0021-972X/99/$03.00/0
The Journal of Clinical Endocrinology & Metabolism
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Vol. 84, No. 12
Printed in U.S.A.

Hormone Replacement Therapy with Estrogen or Estrogen plus Medroxyprogesterone Acetate Is Associated with Increased Epithelial Proliferation in the Normal Postmenopausal Breast*

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ABSTRACT

The relative effects of postmenopausal hormone replacement therapy (HRT) with estrogen alone *vs.* estrogen+progestin on breast cell proliferation and on breast cancer risk are controversial. A cross-sectional observational study was carried out to examine the proliferative effects of HRT with estrogen or estrogen plus the progestin, medroxyprogesterone acetate, in breast tissue of postmenopausal women. Benign breast biopsies from 86 postmenopausal women were analyzed with antiproliferating cell nuclear antigen (anti-PCNA) and Ki67 antibodies to measure relative levels of cell proliferation. Epithelial density and estrogen and progesterone receptor status were also determined. The women were categorized either as users of: 1) estrogen (E) alone; 2) estrogen+medroxyprogesterone acetate (E+P); or 3) no HRT. Compared with no HRT, the breast epithelium of women who had received either E+P or E alone had significantly higher PCNA proliferation indices, and treatment with E+P had a significantly higher index (PCNA and Ki67) than treatment with E alone. Breast epithelial density was significantly greater in postmenopausal women treated with E and E+P, compared with no HRT. Thus, the present study shows that postmenopausal HRT with E+P was associated with greater breast epithelial cell proliferation and breast epithelial cell density than E alone or no HRT. Furthermore, with E+P, breast proliferation was localized to the terminal duct-lobular unit of the breast, which is the site of development of most breast cancers. Further studies are needed to assess the possible association between the mitogenic activity of progestins and breast cancer risk. (*J Clin Endocrinol Metab* **84:** 4559–4565, 1999)

POSTMENOPAUSAL hormone replacement therapy (HRT) with estrogen alone or estrogen+progestin alleviates menopausal symptoms and reduces the risk of osteoporosis, coronary artery disease, and possibly Alzheimer's disease (1–5). However, HRT may increase breast cancer risk (6–10). Because of the prevalence of HRT, understanding the effects of estrogen *vs.* estrogen+progestin on the postmenopausal breast is essential. This, in turn, can help us understand how these hormones may play a role in the genesis of cancer.

Whether estrogen, progestin, or both enhance proliferation of normal breast epithelial tissue is controversial. Progestins decrease endometrial cell proliferation in the uterus (11–13), but their effect on the breast is less clear. In premenopausal women, proliferation of breast epithelial tissue is greatest during the luteal phase of the menstrual cycle, when progesterone levels are maximal (14–22). In animal studies, progestins increase mammary gland epithelial cell proliferation. In the mouse, progesterone, when combined with estrogen, is mitogenic in the adult mammary gland to a greater extent than estrogen alone (23, 24). Similarly, in adult, ovariectomized cynomolgus macaques, estrogen+progestin induces greater breast epithelial cell proliferation than estrogen alone (25, 26). In contrast, in human/mouse xenograft studies, estrogen+progesterone increased proliferation but no more than estrogen alone (27, 28). The doses of estrogen and progesterone used in these xenograft studies, however, have been questioned because of the absence of sex steroid binding globulin in the serum of mice *vs.* its presence in humans (29).

To investigate the effects of HRT more directly, normal breast tissue from postmenopausal women who had taken estrogen, estrogen+progestin, or no HRT was analyzed for cell proliferation, epithelial cell density, and steroid receptor levels. We found that treatment with estrogen+progestin or estrogen alone was associated with significantly higher mitogenic activity in the terminal ductal lobular unit (TDLU) of the breast and greater breast epithelial density than women not receiving HRT.

Materials and Methods

Subjects

A cross-sectional, observational study was carried out to study breast tissue from cycling, premenopausal (n = 56) and postmenopausal (n = 86) women undergoing surgical breast biopsy at Lansing, Michigan area hospitals. Biopsies were carried out to diagnose suspicious palpable lesions upon physical exam or suspicious mammographic densities. Profiles of the study populations of premenopausal and postmenopausal women are summarized in Table 1. Postmenopausal women

Received July 6, 1999. Revision received August 16, 1999. Accepted August 26, 1999.

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* This work was supported by National Institute of Aging Grant R01-AG13059 (to S.Z.H.).

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TABLE 1. Characteristics of premenopausal and postmenopausal subjects

Characteristic	Premenopausal		Postmenopausal		
	Follicular	Luteal	No HRT	E	E + P
Number of subjects	n = 28	n = 28	n = 29	n = 32	n = 25
Mean age (yr) (range)	42.2 ± 7.0[a] (22–50)	41.6 ± 7.8 (24–52)	65.2 ± 9.2 (45–87)	60.8 ± 10.3 (41–82)	59.5 ± 7.8 (47–87)
Body mass index (kg/m^2)	27.8 ± 5.6	24.6 ± 6.9	30.1 ± 8.3	28.6 ± 6.3	28.1 ± 7.7
Time on HRT (yr) (range)	N/A[b]	N/A	N/A	14.2 ± 10.5 (0.25–39)	6.7 ±4.1[c] (2–20)
Menopausal status (%)					
Natural	N/A	N/A	86.2	37.5	88.0
Surgically induced	N/A	N/A	13.8	62.5[d]	12.0
Family history of breast cancer (1° relative) (%)					
Yes	14.3	10.8	24.1	12.5	16.0
No	78.6	82.1	70.0	78.1	68.0
Unknown	7.1	7.1	5.9	9.4	16.0
Oral contraceptives, ever (%)					
Yes	85.7[e]	82.1[f]	31.0	46.9	72.0[g]
No	14.3	17.9	69.0	53.1	28.0
Reproductive history					
Nulliparous (%)	14.3	35.7	10.3	15.6	0.0
Parous (%)	85.7	64.3	89.7	84.4	100.0
Mean no. pregnancies (range)	2.8 (1–6)	2.5 (1–6)	3.8 (1–9)	3.3 (1–6)	3.1 (2–7)
Mean no. deliveries (range)	2.1 (1–4)	1.8 (0–4)	2.9 (0–6)	3.0 (1–6)	3.0 (2–6)
Age at 1st delivery (yr) (range)	26.9 (19–40)	25.3 (17–33)	22.8 (18–47)	22.4 (16–33)	22.0 (17–29)

[a] Means ± SD.
[b] N/A, Not applicable.
[c] Significant difference from other postmenopausal groups (P = 0.0001).
[d] Significant difference from other postmenopausal groups (P = 0.001).
[e] Current users, n = 3.
[f] Current users, n = 3.
[g] Significant difference from other postmenopausal groups (P = 0.009).

were defined as those who had experienced 12 consecutive months of amenorrhea, had a bilateral ovariectomy at least 1 yr before biopsy, or were 55 yr of age or older. Subjects were placed into one of three categories: 1) no HRT, defined as not having taken hormones for 1 yr before surgery; 2) estrogen alone; or 3) estrogen+progestin. HRT subjects were defined as those taking hormones for at least 3 months continuously up to the day of surgery. Premenopausal subjects were divided into two categories depending on the phase of the menstrual cycle: 1) follicular, days 1–14; or 2) luteal, days 15–28.

All hormones were taken on a continuous, daily basis. Subjects had taken E in one of three forms: conjugated equine estrogens (CEEs; dose, 0.3–2.5 mg; n = 27); micronized estradiol (dose, 0.5–1 mg; n = 4); or ethinyl estradiol (dose, 0.05 mg; n = 1). The progestin taken was medroxyprogesterone acetate (MPA; dose, 2.5–5 mg; n = 25), which was taken in combination with CEE (n = 19) or micronized estradiol (n = 6). Herein, all types of estrogens are referred to as E, and MPA is referred to as P.

Tissue samples

The protocol for tissue collection was approved by the University Committee on Research Involving Human Subjects and Institutional Research Review Boards of the participating hospitals; written informed consent was obtained from each patient. Biopsies collected for study were kept on ice, then snap-frozen or fixed in 3.7% buffered formalin within 2 h of surgery for paraffin embedding. Only benign biopsies were used in the study; those diagnosed with atypical hyperplasia or cancer were excluded. Adjacent serial sections (5 μm) were mounted onto 3-aminopropyl-triethoxysilane (Sigma, St. Louis, MO)-coated cover slips, and assayed in parallel, by immunohistochemistry, for proliferating cell nuclear antigen (PCNA, clone PC-10; DAKO Corp., Carpinteria, CA) and Ki67 (MIB-1, Immunotech, Marseille, France). Estrogen receptor (ER) and progesterone receptor (PR; Zymed Laboratories, Inc., San Francisco, CA) were analyzed independently on nonserial sections. The number of tissue samples assayed for proliferation indices (PCNA and Ki67), epithelial density, or receptor status varied because, in some cases, there was not enough tissue for all assays, or some tissue sections only contained lobules or ducts, and not both.

Immunohistochemistry

Adjacent sections were incubated in PCNA antibody (1:100 dilution), Ki67 antibody (1:50 dilution), or normal serum (negative control). Signals were amplified with a biotinylated rabbit antimouse IgG (1:200 dilution, DAKO Corp.) followed by horseradish peroxidase-conjugated avidin-biotin complex (Vector Laboratories, Inc., Burlingame, CA); the chromogen was diaminobenzidine tetrahydrochloride (Pierce Chemical Co. Biotec Co., Rockford, IL). Ki67 antibody immunoreactivity required antigen retrieval, which was achieved by boiling the sections for 10 min in 10 mM citrate buffer (pH 6.0) before antibody treatment. ER and PR were detected as described above for Ki67, including antigen retrieval, using anti-ER (1:2 dilution) or anti-PR (1:1 dilution) antibodies according to the manufacturer's recommendations. Cells with clearly identifiable nuclear staining were considered to be positive for ER or PR; no cytoplasmic staining was observed.

Quantification of immunohistochemical staining

Using a double-blind approach, PCNA, Ki67, ER, and PR were quantified in specific breast structures: inter- and intralobular ducts (ducts) or terminal ductules and lobules (terminal duct-lobular unit, TDLU). Only normal areas of tissues were scored. Areas with fibrocystic changes, hyperplasia, microcalcifications, and metaplasias were not analyzed. Frozen tissue sections were analyzed for PCNA, ER, or PR only when paraffin sections were not available; quantitative comparison of results, obtained with frozen and paraffin sections, revealed no significant differences. Quantification was facilitated by use of a computer-interfaced morphometric digitizing system (Bioquant II, R and M Biometrics, Nashville, TN), as described previously (23). The mean unit area of an epithelial cell was determined by measuring cell areas for over 10,000 cells (mean ± SD, 4.2 ± 0.25 unit area/cell). No significant difference in cell sizes was observed for the

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various study groups. For each section, 1000–4000 cells were scored. It was determined, according to the method of Sadi and Barrack (30), that a minimum of 1000 cells was required to obtain a reliable estimate of the Ki67 proliferation index. All tissue samples were analyzed within 3 yr of collection. Interindividual and intraindividual differences in scoring were monitored for each assay and were calculated to be within 5%.

Epithelial density

The same sections assessed for proliferation and steroid receptors were analyzed for epithelial density, using a video camera (Sony, CCD color camera) attached to a light microscope (Nikon Eclipse E400; Mager Scientific Inc., Dexter, MI). After capture of the image, using an NIH *Image*PC program (Scion Corporation, Frederick, MD), the total area of the tissue and of the epithelium was quantified by digital tracing at 200× magnification. Only the area occupied by epithelial cells was measured; area occupied by lumina or stromal components was excluded. The area of the epithelium was then expressed as a percentage of the total area of the section.

Statistical analysis

Results are expressed as mean ± SEM (except for Table 1, where values are expressed as mean ± SD). Differences between no-HRT, E-alone, and E+P groups were examined using one-way ANOVA and analysis of covariance, adjusting for age. Multiple comparisons between groups were carried out using the Newman-Keuls test and the pairwise comparisons of least-square means, respectively. Differences between premenopausal follicular and luteal groups and between ducts and TDLU were examined using the Student's t test. The above analyses were repeated using nonparametric equivalents, the Kruskal-Wallis test, and the Mann-Whitney test; and the conclusions remained the same. Multiple regression was used to assess the effect of length of HRT use (up to 20 yr) on cell proliferation. All statistical analyses were carried out using the SAS program, with $\alpha = 0.05$ as the statistical significance level.

Results

Population profiles

Table 1 compares the three postmenopausal and the two premenopausal groups analyzed in this study. Some differences were noted among the postmenopausal groups. For the E-alone HRT group, 62.5% had experienced surgical menopause, and their mean time on HRT was twice as long as that of women taking E+P (14.2 *vs.* 6.7yr). As expected, the majority of women receiving no HRT or E+P experienced natural menopause. There was a higher percentage of women in the E+P group (72%) who had taken oral contraceptives, as compared with the no-HRT (31.0%), and E-alone (46.9%) groups.

Effect of HRT on cell proliferation

Anti-PCNA and Ki67 antibodies detect two different proteins present in cycling cells and have both been widely used to measure relative levels of proliferative activity in human tissues (31). Both antibodies were used herein to obtain two measures of proliferation that could be compared to each other and to published results. Although the percentage of epithelial cells detected by the anti-PCNA antibody was significantly greater than that detected with the anti-Ki67 antibody, relative differences between the postmenopausal groups were comparable, regardless of the antibody used. Because breast cell proliferation has been shown to be negatively related to age in premenopausal women (19), all proliferation data were age adjusted by including age as one of the variables in our regression models. This was done for both pre- and postmenopausal women. The results were the same when the means were age adjusted.

The PCNA index (Fig. 1A) was significantly higher in both the E-alone and E+P HRT groups than in the no-HRT group. For the E-alone group, the PCNA index was the same for the TDLU and ducts. In contrast, in the E+P group, the PCNA index was significantly higher in the TDLU than in ducts. The PCNA index in the TDLU of the E+P group was significantly greater than in the TDLU of E-alone group. Results with Ki67 (Fig. 1B) were comparable to the PCNA results, except that the difference between the E-alone *vs.* no-HRT groups did not reach statistical significance.

For premenopausal women, in agreement with past studies (14–22), our results with both anti-PCNA and Ki67 antibodies (Fig. 1) showed that epithelial cell proliferation in the TDLU was greater in the luteal phase of the menstrual cycle as compared with the follicular phase.

Regression analysis was carried out to assess the influence of length of time of HRT use (E and E+P groups) or length of time postmenopause (no-HRT group) on cell proliferation

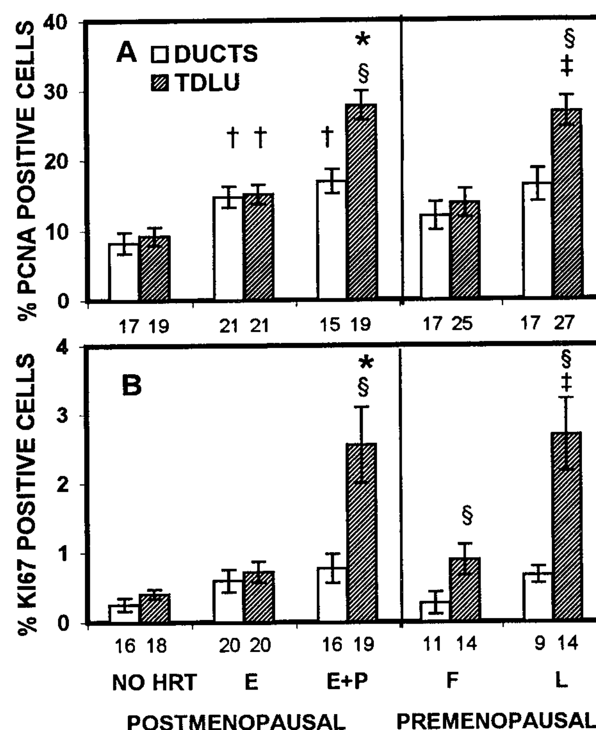

FIG. 1. Epithelial proliferation indices in normal breast tissue of postmenopausal women and cycling premenopausal women. The *number under each bar* represents the number of individuals for whom ducts or TDLU could be analyzed. *, $P = 0.002–0.0001$ that the percentages of PCNA- (A) and Ki67- (B) positive cells in the TDLU of the E+P group were significantly greater than in TDLU of no-HRT or E-alone groups; †, $P = 0.007–0.002$ that the percentages of PCNA-positive cells in the TDLU or ducts of the E group or ducts of the E+P group were significantly greater than in the TDLU or ducts of the no-HRT group; ‡, $P < 0.05$ that the percentages of PCNA- and Ki67-positive cells in the TDLU of the luteal-phase group were significantly greater than in the TDLU of the follicular-phase group; §, $P < 0.05$ that the percentages of PCNA- and Ki67-positive cells were greater in TDLU than in the ducts of the same group.

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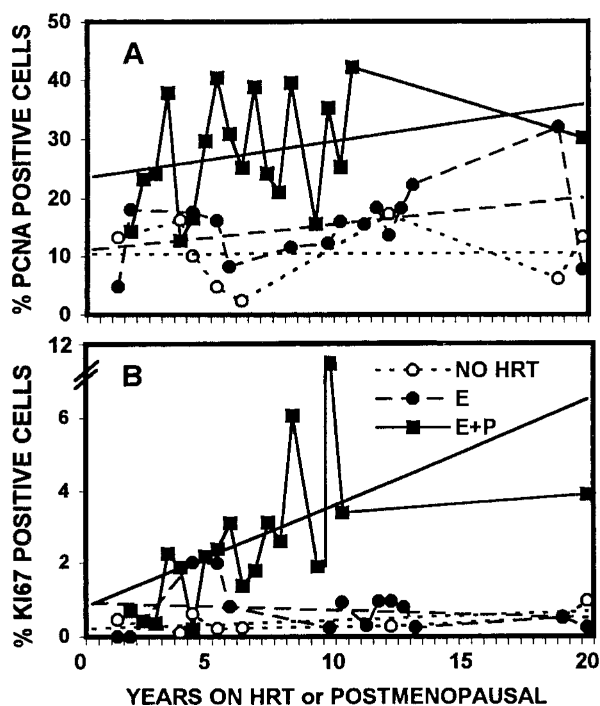

FIG. 2. Effects of length of time on HRT on proliferation in TDLU of postmenopausal women. Labeling indices determined by PCNA (A) and Ki67 (B) in TDLU were regressed on years of HRT or years postmenopause. Each *straight line* represents the best-fit line for each group. $P = 0.03$ that an increase in Ki67 labeling index was observed with each yr of E+P treatment.

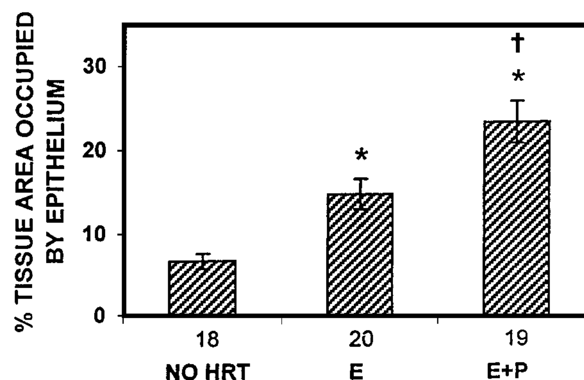

FIG. 3 Effects of HRT on breast epithelial density in postmenopausal women. The *number under each bar* represents the number of individuals for whom epithelial density was determined. *, $P = 0.001$–0.01 that the percentages of epithelial area in the E-alone or E+P groups were significantly greater than that of the no-HRT group. †, $P = 0.02$ that the percentage of epithelial area in the E+P group was significantly greater than that of the E-alone group.

(Fig. 2). For the PCNA index, an increase in slope was observed with each yr of HRT use for both E and E+P; however this did not reach statistical significance. The Ki67 index showed no significant increase in proliferation with time on E alone. However a significant increase ($P = 0.03$) was observed with time on E+P.

Effects of HRT on breast epithelial density and morphology

The number of epithelial cells present in breast tissue at any given time is determined by proliferative cell addition and cell loss caused by cell death. Because anti-PCNA and Ki67 antibodies provide relative measures of proliferative activity, it was of interest to determine the net effects of HRT on breast tissue. The epithelial density was significantly higher in women receiving E alone or E+P, compared with the no-HRT group (Fig. 3). Epithelial density of the E+P group was also significantly higher than the E-alone group. Because large variations in tissue section size could skew results of epithelial density calculations, the mean area (mm^2) per tissue section for each group was determined and found to be similar (mean ± SEM: no-HRT = 41.0 ± 4.0, E = 35.2 ± 4.4, E+*P* = 36.0 ± 3.2).

Histological analysis of breast tissues from the three groups also revealed differences in the appearance of the epithelium (Fig. 4). In the no-HRT and E-treated groups, lobules were small, compact, and similar in appearance. There were more lobules per unit area in the E-treated group than in the no-HRT group (Fig. 4, A and B). In contrast, in the E+P group, lobules were much larger and less compact, and the acini had larger lumina (Fig. 4C). This latter morphology is similar to that observed during the luteal phase in cycling women (32).

Steroid receptor status

The induction of epithelial progesterone receptors (PRs) by estrogen is believed to be the basis for the synergistic effect of estrogen+progestin on epithelial cell proliferation in the rodent (23, 24) and primate mammary gland (25, 26). The current model of progestin-induced proliferation in the mammary gland, based on animal studies, is that estrogen increases PR levels in mammary epithelial cells, and the subsequent binding of progestins to the PR leads to a proliferative response. Progestin binding to PR also results in the down-regulation of PR levels (33). Therefore, we analyzed the effect of HRT on PR content in postmenopausal human breast tissue. Cells were identified as PR positive, based upon the presence of nuclear staining; no cytoplasmic staining was observed. The percentage of PR-positive cells was about 3-fold higher in ducts and TDLU of the E-alone group, as compared with the no-HRT group (Fig. 5A). PR-positive cells were found to be equally distributed in ducts and TDLU. No PR-positive cells were observed in the mammary stroma. The higher number of PR-positive cells in the E-alone HRT group is consistent with the effect of E to up-regulate PR, and it indicates that the postmenopausal breast is responsive to this action of E. Treatment with E+P resulted in lower PR levels than in the E-alone group, consistent with PR down-regulation by P as a consequence of P action (25, 34, 35).

ER-positive cells were identified by presence of nuclear staining. The percentage of ER-positive cells in ducts and TDLU ranged from 4.0 ± 1.4 to 10.0 ± 2.3 (mean ± SEM). No significant differences were observed among the three postmenopausal groups (Fig. 5B).

Discussion

Our results demonstrate, for the first time, that: 1) combination HRT with E+P is associated with higher levels of epithelial proliferation in the TDLU of the postmenopausal

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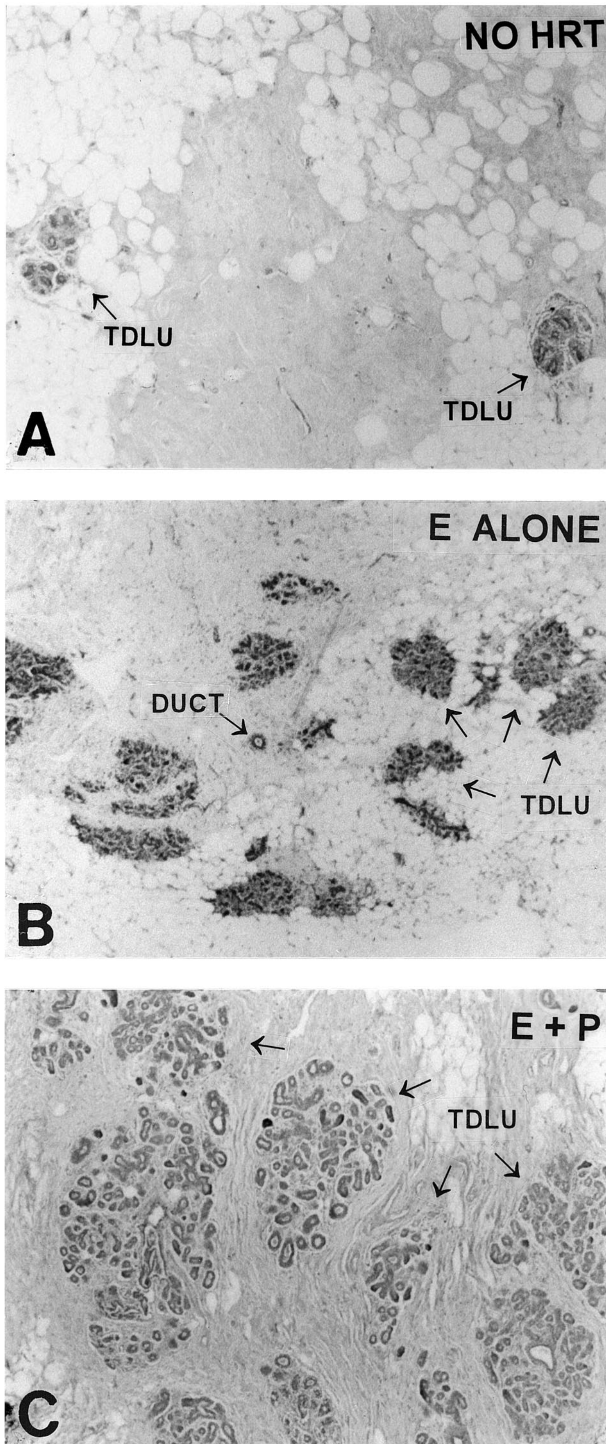

FIG. 4. Photomicrographs of breast tissue from postmenopausal women; no HRT (A), E alone (B), E+P (C). TDLUs and ducts are indicated by *arrows*. Magnification, ×40.

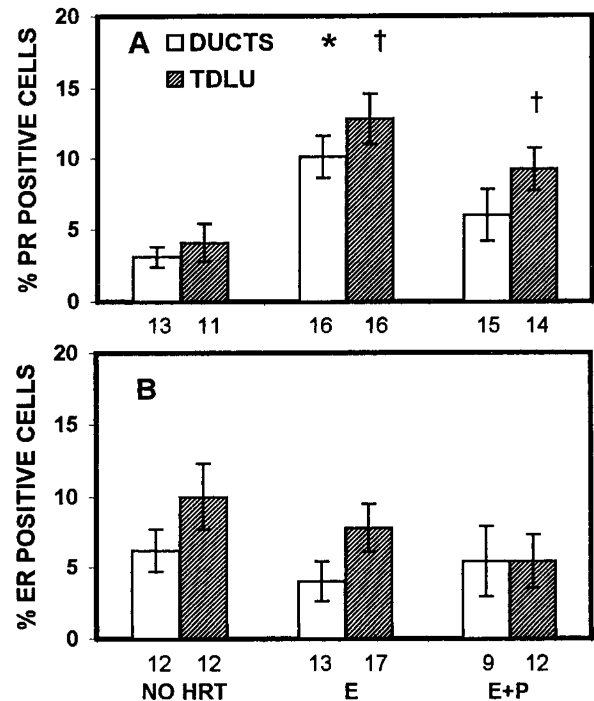

FIG. 5. Effects of HRT on PR (A) and ER (B) in breast tissue of postmenopausal women. The *number under each bar* represents the number of individuals for whom ducts or TDLU could be analyzed. *, $P = 0.002–0.04$ that the percentage of PR -positive cells in the ducts of the E-alone group was significantly greater than in the ducts of the no-HRT or E+P groups. †, $P = 0.0007$ and 0.04, respectively, that the percentages of PR-positive cells in the TDLU of the E-alone and E+P groups were significantly greater than in the TDLU of the no-HRT group.

breast, based on PCNA and Ki67 indices; and 2) E alone and E+P HRT are positively associated with greater epithelial density. In addition, the morphology of the breast epithelium in the E+P group is consistent with the morphology seen in mammary tissue during the luteal phase in cycling women. The amount of epithelial proliferation was also similar to that of luteal-phase premenopausal women. These results also indicate that the mitogenic activity associated with progestins is localized to the TDLU of the human breast.

E-alone HRT was associated with a smaller effect than E+P on proliferation index and epithelial density. Although the proliferation indices for the E-alone group, obtained with PCNA and Ki67, were both higher than the no-HRT group, this difference was statistically significant only for the PCNA index. However, epithelial density, another indicator of proliferative activity in the breast, was also significantly greater in the E-alone group. It is likely that the low percentage of Ki67-positive cells, combined with variability among samples, contributed to the fact that statistical significance was not achieved with the Ki67 antibody in the E-alone HRT group. It has been reported in the literature that the differences between the raw values obtained with PCNA *vs.* Ki67 antibodies could be attributable to the following reasons. First, PCNA has a longer half-life than Ki67 and, therefore, may be detectable in cells that have recently left the cell cycle (36). Second, PCNA has been shown to be detectable in cells undergoing DNA repair (37). However, both methods were used in this paper because both PCNA and Ki67 have been used extensively in the literature as proliferation markers. The observed difference with anti-PCNA and Ki67 antibodies and measurement of epithelial density underscores the importance of using multiple methods to assess cell proliferation.

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There were notable differences in the characteristics of the postmenopausal groups (Table 1). Significantly more women receiving no HRT or E+P had undergone natural menopause *vs.* women in the E-alone group, who were more likely to have experienced surgical menopause and whose time on HRT was twice as long as that of women taking E+P (14.2 *vs.* 6.7 yr). Because E alone is usually prescribed for women lacking a uterus, early surgically induced-menopause is the likely explanation for the longer time on HRT for the E-alone group. Analysis of the influence of length of time of HRT showed a trend of greater proliferation, with increasing length of time on E+P. This suggests that there may be a cumulative effect of E+P on proliferation. However, because the maximum length of time on E+P was 20 yr (*vs.* 39 yr on E) and the numbers of subjects at each individual time point was low, further studies will be required to resolve this question.

There was a significantly higher prevalence of past oral contraceptive use in the E+P group (72%), as compared with the no-HRT and E-alone groups (31% and 46.9%, respectively). We questioned whether this could have influenced our results. We therefore analyzed proliferation indices for women who had ever taken contraceptives *vs.* those who had never taken contraceptives, in each treatment group, and found no significant differences (data not shown). This is not surprising, because contraceptives were taken during their cycling years, and there may not be any residual effects of the hormones after menopause. Previous studies of the effects of current use of oral contraceptives on breast proliferation in premenopausal women have shown either no effect (19, 21) or only a slight effect (17, 18, 22). Furthermore, studies analyzing past use of oral contraceptives have not found any influence on proliferation in the premenopausal breast (15, 17).

Recently, Hargreaves *et al.* (38), using the Ki67 antibody as a proliferation marker, retrospectively examined epithelial cell proliferation indices in postmenopausal breast tissue of women who had taken estrogen alone or estrogen+progestin, in England. They found no association between either estrogen or estrogen+progestin HRT and epithelial proliferation, results different from those observed in the present study. Possible explanations for this difference are: 1) the length of time on HRT; and 2) the progestins used. In our study women had taken estrogen+progestin for up to 20 yr; whereas, in the Hargreaves *et al.* study, women took estrogen+progestin for a maximum of 5.5 yr. In this regard, our analyses of proliferation indices, as a function of time on HRT (Fig. 2), demonstrates that there is a trend of greater proliferation associated with longer time on estrogen+progestin HRT. Interestingly, the epidemiological data indicate that breast cancer risk also increases with length of time on estrogen or estrogen+progestin HRT (7–10). A recent epidemiological study has also shown a positive association with breast cancer risk for estrogen+progestin HRT, which was especially pronounced with continuous combined regimens (10). HRT is hypothesized to play a role in increasing uterine and breast cancer risk through enhanced cell proliferation and potential accumulation of DNA damage (39, 40). In relation to the association of HRT with increased risk of breast cancer, it is of interest to note that, in our study, proliferation associated with E+P was localized to the TDLU, which is the site of origin of most breast cancers.

With regard to the progestins used, in our study, all subjects received MPA, which is a synthetic progestin structurally related to progesterone. In the Hargreaves *et al.* study, 89.7% of the subjects received norethisterone, levonorgestrel, and norgestrel, which are synthetic progestins structurally related to testosterone; 7.7% received tibolone and 2.6% (1 subject) MPA (D.F. Hargreaves, personal communication). Further direct comparative study will be required to determine whether there are differences in the mitogenic activities of various progestins. We have previously shown that estradiol+MPA was highly effective in inducing mammary epithelial cell proliferation in the mouse mammary gland (24). Similarly, in surgically postmenopausal cynomolgus macaques, treatment with CEE+MPA caused a significant increase in breast epithelial cell proliferation, above that obtained with CEE alone (25, 26). Furthermore, the histological appearance of lobules in the no-HRT, CEE-alone, and CEE+MPA-treated monkey glands were strikingly similar to human breast tissues obtained from similar postmenopausal HRT groups herein (26). Thus, the greater proliferation observed in postmenopausal women treated with E+MPA herein is consistent with the mitogenic effect of E+MPA in mouse and monkey mammary glands.

Others have reported that progesterone decreases the proliferative effect of estrogens in the postmenopausal breast. In a prospective study, postmenopausal women were withdrawn from HRT for 12 weeks, then received 14 daily topical treatments with hydroalcoholic gels containing placebo, estradiol, progesterone or estradiol+progesterone applied directly to their breasts (41). Estradiol alone increased the proliferation index (PCNA) of breast epithelium 100-fold, progesterone alone increased proliferation 15-fold and combination treatment with estradiol+progesterone only increased proliferation 13-fold. The results of that study (41) are possibly due to the experimental protocol (pretreatment hormone withdrawal, the use of progesterone or the route and length of hormone administration), compared to the standard protocols of HRT treatment experienced by postmenopausal women in our study.

In summary, the present study provides compelling evidence that postmenopausal HRT with E+P was positively associated with higher levels of epithelial cell proliferation in the TDLU of the breast. There was also a positive association between higher levels of proliferation and increasing length of time on HRT. Both E alone and E+P HRT were associated with greater epithelial density in the postmenopausal breast. The possibility is raised that there may be potential differences in the biological activity of various progestins in breast tissue. Further investigation is needed to assess the possible association between the mitogenic effects of progestins in the breast and breast cancer risk.

Acknowledgments

The authors gratefully acknowledge the expert technical assistance provided by Ms. Jessica M. Bennett, Ms. Bridget M. Mahon, and Ms. Lisa M. Coram and the cooperation and contributions of Drs. Laura L. Morris, Keith Appelgren, L. Rao Kareti, Edward Lanigan, Rafael De Los Santos, Lee Colony, Hugh J. Lindsey, James Harkema, and John Kisala in the

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Department of Surgery, Dr. Jianping He in the Department of Epidemiology, and Dr. Terry L. Woodward in the Department of Physiology at Michigan State University. We also would like to thank Lansing area hospital pathologists, Drs. W. De Mendoca-Calaca, R.N. Horowitz, Ernesto B. Quiachon, Ralph R. Edminster, M. Costa-Fox, N.C. Caliman, Adonica L. Walker, Stanley B. Katlein, Mary A. Flynn, and Alan G. Kaplan.

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