Progestin Treatment Induces Apoptosis and Modulates Transforming Growth Factor-β in the Uterine Endometrium

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Abstract

Background: **Epidemiologic, animal, and human data suggest that progestins are potent endometrial cancer preventive agents. In the ovarian surface epithelium, progestins have been hypothesized to confer a cancer preventive effect via apoptosis and modulation of transforming growth factor-β (TGF-β). Given that the ovarian epithelium and endometrium share a common embryologic origin and similar reproductive and hormonal risk factors for malignancy, we tested the hypothesis that progestins confer biological effects in the endometrium similar to those in the ovary.**

Methods: **Postmenopausal female macaques (n = 78) were randomized into four groups to receive a diet for 36 months containing no hormone versus conjugated equine estrogen (CEE), medroxyprogesterone acetate (MPA), or CEE + MPA. The endometrium was then examined immunohistochemically for treatment-specific changes using antibodies to activated caspase-3 (for apoptosis), Ki-67 (proliferation), and the TGF-β1, TGF-β2, and TGF-β3 isoforms.**

Results: **Percentages of caspase-positive endometrial glandular cells were 3- to 5-fold higher in CEE + MPA–treated animals compared with all others ($P < 0.05$). Caspase-expressing cells were six times more numerous in the endometrial stroma of animals treated with MPA alone relative to other groups ($P < 0.0001$). Induction of endometrial glandular cell apoptosis in the CEE + MPA–treated group was associated with a dramatic increase in expression of TGF-β2 and TGF-β3 in the stromal compartment of the endometrium ($P < 0.0001$).**

Conclusion: **Progestin treatment activates chemopreventive biological effects in the endometrium that are similar to those in the ovarian surface epithelium. These data may facilitate identification of a chemopreventive approach that dramatically lessens the risk of both uterine and ovarian cancer.** (Cancer Epidemiol Biomarkers Prev 2008;17(3):578–84)

Introduction

Approximately 150,000 new cases of endometrial cancer are diagnosed every year worldwide, making endometrial cancer the fifth leading cancer in women globally. In the United States, it is estimated that 41,200 new cases of endometrial cancer were diagnosed in 2006 (1, 2). Several risk factors for endometrial cancer have been identified including nulliparity, early menarche, late menopause, high body mass index, and the administration of unopposed estrogen for the treatment of climacteric symptoms (3-9).

In the past, endometrial cancer has not garnered as much attention as ovarian cancer. This may be secondary to the better overall prognosis of endometrial cancer, as the majority of women diagnosed with endometrial cancer are cured. Yet, stage for stage, survival rates for women with endometrial cancer are similar to those for ovarian cancer (1). In addition, similar to ovarian cancer, highly effective treatments for advanced-stage endometrial cancer are lacking. With an aging and increasingly obese population in the United States, the incidence and mortality from endometrial cancer is likely to become a worsening public health problem, thereby providing a strong rationale for the development of effective methods to prevent the disease (10).

A strong body of evidence suggests that progestins are highly effective endometrial cancer preventive agents. In premenopausal women, use of oral contraceptives that contain both an estrogen and a progestin significantly lowers subsequent endometrial cancer risk. Use of oral contraceptives for a period of at least 12 months confers as much as a 30% to 50% reduced risk of endometrial cancer, a protective effect that lasts for 10 to 20 years (11-15). In addition, progestin-potent oral contraceptives appear to have enhanced endometrial cancer protective effects compared with oral contraceptives containing weak progestins (16, 17), and use of progestin-releasing intrauterine devices, which release potent dosages of progestins locally in the endometrial cavity, leads to a marked reduction in endometrial cancer risk (18, 19). In menopausal women, the addition of a progestin to estrogen replacement therapy decreases the risk of

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precancerous endometrial hyperplasias and abrogates the cancer-causing effect of exogenous estrogen (4, 5, 9), suggesting that progestins have a chemopreventive biological effect on the endometrium. Finally, high-dose progestin therapy has been shown to reverse preexisting PTEN-inactivated endometrial latent precursors as well as endometrial hyperplasias and even low-grade endometrial cancers in some women (20-22).

The biological mechanism(s) that underlie the chemopreventive effect of progestins on the endometrium have not been well characterized. In the ovary, progestins have been shown to induce programmed cell death and differentially regulate transforming growth factor-β (TGF-β) in the ovarian surface epithelium, leading to the hypothesis that progestin-mediated biological effects lead to the arrest or reversal of carcinogenesis via clearance of genetically damaged ovarian epithelial cells. This biological mechanism may explain the marked reduction in ovarian cancer risk associated with oral contraceptive use (23). It is interesting that cancers arising from the ovary and endometrium share common epidemiologic risk factors, including marked risk reduction associated with use of oral contraceptives (11-15, 24, 25) and that both the endometrium and the ovarian surface epithelium share a common embryological precursor (26). It is thus plausible that progestins activate similar molecular pathways relevant to cancer prevention in both of these organ sites. To test this hypothesis, we examined the endometria of primates treated with hormonal interventions, including progestin for evidence of induction of apoptosis and for regulation of expression of the TGF-β isoforms.

Materials and Methods

Animals/Randomization. For this study, we used 78 female adult cynomolgus macaques (*Macaca fascicularis*), with an average age of 7.5 years at the study's end. The cynomolgus macaque is an excellent animal model for yielding experimental results that are pertinent to human reproductive biology. This nonhuman primate has a 28-day menstrual cycle that is similar to that of humans (27, 28). The study was a prospective, randomized, controlled trial designed for the primary endpoint of evaluating the effects of postmenopausal estrogens and progestins on the cardiovascular system, breast, and reproductive tracts. The macaques had previously undergone bilateral oophorectomy 3 months before commencement of the study.

The macaques were randomly assigned to receive one of four hormonal interventions for 3 years: control (n = 19), conjugated equine estrogen (CEE; n = 24; Premarin; Wyeth Ayerst), medroxyprogesterone acetate (MPA; n = 18; Provera; Wyeth Ayerst), and CEE and MPA combined (n = 17). Test compounds were administered daily in the diet throughout the study period at human equivalent doses on a caloric basis to 0.625 mg/d/woman for CEE and 2.5 mg/d/woman for MPA. This dosing strategy is metabolically based and thus results in relative doses similar to those produced by allometric calculations (29); most importantly, it produces serum concentrations in the range of those seen in women taking hormone therapy (30, 31). The duration of treatment was 36 months. The base diet was modeled on a typical moderately atherogenic North American diet (40% of calories from fat and 0.2 mg/kcal cholesterol) and commenced at the beginning of the hormonal interventions, which were given on a continuous basis until the end of the study. At the conclusion of the trial, animals were humanely euthanized by sedation with ketamine (10 mg/kg) and administration of 100 mg/kg pentobarbital i.v. Complete necropsy examinations were done, including examination of multiple organ systems, as reported previously (31). The study was approved by the Animal Care and Use Committee at the Wake Forest University School of Medicine.

Tissue Preparation and Immunohistochemistry. From each animal in the study, the uterus was fixed in 4% formaldehyde for 24 h and then stored at 4°C in 70% ethanol. Thereafter, uterine sections were trimmed to 3 mm thickness and embedded in paraffin.

Immunostaining. Briefly, 5 μm full sections through the uterine wall (endometrium to the serosa) were mounted on charged slides, and tissues were immunostained using our previously published methods (23, 32). Antigen retrieval with heat and citrate buffer (pH 6.0) was used for all antibodies. Appropriate quenching procedures (heat treatment and peroxidase treatment) were used to remove endogenous enzymatic activity. Staining for apoptosis was done using a monoclonal antibody to cleaved caspase-3 (2 μg/mL; Cell Signaling Technology); sections of benign macaque early menstrual phase endometrium were used as a positive control for apoptosis. Staining for TGF-β isoforms was done using antibodies specific for TGF-β1 (0.67 μg/mL anti-TGF-β1 antibody; Santa Cruz Biotechnology), TGF-β2 (4 μg/mL anti-TGF-β2 antibody; Santa Cruz Biotechnology), and TGF-β3 (0.25 μg/mL anti-TGF-β3 antibody; Santa Cruz Biotechnology). For TGF-β staining, normal human umbilical cord was used as a positive control. Staining for proliferating cells was done using a monoclonal antibody to Ki-67 (1.25 μg/mL clone MM1; Novocastra Laboratories). For caspase and Ki-67 staining, the enzyme detection system used alkaline phosphatase and the chromogen used was Vector Red (Vector Laboratories); for TGF-β staining, the enzyme system used was peroxidase and the chromogen was diaminobenzidine (DAKO). Sections were counterstained with Mayer's hematoxylin.

Caspase-stained and Ki-67-stained cells were quantified by counting the percentages of stained and unstained cells in sections by an observer blinded to treatments. The percentages of immunopositive uterine glandular cells as well as stromal cells in both the superficial (functionalis) and deep (basalis) layers of the endometrium were quantitated. TGF-β staining was also assessed in glands and stroma of the functionalis and basalis. Staining was graded for each cell type and endometrial location as 0 (unstained) to 3+ (heavily stained) by three reviewers, including a veterinary pathologist (J.M.C.). All observers were again blinded to treatment.

Statistical Analysis. Both the percentage of caspase-3-expressing cells and the semiquantitative assessments of immunostaining for TGF-β isoforms were analyzed for treatment group as continuous variables using ANOVA

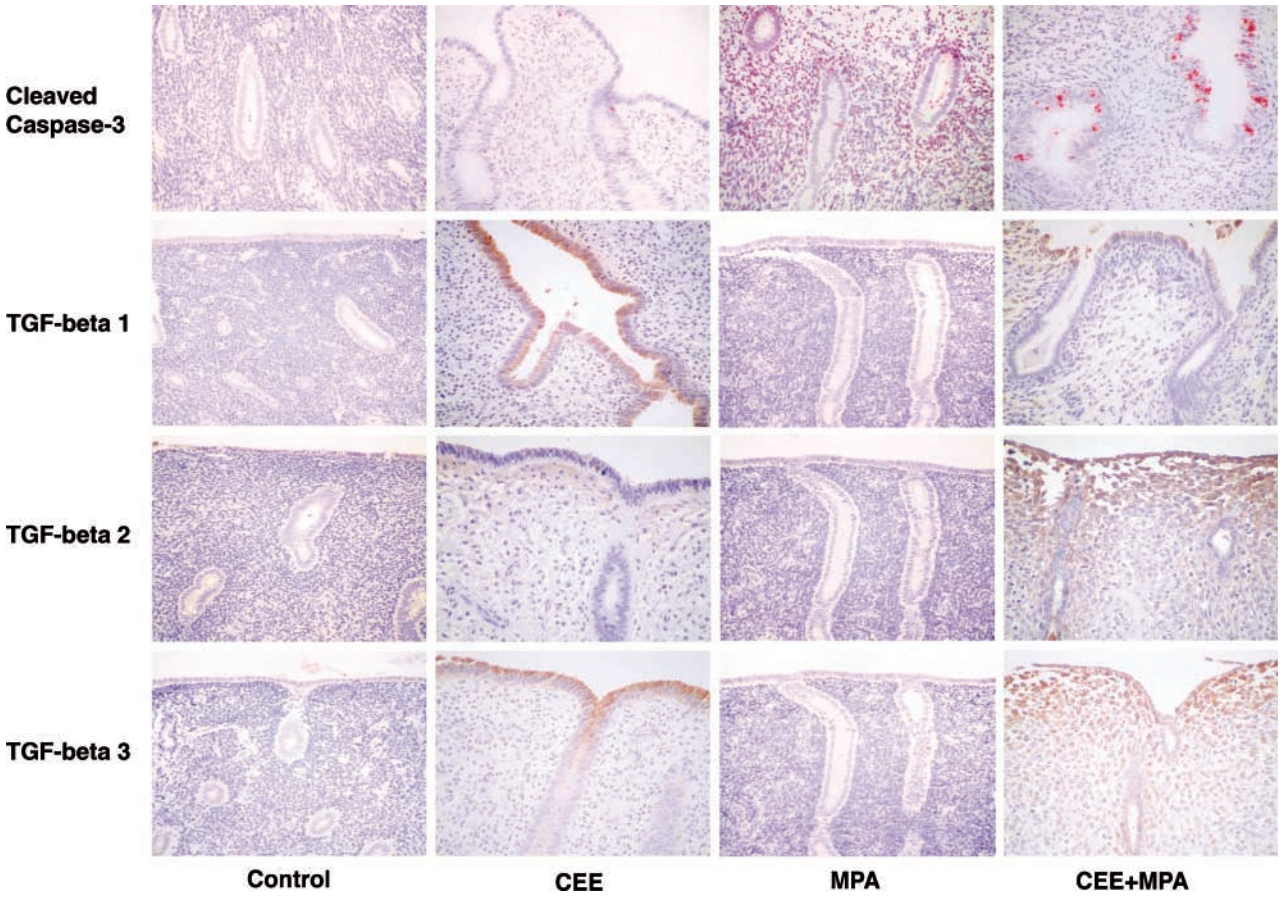

Figure 1. Representative sections of superficial endometrium (functionalis) from each of the treatment groups, immunostained for activated caspase-3 and the TGF-β isoforms.

and a two-tailed significance level of 0.05. Pairwise *t* tests were used to identify specific group differences if the overall ANOVA showed significance. Within-group correlation analysis was done using Pearson correlation coefficients to identify associations between dependent variables. In addition, the data for TGF-β were also analyzed via a binary method. For dichotomized TGF-β [low intensity of staining or low expression (0-1+) versus high intensity of staining or high expression (2-3+)], Fisher's exact test was used to investigate the effects of treatment group to TGF-β expression. Logistic regression modeling was used to determine the importance of the effects (33). Tests were two tailed with significance level of 0.05. Bonferroni-adjusted significance level was applied while the effect to TGF-β was compared between treatment groups.

Results

Hormone treatment-related changes in apoptosis, proliferation, and TGF-β expression were confined primarily to the functionalis (superficial) compartment of the endometrium. The data presented therefore represent findings confined to the superficial endometrial glands and stroma.

Effect of Hormone Treatment on Apoptosis in Endometrium. In general, few apoptotic cells were noted in the endometrium from either the control or CEE-only-treated monkeys. In contrast, progestin treatment with MPA, either in combination with CEE or alone, was associated with significant increases in apoptosis in both glandular and stromal cells (Fig. 1). Percentages of caspase-positive glandular epithelial cells

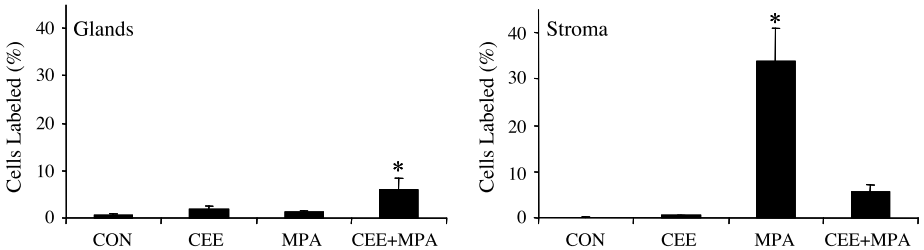

Figure 2. Cleaved caspase-3 immunostaining expressed as a percentage of cells counted in glandular and stromal compartments of the superficial (functionalis) endometrium. *Bars*: SEM; *: groups whose means differ from controls at $P < 0.05$.

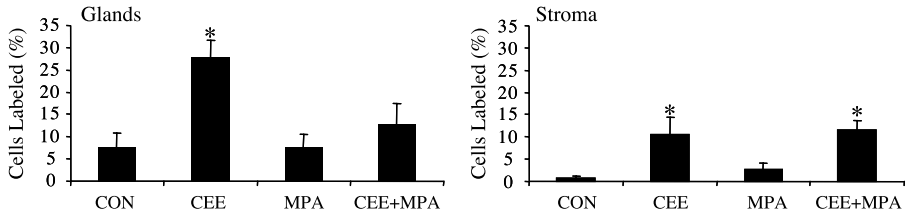

Figure 3. Ki-67 immunostaining expressed as percentage of cells counted in glandular and stromal compartments of the superficial (functionalis) endometrium. *Bars*: SEM; *: groups whose means differ from controls at $P < 0.05$.

were 3- to 5-fold higher in CEE + MPA–treated animals compared with all others ($P < 0.05$). In addition, caspase-3-expressing cells were six times more numerous in the stroma of animals treated with MPA alone relative to other groups ($P < 0.05$; Fig. 2).

Effect of Hormone Treatment on Proliferation in Endometrium. Expression of the proliferation marker Ki-67 was strongly induced in the glandular epithelium by treatment with CEE ($P < 0.05$). This effect was antagonized by the addition of MPA to the treatment regimen. For both CEE and CEE + MPA, proliferation was increased in the stromal compartment ($P < 0.05$; Fig. 3).

Effect of Hormone Treatment on Expression of TGF-β. Expression of the TGF-β isoforms varied throughout the endometrium relative to both treatment group and location within the endometrium. In endometrial glands, expression of TGF-β1 was scant in both the untreated monkeys and those receiving MPA alone. There was a nonsignificant increase in TGF-β1 expression in CEE-treated monkeys, which was abrogated by the addition of MPA ($P < 0.01$). Stromal expression of TGF-β1 was absent across all treatments (Figs. 1 and 4). Expression of TGF-β2 was scant in the glandular compartment and not affected by treatment. However, expression of TGF-β2 was markedly increased in the endometrial stroma of animals treated with CEE + MPA relative to all other groups ($P < 0.0001$; Figs. 1 and 4). For the TGF-β3 isoform, expression in the glandular compartment was generally low and variable. MPA-treated and CEE + MPA–treated animals had lower glandular TGF-β3 expression than CEE-treated animals ($P < 0.01$). In contrast, TGF-β3 expression in the stroma was markedly elevated in CEE + MPA–treated animals, differing from all other groups ($P < 0.0001$; Figs. 1 and 4). Of note, analysis of the TGF-β scoring data as ordinal (stepwise) data rather than continuous variables yielded similar results.

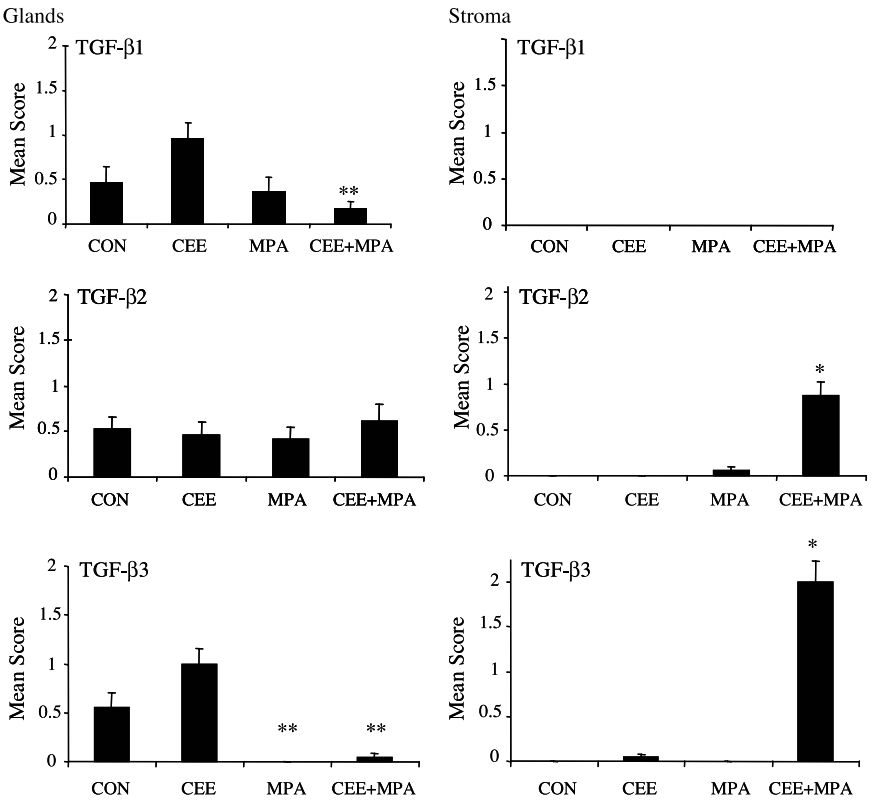

Figure 4. TGF-β1 immunostaining expressed as mean staining score in superficial (functionalis) endometrial glands and stroma. *Bars*: SEM; *: groups whose means differ from controls at $P < 0.0001$ or **: groups whose means differ from CEE at $P < 0.01$.

Discussion

In this study, we have characterized the complex opposing effects of estrogen and progestin on proliferation, apoptosis, and TGF-β signaling in the uterine endometrium. In an ovariectomized menopausal primate model, we have shown that estrogen induces proliferation in endometrial glands while having no effect on apoptosis. In contrast, progestin inhibits estrogen-induced proliferation and differentially regulates expression of TGF-β, causing a decrease in expression of TGF-β3 in endometrial glands while dramatically increasing expression of the TGF-β2 and TGF-β3 isoforms in the stroma. In addition, progestin markedly induces apoptosis in both endometrial glands and superficial stroma. Areas of marked apoptosis in both endometrial glands and stroma associated with progestin treatment occurred concurrently with marked increased expression of TGF-β2 and TGF-β3 in the superficial stroma, suggesting that these two biological events may be related. Finally, progestin-mediated apoptotic effects in endometrial glands occurred only in the presence of estrogen, suggesting the requirement for estrogen priming, whereas estrogen was not required for progestin to induce marked apoptotic effects in the endometrial stroma.

Overall, our findings are consistent with published data regarding the effect of progestins on apoptosis and TGF-β signaling in the endometrium. Progestins have been shown to induce apoptosis in endometrial glands and stroma, an effect that is modulated by several well-known apoptosis-related proteins (22, 34-39). In the mouse, an increase in the Bax/Bcl-2 ratio and associated increase in apoptosis have been shown in endometrial stromal cells in response to progestins, suggesting that progestin-mediated apoptotic effects contribute to the remodeling changes in the uterus associated with decidualization (35). In women, up-regulation of Fas/Fas ligand expression has been shown in endometrial cells in response to progestins; conversely, dysregulation of Fas/Fas ligand expression in hyperplastic endometrium may underlie failure of progestins to reverse endometrial hyperplasia (37). In addition, progestins have been shown to increase the Bax/Bcl-2 ratio and induce apoptosis *in vivo* in women undergoing treatment for endometrial hyperplasia. Moreover, the degree of change in Bax, Bcl-2, and apoptosis has been shown to be correlated with the potency of progestin therapy and in turn with the likelihood of response to therapy (22). Thus, activation of apoptotic pathways may be an important mechanism underlying the chemopreventive effects of progestins in the endometrium. With regard to TGF-β, both TGF-β2 and TGF-β3 have been shown to be increased in the endometrium during the secretory phase or in response to progestins *in vivo* (40-43), whereas expression of TGF-β1 was unchanged (40). Similarly, in the primate endometrium in response to MPA, we found overall levels of TGF-β2 and TGF-β3 to be dramatically increased without a significant effect on TGF-β1.

Our findings in the endometrium bear remarkable similarities to what we described previously in the ovary. Previously, we have shown that progestin induces apoptosis and differentially regulates expression of TGF-β in the ovarian surface epithelium (23). We observed a 4-fold increase in apoptosis in the ovarian epithelium in primates treated with the progestin levonorgestrel. This was associated with a significant increase in expression in the ovarian surface epithelium of the TGF-β2 and TGF-β3 isoforms and a decrease in expression of TGF-β1. In the endometrium, our findings were similar, except for the absence of an effect of progestin on TGF-β1 expression, although the addition of MPA to CEE lessened expression of TGF-β1 in endometrial glands, a trend similar to our prior findings in the ovarian surface epithelium. In addition, in the ovary, changes in expression of TGF-β were localized specifically in the ovarian surface epithelium, whereas the predominant change in expression of TGF-β in the endometrium was primarily in the superficial stroma. Thus, in the ovary, changes in expression of the TGF-β isoforms in the ovarian surface epithelium may have an autocrine and paracrine effect on the ovarian surface epithelium, whereas, in the endometrium, stromal changes in expression of TGF-β may have an autocrine effect in stromal cells and a paracrine effect in adjacent glandular cells. In addition, in the ovarian surface epithelium, increases in expression of TGF-β2 and TGF-β3 were noted with progestin administered alone, whereas, in the endometrium, induction of expression of these two isoforms in the superficial stroma by progestin required the presence of estrogen. It is possible however that estrogen priming is necessary for optimization of progestin effects on TGF-β in both the endometrium and ovary. For example, our earlier study evaluating progestin in the ovarian surface epithelium used premenopausal monkeys who had not undergone ovariectomy and thus received progestin in the setting of an adequate endogenous estrogenic milieu. Conversely, the subtle differences in progestin effect in our prior ovarian study and the current study may be due to differences in the progestins administered. Our prior study involved levonorgestrel, a potent gonane progestin that is a nortestosterone derivative typical in oral contraceptives. However, the current study used MPA, a pregnane progestin typically used in hormone replacement therapy regimens that has significantly less potency than oral contraceptive progestins. To illustrate the potential differences between these classes of compounds, use of oral contraceptives markedly reduces endometrial cancer risk (11-15), whereas the addition of MPA to hormone replacement therapy estrogens mainly abrogates the cancer-causing effect of estrogen replacement therapy rather than markedly reducing endometrial cancer risk (4, 5, 9). It is possible that a hormone replacement therapy regimen that contains a potent progestin such as those in oral contraceptives might actually lower endometrial cancer risk. Nonetheless, the finding that progestins have similar biological effects in the ovarian surface epithelium and endometrium suggests a common biological mechanism that may underlie the cancer preventive effect of progestins in both tissues.

The design of our study does not allow us to prove a causal relationship between changes in TGF-β expression and apoptosis in endometrial glands and stroma. However, it is possible that the two are related. Among the growth factors, TGF-β has been implicated as an important regulator of apoptosis (44-46). In addition, in cells derived from the ovarian and uterine epithelium, TGF-β has been shown to induce apoptosis (47-50).

Furthermore, in hormone-sensitive tissues, such as the breast and prostate, TGF-β has been shown to mediate the apoptotic effects of steroid hormones, including the antiestrogens, retinoids, and vitamin D (51-55). Finally, TGF-β is related to Müllerian inhibitory factor, a peptide that causes complete apoptotic regression of the Müllerian system (the precursor to the uterus, fallopian tubes, and upper vagina) in the developing male embryo *in vivo* (56-59), and which has been shown to inhibit growth and induce apoptosis in cells derived from the ovarian and uterine epithelium *in vitro* (60-62). Given the marked inhibitory effect that the members of the TGF-β family have on the Müllerian system, it is interesting to speculate that the uterine endometrium may be uniquely susceptible to a TGF-β-induced apoptotic response *in vivo*. Moreover, agents which selectively regulate TGF-β in the endometrium, may be excellent candidates for further research as endometrial chemopreventive agents.

A growing body of laboratory and *in vivo* evidence has implicated TGF-β as a potent tumor suppressor and cancer preventive agent (63-66). Transgenic mice have shown both resistance to induced mammary tumors when TGF-β1 is constitutively expressed as well as increased susceptibility to carcinogens when TGF-β genes are deleted (67, 68). In humans, mutations have been described in the TGF-β signaling pathway in a variety of tumors, including cancers of the colon, gastric, pancreatic, uterus, and lymphoid system (65, 69-73). Furthermore, several cellular oncogenes are known to inhibit TGF-β activity. Finally, TGF-β has been implicated as a mediator of the biological effects of several chemopreventive agents, including tamoxifen and retinoids (64, 73). Taken together, these data provide compelling evidence that TGF-β plays an important role as an inhibitor of carcinogenesis and that agents which exploit this pathway may have utility for the chemoprevention of cancer.

Similarly, the apoptosis pathway also holds great promise as a target for chemoprevention. Activation of apoptosis leads to the efficient disposal of cells that have undergone irreparable genetic damage and that are prone to neoplastic transformation (74). Pharmacologic agents that selectively enhance apoptosis have been shown to lower the risk of a variety of cancers in animals and in humans (74, 75). In addition, both in animal models of cancer and in humans, the efficacy of cancer preventive agents has been shown to correlate with the degree of apoptosis induced (22, 74, 76, 77). Conversely, mutations in the genes involved in apoptotic pathways have been shown to be associated with enhanced cancer risk (77).

In light of the known association between activation of TGF-β and apoptotic molecular pathways and cancer prevention, the observation that progestins markedly activate these pathways in the endometrium opens the door toward development of a progestin-based strategy for the effective chemoprevention of endometrial cancer. Moreover, given that progestins have similar chemopreventive biological effects in both the ovary and the endometrium, it is interesting to speculate that a potent progestin-based strategy may effectively prevent both ovarian and uterine cancer, thereby decreasing the incidence and mortality from the two most common gynecologic malignancies.

References

1. Amant F, Moerman P, Neven P, Timmerman D, Limbergan E, Vergote I. Endometrial Cancer. Lancet 2005;366:491–505.
2. American Cancer Society. Cancer facts and figures 2006. Atlanta: American Cancer; 2006.
3. Henderson BE, Casagrance JT, Pike MC, et al. The epidemiology of endometrial cancer in young women. Br J Cancer 1983;47:749–56.
4. McPherson CP, Sellers TA, Potter JD, Bostick RM, Folsom AR. Reproductive factors and risk of endometrial cancer. The Iowa Women's Health Study. Am J Epidemiol 1996;143:1195–202.
5. The Writing Group for the PEPI trial. Effects of hormone replacement therapy on endometrial histology in postmenopausal women: the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial. JAMA 1996;275:370–5.
6. Xu W, Xiang Y-B, Zheng W, et al. Weight history and risk of endometrial cancer among Chinese women. Int J Epidemiol 2006;35:159–66.
7. Trentham-Dietz A, Nichols H, Hampton J, Newcomb P. Weight change and risk of endometrial cancer. Int J Epidemiol 2006;35:151–8.
8. Weiderpass E, Persson I, Adami HO, Magnusson C, Lindgren A, Baron JA. Body size in different periods of life, diabetes mellitus, hypertension, and risk of postmenopausal endometrial cancer (Sweden). Cancer Causes Control 2000;11:185–92.
9. Shulman LP, Yankov V, Uhl K. Safety and efficacy of a continuous once-a-week 17β-estradiol/levonorgestrel transdermal system and its effects on vasomotor symptoms and endometrial safety in postmenopausal women: the results of two multicenter, double-blind, randomized, controlled trials. Menopause 2002;9:195–207.
10. Webb PM. Commentary: Weight gain, weight loss, and endometrial cancer. Int J Epidemiol 2006;35:166–8.
11. Vessey M, Painter R. Oral contraceptive use and cancer. Findings in a large cohort study, 1968-2004. Br J Cancer 2006;95:385–9.
12. Hulka BS, Chambless LE, Kaufman DG, Fowler WC, Greenberg BG. Protection against endometrial carcinoma by combination-product oral contraceptives. J Am Med Assoc 1982;247:475–7.
13. The Cancer and Steroid Hormone (CASH) Study of the Centers for Disease Control and the National Institute of Child Health and Human Development. Combination oral contraceptive use and the risk of endometrial cancer. J Am Med Assoc 1987;257:796–800.
14. The WHO collaborative study of neoplasia and steroid contraceptives. Endometrial cancer and combined oral contraceptives. Int J Epidemiol 1988;17:263–9.
15. Jick SS, Walker AM, Jick H. Oral contraceptives and endometrial cancer. Obstet Gynecol 1993;82:931–5.
16. Maxwell GL, Schildkraut JM, Calingaert B, et al. Progestin and estrogen potency of combination oral contraceptives and endometrial cancer risk. Gynecol Oncol 2006;103:535–40.
17. Rosenblatt KA, Thomas DB; WHO Collaborative Study of Neoplasia and Steroid Contraceptives. Hormonal content of combined oral contraceptives in relation to the reduced risk of endometrial carcinoma. Int J Cancer 1991;49:870–4.
18. Benshushan A, Paltiel O, Rojansky N, Brzezinski A, Laufer N. IUD use and the risk of endometrial cancer. Eur J Obstet Gynecol Reprod Biol 2002;105:166–9.
19. Tao MH, Xu WH, Zheng W, et al. Oral contraceptive and IUD use and endometrial cancer: a population-based case-control study in Shanghai, China. Int J Cancer 2006;119:2142–7.
20. Orbo A, Rise CE, Mutter G. Regression of latent endometrial precancers by progestin infiltrated intrauterine device. Cancer Res 2006;66:5613–7.
21. Randall TC, Kurman RJ. Progestin treatment of atypical hyperplasia and well-differentiated carcinoma of the endometrium in women under age 40. Obstet Gynecol 1997;90:434–40.
22. Vereide AB, Kaino T, Sager G, Ørbo A. Bcl-2, BAX, and apoptosis in endometrial hyperplasia after high dose gestagen therapy: a comparison of responses in patients treated with intrauterine levonorgestrel and systemic medroxyprogesterone. Gynecol Oncol 2005;97:740–50.
23. Rodriguez GC, Nagarsheth NP, Lee KL, et al. Progestin-induced apoptosis in the macaque ovarian epithelium: differential regulation of transforming growth factor-β. J Natl Cancer Inst 2002;94:50–60.
24. Schildkraut JM, Calingaert B, Marchbanks PA, Moorman PG, Rodriguez GC. Impact of progestin and estrogen potency in oral contraceptives on ovarian cancer risk. J Natl Cancer Inst 2002;94:32–8.
25. Whittemore AS, Harris R, Itnyre J; Collaborative Ovarian Cancer Group. Characteristics relating to ovarian cancer risk: collaborative analysis of 12 US case-control studies. IV. The pathogenesis of epithelial ovarian cancer. Am J Epidemiol 1992;136:1212–20.

26. Kurman RJ, editor. Blaustein's pathology of the female genital tract. 5th ed. New York: Springer-Verlag; 2002.
27. Cline JM, Söderqvist G, Register TC, Williams JK, Adams MR, Von Schoultz B. Assessment of hormonally active agents in the reproductive tract of female nonhuman primates. Toxicol Pathol 2001;29:84–90.
28. Brenner RM, Slayden OD. Cyclic changes in the primate oviduct and endometrium. In: Knobil E, Neill JD, editors. The physiology of reproduction. New York (NY): Raven Press; 1994. p. 541–69.
29. Mordenti J. Dosage regimen design for pharmaceutical studies conducted in animals. J Pharm Sci 1986;75:852–7.
30. Yasui T, Uemura H, Tezuka M, Yamada M, Irahara M, Miura M, Aono T. Biological effects of hormone replacement therapy in relation to serum estradiol levels. Horm Res 2001;56:38–44.
31. Cline JM, Söderqvist G, von Schoultz E, Skoog L, von Schoultz B. Effects of conjugated estrogens, medroxyprogesterone acetate, and tamoxifen on the mammary glands of macaques. Breast Cancer Res Treat 1998;48:221–9.
32. Conner P, Register TC, Skoog L, Tani E, von Schoultz Cline JM. Expression of p53 and markers for apoptosis in breast tissue during long-term hormone therapy in cynomolgus monkeys. Am J Obstet Gynecol 2005;193:58–63.
33. Agresti A. An introduction to categorical data analysis. New York: John Wiley & Sons; 1996.
34. Amezcua CA, Lu JJ, Felix JC, Stanczyk FZ, Zheng W. Apoptosis may be an early event of progestin therapy for endometrial hyperplasia. Gynecol Oncol 2000;79:169–76.
35. Akcali KC, Khan SA, Moulton BC. Effect of decidualization on the expression of bax and bcl-2 in the rat uterine endometrium. Endocrinology 1996;137:3123–31.
36. Hanifi-Moghaddam, Gielen SC, Kloosterboer HJ, et al. Molecular portrait of the progestagenic and estrogenic actions of tibolone: behavior of cellular networks in response to tibolone. J Clin Endocrinol Metabol 2005;90:973–83.
37. Wang S, Pudney J, Song J, Mor G, Schwartz PE, Zheng W. Mechanisms involved in the evolution of progestin resistance in human endometrial hyperplasia-precursor of endometrial cancer. Gynecol Oncol 2003;88:108–17.
38. Maruo T, Laoag-Fernandez JB, Pakarinen P, Murakoshi H, Spitz IM, Johansson E. Effects of the levonorgestrel-releasing intrauterine system on proliferation and apoptosis in the endometrium. Hum Reprod 2001;16:2103–8.
39. Vaskivuo TE, Stenback F, Karhumaa P, Risteli J, Dunkel L, Tapanainen JS. Apoptosis and apoptosis-related proteins in human endometrium. Mol Cell Endocrinol 2000;165:75–83.
40. Reis FM, Ribeiro MF, Maia AL, Spritzer PM. Regulation of human endometrial transforming growth factor β1 and β3 isoforms through menstrual cycle and medroxyprogesterone acetate treatment. Histol Histopathol 2002;17:739–45.
41. Rosario GX, Sachdeva G, Manjramkar DD, Modi DN, Meherji PK, Puri CP. Endometrial expression of immunomodulatory cytokines and their regulators during early pregnancy in bonnet monkeys (*Macaca radiata*). Hum Reprod 2005;20:3039–46.
42. Okulicz WC, Ace CI, Longcope C, Tast J. Analysis of differential gene regulation in adequate versus inadequate secretory-phase endometrial complementary deoxyribonucleic acid populations from the rhesus monkey. Endocrinology 1996;137:4844–50.
43. Ace CI, Okulicz WC. Differential gene regulation by estrogen and progesterone in the primate endometrium. Mol Cell Endocrinol 1995; 115:95–103.
44. Haufel T, Dormann S, Hanusch J, Schwieger A, Bauer G. Three distinct roles for TGF-β during intercellular induction of apoptosis: a review. Anticancer Res 1999;19:105–12.
45. Bursch W, Oberhammer F, Jirtle RL, et al. Transforming growth factor-β1 as a signal for induction of cell death by apoptosis. Br J Cancer 1993;67:531–6.
46. Chiarugi V, Magnelli L, Cinelli M. Complex interplay among apoptosis factors: RB, p53, E2F, TGF-β, cell cycle inhibitors and the bcl2 gene family. Pharmacol Res 1997;35:257–61.
47. Berchuck A, Rodriguez G, Olt GJ, et al. Regulation of growth of normal ovarian epithelial cells and ovarian cancer cell lines by transforming growth factor-β. Am J Obstet Gynecol 1992;166:676–84.
48. Hurteau J, Rodriguez GC, Whitaker RS, et al. Transforming growth factor-β inhibits proliferation of human ovarian cancer cells obtained from ascites. Cancer 1994;74:93–9.
49. Havrilesky LJ, Hurteau JA, Whitaker RS, et al. Regulation of apoptosis in normal and malignant ovarian epithelial cells by transforming growth factor β. Cancer Res 1995;55:944–8.
50. Rotello RJ, Lieberman RC, Purchio AF, Gershenson LE. Coordinated regulation of apoptosis and cell proliferation by transforming growth factor 1 in cultured uterine epithelial cells. Proc Natl Acad Sci U S A 1991;88:3412–5.
51. El Etreby MF, Liang Y, Wrenn RW, Schoenlein PV. Additive effect of mifepristone and tamoxifen on apoptotic pathways in MCF-7 human breast cancer cells. Breast Cancer Res Treat 1998;51: 149–68.
52. James SY, Mackay AG, Colston KW. Effects of 1,25 dihydroxyvitamin D3 and its analogues on induction of apoptosis in breast cancer cells. J Steroid Biochem Mol Biol 1996;58:395–401.
53. Kyprianou N, Isaacs JT. Expression of transforming growth factor-β in the rat ventral prostate during castration-induced programmed cell death. Mol Endocrinol 1989;3:1515–22.
54. Roberson KM, Penland SN, Padilla GM, et al. Fenretinide: induction of apoptosis and endogenous transforming growth factor-β in PC-3 prostate cancer cells. Cell Growth Differ 1997;8:101–11.
55. Rendi MH, Suh N, Lamph WW, et al. The selective estrogen receptor modulator arzoxifene and the rexinoid LG100268 cooperate to promote transforming growth factor β-dependent apoptosis in breast cancer. Cancer Res 2004;64:3566–71.
56. Mishina Y, Whitworth DJ, Racine C, Behringer RR. High specificity of Müllerian-inhibiting substance signaling *in vivo*. Endocrinology 1999;140:2084–8.
57. Voutilainen R, Miller WL. Potential relevance of Mullerian-inhibiting substance to ovarian physiology. Semin Reprod Endocrinol 1989;7: 88–93.
58. Cate RL, Mattaliano RJ, Hession C, et al. Isolation of the bovine and human genes for Müllerian inhibiting substance and expression of the human gene in animal cells. Cell 1986;45:685–98.
59. Byskov AG. Differentiation of mammalian embryonic gonad. Physiol Rev 1986;66:71–117.
60. Masiakos PT, MacLaughlin DT, Maheswaran S, et al. Human ovarian cancer, cell lines, and primary ascites cells express the human Mullerian inhibiting substance (MIS) type II receptor, bind, and are responsive to MIS. Clin Cancer Res 1999;11:3488–99.
61. Stephen AE, Pearsall LA, Christian BP, Donahoe PK, Vacanti JP, MacLaughlin DT. Highly purified Mullerian inhibiting substance inhibits human ovarian cancer *in vivo*. Clin Cancer Res 2002;8: 2640–6.
62. Renaud EJ, MacLaughlin DT, Oliva E, Rueda BR, Donahoe PK. Endometrial cancer is a receptor-mediated target for Mullerian inhibiting substance. Proc Natl Acad Sci U S A 2005;3:111–6.
63. Sporn MB, Roberts AB, Wakefiled LM, Glick AB, Danielpour D. Transforming growth factor β and suppression of carcinogenesis. Princess Takamatsu Symposia 1989;20:259–66.
64. Reiss M. Transforming growth factor-β and cancer: a love-hate relationship? Oncol Res 1997;9:447–57.
65. Blobe GC, Schiemann WP, Lodish HF. Role of transforming growth factor in human disease. N Engl J Med 2000;342:1350–8.
66. Narayan S, Thangasamy T, Balusu R. Transforming growth factor β signaling in cancer. Front Biosci 2005;10:1135–45.
67. Pierce DF Jr, Gorska AE, Chytil A, et al. Mammary tumor suppression by transforming growth factor β1 transgene expression. Proc Natl Acad Sci U S A 1995;92:4254–8.
68. Tang B, Bottiner E, Bagnall K, Anver M, Wakefield L. Enhanced liver and lung tumorigenesis in TGF-β1 heterozygous knock-out mice following treatment with diethylnitrosamine and phenobarbital [abstract]. Proc Am Assoc Cancer Res 1997;38:584.
69. Lin HK, Bergmann S, Pandolfi PP. Deregulated TGF-Beat signaling in leukemogenesis. Oncogene 2005;24:5693–700.
70. Izzi L, Attisano L. Ubiquitin-dependent regulation of TGF-β signalling in cancer. Neoplasia 2006;8:677–88.
71. Elliot RL, Blobe GC. Role of Transforming Growth Factor β in human cancer. J Clin Oncol 2005;23:2078–93.
72. Kaklamani VG, Pasche B. Role of TGF-β in cancer and the potential for therapy and prevention. Expert Rev Anticancer Ther 2004;4:649–61.
73. Park SH. Fine tuning and cross-talking of TGF-β signal by inhibitory smads. J Biochem Mol Biol 2005;38:9–16.
74. Sun SY, Hail N, Lotan R. Apoptosis as a novel target for cancer prevention. J Natl Cancer Inst 2004;96:662–72.
75. Ouyang N, Williams JL, Rigas B. NO-donating aspirin isomers downregulate peroxisome proliferator-activated receptor (PPAR)δ expression in APC(min/+) mice proportionally to their tumor inhibitory effect: implications for the role of PPARδ in carcinogenesis. Carcinogenesis 2006;27:232–9.
76. Sinicrope FA, Half E, Morris JS, et al. Cell proliferation and apoptotic indices predict adenoma regression in a placebo-controlled trial of celecoxib in familial adenomatous polyposis patients. Cancer Epidemiol Biomarkers Prev 2004;13:920–7.
77. Reed JC. Mechanisms of apoptosis avoidance in cancer. Curr Opin Oncol 1999;11:68–75.

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