Effects of conjugated estrogens and progestogen in surgically induced endometriosis in oophorectomized rats

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Summary

This study aimed to verify whether estrogen replacement alone or associated with progesterone promotes the recurrence of experimental endometriosis in oophorectomized rats.

The procedure utilized for endometriosis induction was adapted from the one described by Jones (1984). The rats were castrated three weeks after the induction. Hormonal replacement was started 14 days after the castration and was given for 24 days. One group received estrogen alone, another received estrogen associated with medroxiprogesterone acetate, and a last one received placebo.

At the end of this study, the animals who received hormonal medication showed recurrence of the disease. This fact was more evident in the group that received estrogen alone.

We concluded that estrogen alone leads to recurrence of endometriosis in oophorectomized rats with surgically induced endometriosis. The association of medroxiprogesterone promotes involutional changes in the implants, and should be added upon the existence of a past history of endometriosis.

Key words: Endometriosis; Rats; Estrogen; Progesterone.

Introduction

Different types of endometriosis treatment are based on blocking the ovary-released estrogen that results in the atrophy of the ectopic focuses. In some cases, particularity the ones considered advanced, like women with a definite number of children and surgical procedure, etc., bilateral oophorectomy is proposed in order to stop the influence of endogenous estrogens.

The question is to know whether postmenopausal patients should receive estrogen alone or combined with progestins, or whether they should be deprived of any hormonal treatment, taking into account the risk of endometriosis flare-ups [1].

There is a small incidence of endometriosis in postmenopausal women, and only 2-4% of the laparoscopies of patients with endometriosis are performed in the postmenopausal period [2]. There are few investigations into both the incidence of endometriosis in postmenopausal women and the relationship of the disease with hormonal replacement. Most reports on endometriosis in the postmenopausal period deal with complications mainly related to the urinary tract, although respiratory and gastrointestinal complications are also described [3-8]. The women observed by these authors were not necessarily taking hormonal replacement therapy, a fact that challenges most of the authors, and questions if the exogenous administration of estrogen is a mandatory factor for disease progression.

Controlled postmenopausal endometriosis studies in human beings, as well as the course of the disease under hormonal replacement conditions, are very difficult to carry out. Thus, we used an experimental model to study the effects of estrogen, either alone or combined with progestin. Such model comprised the ectopical implantation of endometrium in oophorectomized rats, simulating postmenopausal conditions.

Materials and Methods

Forty adult virgin albino rats (Wistar 1-EPM) weighing about 200 g were kept under routine laboratory care and fed with water and Purina rat chow *ad libitum*. The procedure used for endometriosis induction was adapted from the one described by Jones [9]. Briefly, at the time of surgery, the animals were anesthetized with ethyl ether and their abdomen was shaved; they were submitted to sterile preparation, and opened with a 1.5 cm lower mid-line incision. A cranial segment from the left uterine horn, 4 cm long, was resected. Adequate hemostasis was performed with 6/0 nylon suture. The uterine segment was promptly immersed in sterile isotomic saline at 4°C, opened by longitudinal incision and fragmented in 5 mm squares. Two uterine squares were then fixed with 6/0 nylon suture to the abdominal wall on blood vessels, next to the median suture. The tissues were continuously irrigated with sterile isotonic saline to avoid drying. The abdominal incision was then closed in two layers using 3/0 polyglicolic acid sutures for the muscle and abdominal fascia and 3/0 silk sutures for skin closure. No treatment was given during the postoperative period.

After three weeks the animals were again anesthetized and submitted to bilateral oophorectomy. Length and width of the implant areas were measured. Twenty-one days later, time expected to cause the atrophy of the implants, they were distributed into three groups: Group I (10 rats) was given propylene glycol 0.5 ml/animal/day (oral route); Group II (15 rats) was given conjugated estrogen 50 µg/rat/day (oral route); and, Group III (15 rats) was given conjugated estrogen 50 µg/rat/day combined with medroxyprogesterone acetate 2mg/rat/day (oral route). After 24 days on medication the animals were sacrificed, the implants were measured, sectioned, fixed and processed for light microscopy study (HE).

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Statistical Analysis

Results were analyzed by variance analysis; Student's t-test was also utilized in some instances.

Results

Macroscopic Findings

The development and the growth of the implants were observed in all animals on day 21 of the fixation of the endometrial sections to the abdominal wall. No fragment of the implanted endometrium could be identified, only suture threads in group I at the end of the experiment. However, in groups II and III the sites of the implants were easily detected. Large cystic areas containing serous fluid material were observed in group II.

The mean areas of several implants of the studied groups can be found in table 1 and in figure 1, according to the different periods of the experiment.

Microscopic Findings

Group I (control) - the implant shows only connective tissue, and cells of different shapes can be observed with pyknotic and heterochromatic nuclei in close contact with skeletal muscle fibers. No endometrial glands can be observed.

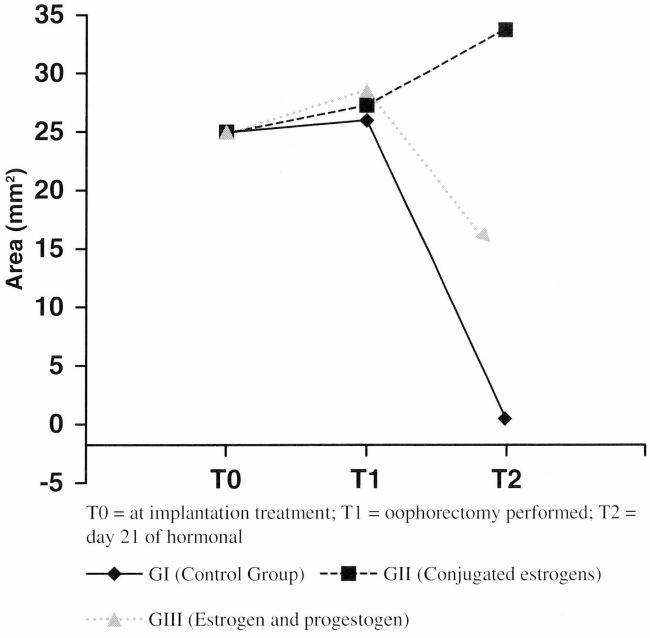
Group II (treated with equine conjugated estrogens) - the implants show large cysts with a smooth thin wall on the striated muscle of the abdominal wall, projecting into the peritoneal cavity. The walls of the cysts are composed of a thin layer of connective tissue, rich in cells, and covered by simple squamous epithelium in the inner part, and by peritoneum in the outer. In some cases the implant architecture resembles the typical endometrium in the estrus phase. The stroma shows numerous fibroblasts, some macrophages with hemosiderin pigment along with plasmocytes and leukocytes. The lining epithelium is made up of cubical or lightly squamous cells depending on the cyst extension status. The epithelial cells show euchromatic nuclei with noticeable nucleoli. Typical mitotic figures are seen in the lining epithelial cells, as well as leukocytes amidst them. Numerous typical endometrial glands lined by a simple cubic or cylindrical epithelium can be observed (Fig. 2).

Group II (treated with estrogen and progestin) - the implants comprise typical endometrial tissue containing

Table 1. — *Mean values (mean±s.d) of endometrium implant in rats observed on day 21 of oophorectomy and hormonal therapy.*

GROUP	T0 (at implantation)	T1 (21 days of implantation)	T2 (21 days of treatment)
I	25.00±0.1	25.61±0.2	0.00**
II	25.00±0.4	25.40±0.4	33.40±0.5
III	25.00±0.5	25.79±0.5	15.06±0.6

*p<0.05 relative to the corresponding 21st day of implantation;
**p<0.01 between groups.



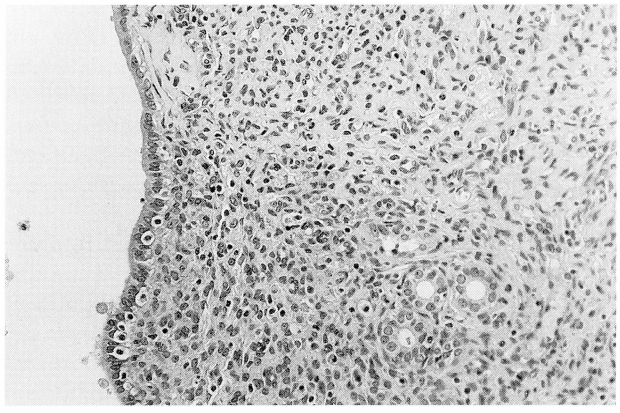


Figure 1. — Photomicrography showing part of the endometrium implant in a group II rat. Note the endometrium covered by a simple cylin epithelium, with endometrium glands (*). Macrophages can be found in the stroma, with hemosiderin pigment. H. E. 210X.

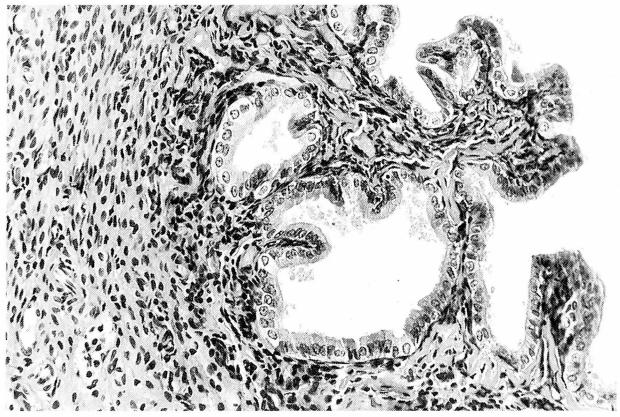


Figure 2. — Photomicrography showing part of the endometrium implant in a group III rat. Observe the superficial epithelium of simple cubic type with several projections into the cyst lumen. H. E. 210X.

surface and glandular epithelium. However, some animals presented small cysts whose lining epithelium exhibited numerous projections into the lumen. The surface and the glandular epithelium are composed of cubic or cylindrical cells containing large euchromatic nuclei with noticeable nucleoli. In the apical area of the cells an eosinophilic material is observed indicating cell secretion. No mitotic figures or leukocyte infiltration are found in this epithelium. The endometrial stroma shows numerous connective tissue cells, and most of them are fibroblasts with large nuclei (Fig. 3).

Discussion

Both the diagnosis and the course of endometriosis implants can only be assessed by invasive procedures, i.e., laparoscopy or laparotomy [10]. Several authors have developed experimental models of endometriosis aiming to study the behavior of ectopic endometrium under different conditions of therapeutic approaches and pregnancy, as well as its relation with fecundity.

Jones, in 1984, used female rats for the first time in the study of endometriosis. He induced the disease by implanting endometrium section extracted from the uterine horn and fixed to the lateral wall of the abdomen. Vernon and Wilson [11], also using rodents, obtained endometriosis by implanting small endometrium sections in the mesenterium and in the utero-ovarian ligament. Sakata *et al.*, in 1990 [12], implanted endometrium sections under the renal capsule of female rats.

In the current study we found significant implant growth three weeks after induction. This finding has also been reported by other authors [9, 13, 14]. No significant difference was found on the surface of the implants in the three groups. All showed homogeneous aspects. At this time, a bilateral oophorectomy was performed in each animal, aiming to cause hypoestrogenism.

In the present study, three weeks was the time period for the hormonal therapy to be started. Other researches have also reported on the length of time hypoestrogenism would take to cause implant atrophy [9, 12, 14, 15].

At the end of hormonal replacement therapy (21 consecutive days), or 42 days after oophorectomy, the groups under equine conjugate estrogen alone or combined with progestin showed recurrence of the disease, more evident when estrogens were given alone. Such result is similar to that or Raikumar *et al.* [14] who, nonetheless, used injectable 17ß-estradiol. Later researchers highlighted that the shorter the time between castration and the start of hormonal replacement, the larger the implants, which shows the importance of a prolonged hypoestrogenism period in preventing recurrence of endometriosis.

On light microscopy, as hormones were added, the implants were found to be composed of endometrium; in the animals with no hormonal replacement, on the other hand, implants were composed of atrophic connective tissue. Following six hormonal therapy cycles clear secretory alterations were noted in the group that received combined therapy, as opposed to the one on estrogen alone. It should be pointed out that the sizes of the implants were also significantly smaller in such group.

There is a debate in the literature regarding the effect of progestins on experimental endometriosis. Jones [9] used levonorgestrel and obtained unexpected results, like an evident remission of the disease with lower doses. Cummings Metcalf [16] used progestins combined with estrone and methoxychlor (pesticide with estrogenic effect) and noticed no benefit with the additional progestin. Following the same line, Dizerega *et al.* [17] noted no advantage in giving progestins combined with estrogens to castrated monkeys.

Castration of animals, as expected, led to implant atrophy, highlighting the importance of estrogens in maintaining the disease.

The hormonal doses used were minimal enough to induce changes in the vaginal epithelium, and compatible with the chosen protocol. Several biological tests (Allan & Doisy test) were performed before the study had started, in order to find the doses of conjugated equine estrogens and medroxyprogesterone acetate by oral route, so that vaginal cytology at the end of the study was compatible with estrus in rats under estrogens alone, diestrus in rats under combined progestins, and atrophy in controls.

Endometriosis flare-ups occurred in rats treated with estrogen alone in the period studied. In the group that also received medroxyprogesterone acetate, the implants suffered involution and exhibited secretory changes. The blockage of endogenous estrogens by means of castration was efficient in promoting regression of the disease.

Therefore, based on the preliminary studies, we suggest that estrogens given alone should be avoided in postmenopausal replacement therapy. If such hormones are needed, they should be combined with progesterone when the patient has a history of endometriosis.

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