Protocol

TITLE: Dual benefits of Vaginal Estriol: improved urogenital health and re-myelination in relapsing remitting multiple sclerosis (RRMS)?

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AIMS:

1. To evaluate the efficiency of vaginal estriol, as a treatment for urogenital symptoms in female patients with RRMS.
2. To evaluate the potential role of vaginal estriol in re-myelination in RRMS patients.

The goal of this pilot project is to determine the effectiveness of 1 mg transvaginal estriol, as an adjunctive therapy for female MS patients. Our goal is to recruit 20 patients from our MS clinic in the neurology department of Texas Tech University Health Sciences Center. This study has been planned in collaboration with reproductive endocrinology, endocrinology, gynecology and basic science. The 1mg transvaginal dose was chosen after careful search of the literature and consultation with our collaborators.

OBJECTIVES:

Aim 1: Define if transvaginal estriol improves urogenital symptoms (urgency, frequency, intermittent incontinence), in female patients with Relapsing Remitting MS (RRMS). Bladder control scale, will be given at baseline, 3, 6, and 9 months after starting transvaginal estriol. Quality of life questionnaire will be given at baseline and at 9 months after starting vaginal estriol.

Aim 2: Assess for evidence of re-myelination associated to the use of transvaginal estriol in woman with MS. Objective measurements for re-myelination will be quantified with brain MRI 3T, Optical coherence tomography (OCT)-and Visual evoked potentials (VEP).

INTRODUCTION / BACKGROUND / SIGNIFICANCE:

More than 2.3 million persons suffer from MS worldwide. Demyelination and axonal loss are the main pathological process in MS. Unfortunately remyelination occurs an incomplete fashion (Mallik, Samson, Wheeler-Kingshott, & Miller, 2014). Experimental models of MS have shown that preservation of myelin and remyelination of axons can increase neuronal survival. Until now, treatment for MS may decrease the relapse rate and progression, without any significant effect on remyelination.

Urinary issues in MS

Urinary dysfunction includes frequency, incontinence, frequent infections, occurs in about 80-90% of MS patients. It causes a major disruption in daily life, imposing economic burdens for patients and their families. Direct costs and indirect costs as psychological distress, loss of productivity are a significant burden for the patient, and increase the risk of unemployment.(Moore, 2001) Besides interfering with quality of life, urinary dysfunctions leads commonly to urinary tract infections which can have a direct impact on MS, worsening neurologic symptoms, and increasing in the number of relapses, which may lead to disability.

Estriol for urogenital health

Urinary dysfunction is frequently treated with antimuscarinic agents; side effects from these drugs (dry mouth, blurred vision, constipation and difficulty with micturition) make compliance an issue (David et.al). Transvaginal estriol decreases urinary symptoms and prevents urinary tract infections.(Rahn et al., 2014) There is less risk of endometrial hyperplasia and endometrial cancer with estriol treatment compared with estradiol treatment (Cody, Jacobs, Richardson, Moehrer, & Hextall, 2012). Furthermore, vaginal delivery offers regional benefits to the lower urogenital tract, it is well absorbed and it is not associated with entero-hepatic recirculation as oral estriol, as some of the adverse effects of oral estrogens are to produce an increase in thromboembolic events. (Rahn et al., 2014)

Estriol in MS, role in remyelination

Estriol is one of the three main estrogens, produced by fetal placenta. Levels increase significantly in the third trimester of pregnancy. It has been postulated as a possible neuromodulator and neuroprotective therapy in multiple sclerosis. (Tiwari-Woodruff & Voskuhl, 2009). Estriol has been studied in animal models and has been demonstrated to have distinctly different modulatory actions on the immune system than estradiol. Moreover, in 1-chloro-2, 4-dinitrobenzene (DNCB) induced contact dermatitis, estriol (Cody, Jacobs, Richardson, Moehrer, & Hextall, 2012)had the strongest suppressive effect when compared to estrone and estradiol (Cody et al., 2012). It has been reported that MS patients have a decreased annualized relapse rate during pregnancy, with a statistically significant decrease in the third trimester. Additionally, in the 3 months postpartum, the rate of relapse increases significantly. (Avila-Ornelas, Avila, Stosic, & Robles, 2011)This effect may be mediated at least in part by estriol, which rises during the third trimester of pregnancy and then falls precipitously postpartum. Furthermore, Estriol has been shown to have a positive effect reducing active brain lesions in multiple sclerosis. Estriol may have a role in remyelination.(Harlow, Honce, & Miravalle, 2015) Oligodendrocytes are the myelinating cells of the CNS. The process of re- myelination represents a major form of plasticity in the brain, unfortunately this process is not complete, leading to partial remyelination in patients with MS. (Mallik et al., 2014) Previous studies have shown that pregnancy leads to an increase in oligodendrocyte precursor cells (OPC) proliferation, oligodendrocyte generation and the number of myelinated axons in the maternal CNS. (Tiwari-Woodruff & Voskuhl, 2009) · If estriol favors remyelination, this could revolutionize current treatment options for MS patients.

Benefit of vaginal estriol

Oral estriol has been shown to produce a significant decrease in the relapse rate in MS patients(Voskuhl et al., 2016) . One of the reasons why vaginal estriol has a better absorption is that oral estriol is associated with entero-hepatic recirculation, which prolongs exposure due to recycling. Furthermore, vaginal estrogen delivery has a regional benefit to the lower urogenital tract, and is well absorbed at a dose of 0.5 mg to 1 mg. In a systematic review of randomized trials comparing vaginal estrogens, it was concluded that the 1mg vaginal estriol, was superior to lower doses for the treatment of urogenital symptoms. (Rahn et al., 2014) In animal models, estriol demonstrated to have distinctly different modulatory actions on the immune system than other form of estrogens, such as estradiol. Vaginal estriol does not increased the risk for heart disease, stroke, or cancer. (Rahn et al., 2014) In summary, patients with RRMS may significantly benefit from estriol as an adjunctive therapy to MS, improving quality of life and restoring myelin

HYPOTHESIS:

Transvaginal estriol offers a safe adjunctive therapy for female MS patients, it improves urogenital symptoms as well as promotes remyelination.

METHODS:

Type of study: Single-group pilot study.

Subjects: Patients with relapsing remitting MS and urogenital symptoms (frequency, urgency, frequent urinary tract infections, incontinence) will be invited to participate in the study. Enrollment will be during their scheduled clinic appointment. Patients will be screened by a member of the study team during their visits to see if they qualify, if so they will be invited to participate in the study.

The clinical trial will be explained to potential participants in detail, reviewing the objectives and methodology of the study. There will be adequate time allotted to answer any questions or concerns from the potential participants. Those patients interested in participating in the study will be asked to sign the consent form. In order to remind patients about their follow-up visits, lab work, etc., they will be contacted routinely. Participants will be instructed to call in case of questions or concerns.
Patients will be evaluated clinically during their scheduled follow up. Patients will be instructed by the primary investigator how to correctly use the vaginal cream. This will be done at their enrolment and reinforced by the principal investigator, during their follow up visits.

Inclusion criteria:

1. Female patients with RRMS between the ages of 35 to 65.
   A) Being prescribed vaginal estriol to treat their urogenital symptoms.
   B) Patients that had underwent menopause or surgical hysterectomy.
2. Patients will continue their disease modifying agent for MS during the trial.

Exclusion criteria:

1. Patients with personal history of breast cancer, uterine or ovarian cancer.
2. Patients with history of fibrocystic breast disease, heart attack or stroke.
3. Patients with history of thromboembolism.
4. Patients with progressive multiple sclerosis
5. Patients who are unable to undergo an MRI
6. Males
7. Patient is already on vaginal or oral or transdermal estrogens
8. Pregnant or breast-feeding patients
9. Patient taking sex hormones eg testosterone for libido
10. Patients taking DHEA or OTC related products that could influence the hormonal milieu.
11. Patient with prolapse uterus or conditions that would impact on transvaginal absorption of estriol

Enrollment in the study will be timed to their follow up brain MRI which will be performed as standard of care. Patients that have no baseline MRI or are unable to undergo MRI will be excluded from the study.

Site of study: The TTUHSC department of Neurology, MS clinic, Lubbock, TX; The TTUHSC Department of Ophthalmology and Visual Sciences, Lubbock, TX

Design:
This is a single-group prospective repeated-measures pilot trial. Duration is 12 months

Intervention:
Estriol vaginal cream will be formulated by Twin Oaks Pharmacy's compounding pharmacy, Regional Pharmacy, by the same compound specialist. 30 mg estriol powder will be mixed with 5 mL of propylene glycol and 22 g of vaginal base cream. The product can be stored at room temperature and has a shelf life of up to 4 months. It comes with an applicator.

The 1 mg dosing was chosen due to evidence in previous trials, and a systematic review of randomized trials comparing vaginal estrogens. It was concluded that the 1mg vaginal estriol, was superior to lower doses for the treatment of urogenital symptoms. (Rahn et al., 2014).

Study visits/Roles of subjects:

Initial visit:
After the informed consent has been obtained and all questions have been addressed
Potential participants will be screened to ensure they are eligible for the clinical trial. Once eligibility has been verified

1. Blood will be collected for baseline laboratory studies serum estriol, FSH and LH level. (about 20 ml of blood)
2. Baseline urogenital status will be evaluated by
    - Bladder Control Scale (BLCS) questionnaire
3. Multiple Sclerosis Quality of Life-54 (l-54) questionnaire will be completed
4. Baseline Visual Evoked Potential (VEP) within 4 weeks of Initial Visit
5. Optical Coherence Tomography (OCT) (occurs at the Ophthalmology clinic) within 4 weeks of Initial Visit

- 3 Tesla brain MRI will be performed as well. (within 3 months of initial visit)
- Dispense vaginal estriol cream once all baselines are complete

The primary investigators will instruct how to correctly use vaginal cream. Patients will be instructed to draw 1 mg of estriol vaginal cream with the applicator and apply intravaginally. They will be instructed to lay on their back for 30 min after vaginal cream application once a day for the duration of the study. The applicator is reusable, cleaning technique will also be given to the patient during their visit and reinforced during follow up. The applicator will be washed with regular soap and water and let air dry.

Patients will be instructed not to start applying vaginal cream until baseline lab work and imaging has been performed.

Three-month visit (90 days +/- 10 days):

- BLCS questionnaire will be completed
- Blood will be collected for serum estriol, FSH and LH level. (about 20 ml of blood)
- VEP and OCT will be also performed. ± 3 weeks of 3 month Visit
- Dispense vaginal estriol cream

Six-month visit (180 days +/- 10 days):

- BLCS questionnaire will be completed
- Blood will be collected for serum estriol, FSH and LH level. (about 20 ml of blood)
- VEP and OCT will be also performed. ±-3 weeks of 6 month Visit
- Dispense vaginal estriol cream

Nine-month visit (270 days +/- 10 days-FINAL VISIT):

- BLCS and MSQOL-54 questionnaires will be completed
- Blood will be collected f serum estriol, FSH and LH level. (about 20 ml of blood)
- 3 Tesla brain MRI will be repeated. ± 3 months of 9 month Visit

During each visit patients will be questioned for adverse events, patients will also have a number to call in case of questions, concerns or adverse events.

Due to the nature of RRMS there might be acute exacerbations in which the patient will be treated with IV methylprednisolone 1gm a day x 3 to 5 days, this is the standard of care for MS exacerbations.

Schedule of events

Procedures	Screening visit	3 month visit	6 month visit	9 month visit FINAL VISIT
Date				
ICF	X			
I&E	X			
Blood work	x	x	x	x
BLCS questionnaire	x	x	x	x
MSQOL-54 questionnaire	x			x
VEP (4 week window for screening visit or 3 week window for 3 and 6 month visits)	x	x	x	
OCT (4 week window for screening visit or 3 week window for 3 and 6 month visits))	x	x	x	
MRI variables (3 month window)	x			X
Vaginal cream instructions	x			
Vaginal cream dispensing (once baselines complete)	x	x	x	
Compliance of vaginal cream use		x	x	x

<u>Materials, instruments or measurements</u>:

Urogenital measurements:

- Bladder control scale (BLCS) questionnaire (validated and used for evaluation of MS bladder symptoms. {Turnbull et al, 1992}). Raw scores on the 4 items are summed to create a Bladder Control Scale (BLCS) total score. Scores can range from 0-22, with higher scores indicating greater bladder control problems. See attached document.

Quality of life:

- Quality of life questionnaire (Multple Sclerosis Quality of Life-54 {MSQOL-54}. Validated and widely used for MS patients. (Vickrey et al, 1995) (Vickrey et al, 1997). The questionnaire includes 54 questions that provide scores on physical and mental health status. Both scores can range 0-100. See attached document.

Blood work:

Levels of estriol, FSH, LH will be performed in Texas Tech Health Science Laboratory (Dr. Prien's certified lab). The assay used will be the Beckman Coulter Access 2 immunoassay system analyzer. This will be run by an established OB/GYN hormone laboratory under the supervision of Dr. Prien and Dr. Penrose.

Re-myelinating measurements:

· Visual evoked potential (VEP) (measurement of the electrical signal recorded at the scalp over the occipital cortex in response to light stimulus) VEP/Prolongation of the P100 latency, is an indirect tool for remyelination in MS. (The test is non-invasive, patients will be looking at a checkboard pattern while the technician is measuring the time it takes to register the image to the brain cortex. This is achieved with scalp electrode in the posterior region.)

· 3Tesla brain MRI (performed as standard of care with no extra cost) will be evaluated for possible changes in white matter lesions, and Diffusion Tensor Imaging (DTI). This will be read by an expert MD collaborator independent from primary investigator.

· Optical coherence tomography (OCT) (noninvasive scan of the retinal layer which provides morphology imagery of the ganglion cell layer which is frequently affected in MS) (Noninvasive test, duration of about 5 to 10 min to perform, patient has to stay still and focus on a small light while we take a picture of the retina layer with the device.)

Data Sheet: A spreadsheet is attached. We will be gathering demographic data, MRI information, EDSS score, and electrophysiologic information, for details please see the spreadsheet attached.

Outcome variables:

1- Urogenital symptoms such as frequency, incontinence, frequent urinary tract infections, and BLCS questionnaire scores.
2- Remyelination (measured by brain MRI 3 DTI, other measurements include presence of T2 lesions, Flair lesions, DWI, T1 black hole, and DTI; Optical coherence tomography (OCT); and visual evoked potentials (VEP).
3- Quality of life, assessed using MSQOL-54 questionnaire.

Analysis:

Patient characteristics will be summarized using frequency (percentage) for categorical variables, and mean (SD) or median (interquartile range) for numerical variables as appropriate. Changes in blood work variables during the course of the study will be assessed using one-way repeated-measures analysis of variance (ANOVA). Compliance assessments will be used as covariate to adjust the differences if necessary, using an analysis of covariance model (ANCOVA). Urinary symptoms, and quality of life data will be analyzed using mixed-effects linear or logistic regression models for continuous or binary outcomes respectively. These models will account for the effect of estriol over time, compliance with treatment, and relevant patient characteristics.

Justification for Sample size:

This is a pilot study. We are planning to include 20 patients. Each patient will be their own control in a single-group repeated measures design. Assuming a significance level $\alpha = 0.05$, and power $1-\beta = 0.80$, a

sensitivity power analysis determined that a moderate effect size (d=0.53 to d=0.66) would be required to detect statistically significant changes in the main outcome.

Risks
Studies suggest that when the lower-potency estrogen, estriol, is administered transvaginal, it does not increase the risk of hormone-dependent cancers of the breast or endometrium (uterine lining) Therefore the risk of breast and endometrial cancer is not increased with vaginal estriol. (Voojis et.al) Another safety concern of estrogens is the risk of thromboembolism. There is a paucity of prospective studies specifically looking at estriol and thromboembolism. Studies have shown that transdermal and transvaginal estrogen have a lower rate of thromboembolism. (Scarabin, Oger, & Plu-Bureau, 2003). Risks associated with venipuncture for blood draws include: discomfort, pain, bleeding, bruising, infection where the needle enters the skin; feeling lightheaded or fainting at the site of the needle or blood. No physical risk with VEP or OCT, these are noninvasive procedures.

Using the applicator or cream may cause vaginal discomfort or infection. Patients will be asked during their visits about vaginal discomfort or vaginal infections. Patients will also be encouraged to call if they are experiencing these. If the patient does experience these symptoms they may be referred to their PCP or Gynecologist for appropriate management.

Benefits:
Patients may experience improvement of her urogenital symptoms as well as improvement in their MS symptoms. Patient may have improved sexual function. Subjects could share satisfaction from potential benefits to other MS patients. May help revolutionize treatment of MS along with urogenital benefits at low cost

Confidentiality:
Patient's information will be de-identified and identifier information will be kept in a safe place.
Electronic files will be locked by password which will be known by the principal investigator.
Study documents will be kept at least 3 years then destroyed.

Study is registered at clinicaltrials.gov

Monitoring
To ensure compliance of the study protocol, GCP guidelines, and TTUHSC Human Research Protection Program research policies and procedures during the conduct of the study, a monitor in the Clinical Research Institute will conduct the monitoring of the study. The first monitoring visit will be conducted within two weeks after the first subject has been enrolled into the study. The succeeding monitoring visits will be scheduled periodically, but no less than every 2 months when there is an active study participant, at a mutually agreed timeframe by the PI and study monitor. All data collected will be 100% source document verified. The study monitor may inspect and audit all study documents, i.e. data collection forms, questionnaires, drug accountability, and medical records within the applicable confidentiality regulations.

Reimbursement:
No reimbursement for the participants

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