A Study of the Role of Sex Hormones in Rat Ovarian Cancer

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Received for publication October 22, 1992

Summary: The effects of estrogen, progesterone, and testosterone on the growth of 7,12 dimethylbenz(а)anthracene (DMBA) induced adenocarcinomas in rats (Wistar strain) were evaluated. Estrogen resulted in the highest acceleration of tumor volume. The histologic features were a solid structure associated with a significant proliferation of connective tissues and with many signet ring cells with intracytoplasmic canaliculi. Progesterone changed the histologic features to a more immature adenocarcinoma associated with a notable solid area with many mitotic figures, although the growth rate of the tumor was the same as the controls. On the contrary, testosterone induced the slowest tumor growth and a histologically scirrhus pattern. The results of this preliminary observation indicate a possible role for sex steroids in the ovarian tumorigenic process.

Key words: ovarian cancer—sex steroid hormones—experimental study—hormone dependence—DMBA

Introduction

During the last three decades, a substantial amount of endocrine research has been devoted to the possible role of steroid hormones in human cancers. More recently, the field has been dominated by the important advances in the area of steroid receptors and their significance in hormone-dependent cancers; e.g., prostate cancer and breast cancer, and these advances have been particularly significant in relation to prognosis and treatment. Since more than half of ovarian epithelial cancers have sex steroid hormone receptors in the tumor tissue (Slotman and Roa, 1988) and the growth of ovarian tumors can be markedly influenced by manipulation of the hormone environment, there is strong circumstantial evidence to suggest that steroids are directly or indirectly involved in the genesis of ovarian tumors (James et al. 1980; Longdon et al. 1990; Peter et al. 1990).

As far as human cancer is concerned, the direct evidence that steroids themselves may be carcinogenic is very limited. Furthermore, Jull (1977) stated in a review on chemical carcinogens that steroids exert only a permissive role. Thus, steroids may operate by allowing or facilitating the primary action of other agents, such as viruses or other hormones; by promoting cell proliferation which provides greater opportunity for carcinogens to initiate a neoplastic change, or by maintaining tumor growth, once it is initiated (James et al. 1980). Despite suggestions from many investigations, the role of steroid hormones in the development of ovarian cancer tissue is still unknown.
To define the hormonal effects on an experimental ovarian cancer, estrogen, progesterone and testosterone were administered to rats with transplanted DMBA-induced cancer tissue. The responsiveness of the DMBA-induced ovarian cancers to hormonal manipulation resembles that of human ovarian cancer, thus indicating a potential usefulness for such studies. This study is concerned with the effects of the hormonal environment on rat ovarian tumor growth.

Materials and Methods

Tumor: The original autochthonous tumor was induced by local application of DMBA to the ovary of a Wistar strain rat according to the "clipping method" of Kato et al. (1974). Transplantation was performed by carefully excising a tumor and mincing it into fragments 2.0 mm in diameter. Tissue for primary grafts was taken from a DMBA-induced autochthonous adenocarcinoma of a rat at the time of sacrifice. Tumor fragments were inserted subcutaneously on the backs of forty-five female rats of the same strain at an age of 2-3 days (Sugiyama et al. 1990). In this study, tumors larger than 500 mm³ were used.

Treatment: The forty tumor bearing rats were randomized into four groups. Each 10 rats received weekly injections of estradiol (0.5 mg/kg/week, 3 weeks, im), hydroxyprogesterone capronate (6.25 mg/kg/week, 3 weeks, im), testosterone enanthate (6.25 mg/kg/week, 3 weeks, im) or 0.1 ml/kg normal saline as a control (Fig. 1). Every week the longest and the shortest diameters of the transplanted tumors were measured using a pair of calipers. The approximate volume of each tumor was expressed as "longest diameter (mm) × shortest diameter (mm)² × 0.5". The mean tumor volume (TV) was plotted on semilogarithmic paper, and the tumor volume ratio (TV of treated group/TV of the controls) was calculated. One week after the last hormonal administration, all the rats were sacrificed and the resected tumors were fixed in Bouin fluid using the maximum diameter section and stained with Hematoxylin and Eosin (H.E.), periodic acid schiff (PAS) for light microscopic observations.

Results

Effects of estrogen, progesterone and testosterone on the increases in tumor volume: The highest tumor volume ratio occurred in the estrogen group, reaching 180% and 150% at the end of the first and the 3rd weeks, respectively. The testosterone group had the lowest ratio; 50% at the end of the 3rd week. The progesterone group had the same ratio as the control at the end of the 3rd week (Fig. 2).
Histological characteristics: The autochthonous adenocarcinoma was a concomitant type adenocarcinoma with both solid and glandular structures. The histology of the serially transplanted original tumor (the control) consisted of an undifferentiated adenocarcinoma, which was surrounded by a scant, delicate fibrovascular stroma (Fig. 3). The tumors exposed to estrogen had remarkably solid structures with many small lumens. Many more mitoses were counted in these tumors than in the controls (Fig. 4). The tumors exposed to progesterone had solid structures with various sized lumens. The tumor cells had abundant cytoplasm, as compared to control cells. Furthermore, more mitoses were also observed in these tumors than in the controls (Fig. 5). Testosterone induced a proliferation and replacement of the fibrous tissue stroma resembling a scirrhus tumor. The tumor cells, however, have less cytoplasm with the same mitotic activity as the controls (Fig. 6).

Fig. 3. Light microscopic section of a control showing an undifferentiated adenocarcinoma with focal necrotic cells and neutrophils (H.E. ×50).

Fig. 4. A tumor from an estrogen-treated rat showing solid structures with many small lumens and high mitotic activity (H.E. ×100).

Fig. 5. Cells with abundant cytoplasm and many mitoses in the progesterone group (H.E. ×50).

Fig. 6. Proliferation and replacement of fibrous tissue stroma in the testosterone group (H.E. ×100).
Discussion

Endocrinological factors in ovarian tumorigenesis and the carcinogenic process are currently receiving considerable attention. A hormone-dependency of human malignant ovarian tumors is supported by epidemiologic studies and therapeutic trials (Tobias and Griffiths, 1976). So far, hormone-dependency has not been demonstrated for any type of neoplasm in the human ovary, but epithelial cancers are derived from Müllerian progenitors and are very likely to be estrogen targets (Lingeman, 1974). Although definite evidence implicating steroid hormones in the development of ovarian epithelial malignancy remains to be established, a possible role of steroids acting on surface epithelial proliferations during fetal and reproductive periods was indicated in previous ultrastructural observations on human and animal ovaries (Gondos, 1975; Motta et al. 1980; Motta and Makabe, 1982). In addition, a previous study demonstrated estrogen receptors in 80% of the rat cancers induced by DMBA, and found that the cancer cells in culture could be induced to proliferate by 17β estradiol (Katabuchi, 1983). Clinically, approximately 50% of epithelial cancers contain elevated levels of cytosolic estrogen receptors and progesterone receptors. After these observation, the effects of estradiol, progesterone and testosterone on ovarian tumor growth were analyzed. In the present study, enlargement of the tumor volume occurred in the estrogen-treated group. The histologic picture was more malignant than the controls.

The epidemiologic evidence for an association between exogenous estrogen and ovarian cancer is mixed and rudimentary. Whereas Hoover et al. (1977) observed a two to three-fold increase in risk for women taking estrogens for menopausal symptoms, Newhous et al. (1977) reported less prior oral contraceptive use among patients with ovarian cancer, as compared to controls. In experiments on dogs, Jabara (1962) has shown that DES induces ovarian tumors. DMBA-induced rat ovarian tumors have hormonal requirements for growth and more estrogen receptors (Katabuchi, 1983).

In the present study, however, the estrogen group had fewer estrogen receptors in the transplanted tumors. Yakushiji (1983) made the same observation. It was suggested that estrogen receptors are essential for hormonally regulated growth and regression of the ovarian tumors, but the absence of estrogen or low levels of estrogen receptors in an ovarian tumor was associated with progression. Estrogen-dependent ovarian tumors should be associated with an increased risk of ovarian cancer among estrogen users, because the presence of estrogen aids the development of estrogen-dependent DMBA-induced mammary tumors in rats (Hugging and Yung, 1962; McGuire and Julian, 1971; McGuire et al. 1977). The progesterone groups had histologically notable solid areas and more frequent nuclear mitotic activities than the controls, although proliferation of the connective tissue was not very remarkable. In spite of the absence of progesterone receptors in the transplanted tumor from progesterone-treated rats, exogenous progesterone promoted the growth of the transplanted tumor, presumably through a hormone receptor. A previous study produced the same result, and found that the DMBA-induced cancer cells in culture proliferated during progesterone application (Kamura, 1983). A number of reports on the apparent benefit from progesterone therapy for some patients provides clinical evidence for possible hormone involvement in some ovarian cancers (Fridman et al. 1978) and led Tobias and Griffiths (1976) to recommend that a trial be initiated with high dose progesterone therapy. Whether the pres-
ence of progesterone receptors, as a marker in ovarian adenocarcinoma, has the importance for predicting probable responses to endocrine therapy as in endometrial adenocarcinoma and as hypothesized for breast cancer remains to be determined (Hoewitz et al. 1975). When considering the therapeutic efficacy of progesterone as a hormone therapy, special attention should be made to the mitogenic activity of progesterone. Testosterone significantly reduced the tumor volume and histologically scirrhus type tumors. The mechanism of action of testosterone in inhibiting the growth of transplanted tumors is not completely understood. This result may be related to an estrogen "antagonistic" action (anti-estrogenic effect) of testosterone. A determination of androgen receptors would increase the likelihood of predicting clinical responsiveness to testosterone therapy (Friberg et al. 1978; Galli et al. 1981; Slotman et al. 1989).

Progesterone and/or testosterone therapy for ovarian carcinoma is worthy of further trials, as it is similar to endocrine therapy for advanced breast cancer or prostatic cancer.

References


Cancer 39, 2934-2947.