Estrogen effects on the vaginal pH, flora and cytology in late postmenopause after a long period without hormone therapy

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Summary

In this report we evaluated the action of conjugated equine estrogens (CEE) on vaginal symptoms, cytology, pH, and flora in late postmenopausal women without any previous hormone therapy. The study was a randomized, double-blind, placebo-controlled trial with 48 late postmenopausal women who received placebo or unopposed CEE (0.625mg/day of CEE orally) during three months of treatment. Vaginal and sexual complaints were evaluated through daily diary cards. We analyzed vaginal changes through cytology and pH measurements. After three months of treatment, 20% of placebo-treated patients and 80% of the CEE-treated patients reported improvement in vaginal dryness and irritation. In the latter group, the vaginal cells and *Lactobacillus* increased and the vaginal pH decreased, without other changes in sexual complaints. We concluded that estrogen ameliorated the genital tract of late postmenopausal women without any previous hormone therapy.

Key words: Estrogen; Menopause; Vagina; Cytology.

Introduction

Postmenopause is a phase during a woman's life which may be affected by low levels of estrogen, resulting in disturbances such as hot flushes, osteoporosis, and genitourinary symptoms. This period can be divided into two stages: early and late. The first stage is defined as five years since the final menstrual period. The participants of STRAW work-shop agreed that this interval was relevant because it encompassed a further dampening of ovarian hormone function to a permanent level as well as accelerated bone loss [1]. The second stage has a definite beginning (after 5 years of menopause), but its duration varies since it ends with death [1].

In addition, postmenopause is associated with a marked reduction in endogenous estrogen production [2]. Low circulating levels of estrogen correlate with same deleterious effects, including those on the vagina. The vaginal epithelium becomes atrophic and dry, which can cause vaginal discomfort, itching, and dyspareunia, resulting in sexual dysfunction and complaints [3]. This epithelium may become inflamed and contribute to urinary symptoms [4]. In fact, these changes occur often in late postmenopause.

The effectiveness of estrogen-based hormone therapy in ameliorating genital symptoms is well established [5]. However, there is a concern whether short-time unopposed estrogen treatment may alleviate the vaginal symptoms as well as sexual complaints, such as dyspareunia in late postmenopausal women who never received postmenopausal hormone therapy. Accordingly, the aim of this study was to evaluate the evolution of vaginal symptoms, cytology, pH, and flora in late postmenopausal women without any previous postmenopausal therapy.

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Material and Methods

Subjects for the present study consisted of women who had vaginal discomfort, itching, and dyspareunia; they attended screening and baseline visits and were subsequently enrolled in the Geriatric Program of the Division of Climacterium at the Department of Gynecology and Obstetrics of the Federal University of Mato Grosso do Sul. Moreover, the patients received psychological assistance before and after the treatment period, including couples' therapy.

The study was a randomized, double-blind, placebo-controlled trial designed to investigate the extent to which the unopposed use of conjugated equine estrogens (CEE, 0.625 mg/day) could decrease vaginal atrophy and/or increase the pH and change the vaginal flora of those patients. Additionally sexual complaints were recorded. To be eligible for this study, women had to be in menopause for at least five years, not on any type of hormonal treatment after menopause, and not currently using lipid-lowering drugs, antidiabetic medications, soybean-derived products, herbal supplements, or antibiotics. Women with a history of uncontrolled hypertension, stroke, transient ischemic attack, cancer diagnosed less than five years before, or previous myocardial infarction were excluded from the study. The Institutional Review Board of the University approved this study. All patients gave informed consent for their participation in the study after reading the protocol of the experiment and being advised about estrogen treatment.

At the screening visit, women responded to a standard questionnaire which ascertained information about demographic characteristics, including age, ethnicity, and education level. Women were also queried about gynecologic history, including age at menopause, use of selected medications, cigarette smoking history, frequency of alcohol use, physical activity, and dietary and nutritional habits. Medication use was validated by examination of prescriptions or pills brought to the clinic for that purpose. Transvaginal sonography (TVS) using a Toshiba SAL-38B real time sonography fitted with a mechanical 5.0-MHz probe was performed in order to evaluate the endometrial cavity to monitor the estrogen effect on the tissue. We used the

thickest endometrial area in one-third of the uterine body. This measure was performed in the anteroposterior direction from the echogenic interface of the endometrium-myometrium junction on both sides. Also, mammography was performed to exclude patients with mammographic changes at risk of breast cancer. The baseline visit occurred one month after the screening visit, and vaginal secretions were collected in order to measure the pH, evaluate flora and cytology (Pap test). This procedure was repeated every month after a 90-day treatment period.

There were 48 women who completed the baseline requirements and received a randomized envelope labeled #1 or #2 corresponding to placebo and unopposed conjugate equine estrogen 0.625 mg/day, respectively. All patients completed the 5-month study (including screening, baseline, and 3 months of treatment). Medication use was validated by examination of prescriptions or pills brought to the clinic for that purpose.

Safety evaluation was based on vital signs, pelvic and breast clinical exams, as well as hematological and biochemical exams, Pap smear, endometrial thickness, mammogram and report of adverse events.

Women were instructed to record the days they took medication, as well as any dates that they missed medication, on a daily diary card which they returned to the investigator at their next visit. The diary cards were also used to record adverse effects and bleeding information, as well as sexual activity and complaints concerning libido, orgasm and sexual intercourse. The severity of disturbances were classified as mild, moderate and severe based on the interference in sexual activity. Also, sexual function of the male partners and the relationship were investigated. To avoid compromising the double-blind design, the occurrence of side-effects or physical changes such as bleeding was recorded by an independent gynecologist.

Ten patients dropped out of the study. Four of them had abnormal bleeding after the treatment period, and the other six left for private reasons. At the end of the study, all patients were subjected to new physical exam and TVS. After one month all patients were evaluated and informed about the received treatment.

Vaginal sample collection

Samples were collected from the posterior fornix or the lateral vaginal wall of nonbleeding women with an Ayre's spatula using a non-lubricated speculum to perform a smear on two glass slides. The smears were then stained according to the Papanicolaou method for vaginal cytology or the Gram procedure for bacterial evaluation. Clue cells and vaginal flora were evaluated on the Gram-stained smear.

Gram-stained smear evaluation

The vaginal flora was determined by evaluation of five different fields under oil immersion (magnification, x 1,000). For vaginal flora analyses we adopted the following score method:

Type I: presence of superficial epithelial cells and absence or low number of leukocytes and Doderlein's lactobacilli representing more than 90% of the vaginal bacteria, other bacteria representing less than 10%.

Type II: Doderlein's lactobacilli representing between 50% to 90% of the bacteria in the vagina, and other bacteria representing less than 50%.

Type III: 100% of the vaginal population built up by bacteria other than Doderlein's lactobacilli.

The other bacteria are Gardnerella vaginalis, Bacteroides spp., Prevotella spp., and Porphyromonas spp, Gram-positive cocci and/or enterobacteriaceae.

All the Gram smear evaluations were performed by two independent investigators, with more than 90% agreement; discrepant readings were reexamined, and a third investigator was consulted in case of disagreement.

Hormonal cytology

The maturation of the vaginal epithelium was assessed manually in Papanicolaou-stained smears by an experienced cytotechnologist and cytopathologist. They counted over 300 cells. The maturation index was calculated as the percentuals of parabasal, intermediate and superficial cells.

Vaginal pH

During the gynecological exam, vaginal pH was measured. A strip of pH paper (Merck 0-14, Darmstadt, Germany) was left for one minute in direct contact with one-third of the left external vaginal wall.

Data analysis

All values in the figures and text are expressed as mean ± SEM. Comparisons of clinical data between the groups and of the baseline versus post-treatment period within groups were made through the unpaired and the paired Student's t-test, respectively. In addition, the distributions of patients in the groups were compared using a contingency table and Fisher's exact test. The results were analyzed by one-way ANOVA followed by a Bonferroni post-hoc test for multiple comparisons. Contingency tables and the chi-square test were performed to

Table 1. — *Clinical features of postmenopausal women enrolled in the study (see Methods for exclusion criteria).*

Feature	Placebo (n = 24)	CEE treated (n = 24)
Age (years)	57.9 ± 0.6	58.2 ± 0.9
Postmenopause status (years)	8.1 ± 0.5	7.4 ± 0.8
Number of pregnancies	5.1 ± 0.4	4.7 ± 0.8
Bone mass index	26.6 ± 0.6	26.5 ± 0.9
Type of delivery		
Vaginal (%)	95	94
Cesarean (%)	5	9
Libido and sexual performance		
Libido disturbances (%)	75	92
Orgasm disturbances (%)	83	71
Dyspareunia (%)	91	88
Male partner dysfunction (%)	8	13
Number of intercourses (per month)	3.7 ± 0.4	4.1 ± 0.9
Vaginal dryness (%)	91	88
Vaginal irritation (%)	91	88
Racial data		
Caucasian (%)	81.8	91.7
African (%)	9.1	8.3
Asian (%)	9.1	0

Absolute values are given as mean ± SEM. No significant differences were detected between the groups. n = number of participants.

Table 2. — *Sexual complaints of postmenopausal women with no previous estrogen treatment.*

Complaint	Placebo (n = 24)						CEE (n = 24)					
	Baseline			90d			Baseline			90d		
	mi	mo	se	mi	mo	se	mi	mo	se	mi	mo	se
Libido disorders	1	13	4	0	11	6	1	10	11	0	9	12
Orgasm disorders	0	9	11	0	8	12	1	6	10	1	5	8
Dyspareunia	1	5	16	0	7	15	0	10	11	4	9	7

n = number of participants. *Abreviations:* mi = mild; mo = moderate; se = serious.

Table 3. — *Hormonal blood levels and vaginal flora of control (placebo) and estrogen (CEE)-treated postmenopausal women at the start ('baseline') and during (30, 60 or 90 days) the treatment period. Hormonal data are given as mean ± SEM.*

	Placebo (n = 24)				CEE-treated (n = 24)			
	Baseline	30d	60d	90d	Baseline	30d	60d	90d
Hormonal data								
FSH (mUI/ml)	61.8 ± 4.4	56.8 ± 3.4	59.4 ± 4.9	60.3 ± 3.9	60.3 ± 3.4	39.8 ± 3.5*	40.2 ± 4.4*	39.2 ± 3.2*
Estradiol (pg/ml)	15.1± 1.4	14.5 ± 1.6	11.8 ± 2.1	13.7± 1.8	14.8 ± 1.6	49.7 ± 4.6*	42.8 ± 4.4*	53.6 ± 5.1*
Vaginal flora								
Type I (%)	0	0	0	0	0	46*	29*	74*
Type II (%)	0	0	0	0	0	0	21*	5
Type III (%)	100	100	100	100	100	54*	50*	21*

*p < 0.01 compared to baseline of CEE group and to placebo group. n = number of participants.

analyze the sexual complaints. Differences were considered significant at the $p < 0.05$ level. All statistical tests were done using the GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego, CA).

Results

Clinical data

To evaluate the clinical characteristics of the patients, we collected data on age, years of postmenopausal status, number of pregnancies, type of deliveries, sexual complaints (libido, orgasm and dyspareunia), male partner dysfunction, frequency of sexual intercourse per month, race, and the calculated bone-mass index (BMI) (Table 1). The data showed no difference between the groups (placebo and CEE). The percentage of patients with sexual complaints and vaginal dryness was high in both groups at baseline. The number of patients that reported male partner dysfunction was low (2 and 3, placebo and CEE, respectively). Also, the referred male dysfunction was mild. After three months of treatment, 20% of the patients in the placebo and 80% in the CEE group reported an improvement in vaginal dryness and irritation ($p < 0.002$), but no differences were detected in sexual complaints (Table 2) and the frequency of sexual intercourse per month.

Endogenous hormones

No differences between the placebo- and CEE-treated groups were detected in the circulating levels of FSH and 17β-estradiol at baseline (Table 3). There was a significant decrease in FSH levels of the CEE group after 30, 60, and 90 days of treatment ($p < 0.01$) as compared to the baseline values and those found in the placebo group. There were no significant changes in FSH levels of the placebo group. The blood levels of 17β-estradiol were significantly increased after 30, 60, and 90 days of treatment in the estrogen group ($p < 0.01$) compared to the baseline and placebo group values. In addition, the endometrial thickness measured by TVS increased in the CEE group compared to baseline and in the placebo group, but the values of patients in both groups were lower than 7 mm after 90 days of treatment.

Vaginal flora

All patients in the placebo and baseline of the CEE group presented vaginal flora type III. However, the vaginal flora changed after the first month of treatment in the CEE group with 46% of patients presenting a type I flora. The percentage of type III flora in the CEE group significantly decreased, but the greatest reduction was detected after 90 days of treatment (Table 3).

Vaginal maturation index

The maturation index was similar in both groups at baseline. There was a significant increase in the intermediate and superficial cells after 30 days of treatment in the CEE group when compared to baseline and to the placebo group. No differences were observed within the placebo group (Figure 1).

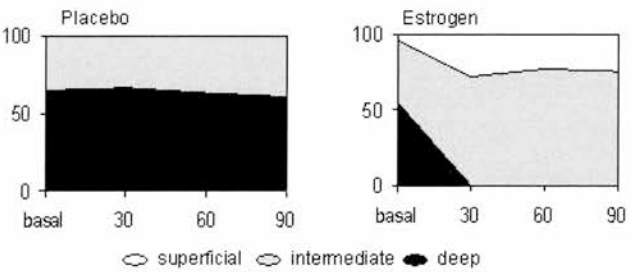

Figure 1. — Vaginal maturation index in the Pap smears of subjects.

Vaginal pH

There was no difference between the two treatment groups at baseline. The vaginal pH decreased significantly from 7.0 to 5.0 (30 days), to 4.7 (60 days), and then to 4.5 (90 days) in the CEE group, but there was not a significant difference between 30 and 60 days of treatment. The pH data of the CEE group were significantly lower than for the placebo group. There were no changes in vaginal pH in the placebo group (Figure 2).

Discussion

Menopause is associated with physiological and psychological changes that influence sexuality. During

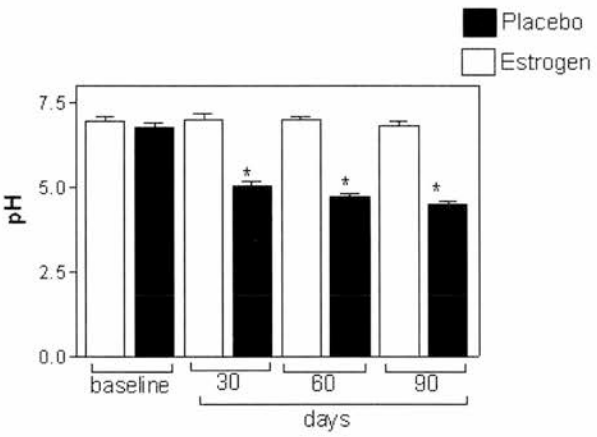

Figure 2. — pH of postmenopausal women compared to baseline and estrogen-treated groups, *p < 0.01.

menopause, the primary biological change is a decrease in circulating estrogen levels. Estrogen deficiency initially accounts for altered bleeding and diminished vaginal lubrication. Continuous estrogen reduction often leads to numerous signs and symptoms, including changes in the vascular and urogenital systems, which can be worse during late postmenopause. Alterations in mood, sleep, and cognitive functions are common as well. These changes may contribute to lower self-esteem, poorer self-image, and diminished sexual responsiveness and sexual desire. Generally, estrogen therapy increases vaginal lubrication and moisture, which may be useful in ameliorating postmenopausal sexual complaints [6]. Despite the observed improvements in vaginal pH, flora, and cytology, our study did not show any enhancement in sexual activity or a reduction in sexual complaints of postmenopausal women without any previous hormone therapy.

Low levels of circulating blood estrogens have various deleterious effects, including those on the lower genital tract. The vaginal epithelium becomes atrophied and dry, which can cause vaginal discomfort, itching, and dyspareunia [3, 4]. These alterations may notably compromise sexual pleasure and ultimately result in the avoidance of sexual intercourse [7]. Our data showed that treatment with estrogens restored vaginal epithelial health, resulting in decreased vaginal pH after one month of therapy. Other studies have shown changes in vaginal fluids and electrolytes with the same period of treatment, but not in vaginal pH, which was reduced only after 18 months of treatment [8].

Some authors have shown that women using estrogens reported less vaginal irritation, pain, dryness, or burning during intercourse [9-12]. Also, relief from urogenital symptoms often leads to an increased sexual desire [13] and arousal [14-16]. However, the late postmenopausal patients in our study did not reduce their sexual complaints upon estrogen treatment. In addition, a study with 60- to 70-year-old women showed that libido is not dependent on female sex hormones; rather, personal relationships and life circumstances are the predominant determinants of sexuality [17]; notwithstanding, the participants of the present study did not report problems regarding their partners.

Doderlein's lactobacilli constitute the vaginal flora during the reproductive age. It is capable of metabolizing the glycogen deriving from the decline of eutrophic vaginal mucosa into lactic acid and the release of hydrogen ions. The final result of that metabolism is an acidic pH with values less than 4.0. Therefore, pathogens become impeded to reside in the lower genital tract. This phenomenon seems to protect the genital tract against opportunistic infections [18, 19]. It is well known that estrogen therapy increases the cellular content of glycogen in postmenopausal women, decreasing the pH [20] besides reducing vaginal discharge and genital irritation in older women. These factors are conceivably important to decrease genital symptoms, facilitating sexual intercourse.

It is important to emphasize that all volunteers denied having any problems with their partners and wanted to be enrolled in the study in order to improve their sexual performance. Also, psychological assistance was provided before and during the treatment period, including advice to couples. In conclusion, if on the one hand estrogenic treatment improved the organic conditions in the lower reproductive tract of late postmenopausal women, on the other hand it was not the only factor involved in overall sexual performance.

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