Effect of long-time administration of tibolone on vaginal cytology of castrated rats

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Summary

Purpose: To evaluate the estrogenic effect of tibolone administered at high-dose and long-term through cytological examination of vaginal epithelium of castrated rats. *Methods*: 15 adult Wistar rats were submitted to bilateral oophorectomy 30 days before starting the experiment. The rats were then randomly divided in two groups. Experimental rats (n = 9) orally received 1 mg tibolone/day; control rats (n = 6) just received carboxymethylcellulose. Vaginal smears were collected from all rats on days 0, 1-6, 30, 60, 90 and 120 of the experiment. *Results:* On day 0, smears from all rats were atrophic, classified as anestrus, and remained this type in the control group until day 120. In the tibolone group, on day 3 all the rats had vaginal cytology similar to estrus and maintained the same aspect till day 90. *Conclusion*: Tibolone has estrogenic action in the vaginal epithelium which is already evident after the first dose and remains without major changes over time.

Key words: Tibolone; Rats; Menopause.

Introduction

Climacterium is a phase of a woman's life which is characterized by the transition between the non-reproductive and reproductive period, characterized by gradual reduction of ovarian function [1]. Menopause marks the end of this period, and therefore is defined as the final interruption of menstrual cycles [2]. The decreased secretion of estrogen observed in climacterium is responsible for several symptoms [1, 3]. Among the most common, one can cite vasomotor symptoms (hot flushes) [1, 3-5], osteoporosis [6], menstrual disorders [1,5], nervousness and irritability [1,5], genitourinary atrophy [5], vaginal dryness [4], and decreased libido [4]. Estrogen deficiency can cause atrophic changes within the urogenital tract with marked reduction of vaginal lubrication (vaginal dryness) and dyspareunia due to vaginal atrophy. Alterations in the urinary tract can arise leading to recurrent infection, urinary incontinence or pollakiuria [7].

Tibolone is a synthetic steroid acting in a tissue-specific mode and used in hormone replacement therapy. It is converted into three active metabolites exerting progestagenic, estrogenic and androgenic effects in vivo and in vitro [8]. By its selective effects, it has been recommended as an excellent alternative to long-term hormone therapy to alleviate postmenopausal symptoms [9]. Tibolone leads to many benefits in sexual function, vaginal atrophy, urogenital symptoms and bone loss. It also has a low incidence of vaginal bleeding and breast pain, symptoms common in other hormone replacement treatments [10]. Therefore, the use of tibolone is beneficial to the urogenital system, since in contrast to absence of endometrial stimulation, the genitourinary tract is stimulated in menopausal women by use of this drug [8].

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The aim of this study was to determine the estrogenic effect of tibolone administered at high dose and long-term through cytological examination of vaginal epithelium of castrated rats.

Material and Methods

Animals

Fifteen Wistar rats, aged 8 weeks and weighing 250 g were used. All rats were produced and maintained in the Animal Facility of the Laboratory of Experimental Nutrition (LABNE-UFF). Rats were housed in individual plastic cages, with controlled temperature (24 ± 2ºC) and artificial illumination alternated in cycles of 12/12 hours. Filtered water and commercial food (FRI-LAB RATOS II, FRI-RIBE) was supplied ad libitum.

Oophorectomy

Bilateral oophorectomy was performed in all rats 30 days before the beginning of the experiment, following the norms of vivisection of animals recommended by the Brazilian School of Animal Experimentation (COBEA). The work was approved by the Ethics Committee in Research of the College of Medicine/Antônio Pedro, University Hospital/Federal Fluminense University. Anesthesia was intramuscular with ketamine (100 mg/kg) and xylazine (20 mg/kg) [11].

Chemicals

Tibolone used in this study was donated by the manipulation pharmacy OFFICILAB. The drug was diluted at 0.2% in solution of 0.5% carboxymethylcellulose (CMC).

Experimental design

After surgery the rats stayed 30 days without medication, receiving only diet and water ad libitum to reduce sex hormone levels and come into surgical menopause [12]. Rats were randomly distributed in two groups. The experimental group (n = 9) received 0.5 ml/rat of tibolone, giving 1 mg/day/rat. The control group (n = 6) received 0.5 ml/day/rat of CMC. Each

group received their treatment by gavage administration for 120 consecutive days.

Vaginal smears were obtained immediately before the oophorectomy (day -30) to ensure that the rats were in the the normal estral cycle. Thirty days after surgery (day 0) new vaginal cytology was performed to verify the status of menopause. After starting the administration of tibolone and CMC, vaginal swabs were collected on days 1-6, 30, 60, 90 and 120 of the experiment to evaluate the vaginal trophism, which is classified as estrus, proestrus, metestrus and diestrus. Smears in estrus, proestrus and metestrus indicate hormonal influence and anestrus points to lack of hormonal influence by atrophic vaginal cytology [13, 14] (Table 1). Smears were immediately fixed in 95% alcohol and stained by the Papanicolaou method.

Table 1. — *Criteria for vaginal cytology classification*.

*Estral cycle phases**	*Vaginal cytology*
Estrus	Cornified cells
Metestrus	Cornified and epithelial nucleated cells (all types), leukocytes and mucus
Diestrus	Leukocytes, mucus and epithelial round cells
Proestrus	Cornified and epithelial nucleated cells (all types)
*Acyclic phase***	
Anestrus	Leukocytes, mucus and epithelial round cells

*Adapted from Baker, 1979 [13]; **Adapted from Dimarco *et al*., 2007 [14].

Table 2. — *Vaginal cytology after tibolone and CMC administration*.

Group	Day 0	n	Day 1	n	Day 2	n	Day 3	n
Tibolone	Anestrus	9	Anestrus	7	~ Metestrus/ Proestrus	1	~ Estrus	9
			~ Metestrus	1	~ Proestrus			
			~ Proestrus	1	~ Estrus	7		
						1		
Control	Anestrus	6	Anestrus	6	Anestrus	6	Anestrus	6

Results

The standard cytology found in both groups until day 3 of the experiment is shown in Table 2. On day 0, i.e., before starting the treatment, all rats showed typical cytology of anestrus in both control and tibolone groups. In the control group (n = 6) this standard cytology lasted until the end of the study. In the tibolone group (n = 9), after administration of one dose, i.e., on day 1, one rat had cytology similar to metestrus and another similar to proestrus. The cytology of the other seven rats remained in anestrus. On day 2, one rat showed standard cytology comparable to the transition from anestrus to proestrus, seven showed cytology similar to proestrus, and one rat already had cytology similar to estrus. On day 3, all rats in the tibolone group had vaginal cytology similar to estrus, which lasted until day 90 of the experiment. On day 120 it was observed that three of nine rats began to show cytology similar to metestrus, two had cytology compatible to the transition from estrus to metestrus and four remained similar to estrus.

Discussion

Tibolone is a highly effective steroid in postmenopause due to the biological activity dependent on its metabolism to 3α and 3β-OH, which displays an affinity for the estrogen receptor (ER), but not to either the progesterone receptor (PR) or the androgen receptor (AR) [15]. The current results demonstrate that 1 mg/day of tibolone administrated for 120 days has an estrogenic action in the vaginal epithelium at the very beginning evidenced by the change in the pattern of vaginal cytology of castrated rats. Smears of the control group continued to have an atrophic pattern.

Tibolone administered to ovariectomized rats for a short period showed a relative potency of 6% compared to ethynyl-estradiol (EE) after daily oral application. The mean number of positive smears (presence of nucleated or cornified epithelial cells) was evaluated and for a full estrogenic response a dose of 1mg/kg tibolone was needed [16]. We used a higher dose of tibolone, equivalent to 16 mg/kg with similar results. However, in adult ovariectomized monkeys treated with tibolone at 0.05 mg/kg or 0.2 mg/kg for two years, no effect of tibolone at either dose was observed on the vaginal maturation index and keratinization [17].

Randomized studies indicate that tibolone normalizes the vaginal karyopyknotic and maturation indexes and alleviates symptomatic atrophic vaginitis. Thus, women treated with tibolone report significant reduction in vaginal dryness and dyspareunia [18]. In a prospective and non-randomized study to assess the effects of six years of tibolone therapy on the genital tract in postmenopausal women both the vaginal karyopyknotic index and maturation index increased significantly in the tibolone treated group, but not in the control group [19]. This result demonstrates estrogenic effects of tibolone on the vagina, which is in agreement with the findings of this study. The vaginal cytological findings and symptoms evaluated in recently postmenopausal women (n = 50), who used 2.5 mg of tibolone daily for two years, showed a significant increase in the karyopyknotic index and maturation value whereas there was no change in the control group (n = 50). Significant symptomatic improvement occurred in vaginal dryness, dyspareunia, sexual enjoyment and libido. Therefore, tibolone has a significant estrogenic effect on the vagina as demonstrated by vaginal cytology [20]. In accordance with this report, an experiment performed with incubation of vaginal tissue from postmenopausal women and oophorectomized rats using radiolabeled tibolone showed stimulatory effects in both women and rats. This effect was due to action of the 3α-OH-tibolone metabolite [8].

According to the literature, tibolone is a tissue-specific compound with favorable effects on the vagina, climacteric symptoms, mood and sexual well being in postmenopausal women [21]. In this study tibolone showed early estrogenic effects on the vaginal tissue of oophorectomized rats. This fact is evidenced by the presence of vaginal cytology similar to estrus in treated rats, indicating estrogen action.

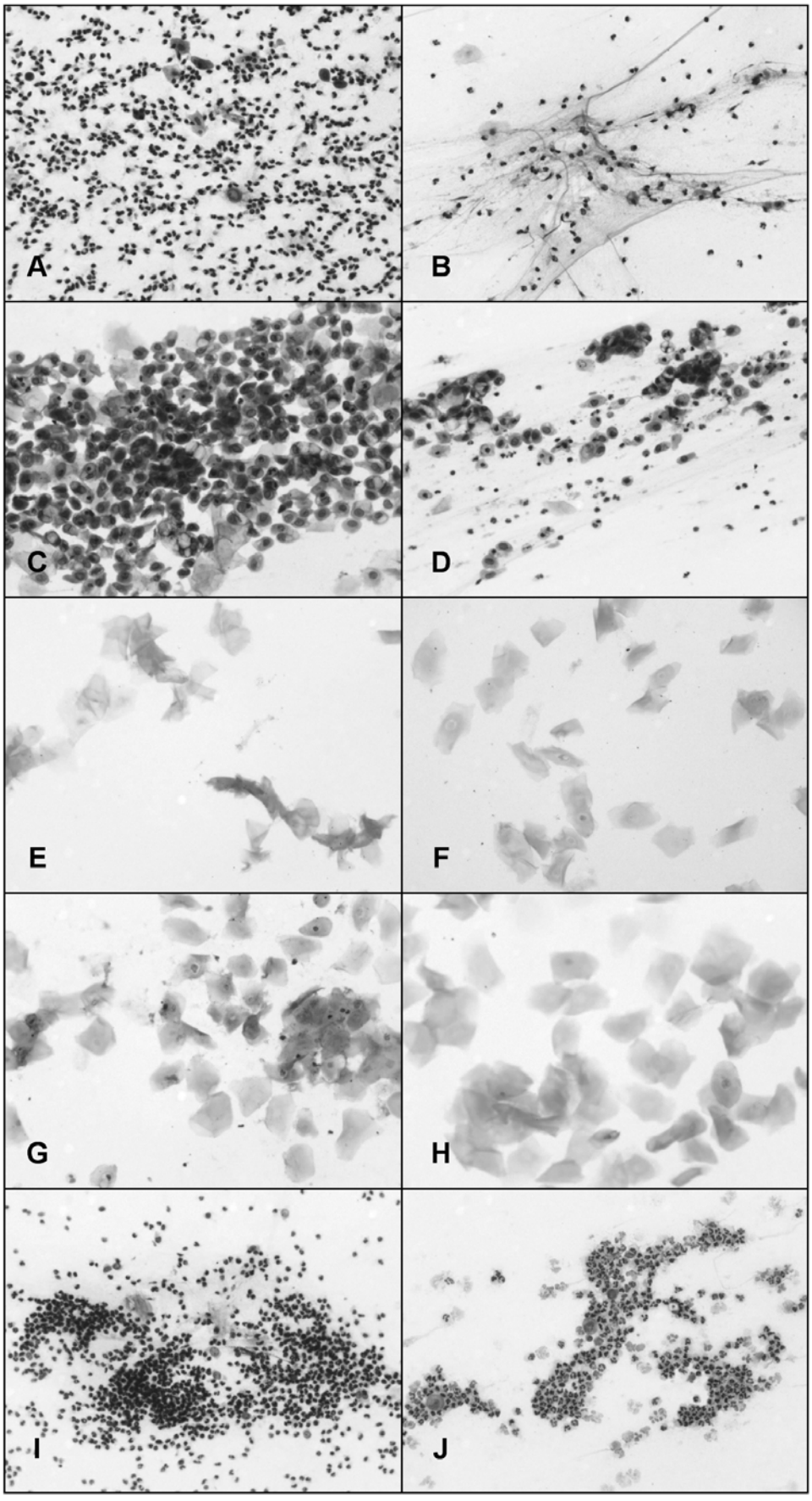

Figure 1. — Vaginal cytology of castrated rats. A, B, C, D, E, F, G, H – tibolone group; I, J – control group. A – Day 0. Cytology compatible to anestrus; B – Day 1. Cytology compatible to anestrus; C – Day 1. Cytology compatible to proestrus; D – Day 2. Cytology compatible to anestrus/proestrus; E – Day 2. Cytology compatible to estrus; F – Day 30. Cytology compatible to estrus; G – Day 120. Cytology compatible to metestrus; H – Day 120. Cytology compatible to estrus; I – Day 0. Cytology compatible to anestrus; J – Day 120. Cytology compatible to anestrus. Papanicolaou, original magnification 200X.

In conclusion, tibolone has estrogenic action in the vaginal epithelium, which is already evident after the first dose and lasts without major changes over time.

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References

[1] Botsis D., Christodoulakos G., Papagianni V., Lambrinoudaki I., Aravantinos L., Makrakis E. *et al*.: "The effect of raloxifene and tibolone on the uterine blood flow and cicatrização de anastomoses de colon endometrial thickness: A transvaginal Doppler a study". *Maturitas,* 2006, *53,* 362.

[2] Shanley D.P., Sear R., Mace R., Kirkwood T.B.L.: "Testing evolutionary theories of menopause". *Proc. R. Soc. B,* 2007, *274,* 2943.

[3] Medeiros S.F., Medeiros M.M.W.Y., Oliveira V.N.: "Climateric complaints among very low-income women from a tropical region of Brazil". *São Paulo Med J.,* 2006, *124,* 214.

[4] Loutfy I., Abdel Aziz F., Dabbous N.I., Hassan M.H.A.: "Women's perception and experience of menopause: a community-based study in Alexandria, Egypt". *East. Mediterr. Health J*., 2006, *12,* S93.

[5] Kloosterboer H.J.: "Tibolone: a steroid with a tissue-specific mode of action". *J. Steroid. Biochem. Mol. Biol.,* 2001, *76,* 231.

[6] Sadarangani A., Salgado A.M., Kato S., Pinto M., Carvajal A., Monso C. *et al*.: "In vivo and in vitro estrogenic and progestagenic actions of Tibolone". *Biol. Res.,* 2005, *38*, 245.

[7] Robinson D., Cardozo L.: "Urogenital effects of hormone therapy". *Best. Pract. Res. Clin. Endocrinol. Metab.,* 2003, *17*, 91.

[8] Blom M.J., Wassink M.G., de Gooyer M.E., Ederveen A.G.H., Kloosterboer H.J., Lange J. *et al*.: "Metabolism of tibolone and its metabolites in uterine and vaginal tissue of rat and human origin". *J. Steroid. Biochem. Mol. Biol.,* 2006, *101*, 42.

[9] Modelska K., Cummings S.: "Tibolone for post menopausal women: systematic review of randomized trials". *J. Clin. Endocrinol. Metab.,* 2002, *87,* 16.

[10] Archer D.F., Hendrix S., Gallagher J.C., Rymer J., Skouby S., Ferenczy A. *et al*.: "Endometrial effects of tibolone". *J. Clin. Endocrinol. Metab*., 2007, *92*, 911.

[11] Piovesan A.C., Júnior J.M.S., Mosquette R., Simões M.J., Simões R.S., Baracat E.C.: "Estudo morfológico e molecular da mama de ratas castradas tratadas com isoflavona ou estrogênios". *Rev. Bras. Ginecol. Obstet*., 2005, *27*, 204.

[12] Jaita G., Candolfi M., Zaldivar V., Zarate S., Ferrari L., Pisera D. *et al*.: "Estrogens upregulate the faz/fasl apoptotic pathway in lactotropes". *Endocrinology,* 2005, *146*, 4737.

[13] Baker D.E.J.: "The Laboratory Rat. Biology and Diseases, Reproduction and Breeding". In: Baker H.J., Lindsey J.R., Weisbroth S.H. (eds.). New York, Academic Press, 1979, 154.

[14] Dimarco N.M., Dart L., Sanborn C.B.: "Modified activity-stress paradigm in an animal model of the female athlete triad". *J. Appl. Physiol*., 2007, *103,* 1469.

[15] Schatz F., Kuczynski E., Kloosterboer H.J., Buchwalder L., Tang C., Krikun G. *et al*.: "Tibolone and its metabolites enhance tissue factor and PAI-1 expression in human endometrial stromal cells: evidence of progestogenic effects". *Steroids*, 2005, *70,* 840.

[16] de Gooyer M.E., Deckers G.H., Schoonen W.G.E.J., Verheul H.A.M., Koosterboer H.J.: "Receptor profiling and endocrine interactions of tibolone". *Steroids,* 2003, *68*, 21.

[17] Cline J.M., Register T.C., Clarkson T.B.: "Comparative effects of tibolone and conjugated equine estrogens with and without medroxyprogesterone acetate on the reproductive tract of female cynomolgus monkeys". *Menopause,* 2002, *9*, 242.

[18] Davis S.R.: "The effects of tibolone on mood and libido". *Menopause*, 2002, *9,* 162.

[19] Morris E.P., Wilson P.O.G., Robinson J., Rymer J.M.: "Long-term effects of tibolone on the genital tract in postmenopausal women". *Br. J. Obstet. Gynaecol*., 2005, *106,* 954.

[20] Rymer J., Chapman M.G., Fogelman I., Wilson P.O.: "A study of the effect of tibolone on the vagine in postmenopausal women". *Maturitas,* 1994, *18*, 127.

[21] Timmer C.J., Verheul H.A.M., Doorstam D.P.: "Pharmacokinetics of tibolone in early and late postmenopausal women". *J. Clin. Pharmacol*., 2002, *54*, 101.

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