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Biological Effects of Various Doses of Vaginally Administered Conjugated Equine Estrogens in Postmenopausal Women*

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ABSTRACT. To determine whether vaginal administration of conjugated equine estrogens (VCE) could provide physiological replacement while avoiding effects on hepatic function, as occurs with oral administration, a study was conducted in which 20 postmenopausal women were evaluated before and after the vaginal administration of CE. The dosages studied were 0.3, 0.625, 1.25, and 2.5 mg/day for 4 weeks. Twenty premenopausal women were also studied, and their values were presumed to reflect normal physiological function. The findings in the postmenopausal women were compared with previously reported results obtained in a similar group of subjects given oral CE (OCE).

Vaginal cytology returned to premenopausal values with 0.3 mg VCE. This response was similar to that exerted with 1.25 mg OCE. Stepwise increases in circulating estrone and estradiol occurred with increasing dosages. The 2.5-mg dosage of VCE raised estrone levels to values similar to those in premenopausal women in the late follicular phase, and estradiol concentrations were similar to early follicular phase concentrations. Limited or no responses of the systemic markers of estrogen action occurred with all doses of VCE. Small decreases in LH and FSH levels occurred, but no dosage significantly reduced the level of either gonadotropin. Although the urinary calcium to creatinine ratio was significantly reduced by the two largest dosages of VCE, the effect of the 2.5-mg dosage was less than that observed with 0.625 mg OCE, the lowest dosage that protects against osteoporosis. Hepatic protein synthesis was significantly increased only by the higher dosages tested. No dosage had a significant effect on circulating levels of triglycerides or total or fractionated cholesterol levels. These data suggest that the vaginal administration of CE exerts mainly a local effect, with limited or no measurable changes in systemic markers of the action of estrogen. (*J Clin Endocrinol Metab* **57**: 133, 1983)

FOR many years, vaginally administered estrogens were believed to exert local effects only. Recently, several studies have shown that estrogens administered by this route are absorbed into the general circulation and, consequently, could exert systemic actions (1–4). Estrogens administered by this route would not pass initially through the liver, as occurs with orally administered hormone (5). Thus, it is possible that vaginally administered estrogens could be prescribed for the treatment of systemic indications, while avoiding some of the side effects related to the action of this class of hormone on hepatic function (6, 7). These side effects include hypertension (8), hyperlipidemia (9), thromboembolism (10), and gallbladder disease (11).

The present study evaluated the local and systemic actions of vaginally administered conjugated equine estrogens (VCE) using specific biological and biochemical markers of the action of estrogen. This vaginal preparation was chosen for study to allow comparison of its effects with those of orally administered conjugated equine estrogens (OCE), the results of which were published previously (6).

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Materials and Methods

Subjects

Twenty postmenopausal patients who had their last menstrual period at least 1 yr before study and 20 premenopausal women in the early and late follicular phases [estrone (E_1) and estradiol (E_2) levels and vaginal cytology only] of their menstrual cycles were studied. None of the subjects had received sex steroids for at least 6 weeks before evaluation. Patients with previous vaginal surgery were excluded from study. Some of the results observed in the premenopausal subjects have been reported previously (6, 7).

Protocol

All subjects were instructed to fast for 12 h before study. At 0800 h, they voided and then drank 250 ml distilled water. After 1 h, a urine specimen was collected. Four blood samples were drawn at 15-min intervals beginning at 0800 h. Vaginal smears 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 VCE. The dosages tested were 0.3, 0.625, 1.25, and 2.5 mg VCE, which corresponded to 0.5, 1, 2, and 4 g VCE cream. The 1-, 2-, and 4-gram dosages were administered according to the markings on the vaginal applicators commercially supplied. The 0.5-g dose was administered by drawing 1 g cream into the applicator and applying a plastic ring around the plunger which allowed only half of the 1-g dose to be inserted. 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 E_1, E_2, LH, FSH, thyroid hormone-binding globulin (TBG), corticosteroid-binding globulin (CBG), and sex hormone-binding globulin (SHBG). Two plasma samples were collected on ice, centrifuged within 30 min, and assayed for renin substrate (RS); triglycerides; total cholesterol; and low density, very low density, and high density lipoprotein cholesterol. 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 (12). Similarly, it has been demonstrated that urinary hydroxyproline in a fasting subject mainly reflects the breakdown of bony matrix (13, 14). Based on these observations, the calcium to creatinine (Ca/Cr) and hydroxyproline to creatinine (OHPr/Cr) 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 four blood samples collected, and the mean concentration was used as the value for that patient. Only one measurement was made for the other parameters.

Measurements

E_1 and E_2 levels were measured by RIA (15). LH and FSH levels were measured by double antibody RIA using reagents supplied by the National Pituitary Agency (16, 17). Results were expressed as nanograms of LER 907 per ml. SHBG levels were measured by a selective ammonium sulfate precipitation technique (18). A binding affinity assay using dextran-coated charcoal was used to measure CBG (19). Serum TBG levels were quantitated by RIA (20). A previously published RIA method was used for RS (21, 22). The urinary calcium concentration was assessed by atomic absorption. Urinary hydroxyproline and creatinine concentrations were measured by autoanalyzer (Technicon Instrument Co., San Francisco, CA). Total plasma cholesterol and triglycerides were measured by the use of AutoAnalyzer (Technicon, San Francisco, CA) procedures, and lipoprotein cholesterols were measured by a combination of preparative ultracentrifugation and heparin-manganese precipitation techniques in a Lipid Research Clinic Laboratory (23).

With the exception of vaginal cytology, all measurements were run in duplicate, while 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.

Analysis of variance and the randomized complete block design were used to determine significant effects of treatment for each parameter. Student's two-tailed t test was used to determine statistical differences between groups. Student's paired t test was employed to determine differences within subjects 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. Biological and statistical significance at a 0.05 level were assumed if the 99.5% confidence intervals did not overlap.

Results

With the exception of RS and E_1, there was no significant difference between the values obtained in the postmenopausal women given an increasing or decreasing dosage schedule. Therefore, the data for all postmenopausal subjects were analyzed together. The possible meaning of the difference for RS is discussed below.

The mean ± SE values for vaginal cytology are shown in Fig. 1. The mean percentage of parabasal cells (PBC; 13.6%) was higher, while the percentage of superficial cells (SC; 2.3%) was lower than the values observed in the premenopausal women either early or late in their follicular phases. The 0.3-mg dosage of VCE significantly lowered the mean PBC to 0.2% and significantly increased the SC to 16.6%. These values fell between those seen in premenopausal controls in the early and late follicular phases. Further increases in the percentage of SC were seen with increasing dosage.

Figure 2 shows the mean ± SE levels of E_1 and E_2 in the patients. The mean levels of both E_1 (26 ± 2 pg/ml) and E_2 (8 ± 1 pg/ml) were significantly lower in postmenopausal than in premenopausal subjects as expected. Stepwise increases in both estrogens occurred with increasing dosages of VCE. With the 2.5-mg dosage, the mean value for E_1 was greater than that in premenopausal women during the late follicular phase, while the mean E_2 concentration was similar to that observed during the early follicular phase. Minimal increases in both estrogens were observed with the 0.3-mg dosage of VCE to levels that were lower than those in premenopausal women in the early follicular phase.

Comparisons were made of the levels of circulating estrogens in subjects given an increasing dosage or de-

SUPERFICIAL CELLS (%)

PB CELLS (%)

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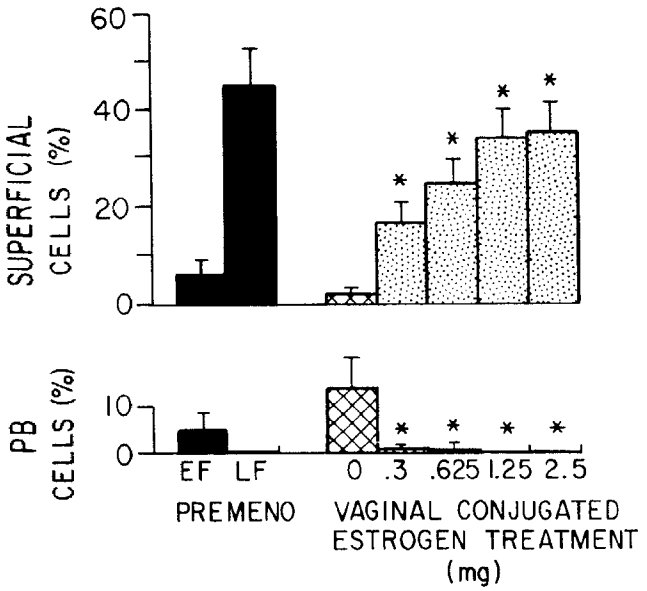

FIG. 1. The mean (±SE) of SC and PB of the vaginal epithelium in premenopausal controls in the early (EF) and late (LF) follicular phases of their cycles compared to values in postmenopausal women before and after vaginal administration of various doses of CE. *, Significantly different ($P < 0.05$) from the untreated postmenopausal value (same for all figures).

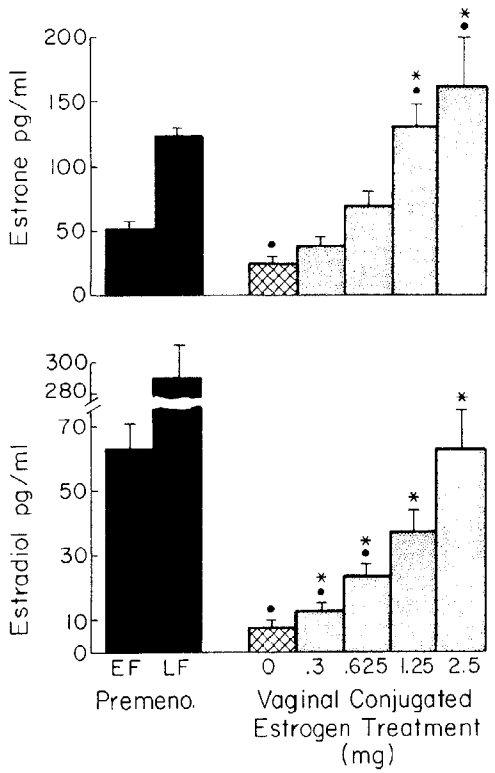

FIG. 2. The mean (±SE) levels of E_1 and E_2 observed in the two groups of subjects. EF, Early follicular phase; LF, late follicular phase. ●, Significantly different ($P < 0.05$) from premenopausal values (same for all figures).

creasing dosage schedule. For the 2.5-mg dosage, the mean E_1 level was significantly higher ($P < 0.05$) in the subjects given this dosage first (247 ± 61 pg/ml) than in those given it last (100 ± 29 pg/ml). For E_2, the level at the 2.5-mg dosage was also higher in those subjects given a decreasing dosage schedule (78 ± 18 pg/ml) than in those receiving the increasing dosage schedule (54 ± 16 pg/ml), but this difference was not statistically significant. There were no differences in circulating estrogen for the other dosages in regard to dosage schedule.

Figure 3 presents data for gonadotropins. As expected, basal gonadotropin levels were significantly elevated in the postmenopausal compared to premenopausal subjects. Step-wise decreases in both gonadotropins were seen with increasing dosages of VCE. LH decreased from 475 to 313 ng/ml, while FSH fell from 2007 to 1414 ng/ml on the 2.5-mg dosage. When analyzed by analysis of variance, these reductions with increasing dosages were significant. However, using the paired t test, no mean value of either gonadotropin was significantly less than baseline, and all values during treatment remained significantly higher than premenopausal levels.

Figure 4 depicts the urinary Ca/Cr and OHPr/Cr ratios. For the urinary Ca/Cr, the mean baseline level in the postmenopausal subjects of 0.124 ± 0.012 was significantly higher than the value of 0.093 ± 0.01 observed in the younger subjects. The lowest dosage of VCE that significantly reduced the ratio was 1.25 mg (0.088 ± 0.011). For the urinary OHPr/Cr, the baseline value of 0.028 ± 0.003 was higher than the mean level observed in the premenopausal subjects (0.020 ± 0.001), but the difference was not significant. There was no demonstrable change in this ratio with VCE administration.

In Fig. 5, the effects of VCE on hepatic protein synthesis are demonstrated. There were no significant differences in the baseline values observed in the older and younger women. In the postmenopausal subjects, RS was significantly increased from baseline (1706 ± 184 ng/ml) by the 2.5-mg dose (2997 ± 291 ng/ml). For the subjects receiving increasing dosages of VCE, the mean for those receiving 2.5 mg was 2571 ± 331 ng/ml, while the patients receiving 2.5 mg as their first dose had a mean value of 3516 ± 466 ng/ml. This difference was statistically significant. SHBG was significantly increased from baseline by the 1.25-mg dosage, but not by the 2.5-mg dosage due to the narrower SE of the 1.25 mg values. Only the 2.5-mg dose of VCE significantly increased the level of TBG from baseline, while no dosage of VCE changed the mean level of CBG.

Figure 6 shows the values for the circulating lipids. Some of the baseline levels of triglycerides and total and fractionated serum cholesterol were higher in post- than

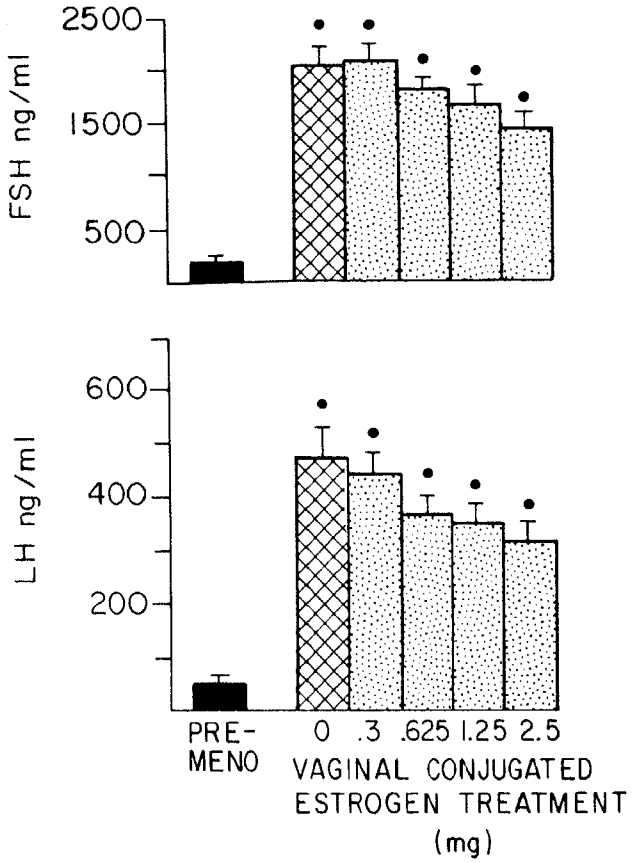

FIG. 3. The mean (±SE) serum concentrations of LH and FSH in the two groups of patients.

in premenopausal women, but the differences were not statistically significant. No dosage of VCE changed the levels of these lipid fractions.

Table 1 compares the effects of VCE to those of OCE for some of the parameters measured. Parallel dose-response curves could not be generated with raw or transformed data. Therefore, single doses were compared. For vaginal cytology, the effect of 1.25 mg CE administered orally on the percentage of SC was comparable to the action of 0.3 mg administered vaginally. For the systemic markers, the effect of 2.5 mg VCE was comparable to that exerted by half to one sixteenth the amount of orally administered hormone.

Discussion

This study was designed to determine the biological effects of VCE using specific markers of the action of estrogens. The values obtained in the premenopausal women during the early and late follicular phases of their menstrual cycles were presumed to reflect normal physiological function. If a dose of VCE was insufficient to alter a specific marker to a mean value that was similar

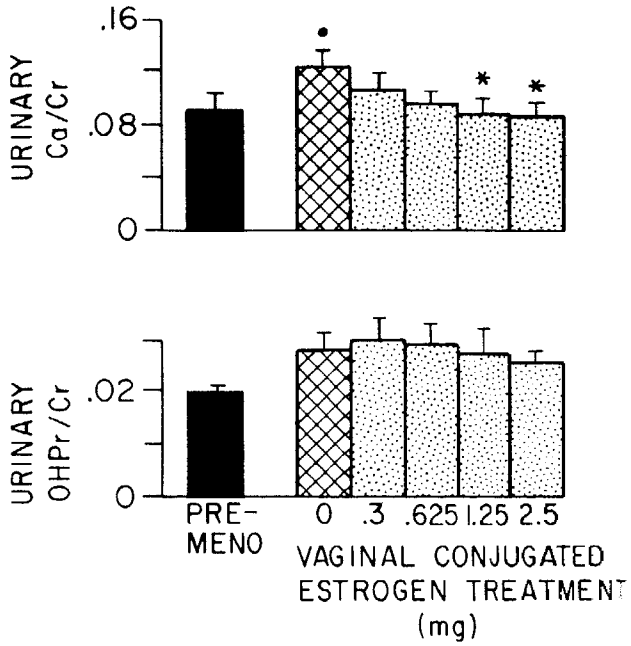

FIG. 4. The mean (±SE) for the urinary Ca/Cr and OHPr/Cr ratios o the two groups. See Figs. 1 and 2 for statistical significance.

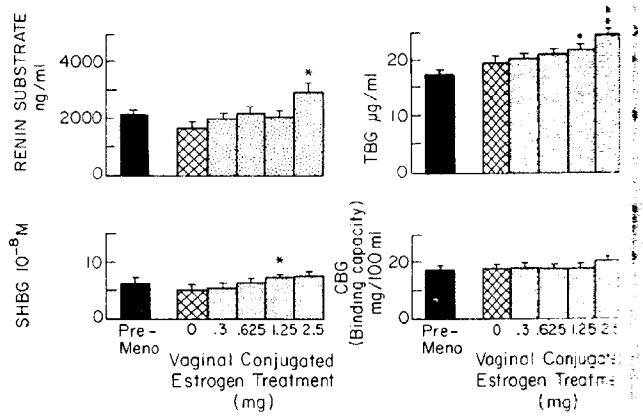

FIG. 5. The mean (±SE) levels of renin substrate, SHBG, and TB and the serum binding capacity of CBG in the two groups studied.

to that found in premenopausal subjects, the dosage wa considered to be subphysiological. If the dose change the marker to a value that was similar to or greater tha the mean premenopausal value, it was considered phys iological or pharmacological, respectively.

Using these criteria, the present results indicate tha the action of VCE is mainly local, with limited or n systemic responses. The 0.3-mg dosage was the lowe amount of VCE that returned the mean percentages o vaginal cells to values that were intermediate betwee those observed in premenopausal women early and lat in their follicular phases. This indicated that this dosag is sufficient to provide physiological replacement to th

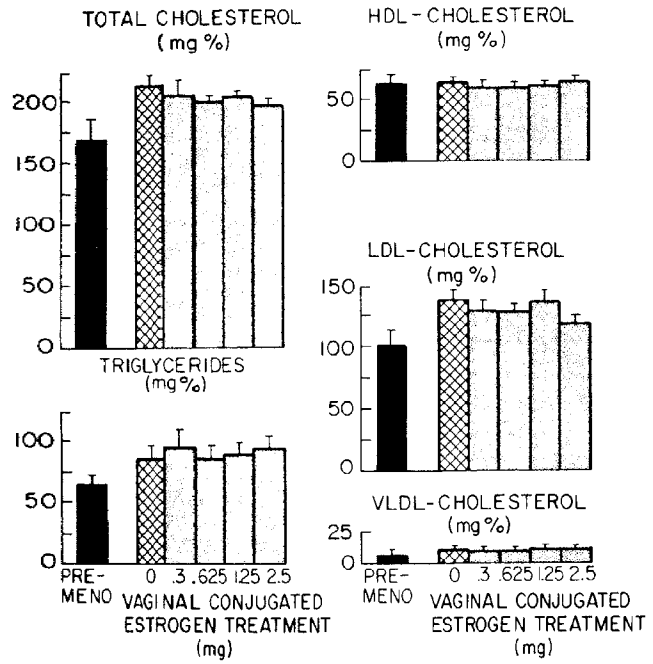

FIG. 6. The mean (±SE) levels of triglycerides and total and fractionated cholesterol in the two groups. HDL-CHOLESTEROL, High density lipoprotein cholesterol; LDL-CHOLESTEROL, low density lipoprotein cholesterol; VLDL-CHOLESTEROL, very low density lipoprotein cholesterol.

TABLE 1. Comparison of relative potency of 2.5 mg VCE with OCE[a]

Marker	Relative potency: oral to vaginal (% superficial cells)
Vagina	<0.25[b]
Systemic	
LH	2–4
FSH	4–8
Ca/Cr	4–8
Renin substrate	4–8
TBG	4–8
SHBG	16

[a] Results were reported by Geola *et al.* (6).
[b] Comparison of 0.3 mg VCE.

vaginal epithelium of the average woman if applied daily. This dosage is one quarter to one eighth the amount currently recommended in the package insert, and cannot be administered easily with the currently available applicator. The effect of 0.3 mg VCE on the vaginal epithelium was similar to that exerted with 1.25 mg OCE. This suggests that on a per mg basis, the potency of VCE is 4-fold greater than that of OCE on vaginal epithelium.

Measurement of circulating E_1 and E_2 levels revealed limited absorption of both hormones, particularly E_2, with the lower dosages of VCE administration. Measurements of E_1 and E_2 levels with the administration of CE by oral and vaginal routes have shown significantly greater levels of both hormones with oral administration (4). It should be noted that the lowest daily dosage (0.3 mg) of VCE that converted vaginal cytology to premenopausal values showed only minimal elevations of circulating E_1 and E_2 levels.

Although several studies have shown substantial absorption of estrogens administered vaginally, including VCE (1–4), this is the first attempt to comprehensively evaluate the systemic actions of vaginally administered hormone. The results indicate that the systemic effects of all doses of VCE studied were substantially less than those of similar doses given orally. For gonadotropins, a significant reduction of both gonadotropins occurred when the effects of all dosages were analyzed using analysis of variance; however, values seen after any single dosage were not significantly different from baseline values. In comparison to orally administered hormone, the 2.5-mg dosage of VCE exerted suppressive effects on gonadotropins which fell between those effects exerted by 0.625 and 1.25 mg OCE for LH and 0.3 and 0.625 mg OCE for FSH.

The probable reason for the difference between the effects of oral and vaginal CE on gonadotropin suppression is the previously mentioned observation that greater blood concentrations of estrogens occur with oral than vaginal administration of CE (4). This conclusion is supported by the observation that with the use of a vaginal ring made of Silastic and impregnated with E_2, blood levels of E_2 in the 100–150 pg/ml range were associated with marked reductions of circulating gonadotropins (24).

For bone, the lowest dosage of VCE that significantly decreased the urinary Ca/Cr ratio was 1.25 mg, while no dosage had an effect on the urinary OHPr/Cr ratio. The latter marker appears to be relatively insensitive to the action of estrogens, since there was no difference between pre- and postmenopausal values, and large doses of ethinyl estradiol have been shown to have limited effects on this parameter (7). In comparison, the effect of 2.5 mg VCE on the Ca/Cr ratio fell between those effects exerted by 0.3 and 0.625 mg orally administered hormone. Studies have demonstrated that estrogen replacement given orally is protective to bone. Diminished rates of loss of bone density (25–28) and reductions in the incidence of forearm, hip, and spinal fractures (29, 30) have been reported with estrogen administration. Based on long term bone density studies, the lowest dosage of OCE that is protective to bone is 0.625 mg (31). The present response of the urinary Ca/Cr ratio suggests that long term use of 2.5 mg VCE probably would not be protective against osteoporosis.

For the liver, VCE exerted variable effects. The most sensitive parameter of the action of estrogen was SHBG, with 1.25 mg VCE significantly increasing its level by an

average of 41%. Previous studies with orally administered estrogens have also shown this marker to be the most sensitive (6, 7). RS and TBG were next, with 2.5 mg VCE significantly increasing the levels by 72% and 25%, respectively. No dosage of VCE had a measurable effect on CBG or plasma lipids. In comparing the hepatic effects of vaginally *vs.* orally administered CE, 2.5 mg VCE exerted actions similar to those of 0.15–0.625 mg OCE.

The effects of estrogens on the liver are important, because altered hepatic function is presumed to be responsible for several adverse effects, including hypertension (8, 32–34), hyperlipidemia (9), hypercoagulability (10), and gallbladder disease (11). An increase of renin substrate in plasma has been proposed as a possible reason for the elevation of blood pressure associated with estrogen administration through increased generation of angiotensin and the synthesis of abnormal and more active forms of this protein (22, 35).

Estrogen administration also influences hepatic lipid metabolism. An increased incidence of gallbladder disease has been reported with oral contraceptive usage (36) and estrogen replacement therapy (11). Because cholesterol saturation of bile is between 75% and 90%, small increases in cholesterol in bile can produce precipitation, leading to stone formation (37). Increased amounts of cholesterol in bile is a common finding in gallbladder disease (37). Oral administration of estrogen increases the cholesterol fraction of bile (38). Proposed mechanisms for this include increased turnover of body cholesterol and increased hepatic synthesis.

Circulating lipids are also influenced by estrogen administration. Lipids are mostly bound to proteins in the circulation, and the concentrations of the various types of lipoproteins correlate with the risk of heart disease. Decreases in low density and very low density lipoprotein cholesterol and increases in high density lipoprotein cholesterol and triglycerides have been reported with oral administration (39, 40). In patients with familial defects of lipoprotein metabolism, estrogen administration has been associated with massive elevations of plasma triglycerides, which have led to pancreatitis and other complications (41). The effects of estrogen on circulating lipids are also believed to be related at least partially to changes in hepatic synthesis, although altered clearance of these substances may be involved.

The enhanced hepatic effects with oral, but not vaginal, administration CE may be explained by the fact that estrogens administered by mouth are absorbed by the intestines and are delivered to the liver before entry into the general circulation. The portal blood concentration of estrogen after oral administration is 4–5 times higher than the concentration in the general circulation (5). This concentration gradient would not occur with vaginal administration.

Three tenths milligrams of VCE (0.5 g cream) was sufficient to provide physiological replacement to the vaginal epithelium. Since this dosage was 4- to 8-fold lower than the threshold amount required to exert a measurable effect on hepatic function, it is probable that 0.3 mg VCE administered daily could be prescribed safely to patients with symptoms of vaginal atrophy, who have liver-related contraindications to estrogen replacement. These contraindications would include chronic impaired liver function, hypertension, familial hyperlipidemias, chronic thrombophlebitis, and gallbladder disease. This is of clinical importance, since there is no good alternative to estrogen replacement for the treatment of symptoms of vaginal atrophy. Long term studies are needed to confirm this point.

The finding of significantly higher RS levels with 2.5 mg VCE in subjects receiving this dosage first compared to those receiving it last may relate to vaginal absorption. Long term therapy leads to increased stratification of the vaginal epithelium and could decrease absorption. This concept is supported by findings of Furuhjelm *et al.* (3), who compared absorption of vaginal estrogen in postmenopausal (atrophic epithelium) and premenopausal (stratified epithelium) women and found significantly greater levels of estrogen in the circulation of post- than in premenopausal subjects. The finding of higher levels of E_1 and E_2 in the subjects given the 2.5-mg dosage first also supports this concept.

Acknowledgments

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