Antiproliferative and proapoptotic effects of raloxifene on uterine leiomyomas in postmenopausal women

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Objective: To study the cell effects of raloxifene on uterine and leiomyoma tissue in postmenopausal women.
Design: Prospective, randomized, double-blind, placebo-controlled study.
Setting: Department of Obstetrics and Gynecology, University "Magna Graecia" of Catanzaro, Italy.
Patient(s): Forty postmenopausal women affected by uterine leiomyomas and selected for hysterectomy.
Intervention(s): Treatment for three cycles of 28 days with raloxifene at a dose of 180 mg/day orally (raloxifene group) or placebo tablets (3 tablets/day orally) (placebo group).
Main Outcome Measure(s): Uterine and leiomyoma dimensions were measured in each subject at entry and before surgery. On leiomyomas and homologous myometrium the proliferating cell nuclear antigen (PCNA)-positive cells/total cells (PCNA/TC) and the Bcl-2-positive cells/Bax-positive cells (Bcl-2/Bax) ratios (%), as proliferation and apoptotic indexes, respectively, were measured.
Result(s): After treatment, uterine and leiomyoma sizes were significantly changed in comparison with baseline and the placebo group. PCNA/TC and Bcl-2/Bax ratios were significantly higher in leiomyomas than in homologous myometrium. A significant difference was detected in PCNA/TC between the myometrium of the raloxifene and control groups, whereas no difference was observed in the Bcl-2/Bax ratio. A significant difference in PCNA/TC and Bcl-2/Bax ratios was detected in leiomyoma tissue between the raloxifene group and controls.
Conclusion(s): In postmenopausal women, raloxifene administration reduces uterine leiomyomas by exerting a cell antiproliferative and proapoptotic action. (Fertil Steril® 2005;84:154–161. ©2005 by American Society for Reproductive Medicine.)

Key Words: Apoptosis, Bax, Bcl-2, leiomyoma, PCNA, raloxifene, SERM

Raloxifene is a synthetic nonsteroidal compound that belongs to selective estrogen receptor modulators (SERMs), agents that interact with estrogen receptors (ERs) and elicit tissue-specific responses (1). It is known that raloxifene acts on the metabolism, the central nervous system, the skeleton, and the cardiovascular system as an estrogenic agonist, whereas it shows an estrogenic antagonist effect on reproductive organs, such as the breast and the uterus (1).

Uterine leiomyomas are the most common benign smooth muscle cell tumors of the myometrium, occurring in at least 25% of women of reproductive age and in 50% of all women when studied postmortem (2). In 20%–50% of cases, uterine leiomyomas cause a clinically relevant symptomatology and treatment is often required (2). Thus, this disease is one of the main causes of health expense in the field of the gynecology (2).

Preclinical data (3–7) showed that raloxifene may have a beneficial effect on uterine leiomyomas. The efficacy of raloxifene on leiomyomas has been confirmed in successive clinical studies as well (8–10). Moreover, at present no experimental data on humans are available in the English literature regarding the cellular effects of raloxifene on leiomyoma tissue.

It has been suggested (11–13) that the growth of uterine leiomyomas is due to an alteration of balancing between cell proliferation and death (apoptosis). There is some experimental and clinical evidence to indicate that the detection of the proliferating cell nuclear antigen (PCNA) and of Bcl-2/Bax proteins can be useful in the analysis of cell-proliferative and apoptotic processes, respectively.

Proliferating cell nuclear antigen is a molecule localized in the nucleus of proliferating cells. In particular, the amount

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of PCNA increases during the late G1 phase, reaching a nadir in the S phase, and then declining during the G2 phase (14, 15). This protein acts as a cofactor for various cellular processes, including DNA replication and repair, cell-cycle regulation, and postreplicative processes (14, 15).

Bcl-2 and Bax are two oncoproteins that belong to the Bcl-2 family. Bcl-2 family members are important regulators of programmed cell death (16–18). Bcl-2 plays a pivotal role in apoptosis by acting as an inhibitor of the apoptotic process (16–18). Bax shares homology with the Bcl-2 oncoprotein in several highly conserved regions. An overexpression of Bax promotes cell death by antagonizing the survival-promoting activity of Bcl-2 (16–18). In particular, when Bax is overexpressed it forms homodimers that can heterodimerize with Bcl-2 and induces cell death (16–18). By this view, the Bcl-2/Bax ratio can predetermine the susceptibility of cells to apoptosis (16–18).

The present study was carried out to evaluate the cellular effects of raloxifene on proliferative and apoptotic processes in uterine leiomyomas of postmenopausal women.

MATERIALS AND METHODS

The procedures used were in accordance with the guidelines of the Helsinki Declaration on human experimentation, and the study was approved by the Ethical Committee of the University "Magna Graecia" of Catanzaro. Before entering the study, the purpose of the protocol was clearly explained to women attending the Department of Obstetrics and Gynecology of the University "Magna Graecia" of Catanzaro, and a printed explanatory consent form was signed by all subjects enrolled.

Subjects

Forty postmenopausal women affected by uterine leiomyomas and selected for gynecological interventions (including hysterectomy) were enrolled in the prospective randomized double-blind placebo-controlled study.

The inclusion criteria were spontaneous postmenopause for at least 1 year and less than 2, presence of at least one and no more than two intramural uterine leiomyomas, and the presence of at least one uterine leiomyoma with a main diameter >2 cm at transvaginal ultrasound (TV-USG). The exclusion criteria were neoplastic, metabolic, and infectious diseases, history of acute or recurrent vascular thrombosis, body mass index (BMI, kg/m^2) >30, use of hormone therapy over the previous 6 months, presence of moderate and severe vasomotor symptoms, and blood coagulation diseases. Women with leiomyomas with a main diameter >5 cm and with hypoechoic or calcified leiomyomas at TV-USG were also excluded (19).

Protocol and Treatment

The subjects were randomly allocated into two treatment groups of 20 women each (raloxifene and placebo groups). The randomization was carried out using online software (www.randomization.it) to generate a random allocation sequence in single blocks as a method of restriction. The random allocation sequence was concealed until the interventions were assigned. The drug and the placebo were packaged in the pharmacy of the University of Catanzaro and labeled according to subject number. The duration of the treatments for both groups was three cycles of 28 days each. For the overall study period, operators and patients were blinded to the treatment allocation.

The raloxifene group received raloxifene hydrochloride (Evista, Eli Lilly, Sesto Fiorentino, Italy) at a dose of 180 mg/day orally, whereas the placebo group received placebo tablets (3 tablets/day orally).

At admission, in all subjects the menopausal status was confirmed by serum FSH and E_2 (FSH >40 mIU/mL, E_2 <20 pg/mL) level detection. At the beginning of the study and after treatment (just before surgery), uterine and uterine leiomyoma dimensions were measured by an expert operator (T.R.) with the use of TV-USG (Toshiba PowerVision 6000, Toshiba Medical System, Rome, Italy) equipped with a 7.5-MHz transvaginal probe. In our ambulatory clinic the intraoperator variation in leiomyoma volumes was <5%. The operator was masked regarding the patient's allocation. The uterine and uterine leiomyoma dimensions were evaluated by measuring the three main diameters and applying the formula of the ellipsoid (8–10). When the presence of two leiomyomas was detected, an arithmetic mean was used (8–10).

Tissue Collection

After a three-cycle course of raloxifene treatment, all women underwent gynecological surgery. All surgical procedures, including hysterectomy, were performed by the same operators (S.P., F.Z.).

In each case, the uterus was sent for pathological examination. Paraffin blocks of representative areas of leiomyomas and of homologous normal myometrium (at least 1 cm away from the tumors) were obtained. Serial samples were cut into 4-μm-thick sections and analyzed after hematoxylin and eosin staining by two independent pathologists to confirm the histological diagnosis of uterine leiomyoma and of normal myometrium. Samples were excluded from the study if there was disagreement between the two pathologists in the histological diagnosis or if an unexpected uterine pathology was found.

Immunohistochemical Study

For each sample, 4-μm-thick sections of uterine leiomyomas and of homologous myometrium were obtained and processed in the same laboratory as detailed in the following steps:

1. Pretreatment (heat-induced antigen retrieval with a 650-W microwave oven) in three sequential steps of 4