P < 0.0001$) and 2 months (lag, $P = 0.005$). Estrone measured at the 2-month lag time point was also marginally lower after oral P_4 treatment ($P = 0.06$). Serum E_2 and P_4 concentrations in the placebo control animals were less than 5 pg/mL and less than 1 ng/mL, respectively. Serum E_2 and P_4 concentrations after oral dosing were similar to those reported for women taking comparable oral hormone doses.[40,41]

TABLE 2. *Treatment effects on serum estrogen and progesterone concentrations*[a]

	Control	E_2 + oral P_4	E_2 + ring P_4	P^b
Estradiol, pg/mL				
2 wk: 2- to 3-h PD	<5	173.6 (151.4-199.0)	235.6 (205.0-270.7)	0.03
2 wk: 24- to 48-h PD	<5	16.4 (14.0-19.1)	31.9 (27.2-37.4)	<0.0001
2 mo: 24- to 48-h PD	<5	9.2 (7.8-11.0)	15.0 (12.6-17.9)	0.005
Estrone, g/mL				
2 mo: 24- to 48-h PD	49.0 (42.9-56.1)	238.6 (218.5-259.4)	288.4 (264.8-314.1)	0.06
Progesterone, ng/mL				
2 wk: 2- to 3-h PD	<1	10.9 (10.0-11.8)	3.8 (3.4-4.1)	<0.0001
2 wk: 24- to 48-h PD	<1	2.8 (2.6-3.0)	4.8 (4.5-5.2)	<0.0001
2 mo: 24- to 48-h PD	<1	2.9 (2.7-3.1)	3.2 (3.0-3.4)	0.19

Values represent mean (90% CI). For conversion to SI units, multiply by the following conversion factors: 3.67 for estradiol (pmol/L), 3.70 for estrone (pmol/L), and 3.18 for progesterone (nmol/L).
[a]Control = placebo; E_2 = 17β-estradiol administered orally; oral P_4 = micronized progesterone administered orally; ring P_4 = micronized progesterone administered via an intravaginal ring. Serum was collected 2 to 3 hours postdosing (PD) 2 weeks into each treatment period and 24 to 28 hours PD at 2 weeks and at the end of each 2-month treatment period.
[b]Oral P_4 and ring P_4 groups were compared using a mixed general linear model; control values are provided for reference only. *P* values indicate differences between E_2 + oral P_4 and E_2 + ring P_4 groups.

Breast epithelial proliferation

The primary endpoint for this study was breast epithelial proliferation, as measured by expression of the Ki67 nuclear antigen. Ki67 expression is an important prognostic marker in human breast cancer[42] and has been used extensively in our model to predict risk associated with various hormone therapies.[18,38,43] Before treatment, Ki67 expression was present in 7.3% ± 1.2% of lobular epithelial cells and 1.4% ± 0.4% of ductal epithelial cells (across all animals); no significant differences were present among groups at baseline($P > 0.1$ for lobular and ductal Ki67). After hormone treatment, breast Ki67 expression was not significantly different between E_2 + oral P_4 and E_2 + ring P_4 groups ($P > 0.1$ for lobular and ductal Ki67) (Fig. 2A). Intramammary mRNA for Ki67 and MCM5, a secondary marker of cell cycle progression, also did not differ after E_2 + oral P_4 and E_2 + ring P_4 treatment ($P > 0.1$ for both) (Fig. 2B).

Breast sex steroid receptor expression

We measured intramammary mRNA of ER-α and PGR, the primary mediators of E_2 and P_4 effects in breast epithelium. No significant differences were seen between E_2 + oral P_4 and E_2 + ring P_4 for either ER or PGR ($P > 0.1$ for both) (Fig. 3).

DISCUSSION

This pilot investigation was designed to compare the effects of oral and intravaginal progesterone on breast proliferation in a postmenopausal primate model. Using dose-calibrated silicone rings and individualized oral dosing, we obtained serum P_4 concentrations similar to those in postmenopausal women taking comparable P_4 formulations. Oral P_4 administration resulted in higher peak P_4 concentrations than did intravaginal administration, whereas oral and intravaginal routes provided similar lag P_4 concentrations at the time of breast biopsy. When added to E_2, oral P_4 resulted in significantly lower serum E_2 concentrations and

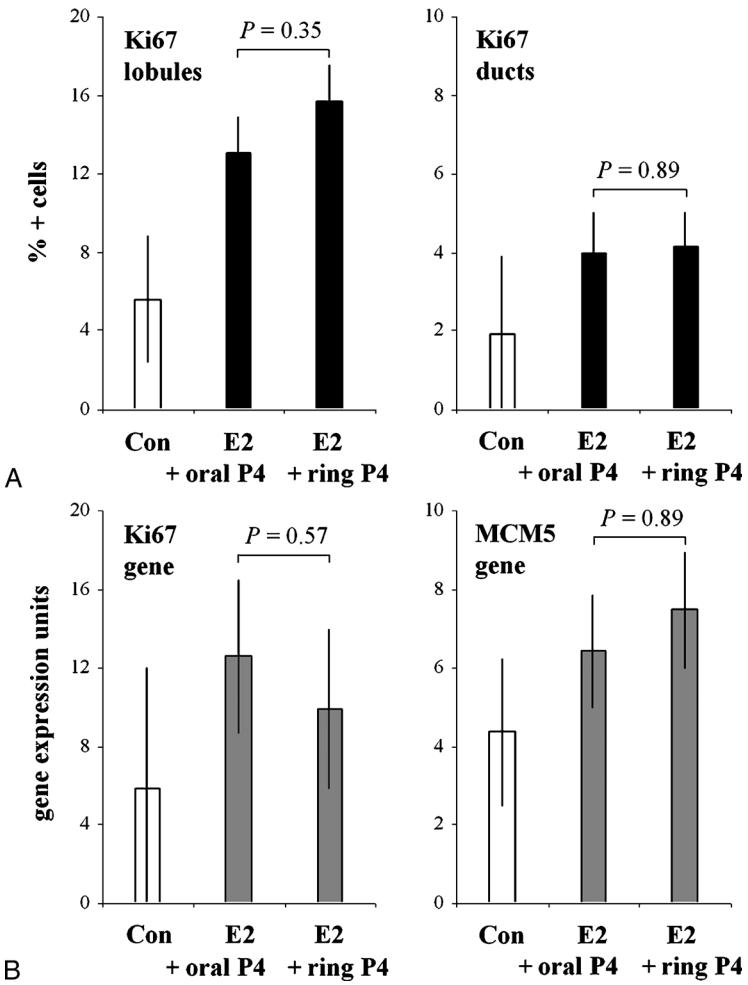

FIG. 2. Hormone treatment effects on cellular proliferation in the breast. **A:** Immunostaining for the proliferation marker Ki67 in lobular and ductal epithelium. **B:** Intramammary expression of mRNA transcripts for Ki67 and MCM5, as determined by quantitative reverse transcriptase–polymerase chain reaction. Treatment groups were placebo (Con), oral estradiol + oral progesterone (E_2 + oral P_4), and oral estradiol + progesterone administered via an intravaginal ring (28 mg/ring) (E_2 + ring P_4). Oral P_4 and ring P_4 groups were compared using a mixed general linear model; control values are provided for reference only. Vertical lines indicate SE.

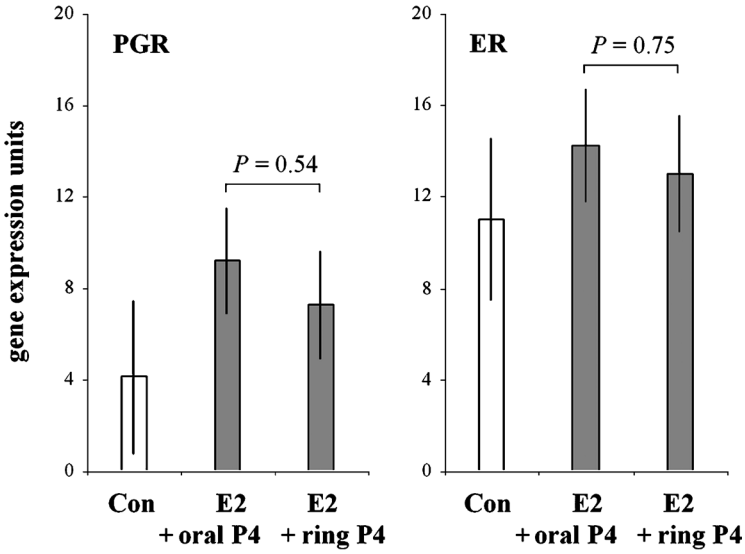

FIG. 3. Hormone treatment effects on gene markers of progesterone receptor (PGR) and estrogen receptor α (ER) in the breast. Intramammary mRNA was determined by quantitative reverse transcriptase–polymerase chain reaction. Treatment groups were placebo (Con), oral estradiol + oral progesterone (E_2 + oral P_4), and oral estradiol + progesterone administered via an intravaginal ring (28 mg/ring) (E_2 + ring P_4). Oral P_4 and ring P_4 groups were compared using a mixed general linear model; control values are provided for reference only. Vertical lines indicate SE.

decreased body weight compared with intravaginal P_4. No significant differences in breast proliferation or sex steroid receptor expression were found, suggesting that standard doses of oral and intravaginal P_4 produce similar effects in the postmenopausal breast.

Previous studies in our model have shown that adding the synthetic progestin MPA to an estrogen enhances overall breast epithelial proliferation by at least 30%,[18,38,43] consistent with breast cancer risk estimates from clinical trials and observational studies of women.[5-9] More recently, we reported that oral micronized P_4 (unlike MPA) does not increase breast proliferation beyond that seen with estrogen alone.[18] In this latter study, oral P_4 resulted in breast epithelial Ki67 expression (13% of lobular epithelial cells and 3% of ductal epithelial cells) very similar to those reported in the current experiment and consistently lower than those of previous studies in our model using estrogen with MPA (18%-24% of lobular epithelial cells and 4%-13% of ductal epithelial cells).[18,38,43] This evidence provides tangential support for the idea that P_4, given either orally or intravaginally, induces less stimulation than MPA in the postmenopausal breast. We should also note that prior reports from our laboratory[18] and others[44] indicate that the duration of hormone treatment used in this study (2 months) is ample time for effects on breast epithelial proliferation to occur.

The optimal serum P_4 concentrations after parenteral P_4 dosing in postmenopausal women has been a controversial issue in recent years.[45,46] Much of the debate has focused on whether adequate endometrial protection can be obtained in the presence of low serum P_4. In the past, serum P_4 concentrations of at least 3 to 5 ng/mL have been considered the minimum required for adequate endometrial protection after oral dosing, although this threshold range may vary considerably for different parenteral formulations based on relative bioavailability and metabolism.[35,45] Our preliminary calibration experiments using rings with high P_4 release rates (>2 mg/d in vitro) clearly demonstrate that intravaginal P_4 is readily absorbed into the systemic circulation and may result in high P_4 concentrations. In the subsequent study we achieved target P_4 levels of 3 to 5 ng/mL and observed no differences in either endometrial area or withdrawal bleeding after oral and intravaginal ring P_4. Further study is needed to evaluate whether intravaginal ring P_4 doses, by taking advantage of the first uterine pass effect, can be lowered further to provide endometrial protection when serum P_4 is less than 2 ng/mL.

In our previous study the addition of oral P_4 to E_2 resulted in 30% to 50% lower serum concentrations of E_2 and estrone compared with E_2 alone and E_2 + MPA.[18] A similar estrogen-lowering effect of oral P_4 was seen in the current experiment, in which E_2 + oral P_4 consistently resulted in 25% to 50% lower serum E_2 values than E_2 + ring P_4. The presence of this E_2-lowering effect with oral but not intravaginal P_4 administration and in both acute and lag samples strongly points to some sort of alteration in enterohepatic recirculation and/or hepatic first-pass metabolism of E_2. It is unclear whether this curious effect occurs in women because no studies, to our knowledge, have directly compared oral and parenteral P_4 alongside oral E_2; if so, this finding suggests that a reduced dose of oral E_2 may be used when given with intravaginal P_4 versus oral P_4.

Differential effects of oral and intravaginal P_4 on body weight were also unexpected. In our previous study we observed significantly greater weight loss with E_2 alone (0.40 ± 0.05 kg) than with E_2 + oral P_4 (0.13 ± 0.06 kg) over a similar 2-month treatment period,[18] suggesting that P_4 may partially antagonize the effects of E_2 on weight loss. Reasons for weight gain associated with intravaginal P_4 in the current

study are not immediately apparent, however. Potential explanations for this observation include greater P_4 bioavailability or higher amounts of particular P_4 metabolites with parenteral P_4 treatment which could have increased appetite and/or lowered physical activity.

CONCLUSIONS

In this study we demonstrate that P_4 delivered by vaginal ring is readily absorbed and provides more stable, sustained serum P_4 concentrations than oral administration. No significant differences were found between the effects of standard doses of oral and intravaginal P_4 on breast epithelium, suggesting that the two routes of administration would provide similar risk profiles for breast cancer promotion in postmenopausal women. Potential differences in exogenous oral estrogen metabolism and body weight effects were observed between oral and intravaginal P_4, although further work is needed to confirm these findings and to identify potential mechanisms. These findings do not support the idea that intravaginal P_4 has an inherently safer risk profile than oral P_4 in the postmenopausal breast. Future studies should focus on whether intravaginal doses of P_4 can be titrated low enough to minimize systemic P_4 absorption while still providing adequate endometrial protection against estrogen stimulation.

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