Effect of hormone replacement and selective estrogen receptor modulators (SERMs) on the biomechanics and biochemistry of pelvic support ligaments in the cynomolgus monkey (*Macaca fascicularis*)

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OBJECTIVE: To evaluate the effect of selective estrogen receptor modulators and ethinyl estradiol on the biomechanical and biochemical properties of the uterosacral and round ligaments in the monkey model of menopause.

STUDY DESIGN: A randomized, double-blind, placebo-controlled study on 11 female macaque monkeys. Ovariectomized monkeys received 12 weeks of placebo, raloxifene, tamoxifen, or ethinyl estradiol. Biomechanical step-strain testing and real-time polymerase chain reaction was performed on the uterosacral and round ligaments.

RESULTS: Tamoxifen and raloxifene uterosacrals expressed differing collagen I /III receptor density ratios, but both selective estrogen receptor modulators showed decreased tensile stiffness compared to ethinyl estradiol and controls.

CONCLUSION: These findings support a possible effect of selective estrogen receptor modulators on biomechanical and biochemical properties of uterosacrals. This may play a role in pelvic organ prolapse.

Key words: macaca fascicularis, pelvic organ prolapse, round ligament, SERMs, uterosacral ligament

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Weakness in the pelvic support structures including the uterosacral ligament (USL), cardinal ligament, and paravaginal connective tissues is thought to result in pelvic organ prolapse (POP).[1,2] Estrogen receptors, ERα and Erβ, are present in the major pelvic support structures in women, including the vaginal wall and uterosacral ligaments, and their expression declines after menopause.[3-5] Changes in pelvic support ligaments tissue quality is thought to be multifactorial.[6-9] One factor having an impact on tissue quality may be hormonal, thus menopause is long suspected to contribute to POP;[10] however, little is understood about the role of hormonal status on pelvic support pathology.

Biomechanical studies with animal models in the orthopedic literature have demonstrated that estrogen can change joint ligament stiffness.[11,12] Both conjugated equine estrogens with medroxyprogesterone acetate and ethinyl estradiol (EE) plus norethindrone acetate have been shown to increase the tensile modulus of the USL and decrease the tensile modulus of the round ligament (RL) in the Macaque monkey model.[13] The tensile modulus is defined as stiffness of the material, or the resistance of the material to uniaxial elongation when a stress is applied to the material in the direction in which it elongates. These changes may allow adaptation to the increase in load imposed by pregnancy without concomitant excessive motion (laxity) of the uterus at the uterosacral junction. This suggests that supportive ligaments may be end-organs for hormonal effect. It may therefore be possible that selective estrogen receptor modulators (SERMs) likewise have an effect on tissue quality.

Currently, SERMs such as raloxifene and tamoxifen are widely used in clinical practice for the treatment of and the prevention of osteoporosis and breast cancer.[14] The use of SERMs has increased after

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2002 when the Women's Health Initiative Study[15] reported a greater risk for cardiovascular adverse effects and breast cancer in women taking estrogen/progesterone supplementation.[16-18] SERMs include a relatively large number of compounds, each with different profiles of estrogenic/antiestrogenic actions on the genital tract. Two classes of SERMs approved for use by the Food and Drug Administration (FDA) are as follows: (1) raloxifene, a benzothiophene that has estrogen-antagonistic effects in both the breast and uterus and estrogen-like agonistic effects in bone and on serum lipid levels; and (2) tamoxifen, a triphenylethylene that has estrogen antagonistic effects in breast tissue, and stimulatory effects in endometrial tissue.[19] Little is understood about their potential clinical effects on the urogenital system with the exception of the endometrium.[19-22] Specifically, little is known about the potential effect of SERMs on uterovaginal prolapse and urinary incontinence.[19-22]

In 2002, our colleagues published a randomized, placebo controlled, double-blind study of 57 postmenopausal women suggesting worsening prolapse with raloxifene and tamoxifen compared with conjugated equine estrogen or placebo, when evaluated by standardized POP quantification methodology.[23] The findings suggested that both tamoxifen and raloxifene increase the incidence of pelvic floor prolapse, although this was not apparent from the licensing studies data for either of the drugs. Increase uterine prolapse and incontinence were also adverse events reported to the FDA in prematurely terminated clinical trials of 2 SERMs that are tamoxifen derivatives, levoremeloxifene and idoxifene.[24,25] It may therefore be possible that SERMs may likewise have an effect on tissue quality. These studies suggest a relationship between SERMs and POP; however. There are no preclinical studies showing a mechanistic basis for SERMs induction of POP. The effect of SERMs on the tissue properties of pelvic supportive ligaments has been suggested but never evaluated through formal biomechanical and biochemical testing.[26-28]

The cynomolgus monkey has served as important nonhuman primate models in several studies of aging, including menopause.[13,29-31] The pelvic anatomy of the macaque species is almost identical to that of the human, providing opportunities for studying an analogous support system.[32-34] The animal model allows hormonal manipulation, isolation, and testing of supportive tissues in a way not feasible in human subjects.[35] We used this model to evaluate quantitatively the effects of estrogen deprivation, and the effects of SERMs on ligament composition and tensile properties of the pelvic support ligaments. We compared ovariectomized controls to animals fed raloxifene, tamoxifen, and EE. Supportive ligament receptor density, load deformation and elastic modulus were measured.

We hypothesized that SERMs such as tamoxifen and raloxifene, commonly used in postmenopausal and breast cancer prevention therapy, enhance the hypoestrogenic effects of menopause and may reduce tissue stiffness. The impact of SERMs on biomechanical properties may be correlated to changes in estrogen and progesterone receptor density and collagen type ratio. These translational studies may help elucidate the hormonal factors that impact POP in the postmenopausal female.

Materials and Methods

Experimental design

Randomized, double-blind, placebo-controlled pilot study with outcome measurements performed by persons blinded to animal status (Figure 1).

Animals and treatment protocol

Eleven Indonesian-origin (Bogor, Indonesia) adult female cynomolgus macaques (*Macaca fascicularis*) were obtained from a licensed vendor (Primate Products, Miami, FL). Age as estimated by dentition was 7-13 years with a mean of 9.85 years. The typical life span for this species is 30 years and the average age at menopause is approximately 20 years. Ovariectomies were performed under aseptic conditions using isoflurane anesthesia and appropriate postoperative care and monitoring. Ovariectomized animals were randomly assigned into 4 groups: a control placebo group that received only the vehicle (n = 3); raloxifene 3 mg/kg/d (n= 3); tamoxifen 1 mg/kg/d (n = 2); and EE 3 μg/kg/d (n = 3). Duration of administration was 12 weeks. The doses were scaled from those doses used in women. Drug doses were calculated based on the assumption that an average woman consumes approximately 1800 calories per day. The animal (subjects) were fed a 120 calories/kilogram of body weight atherogenic diet and kept in individual cages. All drugs were administered by training the animals for cooperative oral dosing with the drugs in a flavored aqueous vehicle (Crystal Lite; Kraft Foods Inc, Winnetka, IL; Splenda; Johnson and Johnson/McNeil, New Brunswick, NJ). Serum samples were drawn at 1.5 hours and 24 hours postdosing. After 12 weeks of treatment, animals were sedated with ketamine (10 mg/kg) and humanely euthanized by intravenous injection of pentobarbital (100 mg/kg), followed by assessment of multiple organ systems. The Institutional Animal Care and Use Committee of Wake Forest University approved all procedures involving animals, under accreditation by the Association for the Assessment and Accreditation of Laboratory Animal Care.

Ligament preparation

After necropsy the USL and RLs were harvested. All USLs were ligated with 0 silk in situ at the sacral end to within 0.3 cm to the sacrum, and dissected around the sutures (Figure 2). All tissues for biomechanical testing were wrapped in saline-soaked gauze, sealed in airtight bag, and stored at −80°C. The ligaments were then shipped to Columbia University Center for Orthopaedic Research Biomechanics Laboratory and were stored in −20°C freezers. Before testing, each ligament was thawed for 30 minutes in normal saline solution. During storage, dissection, preparation, and geometric measurement, all specimens were moistened with 0.15 M physiologic saline solution to maintain hydration. Ligaments were tagged with emery cloth at each end with cyanoacrylate and mounted in grips on the Instron 5848 Micro Tester material testing apparatus. All testing was performed with approval from the Institu-