Influence of Estrogens and Endocrine Ablation on Duration of Remission Produced by Ovariectomy or Androgen Treatment of 7,12-Dimethylbenz[a]anthracene-induced Rat Mammary Tumors

**Morris N. Teller, Richard J. Kaufman, Matthew Bowie, and C. Chester Stock**

*Divisions of Experimental Chemotherapy and Chemotherapy Research, Sloan-Kettering Institute for Cancer Research, New York, New York*

**SUMMARY**

The 7,12-dimethylbenz[a]anthracene-induced rat mammary tumor system was used to determine (a) the influence of hormonal therapy supplementary to ovariectomy and (b) the influence of endocrine ablation following androgen-induced remission on the incidence and duration of remission. Administration of 17α-thioestradiol, 17α-estradiol, or 17β-estradiol did not increase or decrease the duration of response of ovariectomy-induced remissions. Likewise, duration of remission was not significantly altered when ovariectomies and/or adrenalectomies were performed on rats with androgen-induced remissions. Large doses of the estrogens converted 37.5% of the ovariectomy failures to complete remissions. The results suggest that the concept of estrogen dependency does not by itself explain the beneficial effects of the different therapies and that the cell population responding to the several treatments are one and the same.

**INTRODUCTION**

Human mammary cancer is thought to be hormonally dependent in perhaps a third of the female patients with this disease. The treatment of choice of recurrent systemic mammary carcinoma in premenopausal patients and those less than 1 year postmenopausal is bilateral oophorectomy. That perhaps a third of the patients so treated show some evidence of objective regressions, indicates that the removal of some ovarian hormone(s) (probably estrogen) has a deleterious effect on the tumor cell. Attempts have been made to alter the progression of the disease by further surgical procedures or by the administration of hormones (4, 12, 13). The 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary carcinoma in the rat has been shown to share some of the characteristics of human mammary carcinoma including its reaction to changes in the hormonal environment (1, 2, 5, 6, 15, 17, 20, 21). This experimental system, therefore, has been used to determine (a) the influence of hormonal therapy supplementary to ovariectomy and (b) the influence of endocrine ablation following androgen-induced remission on the incidence and duration of remission.

**MATERIALS AND METHODS**

The induction of mammary tumors in Sprague-Dawley rats was carried out according to the method of Huggins et al. (6). Chemotherapy procedures and dosages followed those described previously (19, 20). 17α-Thioestradiol was generously supplied by Dr. V. A. Drill, G. D. Searle and Co., and also by the Endocrine Evaluation Branch, Cancer Chemotherapy National Service Center, National Cancer Institute, NIH, USPHS, Bethesda, Maryland, which also supplied 17α-estradiol and 17β-estradiol.

Bilateral ovariectomy and/or bilateral adrenalectomy were performed on rats bearing 1 or more growing tumors ≥1 cm in average diameter. Surgery was performed in a sterile environment on rats anesthetized with sodium pentobarbital. Ovaries and, when applicable, also adrenals were removed through 2 lateral incisions made in shaved areas. Silk thread was used to tie off ovaries, but not adrenals, before removal. The musculature was sutured with surgical silk before clamping the skin with wound clips. Drinking water for adrenalectomized rats contained 1% saline.

Steroids were dissolved in sesame oil by stirring. The dose in 0.2 ml of oil was administered i.m. in alternate thighs once a day, 5 times/week for 8 weeks, unless otherwise specified. Tumor-bearing rats treated with sesame oil alone were observed routinely as controls for tumor growth. The anabolic activity of the androgen 2α-methyldihydrotestosterone propionate permitted administration of full doses. Steroids with catabolic activity (estrogens) were reduced in dose and/or in number of injections after the 1st week, and when necessary to prevent a host weight loss greater than 40 gm (approximately 15% of starting weight). Rats with larger weight losses often died early or failed to regain the loss by the end of the 12th week after initiation of therapy. Treatment on postoperative rats began after complete regression of all tumors or, when this was not achieved, within 4 weeks after surgery.

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Tumors were measured biweekly and recorded as averages of 2 perpendicular diameters, 1 across the greatest width. Host weight changes were recorded as percentages at the same time.

The effect of oophorectomy was evaluated at the end of 4 weeks, or earlier when complete remission occurred. A rat was in complete remission only if no tumors were palpable within this time limit. A rat was in partial remission if: (a) the average diameter of all tumors decreased 25% or more during the first 2 postoperative weeks; (b) all tumors 0.5 cm in average diameter decreased; and (c) no palpable mass less than 0.5 cm in average diameter increased. A rat was a nonresponder if the average diameter of tumors did not decrease at least 25% from the maximum size attained during the 4-week period. Ovarietomy failures treated with estrogens and intact rats treated with androgen were evaluated by similar criteria at the end of an additional 4-week observation period following 8 weeks of therapy (19). Evaluations of other treatments were made as indicated. Regardless of treatment, it was considered that remission commenced when all tumors became impalpable and terminated when a tumor appeared at a new site or recurred at a previous site. Death also terminated a remission when it was spontaneous, or induced because of ulceration (usually ear duct tumors) or from other causes. The number of such deaths was similar for all groups. Student's "t" test was used to calculate the significance of difference.

RESULTS

Ovarietomy-induced Remission

Bilateral Oophorectomy. As a baseline for the various experiments, 42 ovariectomized rats with a total of 57 tumors at the time of surgery were held for long-term observation. At the end of the 4th postoperative week, 34 rats (81%) were in complete remission, 4 (9.5%) in partial remission, and 4 (9.5%) were nonresponders. Duration of complete remission ranged from 4 to 59 weeks and was independent of the number of treatments, 42 ovariectomy failures treated with estrogens and intact rats treated with androgen were evaluated by similar criteria at the end of an additional 4-week observation period following 8 weeks of therapy (19). Evaluations of other treatments were made as indicated. Regardless of treatment, it was considered that remission commenced when all tumors became impalpable and terminated when a tumor appeared at a new site or recurred at a previous site. Death also terminated a remission when it was spontaneous, or induced because of ulceration (usually ear duct tumors) or from other causes. The number of such deaths was similar for all groups. Student's "t" test was used to calculate the significance of difference.

Androgen-induced Remission

Androgen Therapy. A group of 78 rats with DMBA-induced, growing mammary tumors were treated i.m. with 2α-methyl沿海derosterone propionate (2α-MDTP) at a dose of 60 mg/injection once weekly for 8 weeks. At the end of 4 additional weeks of observation, 67% of the rats were in complete remission.

![Chart 1. Effect of estrogen treatments on duration of complete remission resulting from bilateral oophorectomy. Each dot represents a single rat. Numbers in parentheses refer to medians.](image-url)
Effect of Endocrine Ablation. To determine the effect on duration of remission, 4 groups of rats with 2α-MDTP-induced complete remissions were treated as follows: ovariectomy, adrenalectomy, ovariectomy + adrenalectomy, or sham operation. A 5th group, nonoperated controls, was composed of rats in complete remission induced by various doses of 2α-MDTP. The durations of remission among the rats treated with the different dosages did not differ significantly (18); they were considered, therefore, as a single unit. Surgery was performed the same week complete remission was attained. Chart 2 shows the duration of complete remission for each evaluated rat. The medians for the variously treated groups ranged from 12.5 to 28 weeks. Ovariectomy and/or adrenalectomy did not significantly prolong duration of CR beyond that of the unoperated rats ($P > 0.05$).

DISCUSSION

The DMBA-induced mammary carcinoma-rat system was used to explore procedures clinically uncommon in the therapy of breast cancer. These involved different but allied modes of treatment in an attempt to prolong remission. Although the neoplasms induced in the rats arise from mammary tissue, are mainly carcinomas and are hormonally responsive, some dissimilarities from the clinical picture are clear. Multiple tumors are produced in the DMBA-treated rats, and the tumors rarely metastasize (3, 10). In addition, remission can be considered terminated when a rapidly growing tumor appears at a new site, rather than when the tumor recurs at the site of regression. The degree this factor influences the real median of duration of remission cannot be ascertained but must be considered when comparisons are made.

Comparisons between medians for duration of remission for ovariectomy-alone and the estrogen-treated groups, as well as the lack of correlation between host weight change and duration of remission, indicated that temporary drug toxicity did not affect the duration of complete remission.

The estrogens, 17α-thioestradiol, 17α-estradiol, and 17β-estradiol, were used because of their different estrogenic potencies (0.02, 0.3 and 5–10 times respectively that of estrone; see Ref. 19) and because their antitumor effects have been compared in experimental chemotherapeutic studies (19). Chart 1 shows there was no advantage in estrogen treatment soon after castration. No significant difference was found between the medians for duration of remission following administration of compound, nor between these medians and that for ovariectomy alone. These results can be compared with those reported for intact, estrogen-treated rats (19): medians for duration of complete remission were 12, 18, and 12 respectively for 17α-thioestradiol, 17α-estradiol, and 17β-estradiol at the same dosages used in the present experiments. These medians likewise did not differ significantly from each other nor from those in the present experiment.

Apparently, estrogen treatment did not increase or decrease (by reactivation of tumor cell growth) the duration of response of ovariectomy-induced remissions (Chart 1). As reported (19), estrogen therapy alone is effective in inducing complete remission of this rat mammary tumor. If the cell population involved in castration-induced remission is different from the cell population sensitive to estrogen treatment, one would expect an increase in duration of response when both methods of treatment are combined. Since this did not occur, the data strongly suggest that the castration-responsive cells and the estrogen treatment-responsive cells are one and the same.

Apparently, it is consistent with this finding that the duration was not decreased in the castration-induced remissions when estrogen therapy was added. If the castration-responsive cells are estrogen dependent, then the effects of castration would have been antagonized by the addition of estrogens. Actually, hormone therapy involved massive amounts of estrogen, whereas ovariectomy removed small amounts.

Thus, the point is raised regarding the known dual aspects of the activity of estrogen. This dual activity was noted by Huggins et al. (7) and Kim et al. (11), among others, and reviewed by Kim (9). Kim et al. (7) observed that small amounts of estrogen stimulated the growth of rat mammary tumors (by an increase of pituitary prolactin), whereas large amounts of estrogen initially stimulated, but eventually inhibited, tumor growth in intact rats. In the latter case, they found the pituitary was enlarged and contained large amounts of prolactin, but plasma prolactin levels decreased. Based on these findings and those of others, Kim (9) suggested that a direct relationship exists between mammary tumor size and plasma prolactin levels and that high doses of estrogen prevent the release of prolactin from the pituitary. The mediatary effect of the pituitary in rat mammary tumor growth was confirmed by Sterental et al. (16). They demonstrated that estrogen administration reactivated tumor growth after adrenalectomy-ovariectomy but not after hypophysectomy. The failure of estrogens to stimulate regrowth of tumors in castration-induced remissions in the present experiments, as shown by the medians for duration of remission (Chart 1), is seen to be consistent with the foregoing when it is realized that the doses of estrogen were massive.

It is also relevant that large doses of estrogens produced complete remission of tumors in approximately 35% of the castration failures (partial responders and nonresponders).
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Based on Kim's conclusion (9), the reason for these conversions becomes clear if it is hypothesized that the failure to respond to castration results from a less-than-critical drop in circulating prolactin secondary to removal of small amounts of estrogen (oophorectomy). The subsequent administration of large amounts of estrogen would cause a reduction in plasma prolactin to below the critical level necessary for growth.

Kennedy (8) induced remissions in premenopausal patients with advanced breast cancer by administration of large doses of estrogen. Pearson et al. (14) reported that small amounts of estrogen reactivate breast cancer in women who previously responded to therapeutic castration and may stimulate growth with advanced breast cancer by administration of large doses of estrogen (oophorectomy). The subsequent administration of large amounts of estrogen would cause a reduction in plasma prolactin secondary to removal of small amounts of endogenous estrogen, whereas the dosages in estrogen therapy are generally 30-50 times the physiologic amounts. All these data pertaining to animals and humans suggest that, following endocrine ablation or hormonal therapy alone, the same cell population responds, and by the same mechanism. Consistent with this observation, there were no increments in median duration of remission when ovariectomies and/or adrenalectomies were performed on the rats with androgen-induced complete remissions. If the concept that more is involved than mere estrogen dependency applies to human mammary cancer, then current thoughts on the management of patients in the advanced stages require modification.

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