Experimental Research

Effects of simultaneous treatment with estrogen and testosterone on the uterus of female adult rats

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Summary

Background: Testosterone (T) associated with estrogen (E) has been used in hormonal replacement therapy in postmenopause women and the effects of this hormonal association on the uterus are not known. *Objective:* To study the effect of long-term simultaneous exposure to testosterone and estrogen on the uterus of non-castrated adult female rats. *Methods:* Groups of ten adult non-castrated female Wistar rats were treated with non-esterified testosterone and beta estradiol (subcutaneous implants with 50 mg of each hormone) or with testosterone cipionate and estradiol valerate (weekly intramuscularly or by subcutaneous injection of respectively, 2.85 mg/kg and 0.166 mg/kg). Control groups received no treatment (10 rats) or injections of diluents (6 rats). All animals were killed six months after hormonal exposure. *Results:* All rats treated with T+E developed hyperplasia and hyperkeratosis of the vaginal and cervical epithelium and focal metaplasia with keratinization of the endocervical and endometrial epithelium. Ascending pelvic inflammatory disease with pyometra and tuboovarian abscesses were frequent (25% mortality until the end of the experiment). *Conclusions:* Testosterone associated with estrogen induced metaplasia of the genital epithelium but did not induce neoplastic lesions. The metaplasic lesions reduced the mucosal defense mechanisms enhancing ascending genital inflammatory disease. Although metaplasia of the cervical and endometrial epithelium has been observed after estrogen exposure in rats, testosterone does not appear to inhibit these estrogen effects.

Key words: Testosterone; Estrogen; Inflammatory pelvic disease; Uterus; Rats.

Introduction

In recent years testosterone has been used in association with estrogen and progesterone in hormonal replacement after menopause [1-4]. Although no relationship between blood androgens levels and endometrial carcinoma has been reported [5, 6], there is not any information about the effects of androgens in association with estrogens on the uterus. It has been reported that estrogens can induce endometrial carcinoma, as demonstrated in epidemiological and experimental observations [7, 8]. In adult rats, chronic estrogen administration induced epidermoid metaplasia of the endometrium [9], but endometrial cancer has only been observed in some specific rat strains [10]. In adult female rats, chronic administration of testosterone induced endometrial hyperplasia [11] and had a promoter action in DMBA-induced endometrial cancer [12].

As there are no reports on the effects of androgens associated with estrogens on the uterus, we studied the effects of these hormones administered together on the endometrium of adult non-castrated female rats.

Material and Methods

Adult females wistar rats (body weight 110 g, around 90 days old) maintained in plastic cages with eight to ten animals per cage, receiving a commercial diet and tap water ad libitum, were used in the experiments.

Testosterone was used in two forms: (a) non-esterified testosterone (Akzo Nobel, Holland), 50 mg/kg in silicone tubes implanted subcutaneously and, b) testosterone cypionate (Deposteron, Sigma Pharma Ltda, São Paulo), 2,85 mg/kg, administered weekly intramuscularly. Estrogen was used as subcutaneous pellets of beta estradiol (Akzo Nobel, Netherlands), 50 mg/kg and estradiol valerate (Smart Chemishe Produkte Handels, Germany), 0,166 mg/kg in corn oil, administered weekly by subcutaneous injection. The doses of testosterone propionate and estradiol valerate were based on doses used for therapeutic purposes in humans [13].

In experiment I, ten rats received one subcutaneous implant of silicone tube with testosterone and one pellet of estrogen on each side of the dorsal line. Ten rats without treatment formed the control group. In experiment II, ten rats received a weekly injection of testosterone cypionate (in the thigh muscle) and of estradiol valerate (subcutaneously). Six control rats received corn oil in the same volume, by the same route.

All the animals were weighed at two-week intervals and observed daily for intercurrences.

All surviving animals were killed after pentobarbital anesthesia, 180 days after the beginning of the hormonal treatment. After inspection of the thoracic and abdominal cavities, the ovaries, uterus and vagina were dissected in one block and fixed in Bouin fixative, diluted 1:4 in 0.15M NaCl solution. After fixation, fragments of the uterine corns and vagina were paraffin embedded and the sections were stained by hematoxylin and eosin and by the picrosyrius method for collagen [14].

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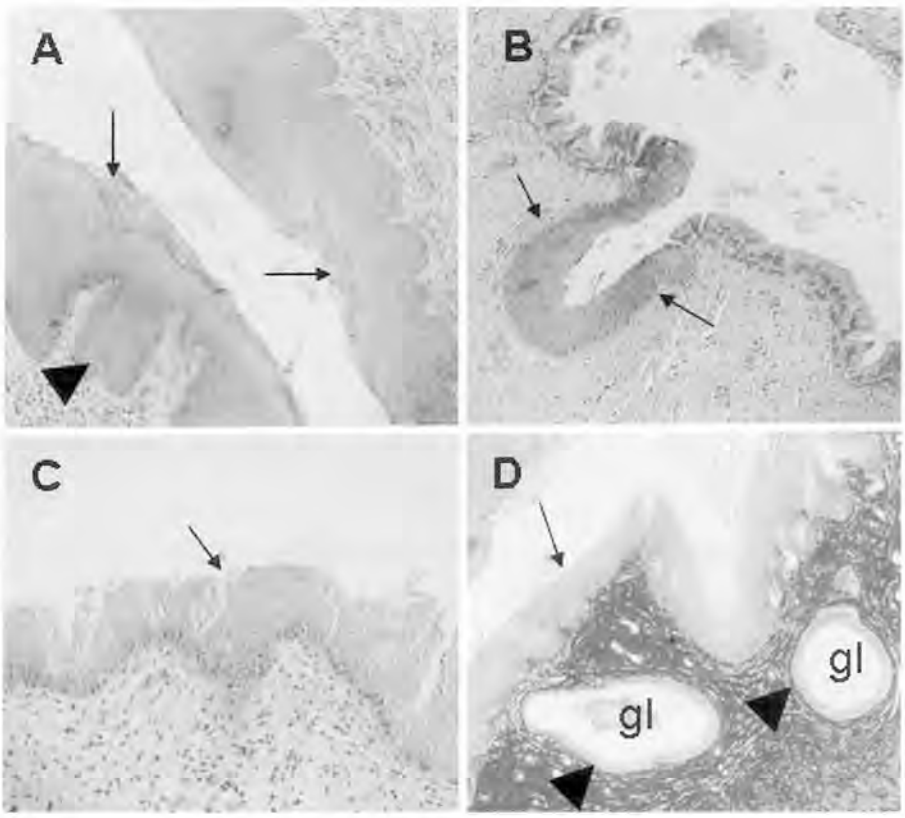

Figure 1. — Microscopic patterns of the vagina, uterine cervix and endometrium of adult rats treated simultaneously with estrogen and testosterone for 180 days. A) Longitudinal sections of the vagina showing severe epithelial hyperplasia (head of arrows), hyperkeratosis (black arrows) and presence of a great number of eosinophils in the stroma (hematoxylin and eosin x 100); B) Uterine cervix with area of epidermoid metaplasia in a cervical gland (arrows); C) Area of epidermoid metaplasia of the endometrium (arrow) with eosinophils in the stroma (hematoxylin and eosin x 100); D) Area of epidermoid metaplasia of the endometrium (black arrow) and normal columnar epithelium lining the glands (heads of arrows) (picrosyrius x 100).

Results

The animals treated with testosterone and estrogen had a lower weight gain than the control group in both experiments.

After three months of hormonal treatment some rats presented severe weight and hair loss and five animals (three receiving the hormones by subcutaneous implants and two in the group receiving the hormones by weekly injection) died before the end of the experiments. The necropsy of all five spontaneously dead rats demonstrated inflammatory pelvic disease with uterus-tubo-ovarian abscesses.

The macro and microscopic pattern of changes observed at autopsy performed after 180 days of hormonal treatment were similar in the two experimental groups and will be described together. All seven surviving rats from experiments I and 6 out of eight survivors from experiment II presented utero-tubo-ovarian abscesses at the end of the experiment. Two animals from experiment II had the uterine horns dilatated and filled with a cloudy fluid.

None of the 16 animals in the two control groups presented macroscopic evidence of inflammatory pelvic disease. The uterine horns had different degrees of dilatation because of the changes in consequence of the estrous cycle.

Microscopy of the uterine cervix and horns did not show any evidence of inflammatory pelvic disease. Some changes in the height of epithelial cells and in the cellularity of the stroma were observed and represented variations of the estrous cycle.

In the animals treated with estrogen and testosterone microscopy of the vagina showed hyperplasia with hyperkeratosis of the epithelium and a great number of eosinophils in the stroma (Figure 1A). In the uterine cervix there was hyperplasia with hyperkeratosis in the first portion of the cervix and epidermoid metaplasia in some areas of the lining epithelium in the second portion (corresponding to the endocervix), sometimes extending to the cervical glands (Figure 1B). In the areas of the uterine horns, without inflammation the endometrial epithelium was columnar and presented focal areas of epidermoid metaplasia, randomly distributed but observed in all animals (Figure 1C). The endometrial gland presented columnar epithelium but without metaplasia (Figure 1D). In some animals the endometrial glands were dilated and lined by cuboid epithelium. Focal areas of endometritis with a great number of neu-

trophils into the lumen of the gland were frequently observed. In the endometrium compromised by the abscess there was the typical pattern of this inflammation: a massive infiltration of neutrophils and macrophages that destroyed the endometrium and the muscle. Gram-stained smears of the purulent material showed a great number of gram-positive and gram-negative microorganisms that were not isolated by culture.

In the two animals without inflammatory pelvic disease the uterine horns presented cuboid epithelial cells and a thin stroma with glands compressed as a consequence of dilatation induced by fluid accumulation.

In all animals the endometrial stroma presented a great number of eosinophils.

Discussion

The results demonstrated that the simultaneous treatment of adult female rats with estrogen and testosterone, administered by subcutaneous implants or by injection, over six months induced in all animals: (a) hyperplasia and epidermoid metaplasia of the vaginal epithelium and focal areas of epidemoid metaplasia of the cervical and endometrial epithelium, and (b) stromal eosinophilia in the vaginal, cervical and endometrial mucosae. In addition the hormonal treatment enhanced severe inflammatory pelvic disease in 90% of the animals.

These effects seem to be a consequence of estrogen action. In fact epidermoid metaplasia has been reported in the endometrium of castrated and non-castrated rats chronically treated with estrogen [9] but was not observed in castrated or non-castrated rats treated by testosterone alone [12]. Chronic administration of testosterone induced adenomatoid endometrial hyperplasia [12]. As this kind of hyperplasia was not observed in any of the rats treated simultaneously with testosterone and estrogen it is possible to conclude that the effect of testosterone was blocked by the presence of estrogen.

In contrast, testosterone did not impair the induction of epithelial metaplasia by estrogen that occurred in all the animals that received the simultaneous treatment with the two hormones. Moreover, it has been reported that in adult female rats, estrogen induced eosinophilia of the stroma, glandular dilatation and increased the length of epithelial cells [15], and all these changes were observed in rats treated with the hormonal association. Thus we can conclude that testosterone did not antagonize the effects of estrogen.

The high frequency of inflammatory pelvic disease observed in the two experiments is another effect of estrogen that was not antagonized by the simultaneous infection of testosterone. In rats treated with estrogen inflammatory pelvic disease was observed 90 days after hormonal treatment, but was not observed in rats treated with testosterone alone for six or more months [16].

The inflammatory pelvic disease observed had the pattern of an ascendant inflammatory disease and could be a consequence of impairment of mucosal defense mechanisms and metaplastic changes of the cervical and endometrial epithelium that enhanced the invasion of microorganisms through the vagina [16].

Conclusion

In conclusion, the simultaneous administration of estrogen and testosterone (subcutaneous implants or injection) in adult non-castrated rats over six months induced hyperplasia with hyperkeratosis of the vaginal epithelium, random focal areas of epidermoid metaplasia of the cervix and endometrium and infiltration of eosinophils in the vaginal and endometrial stroma in all animals enhancing spontaneous inflammatory pelvic disease in 80% of the animals. As all these effects have been described in rats treated with estrogen alone, it is possible to conclude that estrogen action is not blocked by testosterone.

References

[1] Sherwin B.B., Gelfand M.M.: "Differential symptom response to parenteral estrogen and/or androgen administration in the surgical menopause". *Am. J. Obstet. Gynecol.*, 1985, *151*, 153.
[2] Carsson PR., Carson S.A.: "Androgen replacement therapy in women: myths and realities". *Int. J. Fertil.*, 1996, *4*, 412.
[3] Wang C., Swerdloff R.S.: "Androgen replacement therapy". *Ann. Med.*, 1997, *29*, 365 .
[4] Burd I.D., Bachmann G.A.: "Androgen replacement in menopause". *Curr. Women's Health Rep.*, 2,001, *1*, 202.
[5] Judd H.L., Davidson B.J., Frumar A.M., Shamonki I.M., Lagasse L.D., Ballon S.C.: "Serum androgens and estrogens in postmenopausal women with and without endometrial cancer". *Am. J. Obstet. Gynecol.*, 1980, *136*, 859.
[6] Bomans K., Strang P., Backstrom T., Stendahl U.: "The influence of progesterone and androgens on the growth of endometrium carcinoma". *Cancer*, 1993, *71*, 3565.
[7] Herrinton L.J., Weiss N.S.: "Postmenopausal unopposed estrogens. Characteristics of use in relation to the risk of endometrial carcinoma". *Ann. Epidemiol.*, 1993, *3*, 308.
[8] Brinton L.A., Hoover R.N.: "Estrogen replacement therapy and endometrial cancer risk: unresolved issues". *Obstet. Gynecol.*, 1993, *81*, 265.
[9] Baba N., Von H.E.: "Experimental carcinoma of the endometrium, adenocarcinomna in rabbits and squamous cell carcinoma in rats and mice". *Progress Exp. Tumor Res.*, 1967, *9*, 192.
[10] Deerberg F., Kaspareit J.: "Endometrial carcinoma in BD II/Han rats: model of a spontaneous hormone-dependent tumor". *J. Natl Cancer Inst.*, 1987, *6*, 1245.
[11] Laqueur G.L., Fluhmann C.F.: "Effects testosterone propionate in immature and adult female rats". *Endocrinology*, 1942, *30*, 93.
[12] Terada S., Suzuki N., Uchide K., Akasofu K., Nishida E.: "Effects of testosterone on the development of endometrial tumors in female rats". *Gynecol. Obstet. Invest.*, 1993, *36*, 29.
[13] Hardman J.G., Limberd L.E., Molinoff P.B., Ruddon R.W., Goodman & Gilmans: "Pharmacological Basis of Therapeutics", 9th editon, New York, McGraw Hill, 1996, 1415.
[14] Prophet E.B., Mills B., Arrington J.B., Sobin L.H.: "AFIP Laboratory Methods in Histotechnology". AFIP, 1992.
[15] Ramos A.M.G., Pereira F.E.L., Camargos A.F.: "Ação do valerato de estradiol no útero da rata adulta não castrada: análise morfológica". *Revista Médica de Minas Gerais*, 1995, *5*, 30.
[16] Ramos A.M., Perazzio S., Camargos A.F., Pereira F.E.: "Spontaneous inflammatory pelvic disease in adult non-castrated female rats treated with estrogen". *Braz. J. Infect. Dis.*, 2005, *9*, 6.

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