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Raloxifene therapy does not affect uterine blood flow in postmenopausal women: a transvaginal Doppler study

George Dantas de Azevedo [a,b,*], Maria Fernanda Massoni do Prado [a], Rui Alberto Ferriani [a], Rosana Maria dos Reis [a], Aderson Tadeu Berezowski [a], Tatiane Flores Ribeiro [a], Ester Silva [a], Técia Maria de Oliveira Maranhão [b], Marcos Felipe Silva de Sá [a]

[a] *Department of Obstetrics and Gynecology, Faculty of Medicine of Ribeirão Preto, University of São Paulo, BR 101, Ribeirão Preto, SP, Brazil*
[b] *Department of Morphology, Federal University of Rio Grande do Norte, Lagoa Nova, Natal, RN, Brazil*

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Abstract

Objective: To monitor the effects of raloxifene therapy on the uterus of postmenopausal women by transvaginal ultrasonography and color flow Doppler. *Methods*: Twenty-five healthy postmenopausal women were enrolled in this prospective longitudinal study performed at Department of Gynecology and Obstetrics, Faculty of Medicine of Ribeirão Preto. The patients were treated with raloxifene hydrochloride (60 mg per day) for 6 months. All were submitted to transvaginal ultrasound examination with color flow Doppler (ATL-HDI 3000 equipment) before the beginning and after 1, 3 and 6 months of treatment. Resistance index (RI) and pulsatility index (PI) of the uterine arteries were determined by the Doppler method, being considered as indicators of uterine perfusion. The following variables were analyzed: endometrial thickness, uterine volume, RI, and PI. Data were analyzed statistically by repeated-measures analysis of variance. *Results*: Before treatment, endometrial thickness was 3.38 ± 0.73 mm, and similar values were observed after 1, 3 and 6 months of treatment (3.04 ± 0.82; 3.3 ± 0.83; and 3.37 ± 0.79, respectively) ($P > 0.05$). No significant differences in uterine volume were observed between the pre- and post-treatment periods. Uterine artery perfusion as indicated by RI and PI measured by Doppler also showed no significant variation, with a high impedance flow being maintained throughout treatment. *Conclusions*: In the group studied here, raloxifene treatment at the dose of 60 mg per day for 6 months did not induce significant changes in endometrial thickness, uterine volume or uterine artery perfusion, confirming that short-term raloxifene treatment does not affect the uterus of postmenopausal women.

Keywords: Menopause; Climactery; Selective estrogen receptor modulator; Raloxifene; Doppler; Endometrium

1. Introduction

Over the last decades, great emphasis has been placed on hormonal replacement therapy (HRT)

* Corresponding author. Tel.: +55-84-215-3431; fax: +55-84-211-9207.
E-mail address: georgedantas@uol.com.br (G.D. de Azevedo).

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during the postmenopausal period as an effective alternative for the control of the effects of estrogen deficiency. Studies have indicated that HRT relieves vasomotor and urogenital symptoms, protects against colorectal cancer, and improves bone density and reduces the incidence of fractures [1–3]. However, estrogen administration for many women has side effects and risks, a fact that impairs patient compliance with the therapy proposed. Together with breast symptoms and the fear of cancer, genital bleeding is one of the major causes of low compliance to treatment.

Other therapeutic options like selective estrogen receptor modulators (SERMs) have been used to avoid these inconveniences and to improve patient compliance, keeping the benefits of estrogens and minimizing their undesired effects. Raloxifene hydrochloride is a so-called second-generation SERM, with an action profile involving an estrogen agonist activity on the bone tissue, but having no activity on breast [4,5]. In addition to beneficial effects on bone mineral density, raloxifene causes favorable changes in lipid profile, with reduction of total cholesterol and low-density lipoprotein cholesterol [6,7]. With respect to its effects on the endometrium, several studies have indicated the absence of undesirable stimulatory effects, with no observation of significant changes in endometrial thickness during the treatment with raloxifene [4,8]. However, the effects of raloxifene on blood perfusion of the uterine arteries have not been well defined in human. Therefore, the aim of the present study was to establish the effects of raloxifene therapy on the uterine arteries, endometrial thickness and uterine volume in postmenopausal women, assessed by transvaginal ultrasound and color flow Doppler.

2. Materials and methods

2.1. Patient population

Twenty-five healthy postmenopausal women were enrolled in an open prospective longitudinal study. The study was performed at the outpatient clinic of the Department of Gynecology and Obstetrics, School Hospital of Faculty of Medicine of Ribeirão Preto, Brazil. Inclusion criteria were: age between 50 and 70 years, last spontaneous menstrual cycle occurring more than 24 consecutive months before the study, presence of an intact uterus without anomalies upon initial ultrasonographic evaluation, no hormone replacement therapy during the 90 days before the study, and agreement of the patient to participate in the study after giving written informed consent. The study was analyzed and approved by the School Hospital Ethics Committee.

All subjects had a normal cardiac profile, no serious chronic disease, and were not taking any medication that could interfere with Doppler evaluation. Exclusion criteria were a previous or present history of thromboembolic disorders, myocardial infarction, coronary disease, stroke, hypertension, diabetes mellitus, hepatic or cholestatic diseases, severe depression, blood coagulation disorders, renal failure, and estrogen-dependent neoplasia. Individuals with abnormal genital bleeding of unknown cause were also excluded from the study. Other exclusion criteria were smoking, alcoholism, obesity (body mass index equal to or higher than 30 kg/m^2), and the presence of significant laboratory alterations in hepatic or renal functions or any other clinically significant abnormal pretreatment result.

Before inclusion in the study group, the volunteers were submitted to a complete clinical evaluation consisting of anamnesis and detailed physical examination, as well as complementary screening exams as part of normal routine care during the climacteric period. All patients presented serum 17β-estradiol <20 pg/ml, follicle-stimulating hormone >30 mIU/ml and blood biochemistry (fasting glycemic, urea, creatinine, bilirubins, lipid profile) within normal limits.

2.2. Study protocol

Eligible women were assigned to a daily dose of 60 mg of raloxifene hydrochloride (Eli Lilly and Company, Indianapolis, IN), 1 tablet per day. The following variables were measured: endometrial thickness, uterine volume, and the resistance and pulsatility indexes of the uterine arteries using Doppler ultrasonography. Ultrasonographic parameters were assessed before the beginning and after 1, 3 and 6 months of treatment.

2.3. Ultrasonographic examination and hemodynamic measurements

The ultrasonographic exams were carried out by the same investigator who had a competence certificate

and was experienced in the Doppler methodology. An ultrasound system (HDI 3000; Advanced Technology Laboratory, USA) was used with a 9–5 MHz endovaginal probe. In order to avoid possible circadian interferences in the Doppler evaluation, the tests were carried out during the same period of the day (morning) in a silent, moderately illuminated and air-conditioned room at a temperature of 23 °C. The patients were told to completely empty the bladder and to rest in dorsal decubitus for 10 min before examination. Patients were examined in the gynecological position with the back elevated 30° above the horizontal plane.

The first part of the transvaginal ultrasonographic examination consisted of Doppler analysis, since prolonged handling of the uterus may activate pelvic circulation, thus compromising the evaluation. The uterine artery was visualized laterally to the uterine wall at the level of the internal ostium, by amplitude Doppler. A 2-mm sample above each of the ascending branches of the uterine arteries was used. The Doppler indexes were measured after obtaining at least five similar flow rate waves of satisfactory quality. The impedance indexes used were resistance index (RI = peak systolic-shifted frequency minus the end diastolic-shifted frequency divided by the peak systolic) and pulsatility index (PI = peak systolic-shifted frequency minus the end diastolic-shifted frequency divided by the mean Doppler shift). Since the technique and experience of the investigator in obtaining the Doppler indexes play an important role, intra-observer variability of the method was determined by performing at least three measurements of each index in each of the arterial segments studied. The mean and standard deviation (SD) of each index were calculated and used for statistical analysis. The intra-observer coefficient of variation (CV = [S.D./mean] × 100%) for each of the Doppler indexes was calculated as the mean of the individual CV obtained.

The second part of the transvaginal ultrasonographic examination consisted of the analysis of the endometrium and uterine volume. Endometrial thickness, in mm, was determined as the distance between an endometrium–myometrium interface and the opposite one. The uterine diameters were measured on the longitudinal ($D1$), anteroposterior ($D2$) and transverse ($D3$) planes, and the uterine volume (in cm^3) was calculated using the following formula: $D1 \times D2 \times D3 \times 0.52$.

2.4. Statistical analysis

The non-parametric Kolmogorov–Smirnov test was used to determine the normality of the distribution of the values. The normality hypothesis was not rejected for any of the variables. Since all variables showed a normal distribution, analysis of variance for repeated measures (ANOVA) was used to compare the values obtained during the pretreatment period and those obtained during the subsequent phases of the study, with the level of significance set at 5%.

3. Results

The mean age of the patients was 56.0 ± 4.8 years and the mean time after menopause was 8.3 ± 5.1 years. During pretreatment evaluation, the patients showed a mean body mass index of 25.2 ± 3.7 kg/m^2, a waist/hip ratio of 0.81 ± 0.04 and mean systolic and diastolic pressures of 125.6 ± 17.2 and 80.5 ± 8.0 mmHg, respectively. Clinical follow-up showed good treatment compliance in the study group.

Mean endometrial thickness at the pretreatment period was 3.38 ± 0.73, a value considered to be within the normal limit for postmenopausal women not using HRT [9]. Raloxifene treatment did not show any significant stimulatory effects, since endometrial thickness was practically unaltered at 1, 3 and 6 months of treatment (3.04 ± 0.82, 3.30 ± 0.83 and 3.37 ± 0.79, respectively) ($P > 0.05$).

Uterine volume also did not show any significant alterations during the 6-month follow-up period. Mean uterine volume was 40.4 ± 17.8 cm^3 at baseline and similar values were observed at 1, 3 and 6 months of treatment (40.4 ± 16.8, 40.2 ± 14.6 and 39.9 ± 14.4, respectively).

Analysis of the blood perfusion parameters in the uterine arteries by the Doppler method showed a high flow impedance, as demonstrated by the elevated RI and PI in these arteries during pretreatment evaluation. Table 1 shows the RI and PI values of the uterine arteries during the 6-month treatment with raloxifene. No significant alterations were observed, with the high impedance standard observed during the pretreatment phase being maintained.

Table 1
Resistance (RI) and pulsatility (PI) indexes of the uterine arteries (UA) of postmenopausal women evaluated before and after 1, 3 and 6 months of raloxifene treatment

	Pretreatment	1 month	3 months	6 months
Right UA				
RI	0.92 ± 0.05	0.93 ± 0.05	0.93 ± 0.05	0.91 ± 0.04
PI	3.03 ± 0.81	3.27 ± 1.05	3.08 ± 0.73	2.98 ± 0.43
Left UA				
RI	0.91 ± 0.06	0.92 ± 0.05	0.92 ± 0.05	0.91 ± 0.04
PI	2.85 ± 0.68	3.11 ± 1.06	3.03 ± 0.75	3.02 ± 0.68

No significant differences were observed between studied periods.

The Doppler method showed good reproducibility, as indicated by the low intra-observer CV in terms of RI (2.36%) and PI (7.5%). No statistically significant differences were found between the measurements on the right and left uterine arteries.

4. Discussion

Transvaginal ultrasonography provides high resolution images, substantially improving the ability to evaluate pelvic organs and to study morphological changes in detail. With the use of high frequency transducers, the endometrium can be assessed in terms of echogenicity, regularity and thickness, so that this method has become universally accepted as the major tool for endometrial screening during the postmenopausal period even in women who do not use HRT. Several studies have been conducted to determine the normal ultrasonographic pattern of the endometrium in postmenopausal women and an endometrial thickness of 4 mm or less is accepted by most investigators as being associated with a minimum risk of endometrial disease [9,10].

In the present study, no significant changes in endometrial thickness were observed during a period of 6 months of raloxifene use. Reports from literature also show the safety of raloxifene with respect to the endometrium [6,8]. Data from Multiple Outcomes of Raloxifene Evaluation (MORE) trial confirmed the hypothesis that raloxifene does not increase the risk of endometrial hyperplasia or cancer [5]. Even when higher doses than normally used were employed and the effects on the uterus were evaluated using a more extensive methodology, the results obtained for the group treated with raloxifene did not differ from those obtained for the placebo group [11,12].

With respect to uterine volume, again no significant differences were observed between basal values and the values determined during the study. Goldstein et al. [11] demonstrated that raloxifene did not cause significant changes in uterine volume, whereas a 22% increase was observed in the group treated with estrogen. The increase in endometrial thickness and uterine volume has also been reported after the use of tamoxifen [13–15].

Estrogen has a direct effect on the arteries, causing vasodilation [16]. This effect can be detected by Doppler analysis of blood flow [17]. After menopause, because of the hypoestrogenism occurring in women, there is a reduction of the blood supply to pelvic organs, resulting in important atrophy of the vaginal mucosa and of the lower urinary tract, as well as reduction of uterine and ovarian volume. During this phase in a woman's life, Doppler analysis of the uterine arteries demonstrates high RI and PI, reflecting the existence of high vascular resistance [18].

Several studies have demonstrated that estrogen therapy during menopause causes significant changes in blood perfusion of the uterine arteries and of other arterial beds such as the carotids and cerebral arteries. Thus, after the introduction of HRT, an increase in uterine perfusion can be identified by the Doppler technique, as demonstrated by marked reduction of RI and PI of the uterine arteries [19,20]. In contrast to the large amount of information about the vascular actions of estrogens, little is known about the effects of SERMs and of raloxifene in particular on the different vascular beds of the organism. Most available data about SERMs exclusively refer to tamoxifen, indicating an estrogen-like effect of the drug on uterine perfusion [21,22].

An in vitro study in rabbit coronary arteries demonstrated that raloxifene induces vasodilation by an endothelium-dependent mechanism mediated by the estrogen receptor, suggesting that this drug may activate the release of nitric oxide from endothelial cells [23]. Another experimental study in sheep demonstrated that intra-vaginal administration of raloxifene provoked an acute increase in uterine blood flow from 20 to 220 ml/min, an effect also observed in the coronary arteries and partially mediated by nitric oxide [24]. An in vitro study using human umbilical vein

cells demonstrated a stimulatory effect of raloxifene on nitric oxide production in endothelial cells by an acute mechanism of activation of the enzyme nitric oxide synthetase depending on the estrogen receptor [25].

However, few data are available in the literature about studies in which human uterine perfusion was evaluated by the Doppler method during treatment with raloxifene. In recent report, Post et al. demonstrated that compared with placebo, no significant changes in the PI value of the uterine artery were observed in the women using the currently marketed dosage of 60 mg of raloxifene [26]. In the present study, during treatment with raloxifene (60 mg per day) for 6 months, we also did not observe significant changes in any of the Doppler indexes, with the maintenance of a high vascular resistance in the uterine arteries. The maintenance of high RI and PI suggests that the treatment was virtually free of vasodilating effects on the uterine arteries.

Although apparently opposing, our results do not contradict those previous studies since they were conducted using different methodologies, vascular beds and animal species [23–25]. Within the SERM concept, raloxifene presents variable effects according to the region considered. On this basis, the vascular effects of the drug would also be expected to be tissue-selective. It is also possible that the differences observed between the effects of estrogen and raloxifene on the uterine blood vessels and on the endometrium and myometrium may be related to the differential expression of alpha and beta estrogen receptors in different tissues, as well as to changes in the intra-cellular processing of the ligand–receptor complex.

The results obtained on uterine perfusion is in agreement with those concerning endometrial thickness and uterine volume, since the absence of vascular effects is assumed to have as consequence the absence of effects on the development of endometrium and myometrium. However, this apparently logic relation is not universally accepted by researchers. We still do not know whether there is a direct relationship between the reduction in vascular resistance, increase on uterine blood flow and the subsequent development of endometrial hyperplasia and cancer, as it may occur in postmenopausal women using non-opposed estrogen.

In conclusion, our results support the hypothesis that raloxifene has no estrogen agonist activity on uterine tissue when administered on a short-term and at regular dose basis (60 mg per day).

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