Effects of tamoxifen and raloxifene on body and uterine weights of rats in persistent estrus

D.M. Rodrigues-Junior, P.V. Lopes-Costa, A.R. dos Santos, B.B. da Silva

Department of Gynecology, Piaui Federal University, Teresina, Piaui (Brazil)

Summary

Purpose: To evaluate the change in body and uterine weights of rats in persistent estrus, a model developed to mimic polycystic ovary syndrome treated with selective estrogen receptor modulators (SERMs) tamoxifen and raloxifene. *Methods:* Sixty Wistar-Hannover rats induced by a single subcutaneous dose of 1.25 mg testosterone propionate were divided into three groups of 20 animals: Group I (placebo); Group II (tamoxifen, 250 µg/day) and Group III (raloxifene, 750 µg/day). At 90 days of life, the treatment began for 30 consecutive days, in which the animals were weighed weekly. On the 31st day, the animals were sacrificed and the uterus removed. Data were analyzed statistically by analysis of variance and by the Tukey-Kramer multiple comparisons test ($p < 0.05$). *Results:* Means of body and uterine weights (g) after treatment were: 227.3 ± 2.20 and 0.40 ± 0.01; 185.3 ± 2.45 and 0.25 ± 0.01; 186.4 ± 2.20 and 0.27 ± 0.01 in Groups I, II and III, respectively ($p < 0.001$). There was no statistical difference between groups II and III for body and uterine weight (p = 0.727 and p = 0.646, respectively). *Conclusion:* The present results indicate that, at the doses and during the time of treatment used, both tamoxifen and raloxifene reduce in a similar way the body and uterine weights of rats in persistent estrus showing a possible antiestrogenic effect of SERMs under high levels of estrogens.

Key words: Animal models; Estrogen; Tamoxifen; Raloxifene.

Introduction

Selective estrogen receptor modulators (SERMs) are drugs that have attracted the attention of many investigators studying primary chemoprevention of breast cancer in women at high risk for the disease [1, 2]. Tamoxifen was the first drug to be approved in the United States for chemoprevention in women at high risk for breast cancer. However, long-term use of tamoxifen may lead to significant side-effects, especially endometrial carcinoma [3]. For this reason, there has been great interest in identifying other SERMs that may serve as an alternative to tamoxifen [4, 5].

Raloxifene, a second-generation SERM approved in the United States and in other countries for the prevention and treatment of osteoporosis in postmenopausal women, has been shown to exert an antiestrogenic action on the breast without, however, stimulating the endometrium [4]. This fact was confirmed in a recently published study of tamoxifen and raloxifene (STAR) trial, which showed raloxifene to be almost as effective as tamoxifen in reducing invasive breast carcinoma [6].

Little is known about the behavior of SERMs during chronic anovulation in which obesity and constant estrogenic stimulation are a common finding in these patients [7]. Due to ethical issues in humans, studies are required to be conducted in animal models. Female rats receiving steroids, particularly at birth, enter a condition of persistent estrus upon reaching adulthood. Furthermore, these animals develop characteristics that mimic women with polycystic ovary syndrome (PCOS) [8, 9].

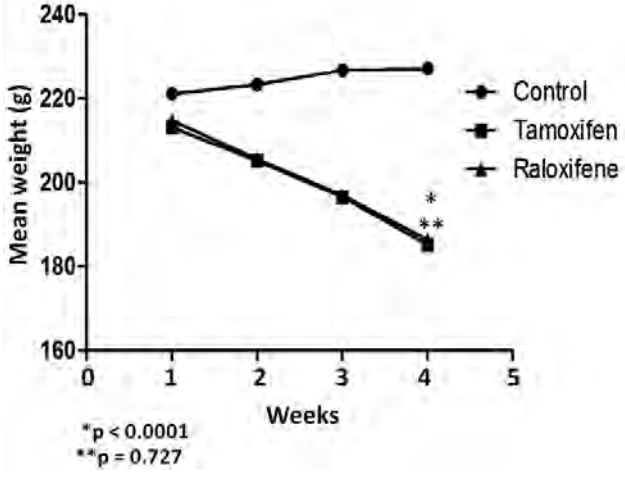
Therefore, in view of the scarcity in the literature, studies which assessed SERMs behavior on body and uterine weights in states of chronic anovulation and hyperandrogenism are scarce. Thus, to best of our knowledge, we have used a biological model mimicking states of chronic anovulation presenting excess of body and uterine weights to better understand tamoxifen and raloxifene behavior in these conditions.

Material and Methods

The study protocol was carried out in accordance with the ethical principles of the Colégio Brasileiro para Experimentação Animal (COBEA), also by a local Committee on Ethics and Animal Experimentation of the Federal University of Piaui (registers numbers 35/2010 and 54/2010). Sixty female, virgin Wistar-Hannover rats obtained from the animal laboratory of the Federal University of Piaui were used in this study. The animals were kept under controlled temperature (25°C) and lighting conditions (7:00 a.m. to 7:00 p.m.) in individual cages with free access to water and standard laboratory rodent chow (SUPRA-LAB, São Paulo, Brazil). Androgenization or persistent estrus was achieved in the animals by administering a single dose of subcutaneous injection of 1.25 mg of testosterone propionate diluted in 0.1 ml of corn oil on the second day of life. The state of persistent estrus was confirmed in the animals based on the presence of obliteration of the distal third of the vagina and on keratinization of the vaginal epithelium, the principal characteristic of persistent estrus [8, 10], on the presence of polycystic ovaries as observed during histology performed at the end of the study. The rats were randomly distributed into three groups of 20 animals each: Group I (persistent estrus, control) received only vehicle (propylene glycol), whereas Group II (persistent estrus) received tamoxifen and Group III (persistent estrus) received raloxifene.

The animals in Group I received 0.5 ml/animal/day of propylene glycol (placebo), while those in Group II received 250 µg/animal/day of tamoxifen diluted in 0.5 ml of propylene glycol

Revised manuscript accepted for publication September 26, 2011



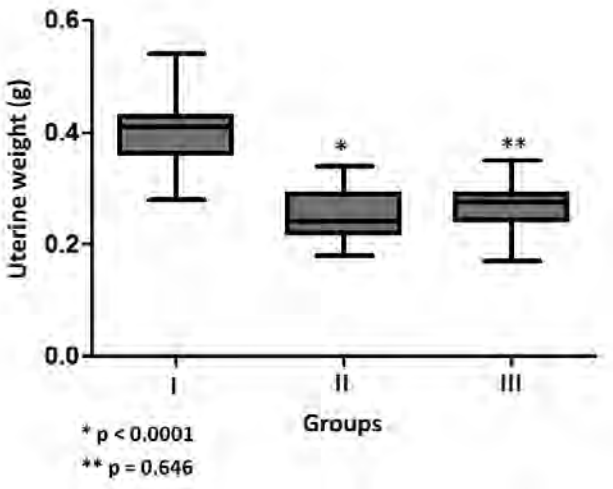


Figure 1. — Change in body and uterine weights of rats in persistent estrus treated with placebo (I), tamoxifen (II) and raloxifene (III).

Figure 2. — Boxplot of the uterine weight of rats in persistent estrus treated with placebo (I), tamoxifen (II) and raloxifene (III).

Table 1. — *Mean body weight (g) of control and experimental groups throughout treatment*.

Weeks	Control	Tamoxifen	Raloxifene
I	221.3 ± 2.26	213.3 ± 1.73	214.9 ± 2.05
II	223.5 ± 2.24	205.2 ± 1.98	205.6 ± 2.09
III	226.9 ± 2.21	196.7 ± 2.23	197.1 ± 2.16
IV	227.3 ± 2.20*	185.3 ± 2.45**	186.4 ± 2.20**

Tukey test was conducted to compare group by group; SEM: standard error of the mean. *(p < 0.001); **(p = 0.727).

Table 2. — *Mean uterine weight (g) of rats in persistent estrus receiving placebo (I); tamoxifen (II) or raloxifene (III)*.

Groups	Mean	SEM	Minimum	Median	Maximum
I	0.40	0.01	0.28	0.24	0.5
II	0.25*	0.01	0.18	0.24	0.34
III	0.27**	0.01	0.17	0.27	0.35

Tukey test was conducted to compare group by group; SEM: standard error of the mean. * I > II and III ($p < 0.0001$); ** II = III ($p = 0.646$).

and Group III received 750 µg/animal/day of raloxifene also diluted in 0.5 ml of propylene glycol. At 90 days of life, the treatment began for 30 consecutive days by gavage using specific metal tubing.

At the beginning of the treatment the animals were weighed weekly with a digital scale and with the aid of a plastic beaker up to the end of the treatment. On the 1st day after the treatment, the animals were sacrificed and the uterus was removed through a longitudinal abdominal incision and then weighed.

The ANOVA test made comparisons between groups. Post hoc pair wise comparisons between means in groups were conducted using the Tukey method with an overall significance level at 5% [11].

Results

The body and uterine weights of experimental animals treated with placebo (Group I) were significantly higher than the body and uterine weights (g) of animals treated with tamoxifen (Group II) and raloxifene (Group III) ($p < 0.001$). The mean of body weights of the control and experimental groups are listed in Table 1 and uterine weights are listed in Table 2. There was no statistical difference between Groups II and III for body weight (p = 0.727) (Figure 1) or for uterine weight (p = 0.646) (Figure 2).

Discussion

In the current study, female rats exhibiting persistent estrus were used to evaluate the effects of SERMs directly on body and uterine weights. Tamoxifen, administered at a dosage of 250 µg/animal/day, and raloxifene, administered at a dosage of 750 µg/animal/day, for 30 days, significantly reduced body and uterine weights compared to the group treated with placebo.

The metabolism of rats is faster than in humans, hence the weight-equivalent dose would constitute under-dosage and would not therefore mimic the serum and tissue concentration of SERMs in humans [12]. There are reports of experimental studies in the literature in which rats were given daily oral doses of raloxifene that varied from 1 to 10 mg/kg, without any toxicity for the animals. Therefore, the dosages in this study were chosen because they were sufficient to reproduce tissue levels of the drugs observed in adult women on using this medication [13, 14].

Chronic anovulation syndrome was induced and characterized by keratinization of the vaginal epithelium in this animal model [8, 10]. The adult animals develop characteristics that mimic PCOS in women such as chronic anovulation, sterility and polycystic ovaries [9, 15]. Furthermore, this model of rats in a state of persistent estrus manifested high food intake, elevated body weight and obesity [16, 17] associated with increased of hyperinsulinemia, which in turn is significantly correlated with the risk of cardiovascular diseases [18, 19] and seems to induce hyperandrogenemia [20]. Nevertheless, PCOS is a multifactorial syndrome and probably no animal model has the capacity to reproduce all aspects of this syndrome.

In previous reports, estrogen increased the production of leptin in ovariectomized rats, the *ob* gene product, which has an important role in regulating body weight [21, 22]. A study performed by Hozumi *et al*. [23] in ovariectomized rats mimicking postmenopausal women treated with tamoxifen, showed a significantly reduced weight gain, food intake, weight of adipose tissue and leptin concentration. In addition, these animals treated with SERMs become resistant to insulin [23]. Most of the additional effects of tamoxifen on lipid metabolism as changes in body weight and composition, or endometrium are likely to be due to estrogenic effects [24]. However, ovariectomized rats have low levels of circulating estrogen, while the influence of SERMs on adipose tissue under higher levels of estrogen remains unknown.

Our data is the first to evaluate the effect of raloxifene compared to tamoxifen in body mass in this animal model. Nevertheless, these data differ from the study of Patriarca *et al*. [25], which found no differences in body weight of rats in persistent estrus treated with tamoxifen compared to a control group, probably due to differences in dosages used in trials, with our concentration being more than double that used by the authors.

Similar to this study, Patriarca *et al*. [25] observed, due to an anti-uterotrophic effect, a reduction in uterine weight in animals receiving tamoxifen. After macroscopic examination of the uterus, lower uterine weights in animals treated with the antiestrogens were likely caused by the inhibitory effect of tamoxifen and raloxifene on fluid retention mediated by estrogen [25], resulting in decrease of mass in this organ.

Conclusion

In conclusion, use of 250 g of tamoxifen and 750 g of raloxifene in rats with persistent estrus significantly reduced body and uterine weights in this experimental model. Even though the results are limited in extrapolating the present study to humans, these findings strengthens the literature and have provided a wider perspective on further studies aimed to elucidate the tamoxifen and raloxifene effect on body and uterine weights in the presence of high circulating levels of estrogen.

References

[1] Goldstein S.R., Siddhanti S., Ciaccia A.V., Plouffe L. Jr.: "A pharmacological review of selective oestrogen receptor modulators". *Hum. Reprod. Update,* 2000, *6,* 212.

[2] Lewis J.S., Jordan V.C.: "Selective estrogen receptor modulators (SERMs): mechanisms of anticarcinogenesis and drug resistance". *Mut. Res.,* 2005, *591,* 247.

[3] Fisher B., Costantino J.P., Wickerham D.L., Redmond C.K., Kavanah M., Cronin W.M. *et al.*: "Tamoxifen for the prevention of breast cancer: current status of the national surgical adjuvant breast and bowel project p-1 study". *J. Natl. Cancer Inst*., 1998, *97,* 1636.

[4] Cummings S.R., Eckert S., Krueger K.A., Grady D., Powles T.J., Norton L. *et al*.: "The effect of raloxifene on risk of breast cancer in postmenopausal women: results from the MORE randomized trial. Multiple Outcomes of Raloxifene Evaluation". *JAMA,* 1999, *16,* 2189.

[5] Osborne C.K.: "Aromatase inhibitors in relation to other forms of endocrine therapy for breast cancer". *Endocr. Relat. Cancer,* 1999, *6,* 271.

[6] Vogel V.G., Costantino J.P., Wickerham D.L., Cronin W.M., Atkins J.N., Bevers T.B. *et al*.: "Update of the National Surgical Adjuvant Breast and Bowel Project Study of Tamoxifen and Raloxifene (STAR) P-2 Trial: Preventing breast cancer". *Cancer Prev. Res. (Phila)*. 2010, *3*, 696.

[7] Yen S.S., Vela P., Rankin J.: "Inappropriate secretion of follicle-stimulating hormone and luteinizing hormone in polycystic ovarian disease". *J. Clin. Endocrinol. Metab.,* 1970, *30,* 435.

[8] Barraclough C.A.: "Production of anovulatory, sterile rats by single injections of testosterone propionate". *Endocrinology*, 1961, *68,* 62.

[9] da Silva B.B., Lopes-Costa P.V., Dos Santos L.G., Pires C.G., Gontijo J.A.: "Evaluation of Ki-67 antigen expression in the zona reticularis of the adrenal cortex of female rats in persistent estrus". *Hum. Reprod*., 2009, *24,* 705.

[10] Wrenn T.R., Wood J.R., Bitman J.: "Oestrogen responses of rats neonatally sterilized with steroids". *J. Endocrinol*., 1969, *45,* 415.

[11] Kutner M.H., Nachtshein C.J., Neter J., Li W.: "Applied linear statistical models". Fifth ed., Irwin, Boston McGraw-Hill, 2005.

[12] Lien E.A., Solheim M.E., Ueland P.M.: "Distribution of tamoxifen and its metabolites in rat and human tissues during steady-state treatment". *Cancer Res.,* 1991, *51,* 4837.

[13] Dodge J.A., Lugar C.W., Cho S., Short L.L., Sato M., Yang N.N. *et al*.: "Evaluation of the major metabolites of raloxifene as modulators of tissue selectivity". *J. Steroid Biochem. Mol. Biol.,* 1997, *61,* 97.

[14] Decensi A., Bonanni B., Guerrieri-Gonzaga A.: "Biologic activity of tamoxifen at low doses in heathy women". *J. Natl. Cancer Inst.,* 1998, *18,* 3172.

[15] da Silva B.B., Gebrim L.H., Simões M.J., Baracat E.C., Rodrigues de Lima G.: "Morphologic and morphometric study of the breast parenchyma of rats in persistent estrus treated with tamoxifen and conjugated estrogens". *Clin. Exp. Obstet. Gynecol*., 2001, *28*, 60.

[16] Sun F., Yu J.: "The effect of a special herbal tea on obesity and anovulation in androgen-sterilized rats". *Proc. Soc. Exp. Biol. Med*., 2000, *223,* 295.

[17] da Silva B.B., Rocha Gontijo J.A., Créte A.R., de Jesus Simões M.: "Evaluation of body weight in androgenized female rats". *Clin. Exp. Obstet. Gynecol*., 2002, *29,* 97.

[18] Nestler J.E., Jakubowicz D.J., Evans W.S., Pasquali R.: "Effects of metformin on spontaneous and clomiphene-induced ovulation in the polycystic ovary syndrome". *N. Engl. J. Med.,* 1998, *25,* 1876.

[19] Ginsberg H.N.: "Insulin resistance and cardiovascular disease". *J. Clin. Invest*., 2000, *106,* 453.

[20] Velazquez E.M., Mendoza S., Hamer T., Sosa F., Glueck C.J.: "Metformin therapy in polycystic ovary syndrome reduces hyperinsulinemia, insulin resistance, hyperandrogenemia, and systolic blood pressure, while facilitating normal menses and pregnancy". *Metabolism*., 1994, *43,* 647.

[21] Shimizu H., Shimomura Y., Nakanishi Y., Futawatari T., Ohtani K., Mori M.: "Estrogen increases in vivo leptin production in rats and human subjects". *J. Endocrinol*., 1997, *154,* 285.

[22] Tanaka M., Nakaya S., Kumai T., Watanabe M., Tateishi T., Shimizu H., Kobayashi S.: "Effects of estrogen on serum leptin levels and leptin mRNA expression in adipose tissue in rats". *Horm. Res*., 2001, *56,* 98.

[23] Hozumi Y., Hakamata Y., Nagai H.: "Plasma leptin concentration in tamoxifen-treated ovariectomized rats". *Gynecol. Obstet. Invest*., 2005, *59,* 70.

[24] Grainger D.J., Metcalfe J.C.: "Tamoxifen: teaching an old drug new tricks?". *Nat. Med*., 1996, *2,* 381.

[25] Patriarca M.T., Simões R.D., Smaniotto S., De Teves D.C., Simões D., Evencio-Neto J. *et al*.: "Morphological action of tamoxifen in the endometrium of persistent estrous rats". *Acta Obstet. Gynecol. Scand*., 1996, *75,* 707.

Address reprint requests to:
D.M. RODRIGUES Jr., M.D.
Rua Jose Camargo, 186 - apto 34
04139-010, Sao Paulo, Sao Paulo (Brazil)
e-mail: dorivalmrjr@gmail.com