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Biologic Effects of Various Doses of Ethinyl Estradiol in Postmenopausal Women

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To determine which dosage of estrogen might provide physiologic replacement while minimizing adverse effects, 20 postmenopausal women were studied before and after oral administration of ethinyl estradiol. Twenty premenopausal women studied in the early and late follicular phases of the menstrual cycle were presumed to reflect normal physiologic function. Variable responses of the different biochemical and biologic markers to the actions of ethinyl estradiol were observed. Liver protein synthesis was the most sensitive measure of the action of ethinyl estradiol. In comparing the relative potencies of ethinyl estradiol with previously reported results observed with the usage of conjugated equine estrogens, the actions of 10 μg ethinyl estradiol were approximately equivalent to the biologic effects of 1.25 mg conjugated estrogens. The results suggest that ethinyl estradiol is far more potent than previously believed and that the daily administration of 10 μg, a dose lower than currently available commercial preparations, may be adequate for relief of symptoms of vaginal atrophy and may provide protection from the occurrence of osteoporotic fractures. *(Obstet Gynecol 59:673, 1982)*

The postmenopausal syndrome is characterized by a constellation of symptoms which are endocrinologic, somatic, and psychologic in nature. Estrogen replacement therapy has been shown to be effective for the relief of hot flashes and symptoms associated with vaginal atrophy and for the prevention of osteoporosis.[1–6] Adverse effects include hypertension,[7] cardiovascular illness,[8–11] gallbladder disease,[12] endometrial cancer,[13–17] and, less well documented, breast cancer.[18,19] Some of these complications have been shown to be dose dependent.[15,20,21]

Recently Geola et al[22] reported the effects of various doses of conjugated equine estrogens on several biochemical or biologic parameters of the action of estrogen and observed different sensitivities of the various target organs. To date, similar information is not available concerning the most commonly used synthetic estrogen, ethinyl estradiol (EE). The present study was undertaken to examine the biologic effects of various doses of EE and to compare the results with the values reported previously for conjugated equine estrogens.

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Materials and Methods

Subjects

Twenty postmenopausal patients who had experienced their last menstrual period at least 1 year before study and 20 premenopausal women in the early and late follicular (vaginal cytology only) phases of the menstrual cycle were studied. Some of the results observed in the premenopausal subjects have been reported previously.[22] None of the subjects had received sex steroids for at least 6 weeks before evaluation.

Protocol

All subjects were instructed to fast for 12 hours before the study. At 0800 hours they voided and then drank 250 ml distilled water. After 1 hour a urine specimen was collected. Four blood samples were drawn at 15-minute intervals beginning at 0800 hours. Vaginal smears then obtained from the middle third of the side wall of the vagina were immediately fixed with Spray-Cyte. Repeat studies were performed on the last day of administration of each 4-week dosage cycle of EE.* The dosages tested were 5, 10, 20, and 50 μg; the 5- and 10-μg doses were prepared in the University of California, Los Angeles, Pharmacy and the 20- and 50-μg doses were obtained from the manufacturer. Ten subjects used an increasing dosage schedule beginning with the lowest dosage, and the remainder used a decreasing dosage schedule beginning with the highest dosage. Treatment was cyclic with a 1-week interval between test doses.

The serum samples were assessed for luteinizing hormone (LH), follicle-stimulating hormone (FSH), thyroid-binding globulin (TBG), corticosteroid-binding globulin (CBG), and sex hormone–binding globulin (SHBG). A plasma sample was collected on ice, centrifuged within 30 minutes, and assayed for renin substrate. Calcium, hydroxyproline, and creatinine were measured in the urine and the ratios of calcium and hydroxyproline to creatinine were calculated. It has been shown that in a fasting subject urinary calcium comes mainly from bone.[23] Similarly, it has been demonstrated that urinary hydroxyproline in a fasting subject mainly reflects the breakdown of bony matrix.[24,25] Based on these observations the calcium:creatinine and the hydroxyproline:creatinine ratios were used as indices of bone resorption reflecting loss of mineral and matrix, respectively. To minimize the effects of pulsatile release of gonadotropins on serum levels, LH and FSH were measured in all 4 blood samples collected, and the mean concentration was used as the value for that patient. Only 1 measurement was made for the other parameters.

Measurements

LH and FSH levels were measured by double antibody radioimmunoassay using reagents supplied by the National Pituitary Agency.[26,27] Results were expressed as nanograms of LER 907 per milliliter. SHBG levels were measured by a selective ammonium sulfate precipitation technique.[28] Serum TBG levels were quantitated by radioimmunoassay using a Corning kit (Corning Glass Works, Corning, NY).[22] Previously published radioimmunoassay methods were used for CBG and renin substrate.[29–31] Urine calcium concentration was assessed by atomic absorption. Urine hydroxyproline and creatinine concentrations were measured by autoanalyzer (Technicon Instrument Co, San Francisco, CA). With the exception of vaginal cytology, all measurements were run in duplicate. All measurements in a given subject were run in the same assay. The mean coefficient of variation was less than 17% for all assays measured in 2 samples obtained 6 weeks apart in 15 untreated postmenopausal women.[22]

The Student 2-tailed t test was used to determine statistical differences between groups. The Student paired t test was employed to determine differences of values within subjects who were studied repetitively.

A 99.5% confidence interval was calculated for the means of each parameter at each dosage and was compared to the confidence intervals of means for the premenopausal group and baseline measurements. A 99.5% confidence level was selected as an adjustment for repeated measures. Biologic and statistical significance at a .05 level was assumed if the 99.5% confidence intervals did not overlap.

Results

For all parameters of the action of EE there were no significant differences between the results observed in subjects given an increasing and those given a decreasing dosage schedule. Therefore, the data for all postmenopausal subjects were analyzed together.

The data for LH and FSH are shown in Figure 1. In premenopausal controls, mean values (± SEM) for LH and FSH were 55 ± 5 ng/ml and 195 ± 12 ng/ml, respectively. The baseline concentrations of both were significantly elevated in the postmenopausal patients (LH, 456 ± 38 ng/ml; FSH, 2064 ± 152 ng/ml). The levels of both hormones showed a stepwise decline with increasing dosages of EE. With the 50-μg dosage

* Supplied by Schering Corporation, Kenilworth, NJ.

both LH (181 ± 23 ng/ml) and FSH (509 ± 52 ng/ml) were still significantly greater than the values observed in the premenopausal subjects. However, in 1 and 6 subjects of the postmenopausal group, the levels of LH and FSH, respectively, fell into the early follicular phase range at this dosage. The smallest dosages of EE that elicited a significant reduction of the gonadotropins from baseline were 20 and 10 μg for LH and FSH, respectively.

Figure 2 shows the mean (± SEM) percentage of superficial and parabasal cells by vaginal cytology. Values observed in the premenopausal women early and late in the follicular phase are also given. In the postmenopausal subjects the baseline percentage of parabasal cells (28.1 ± 11.4%) was significantly greater and the percentage of superficial cells (0.8 ± 0.4%) was significantly less than the values observed in the premenopausal subjects either early (parabasal, 4.9 ± 3.3%; superficial, 6.3 ± 2.0%) or late (parabasal, 0; superficial, 42.8 ± 9.2%) in the follicular phase. Five micrograms of EE significantly reduced the number of parabasal cells (2.8 ± 1.6%) and increased the percentage of superficial cells (11.8 ± 3.7%) to values similar to those observed during the early follicular phase in the ovulatory subjects. Increasing dosage had no demonstrable effect on parabasal cells but progressively increased the percentage of superficial cells. At the 50-μg dosage the mean percentage of superficial cells (46.4 ± 8.1%) was similar to the value (42.8 ± 9.2%) observed in the late follicular phase of the younger subjects.

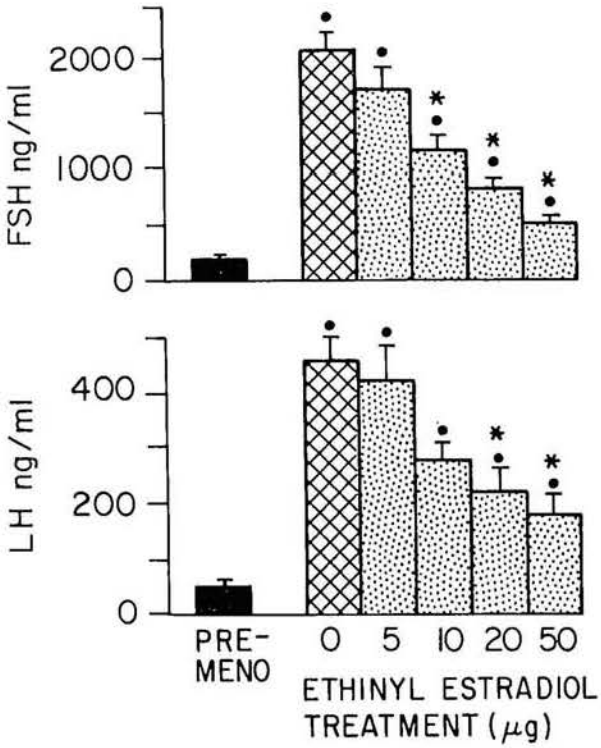

Figure 1. Mean (± SEM) serum concentrations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in the premenopausal controls (solid bars) and postmenopausal group before and after oral administration of various doses of ethinyl estradiol. Dot = significantly different ($P < .05$) from the premenopausal value. Asterisk = significantly different ($P < .05$) from the untreated postmenopausal value.

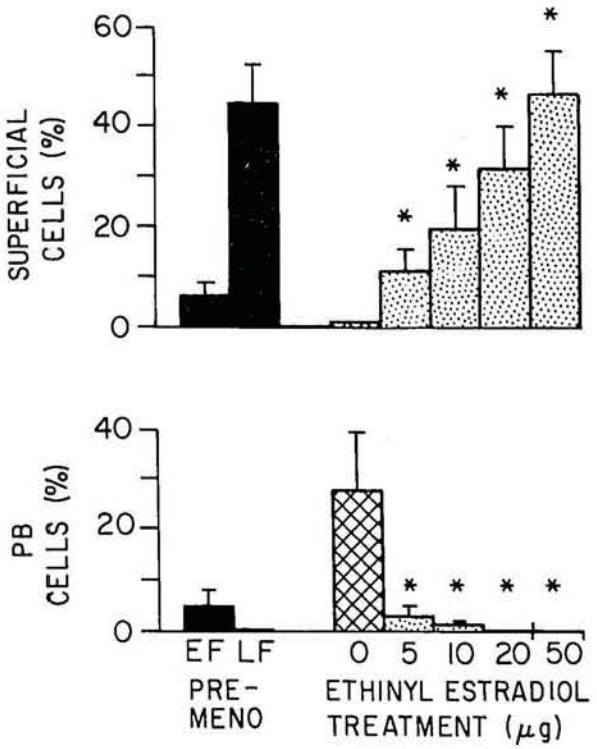

Figure 2. Mean (± SEM) for percentage of superficial and parabasal (PB) cells by vaginal maturation index in the 2 groups of patients. Asterisk = significantly different ($P < .05$) from the untreated groups; EF = early follicular phase; LF = late follicular phase.

The urinary calcium:creatinine and hydroxyproline:creatinine ratios are depicted in Figure 3. The mean ratios were significantly elevated in the untreated postmenopausal patients (0.131 ± 0.02 and 0.028 ± 0.02, respectively) over the values in the younger women (0.093 ± 0.01 and 0.02 ± 0.0002, respectively). EE administration lowered both ratios. For calcium:creatinine, 5 μg EE decreased the ratio to a value similar to that observed in the younger women, but 10 μg was the lowest dose that significantly reduced the ratio from baseline. Increasing amounts of EE had a minimal effect on this ratio. All doses of EE lowered the hydroxyproline:creatinine ratio to values intermediate between those observed in the premenopausal and untreated postmenopausal women.

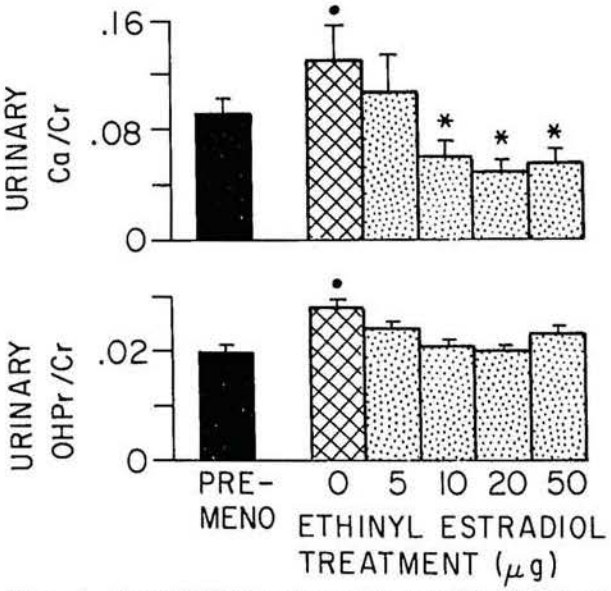

Figure 3. Mean (± SEM) for urinary calcium:creatinine (Ca/Cr) and hydroxyproline:creatinine (OHPr/Cr) ratios in the 2 groups. See Figure 1 for symbols.

The data on hepatic effects are shown in Figure 4. Baseline values of the 4 parameters were not significantly different from the levels observed in the premenopausal subjects. With the 5-μg dosage renin substrate, TBG, and SHBG were significantly elevated above the levels observed in the premenopausal subjects, ie, pharmacologic effects. The 10-μg dosage significantly increased the CBG-binding capacity. With increasing dosages stepwise increases were observed for all parameters. With the 50-μg dosage, the levels of renin substrate and SHBG were more than 3 times higher than in the premenopausal women, and the concentrations of TBG and CBG were more than twice as high.

Table 1 compares the biologic effect of 10 μg EE with the response reported previously[22] from the oral administration of 0.625 and 1.25 mg of conjugated equine estrogens (CE). Responses of each parameter are expressed as the percentage of change from the baseline value. Relative potencies of CE to EE per weight were calculated for each parameter and are also listed. Parallel dose-response relationships could not be established between the effects of EE and CE using the raw data or single or double log transformations of the results. For this reason comparisons were made only between the effects of 10 μg of EE and those of 0.625 and 1.25 mg of CE. For LH, renin substrate, and SHBG, the percentage of changes with EE fell between the values found for 0.625 and 1.25 mg of CE. For all other parameters the effects of 10 μg of EE were greater than those exerted with 1.25 mg of CE.

Discussion

The study was designed to identify a dose of EE that would provide physiologic estrogen replacement for postmenopausal women and to compare the relative potency of this synthetic estrogen preparation with the previously reported responses to oral CE administration. EE was studied because it is a commonly used synthetic estrogen preparation.

The values obtained in the premenopausal women during the early and late follicular phases (vaginal cytology only) of their menstrual cycles were presumed to reflect normal physiologic function. If a dose of EE was insufficient to alter a specific marker to a mean value which was similar to that found in premenopausal subjects, the dosage was considered subphysiologic. If the dose changed the marker to a value that was similar or greater than the mean premenopausal value, it was considered physiologic or pharmacologic, respectively.

Using those criteria, no dosage of EE provided physiologic estrogen replacement for all parameters studied. In fact, each dosage was associated with subphysiologic, physiologic, and pharmacologic responses depending on which parameter was considered. Therefore, no one parameter could be used to determine the potency of EE at all of its sites of action.

For gonadotropins, increasing doses of EE progressively reduced LH and FSH levels, but even the 50-μg dosage resulted in a subphysiologic response. There are several possible explanations for this. First, 50 μg EE may truly be a subphysiologic dose at the level of the hypothalamus and pituitary. Second, blood was sampled 24 hours after the last dose of medication;

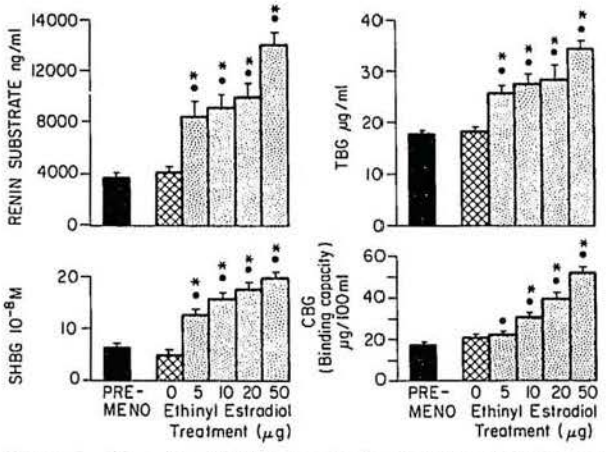

Figure 4. Mean (± SEM) serum levels of renin substrate, sex hormone–binding globulin (SHBG), and thyroxine-binding globulin (TBG) and the serum binding capacity of corticosteroid-binding globulin (CBG) in the 2 groups. See Figure 1 for symbols.

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