Lack of effect of isoflavonoids on the vagina and endometrium in postmenopausal women

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Objective: To determine the effects of soy-derived isoflavones on vaginal epithelium and the endometrium.
Design: Double-blind, randomized, placebo-controlled crossover trial.
Setting: Outpatient clinic of a university hospital.
Patient(s): Sixty-four postmenopausal women with a history of breast cancer.
Intervention(s): The women took (in a randomized order) 114 mg of isolated isoflavonoids or placebo in tablets daily for 3 months; the treatment regimens were crossed over after a 2-month washout period. The subjects were studied before and on the last day of each treatment period.
Main Outcome Measure(s): Vaginal dryness, maturation index (MI) of vaginal epithelium, endometrial thickness, histology, and expression of estrogen (E) and progesterone (P) receptors and the proliferation marker Ki-67 in the endometrium.
Result(s): Isolated isoflavones did not relieve vaginal dryness. Maturation index values remained unchanged during the isoflavone regimen, but decreased during the placebo regimen. No changes were found in any of the variables measured in the endometrium.
Conclusion(s): Daily administration of 114 mg of isolated isoflavones for 3 months had no effect on the subjective perception of vaginal dryness or on objective findings in the vagina or endometrium. This implies safety with regard to the endometrium. (Fertil Steril® 2005;83:137–42. ©2005 by American Society for Reproductive Medicine.)

Key Words: Phytoestrogen, endometrium, estrogen receptor (ER), P receptor (PR), Ki-67, vaginal cytology, maturation index

Phytoestrogens are plant-derived compounds that structurally and functionally resemble E_2 (1). Isoflavonoids, such as daidzein and genistein, are the most common phytoestrogens found in various fruits and vegetables, especially soy (1). As they bind weakly to estrogen (E) α-receptor and more strongly to E β-receptor and compete with E_2 for the same receptor sites, they may possess organ-specific estrogenic and antiestrogenic effects (2, 3). Women who have contraindications for traditional hormone therapy (HT) or who want a "natural" alternative, widely use phytoestrogens for treatment of climacteric complaints, although scientific evidence of their benefits or hazards is often insufficient (4–7).

Vaginal atrophy is commonly seen in postmenopausal women not using HT. The use of HT strengthens vaginal epithelium as soon as in 1 month (8), whereas phytoestrogens have failed to affect vaginal epithelium in most (9–12), although not all studies (13, 14). With regard to the endometrium, phytoestrogens have had no effect on endometrial thickness when given alone (11, 15) or in combination with soy protein (16). Neither have phytoestrogens had an estrogenic effect on endometrial histology (17, 18).

The effects of phytoestrogens on E receptors (ERs) and progesterone (P) receptors (PRs) in humans have not been studied, although these receptors are necessary for steroid hormone actions and they are, at least partly, regulated by E (19). The nuclear antigen Ki-67, which is closely related to proliferation, can reflect the estrogenicity of a given compound on the endometrium (20). Isoflavone-rich soy protein isolate is known to stimulate the expression of Ki-67 in the endometrium in monkeys (21) and in perimenopausal women (22), but no data exist on the effect of purified isoflavonoids on Ki-67 expression in postmenopausal women. Therefore, we studied the effects of isolated isoflavones on vaginal dryness and maturation index as well as on endometrial thickness, histology, and expression of ER, PR and Ki-67 in postmenopausal women.

MATERIALS AND METHODS

With approval from the Institutional Review Board and the Ethics Committee, we studied postmenopausal women who had undergone operation for breast cancer more than 6 months earlier (Table 1). The volunteers received thorough written and verbal information on the purpose and the con-

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TABLE 1

Clinical characteristics of the study population (n = 56).

Characteristics	
Age (y)	54 ± 6 (35–69)
Time since menopause (y)	5.3 ± 5.5 (0.6–27.0)
Body Mass Index (kg/m^2)	26.3 ± 3.3 (21.2–33.6)
Previous use of HT	22 (39%)
History of hysterectomy	10 (18%)
FSH (U/L)	80.6 ± 32.0 (30.9–166.5)

Note: Values are mean ± SD (range) or n (%). HT = hormone therapy; FSH = follicle-stimulating hormone.

Nikander. Isoflavinoids, vagina, and uterus. Fertil Steril 2005.

duct of the study, and informed consent was obtained from all of them.

Thirteen women (11 premenopausal and 2 postmenopausal) had received chemotherapy, at a mean of 5 years before recruitment. Three had used tamoxifen for 2 months to 4 years, but this treatment had been discontinued 5 months to 4 years before recruitment. No woman showed signs of metastasis at recruitment, which took place between September 1, 1999 and October 10, 2000. All women had incapacitating hot flashes and other climacteric symptoms; menopausal status was confirmed by a level of serum FSH exceeding 30 U/L.

The women were not using HT, statins, natural products with presumed estrogenic activity, or drugs possibly affecting climacteric symptoms, or metabolism and absorption of phytoestrogens (e.g., antibiotics during the previous 3 months). None had a history of a thromboembolic or hepatic event. Eight women took antihypertensive drugs. Before the diagnosis of breast cancer, 25 of the 64 women (39.1%) who were screened had used some form of HT. Thirteen of the screened women had undergone hysterectomy for benign conditions, 4 of them undergoing bilateral oophorectomy.

After a double-blind, crossover technique, the women were treated in computer-randomized order with either isoflavonoids or placebo. Each treatment period lasted 3 months, and the treatment phases were interrupted by a 2-month washout period. Isoflavonoid tablets and similar-looking placebo tablets were to be taken every 12 hours (3 tablets) with a glass of water. Each isoflavonoid tablet (Bonette, Novomed, Helsinki, Finland) consisted of glycitein (11 mg, 58%), daidzein (7 mg, 36%), and genistein (1 mg, 6%), the total dose of isoflavonoids being 114 mg/d (23).

The women visited the research center immediately before and on the last day of each treatment period. General and pelvic examinations including transvaginal ultrasonography were performed and appropriate blood samples, vaginal and cervical smears, and endometrial biopsies were collected. During the study the women were encouraged to lead normal lives with no changes in dietary habits, alcohol consumption, or physical activity, which were all recorded by means of questionnaires before and at the end of each treatment period. They kept weekly diaries concerning their general health, possible side effects, bleeding, and use of antibiotics or other concomitant drugs. Compliance with use of the study medication was confirmed by checking diaries and by analyzing serum levels of the isoflavonoids daidzein, genistein, and equol, as reported before (23).

At each visit the women were asked to rate their vaginal dryness on a scale from 0 (= no dryness) to 3 (= severe dryness). To evaluate vaginal epithelium objectively, vagina, cervix, and endocervix Papanicolaou smears were collected. Epithelial cells from the vagina were collected with a wooden spatula and cells from the cervix with a brush. The smears were fixed in 95% ethyl alcohol and the slides stained by means of the modified Papanicolaou method. Vaginal cells (n = 200) from each slide were examined to determine the percentages of parabasal, intermediate, and superficial squamous cells in the vagina. The maturation index (MI) was calculated as the sum percentage of superficial cells plus half the percentage of intermediate cells. All cytological samples were evaluated by the same cytologist, who was blind to the treatment.

Endometrial thickness was measured by transvaginal ultrasonography (Hitachi EUB-525 with a 5-MHz transvaginal transducer, Tokyo, Japan) in the anteroposterior direction from the echogenic interface of the endometrial–myometrial junction on both sides. After this examination, endometrial samples were taken with disposable biopsy curettes (Pipelle, Laboratoire CCD, Paris, France). The samples were fixed in formalin and embedded in paraffin for histological and immunohistochemical analyses. All biopsies were examined by the same pathologist. The estrogenic status of the endometrium was evaluated on a four-point scale: 0 = totally atrophic, 1 = minimal estrogenic effect, 2 = modest estrogenic effect, and 3 = proliferation. Scores of 0 and 1 together were considered atrophic.

Expression of ER, PR, and the proliferation marker Ki-67 in the endometrial samples was examined by immunohistochemistry using monoclonal mouse antibodies that detect human ER α (DAKO ER, Dako, Denmark), PR A and B (Novocastra Laboratories Ltd., Newcastle-upon-Tyne, UK), and Ki-67 (Pharmingen, San Diego, CA) as previously described (24). The immunohistochemical data were analyzed by two independent observers by means of subjective semiquantitative scoring. In negative controls the primary antibody was replaced with nonimmune mouse serum of equivalent concentrations. Ten fields of cells of a tissue section (magnification ×200) were examined, and only nuclear staining was considered specific for ER, PR, and Ki-67. The expression levels of ER and PR were based on staining