Published in final edited form as:

Maturitas. 2015 August ; 81(4): 475–479. doi:10.1016/j.maturitas.2015.05.010.

Low-Dose 17- β -Estradiol Cream for Vaginal Atrophy in a Cohort without Prolapse: Serum Levels and Vaginal Response Including Tissue Biomarkers Associated with Tissue Remodeling

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Abstract

Objectives—Describe the effect of 50mcg vaginal 17- β -estradiol (E2) cream on vaginal maturation, serum estrogen levels, atrophic symptoms, and biomarkers of oxidative stress and tissue remodeling in postmenopausal women without prolapse.

Methods—Seventeen women, 65 years or older, applied intravaginal E2 cream nightly for eight weeks, then twice weekly for eight weeks. Vaginal biopsies, serial blood draws, and atrophic symptoms were obtained at baseline, eight, and sixteen weeks. Changes in atrophic symptoms, vaginal maturation indices (VMI), and serum E2 were measured. Immunohistochemical staining characterized levels of transforming growth factor-beta (TGF- β), nuclear factor kappa B (NFkB), inducible nitric oxide synthase (iNOS), endothelial nitric oxide synthase (eNOS), and thrombospondin (TSP).

Results—Serum E2 levels (pg/mL) were unchanged from baseline (mean (SD)) 7.7 (3.3) to eight 9.7 (5.7) and sixteen 8.7 (5.8) (p=0.24) weeks. VMI (mean (SD)) improved from baseline 34.2 (18.3) to eight 56.7 (13.1) and sixteen 54.5 (11.3) (p<0.001) weeks with no difference between eight and sixteen weeks. Vaginal dryness (p=0.03) and itching (p=0.02) improved. Tissue biomarker levels did not change (TGF- β p=0.35, NFkB p=0.74, eNOS p=0.80, iNOS p=0.24, TSP p=0.80).

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Disclosures

JDI, MGC, and PSG do not have any conflicts of interest.

Discussion—Vaginal E2 improved atrophic symptoms and VMI without elevating serum E2. Tissue remodeling biomarkers did not change.

Keywords

vaginal estrogen; vaginal atrophy; estradiol; tissue biomarkers

1. Introduction

Vaginal atrophy affects many older women with and without prolapse [1]. Changes in the biochemical and molecular structure of the vagina have been identified in women with pelvic organ prolapse (POP)², and it has been hypothesized that these changes may be involved in the pathogenesis of POP [2–4]. Estrogen has been shown to modulate many biomarkers of oxidative stress and tissue remodeling, including transforming growth factor-beta (TGF- β), nuclear factor kappa B (NF κ B), inducible nitric oxide synthase (iNOS), endothelial nitric oxide synthase (eNOS), and thrombospondin (TSP) [5–13]. Knowledge about how these biomarkers change in response to vaginal estrogen in women without POP is limited.

Findings of the Women's Health Initiative created confusion, debate, and fear over the general use of estrogen [14]. Consequently, even though current guidelines support the use of vaginal estrogen to treat vaginal atrophy, many women have been reluctant to use any form of estrogen [15–18]. Treatment of vaginal atrophy to optimize vaginal health has also become common in the surgical and nonsurgical management of pelvic floor complaints including prolapse and urinary incontinence.

The objective of this pilot study was to describe the effect of topical 17- β -estradiol (E2) on vaginal maturation index, serum estrogen levels as measured using a highly sensitive radioimmunoassay, atrophic symptoms, and biomarkers of oxidative stress and tissue remodeling in postmenopausal women without prolapse.

2. Materials and Methods

After IRB approval was obtained, seventeen patients were recruited from an outpatient Urogynecology clinic for this pilot study. Postmenopausal women aged 65 or older, status post hysterectomy with signs of vaginal atrophy, such as thinness, pallor, petechiae, and decreased rugation, were included. Symptoms of atrophic vaginitis were not required.

²Abbreviations:

POP – Pelvic Organ Prolapse
TGF- β – Transforming Growth Factor-Beta
NF κ B – Nuclear Factor Kappa B
iNOS – Inducible Nitric Oxide Synthase
eNOS – Endothelial Nitric Oxide Synthase
TSP – Thrombospondin
E2 – Estradiol
VMI – Vaginal Maturation Index
Tukey's HSD - Tukey's Honestly Significant Difference
SD – Standard Deviation

Women with prolapse beyond the hymen, a contraindication to estrogen, or estrogen therapy in the last 6 months were excluded.

Patients were evaluated at baseline for symptoms of vaginal atrophy and to confirm no other pathology. The question was phrased "How often do you have any of the following problems in your vagina?" and asked for dryness, itching, discomfort, discharge, pain with starting sex, and pain during sex. The response options were never, rarely, sometimes, or often. Patients also underwent blood sampling, vaginal smears, and vaginal biopsies.

Low-dose intravaginal estrogen therapy was then initiated with 50mcg of 17- β -Estradiol cream nightly. Patients used 0.5g of 0.01% 17- β -Estradiol cream applied with a finger to the inside of the vagina to achieve the 50 mcg dose. All patients were instructed to apply the cream at night, which resulted in any study evaluation the next day being at least 8 hours later. After eight weeks, patients were re-evaluated with the same procedures and atrophy questions. Estrogen therapy frequency was then decreased to twice weekly and continued for eight additional weeks. Patients were then reevaluated at the 16-week endpoint with the same atrophy questions and procedures.

Vaginal biopsies to the depth of the subepithelium were obtained from POPQ point Bp at each time point to investigate changes in biomarkers TGF- β , NF κ B, iNOS, eNOS, and TSP. Vaginal smears were obtained, placed on glass slides, and subjected to the standard Papanicolaou polychrome staining procedure [19]. The slides were then read by a pathologist (MGC). Three distinct cell types from least to most mature--parabasal cells, intermediate cells, and superficial cells--were distinguished in the smears and used to calculate the Vaginal Maturation Index (VMI) (range 0–100) [20]. The numbers of each type of cell were counted on the individual slides and then extrapolated to a relative percentage and expressed in a formula as follows: $(0 \times \% \text{ parabasal}) + (0.5 \times \% \text{ intermediate}) + (1 \times \% \text{ superficial})$.

Serum estradiol was measured by a highly-sensitive radioimmunoassay method. The antiserum used in this assay cross-reacts with estradiol 100% and with estrone 2.4%; the antiserum cross-reacts <1% with other steroids commonly found in humans. The theoretical sensitivity of the assay standard curve is 2.2pg/ml. The reported within-assay coefficients of variation for the assay varied from 6.9–8.9% for quality control samples over the range of 5.3 – 92.6 pg/ml. The estradiol assay standards ranged from 2.5pg/ml to 250pg/ml. To limit variability, all samples from this study were analyzed in the same assay procedure.

Tissue biomarker staining was assessed at baseline, eight weeks, and sixteen weeks. Formalin fixed, paraffin-embedded tissue sections were subjected to immunohistochemical staining by the peroxidase method following antigen retrieval in Citrate Buffer, pH 6.0. Reaction product was obtained after incubation of the slides with diaminobenzidine. Slides were counterstained with hematoxylin. Four investigators, blinded to slide type and time point, scored the intensity of staining from 0 to 4, with 0 being none and 4 being strong, such that the sum of scores for each slide could range from 0 to 16.

No sample size calculation was performed for this pilot study. Repeated measures analysis, which accounts for the dependency of values across all time points, assessed for changes in

VMI, serum E2 level (as measured by highly-sensitive radioimmunoassay), growth factors, and responses to atrophy questions at baseline, eight weeks, and sixteen weeks. Tukey's Honestly Significant Difference (HSD) corrected for multiple testing. Analysis was performed on JMP IN 8.1 (Cary, NC).

3. Results

The mean participant age was 72.4 years (SD 6.3, median 70, range 65–84), and mean BMI was 24.9 kg/m² (SD 3.0, median 25.3, range 18.9–29.8). Using Tukey's HSD, no statistical difference was found in serum E2 levels (pg/mL) across time points (mean (SD)): Baseline 7.7 (3.3); 8 weeks 9.7 (5.7); 16 weeks 8.7 (5.8); $p=0.24$. VMI score improved from baseline, but there was no difference found between eight and sixteen weeks (Table 1). There was marginal evidence that serum E2 level correlated with VMI ($p=0.06$).

Symptoms of vaginal dryness and itching did improve, but vaginal discomfort, discharge, and dyspareunia did not (Table 1). No significant change from baseline was found for biomarker staining at any time point (Table 2).

4. Discussion

Low dose vaginal estrogen is an effective treatment for atrophy symptoms [17–18]. This study showed that older women using 50mcg of intravaginal estradiol nightly for 8 weeks followed by twice weekly for 8 weeks had improvements in their VMI and vaginal symptoms of dryness and itching. Importantly, these improvements occurred without significantly elevating their serum estradiol levels when sampled with a highly-sensitive radioimmunoassay. This information is consistent with the published literature and can be used when counseling older women with vaginal atrophy who may be concerned about initiating intravaginal estrogen therapy [1, 15–18].

Lack of improvement in vaginal discomfort, discharge and dyspareunia is likely due to the less frequent occurrences of these symptoms at baseline, which may make it difficult to have a detectable change. Patients were not required to have symptoms to participate in the study, which may have biased against finding changes in symptoms. Further, the ability to detect changes in symptoms, like dyspareunia, which are multifaceted and confounded by other factors, may be limited with intravaginal E2 treatment alone. These conditions may also require a longer period of treatment to affect symptoms.

Estrogen has been shown to influence tissue biomarker/growth factor expression in rat models and in human skin remodeling [5, 7, 9, 12, 22]. Even though the biomarkers selected for this study have been previously noted to play a role in skin remodeling, in this pilot study, immunohistochemical staining levels did not change in response to treating vaginal atrophy with low-dose estradiol cream. This pilot study with its small sample size, however, may have been underpowered to detect changes. Additionally, the relevance of biomarker immunohistochemical staining is unknown. It could be that these biomarkers are involved in the vaginal response to re-estrogenization but that changes in biomarker staining were too subtle to detect or take longer to occur. More sensitive measures that reflect active production of these biomarkers may have more accurately reflected any changes. It is also

possible that E2 treatment influences different biomarkers than were investigated in this pilot study.

Strengths of this study included obtaining biopsies to perform a novel exploration into markers of tissue remodeling and analyzing them at multiple clinically relevant time points. Further, an ultrasensitive radioimmunoassay was utilized to monitor serum E2 levels.

The major weakness of the study was the small sample size inherent to a pilot study, which may have affected our power to detect changes in some outcomes. As an uncontrolled observational study, it is possible that any improvements or changes noted were the result of a placebo effect from the cream itself rather than the estrogen it contained. Also, the 16 week study period may not have been long enough to capture changes in biomarker staining. Further, while there are now validated instruments to assess the impact of vaginal atrophy, there were no validated measures published at the time this study was conducted [23–24].

Systemic estradiol levels have been shown to peak about 4 hours after vaginal administration of estradiol cream [25–26]. Because patients in this study applied the cream at bedtime and blood samples were obtained during routine (daytime) clinic hours which resulted in a minimum of an 8 hour interval, the serum values in this study are likely not representative of maximum systemic values. Sampling would need to be performed at multiple intervals closer to administration to more accurately measure maximum absorption.

Along with VMI, vaginal pH has also been used as an important objective measure in the treatment of vaginal atrophy [27–28]. Improvements in vaginal pH and VMI have been shown to correlate in patients treated for vaginal atrophy, which makes sense considering that vaginal pH improves (e.g., lowers) as result of lactic acid produced by lactobacilli which are better able to thrive in the glycogen-rich, estrogenized vagina with a higher VMI [29–30]. Even so, a recent study showed that vaginal pH correlated with atrophic symptoms [31]. Vaginal pH measurement would have enriched our study findings but is reflected in the improvement found in VMI.

Studies in Europe have evaluated various low and ultra-low dose estriol preparations and shown them to be safe and effective [30, 32]. Since estriol is a weaker estrogen, has a shorter duration of binding to nuclear receptors, and cannot be converted to estradiol, it has no appreciable effect on the endometrium when vaginally administered once daily and in low doses [1, 30, 32–33]. This balance of efficacy and minimized untoward effects makes estriol a very appealing possibility for the future. The lack of any FDA-approved estriol preparations, however, limits its availability in the United States to custom compounded preparations.

4.1 Conclusions

A 50mcg vaginal dose of E2 cream appears to be effective for relieving symptoms of vaginal atrophy without raising systemic hormone levels. This finding contributes to the evidence that low dose vaginal E2 is likely safe and effective in treating atrophy. Additionally, despite effective treatment of vaginal atrophy, there were no changes in

staining for TGF- β , NF κ B, iNOS, eNOS, or TSP, biomarkers associated with oxidative stress and tissue remodeling.

Acknowledgements

Funding provided by National Institutes of Diabetes and Digestive and Kidney Diseases, 2K24-DK068389, to HER and the Southeast Center for Excellence in Geriatric Medicine to TLW. These funding sources played no role in design or conduct of the study.

HER serves on the Board of Directors for the Society of Gynecologic Surgeons, is a consultant for Pelvalon and Kimberly Clark, receives royalties from UpToDate, speaks for Symposia Medicus, and has received grants from Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institute of Diabetes and Digestive and Kidney Diseases/Yale University, National Institutes of Health, National Institute of Allergy & Infectious Diseases, and Department of Health and Human Services. TLW received a grant from the Southeastern Center for Excellence in Geriatric Medicine. CRP serves on the Institutional Review Board at the University of Alabama at Birmingham. KLB has served as a consultant and received grants from Pfizer.

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Table 1

Symptomatic and Objective Outcomes with Intravaginal Estradiol Cream*

Parameter	Baseline	8 Weeks	16 Weeks	p-value
Serum Estradiol ^a pg/mL (mean, SD ^b)	7.7, 3.3	9.7, 5.7	8.7, 5.8	0.24
Vaginal Maturation Index Score (mean, SD)	34.2, 18.3	56.7, 13.1	54.5, 11.3	<0.001
Vaginal Dryness (%)				
Never	25	21	35	0.03
Rarely	5	26	15	
Sometimes	45	16	35	
Often	25	37	15	
Vaginal Itching (%)				
Never	30	16	25	0.02
Rarely	30	26	50	
Sometimes	30	47	25	
Often	10	11	0	
Discomfort (%)				
Never	40	42	36	0.60
Rarely	35	37	29	
Sometimes	20	16	29	
Often	5	5	6	
Discharge (%)				
Never	40	37	36	0.92
Rarely	20	37	29	
Sometimes	40	16	29	
Often	0	10	6	
Pain when starting sex (%)				
Never	46	54	54	0.56
Rarely	8	15	15	
Sometimes	38	23	23	
Often	8	8	8	
Pain during sex (%)				
Never	46	61	62	0.28
Rarely	15	8	15	
Sometimes	31	23	15	
Often	8	8	8	

* Estradiol 50 mcg nightly for 8 weeks, then twice a week for 8 more weeks.

^a Approximate postmenopausal levels 10–20 pg/mL [21]

^b SD (Standard Deviation)

Table 2Biomarker Staining Intensity with Intravaginal Estradiol Cream^{*}

Growth Factor	Baseline	8 Weeks	16 Weeks	p-value
TGF-β	10.6 $\pm$ 2.1	11.5 $\pm$ 1.8	11.4 $\pm$ 2.0	0.35
NFKB	8.4 $\pm$ 3.4	9.1 $\pm$ 3.1	8.6 $\pm$ 2.8	0.74
eNOS	8.2 $\pm$ 3.3	7.6 $\pm$ 3.0	8.0 $\pm$ 2.4	0.80
iNOS	10.6 $\pm$ 2.9	11.9 $\pm$ 1.5	11.1 $\pm$ 2.6	0.24
TSP	5.7 $\pm$ 3.7	6.6 $\pm$ 4.3	6.3 $\pm$ 4.1	0.80

^{*} Estradiol 50 mcg nightly for 8 weeks, then twice a week for 8 more weeks.

Range of scores, 0–16 (least intense – most intense)