BASIC SCIENCE: GYNECOLOGY

Effects of lasofoxifene on the uterus, vagina, and breast in ovariectomized cynomolgus monkeys (*Macaca fascicularis*)

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OBJECTIVE: The purpose of this study was to assess the effects of lasofoxifene on the reproductive system in ovariectomized nonhuman primates.

STUDY DESIGN: This was a 2-year, randomized study. Adult female macaques (*Macaca fascicularis*) were assigned randomly into 5 groups: ovariectomized, placebo-treated controls (n = 22); sham-ovariectomized, placebo-treated controls (n = 24); ovariectomized animals given 0.021 mg Premarin kg/d (conjugated equine estrogen; n = 25); lasofoxifene at 1.0 mg/kg/d (n = 23); or lasofoxifene at 5.0 mg/kg/d (n = 25). Outcomes included organ weights and histopathologic findings.

RESULTS: Lasofoxifene did not increase uterine weight or endometrial thickness and did not change mammary, vaginal, or cervical histologic condition. Mild endometrial fibrosis and cystic change were seen in lasofoxifene-treated animals, in contrast to significant uterine weight increases and endometrial hyperplasia induced by conjugated equine estrogen.

CONCLUSION: Lasofoxifene did not increase uterine weight and produced minor histologic uterine changes at the doses that were given and had no effect on the breast, vagina, or cervix.

Key words: endometrium, estrogen, *Macaca fascicularis*, mammary gland, selective estrogen receptor modulator

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Postmenopausal estrogen replacement therapy is a commonly used treatment for climacteric symptoms in the short term and has long-term benefits including prevention of osteoporosis and other chronic illnesses.[1] However, estrogen replacement therapy is associated with increased breast and endometrial cancer risk[2,3] and the need for concurrent progestin treatment (hormone replacement therapy) to offset endometrial cancer risk carries with it a spectrum of adverse effects that include changes in mood, endometrial bleeding, and adverse effects on breast cancer risk and cardiovascular disease risk.[4] Because of these disadvantages to the use of conventional hormone replacement therapy for the treatment of climacteric symptoms and bone loss, interventions with tissue selectivity have been sought, which include the selective estrogen receptor modulators (SERMs).[5,6] SERMs such as raloxifene (Evista) produce bone-protective effects like hormone replacement therapy but without the increase in breast and endometrial cancer risks that are associated with hormone replacement therapy. Other agents such as levormeloxifene have produced adverse effects on the female reproductive tract,[7] which necessitates careful assessment of reproductive outcomes in the development of new SERMs. Lasofoxifene (CP-336,156) is a potent SERM with efficacy for the prevention of bone loss in rats with little effect on uterine weight[8] and was explored in this study with the use of the nonhuman primate model of postmenopausal women's health. The primary outcome for this study was prevention of osteoporosis; those data are reported elsewhere.[9]

Cynomolgus macaques are uniquely similar to women in many aspects of reproductive biology. Many investigators have used macaques for a wide variety of studies of reproductive physiology and menopausal health, clearly demonstrating the usefulness of the model.[10] Using this model, we have evaluated breast and endometrial effects for conventional hormone replacement therapy and have found effects of estrogens and progestins that are analogous to those seen in women.[11] We have shown previously that endometrial hyperplasia is induced by conjugated equine estrogens (CEE)[12,13] or estradiol[14] that was given orally to macaques and that this effect is antagonized by the addition of a progestin such as medroxyprogesterone acetate[13] or nomegestrol acetate.[15] We have also validated previously vaginal keratinization as an indicator of estrogenic effect in macaques.[16] Furthermore, we have assessed tamoxifen, a first-generation SERM, in this model[11,17] and a variety of other SERMs.[18]

In this study, we sought to evaluate the effects of a next-generation SERM on the reproductive tissues of ovariectomized

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TABLE 1
Body weight and uterine weight by treatment group

Variable	Group				
	Ovariectomized	Intact (sham-ovariectomized	CEE	Lasofoxifene 1.0 mg/kg	Lasofoxifene 5.0 mg/kg
Bodyweight (kg)	2.81 ± 0.40	3.06 ± 0.51	2.86 ± 0.42	2.89 ± 0.41	2.79 ± 0.44
P value, Kruskal-Wallis test (difference from ovariectomized)		NSD	NSD	NSD	NSD
Uterine weight (g)	3.00 ± 1.99	10.04 ± 5.93	15.71 ± 21.39	3.33 ± 1.67	4.20 ± 6.09
P value, Kruskal-Wallis test (difference from ovariectomized)		<.0001	<.0001	NSD	NSD
No evaluated	22	24	25	23	25

NSD, no significant difference.

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primates and to compare those effects with those of CEE. We hypothesized that lasofoxifene would not increase uterine weight or endometrial thickness, when compared with CEE.

MATERIALS AND METHODS

Study design

The work reported here was a randomized 24-month efficacy study of lasofoxifene (CP-336,156) in mature ovariectomized cynomolgus monkeys (*Macaca fascicularis*) for evaluation of bone quality, as recommended in the 1994 Food and Drug Administration Guidelines for Preclinical and Clinical Evaluation of Agents Used in the Prevention or Treatment of Postmenopausal Osteoporosis,[19] with the secondary goal of assessing effects on the reproductive tract and breast. The age of the animals was estimated from dentition; animals were 19.9 ± 3 years of age at the end of the study. Bodyweights did not differ by group (Table 1). Groups included ovariectomized, placebo-treated controls (n = 22); sham-ovariectomized, placebo-treated controls (intact; n = 24); and animals that were given 0.021 mg Premarin kg/d (CEE; n = 25), lasofoxifene at 1.0 mg/kg/d (n = 23), or lasofoxifene at 5.0 mg/kg/d (n = 25). The 2 lasofoxifene doses that were used in this study exceeded the phase III doses in human clinical trials by 140- and 700-fold.

Ovariectomies were performed under standard aseptic conditions with isoflurane anesthesia and appropriate veterinary postoperative care and monitoring. The drug was administered by training the animals for cooperative oral dosing with the drugs in a flavored aqueous vehicle (Crystal Lite; Kraft Foods, Inc, Rye Brook, NY). After 2 years of daily treatment, all animals were sedated with ketamine and humanely killed by intravenous injection of pentobarbital (100 mg/kg), followed by assessment of multiple organ systems. For the work described here, the entire reproductive tract and mammary tissue were collected for pathologic evaluation that consisted of histopathologic evaluation and histomorphometry of the endometrium, mammary gland, ovaries, vagina, and cervix. Pathologists were blinded to the treatment group. All procedures that involved animals were approved by the Institutional Animal Care and Use Committee of Wake Forest University. Wake Forest University is accredited by the Association for the Advancement and Accreditation of Laboratory Animal Care.

Pathologic evaluations

Gross abnormalities were noted and photographed at the time of necropsy, and uterine weight was determined. All tissues were fixed in 10% neutral buffered formalin. Uterus (sectioned transversely), cervix/vagina (sectioned longitudinally), mammary gland (sectioned sagittally through the nipple), and ovaries, when present, were processed into paraffin by standard histologic techniques and sectioned at 5 μm. Sections were stained with hematoxylin and eosin. Histologic evaluation of the endometrium was performed by a board-certified veterinary pathologist (J.M.C.) for evidence of hyperplasia and other histopathologic abnormalities; all histologic findings were graded subjectively as 0 (not present), 1 (minimal), 2 (mild), 3 (moderate), or 4 (marked).

Endometria were classified qualitatively as cyclic, atrophic, inactive, or hyperplastic. Endometria that were not thickened and had small, straight, tubular glands with low-cuboidal glandular epithelium were considered to be *atrophic*; those with more open to cystic prominent glands, but lacking epithelial mitoses, were termed *inactive*; and those with glandular pseudostratification and mitoses with endometrial thickening were termed *hyperplastic*. *Cystic change* was defined as focal to extensive dilation of endometrial glands within either the basalis or functionalis, which resulted in a round rather than tubular contour. In addition to qualitative morphologic diagnoses, the proportion of endometrial glands that had pseudostratified epithelium was estimated as a percentage of all glands in the section. Also, the numbers of mitoses among glandular epithelial cells were counted in ten fields at 40× magnification in 1 endometrial section for each animal. Previous pregnancy status was assessed by histologic assessment