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HORMONAL TREATMENT OF ENDOMETRIAL CARCINOMA: AN OVERVIEW AND NEW DEVELOPMENT IN BIOLOGY

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Summary—Progestins are routinely used in the treatment of endometrial carcinomas with about 30% response rate. After a 10–12 month mean response time, the tumors begin to regrow. This clinical situation has been reproduced in the experimental model for human endometrial carcinomas, developed by us. The model consists of growth and maintenance of human endometrial carcinomas of different histologic grade and sex steroid receptor content, in defined hormonal milieu, by serial transplantation in athymic nude mice. Biologically and clinically relevant information on the role of steroid receptors in eliciting hormonal responses, the effect of combination treatment with tamoxifen and progestin and the mechanism of resistance to this treatment after an initial response have been obtained. These studies form the basis for designing and testing rational treatment strategies for human endometrial carcinomas.

INTRODUCTION

Endometrial carcinoma is the most common gynecologic malignancy in the United States [1]. Although 85–90% of patients with endometrial carcinoma are cured by surgery alone, remaining patients have recurrent or metastatic disease. Progestins are routinely used in the treatment of recurrent or metastatic disease with about 30% response rate and a 10–12 month mean duration of response [2]. The mechanism underlying the resistance phenomenon after an initial response to progestin therapy is poorly understood. An experimental model for human endometrial carcinoma where the hormonal manipulation of tumor growth can be systematically examined is essential for a critical evaluation of the resistance phenomenon and to eventually design effective treatment strategies for this disease.

We have previously reported on the development of an experimental system where human endometrial carcinomas could be established by subcutaneous growth in castrated nude mice followed by serial transplantation under varying hormonal milieu [3]. Using this experimental model we have reproduced the resistance and regrowth of human endometrial carcinomas after an initial response to progestin therapy [4]. A series of nude mouse grown endometrial carcinomas of different histologic grade, sex steroid receptor status and growth characteristics available for use in our laboratory is shown in Table 1.

Detailed investigations on the effects of estradiol-17β, progestin and the non-steroidal synthetic agent tamoxifen indicated that: (1) estradiol-17β accelerates endometrial tumor growth and enhances progesterone receptor concentrations in steroid receptor-positive tumors while it had no effect on steroid receptor-negative tumors [3]; (2) in contrast to its antiestrogenic effect on the breast carcinoma, tamoxifen exhibits a true estrogen-agonistic effect viz. accelerates tumor growth and increases PR concentrations in the steroid-receptor positive endometrial carcinoma while having no influence on receptor-negative tumors [4]; and (3) progestin administration after exposure to estradiol-17β or tamoxifen results in typical progestational responses characterized by increased activity of the progestin sensitive enzyme, estradiol 17β-dehydrogenase, induced subnuclear vacuolization and enhanced glycogen and glycoprotein synthesis [4, 5]. These biochemical and morphologic responses of steroid-receptor positive endometrial cancer led us to predict that a combination treatment with tamoxifen and progestin may result in better control of tumor

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Table 1. Characterization of human endometrial carcinomas established by serial transplantation in nude mice

Tumor	Histologic grade	Differential growth with E_2	Passage number	Steroid receptors	Ploidy
EnCa-X	I	+	27	+	Diploid
EnCa-V	III	–	12	–	Diploid
EnCa-K	II	–	31	–	Diploid
EnCa-101	I	+	132	+	Diploid
EnCa-115	II	+	15	+	Diploid
EnCa-117	II	+	19	+	Diploid
EnCa-138	III	–	36	–	Diploid
EnCa-139	III	–	11	–	Diploid
EnCa-143	III	–	7	–	Aneuploid
EnCa-144	II	+	10	+	Diploid
EnCa-154	III	–	9	–	Aneuploid

growth in this experimental model. Since progesterone receptors are essential for the growth inhibitory response of progestin, we postulated that any agent that increased progesterone receptor concentrations within the tumor may increase the degree and duration of response of endometrial carcinomas to progestin therapy. Hence, a treatment protocol using combined tamoxifen and progestin (medroxyprogesterone acetate, MPA).

Indeed, administration of both tamoxifen and MPA proved superior to either tamoxifen or MPA alone in controlling EnCa-X growth [6]. EnCa-X growth was suppressed for a 4 week period following which there was a decrease in tumor size which lasted for a 5–6 week, period. Representative EnCa-X mice treated with tamoxifen alone or tamoxifen + MPA at 9–10 weeks of treatment are shown in Fig. 1. However, after this initial growth suppression phase the tumors became resistant to continued progestin administration and began to regrow at a rate approximating those treated with tamoxifen alone. This phenomenon of resistance to progestin therapy after an initial response is reminiscent of that seen in patients who undergo hormonal treatment for recurrent or metastatic endometrial carcinoma.

The reason for regrowth of tumors following a period of response and regression is unknown. Potentially at least two mechanisms underlying this phenomenon could be visualized: (1) selective growth of steroid receptor-negative tumor cell subpopulations and emergence of progestin-nonresponsive tumor; and/or (2) progesterone receptor downregulation due to continued MPA administration thereby resulting in a progestin-nonresponsive tumor. If the former was true, then serial transplantation of tumors excised during continued progestin administration (resistant/regrowth phase) would be expected to show the lack of accelerated growth in the presence of estradiol-17β. Histological examination of resistant tumor may also be expected to reveal any alteration in morphologic characteristics. If, on the other hand, progestin-insensitivity resulted from lack of progesterone receptor during the regrowth phase it should be evident from receptor analysis. The development of a series of monoclonal antibodies to progesterone receptor using the nude mouse system [7] enabled us to determine the tumor PR status by Western blot analysis, circumventing the technical problems of performing ligand binding assays during progestin treatment.

The properties of tumors during growth arrest and regrowth phases in the tamoxifen + progestin combination treatment group was compared with tumors from control, tamoxifen or progestin alone group using EnCa-101, another well-differentiated, steroid-receptor positive, tamoxifen-sensitive endometrial carcinoma [8]. Administration of MPA to tamoxifen-exposed EnCa-101 resulted in the suppression of tumor growth (Fig. 2). Starting at week 4 of

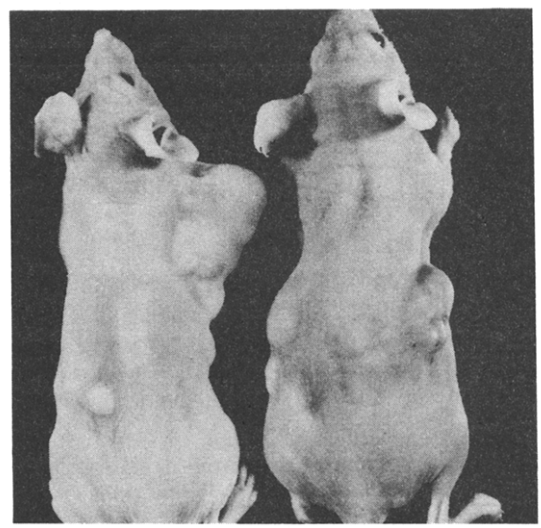

Fig. 1. Representative nude mice bearing EnCa-X grown in the presence of tamoxifen. The animal on left was administered saline and that on right received 1 mg depo Provera i.m. at weekly intervals for 9–10 weeks.

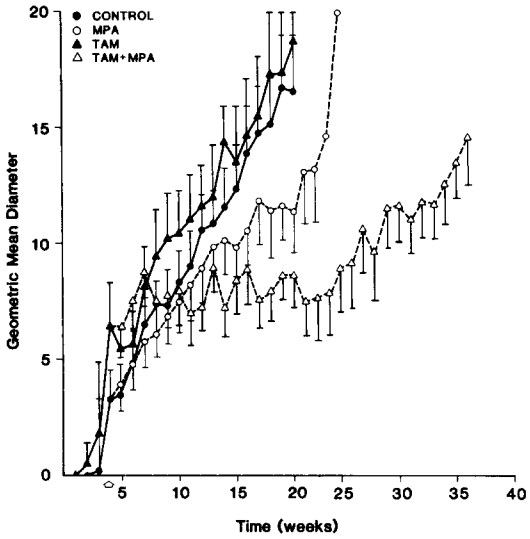

Fig. 2. Combination treatment with tamoxifen and depo Provera on the growth of EnCa-101 grown in nude mice. The geometric mean diameter in millimeters are shown as mean ± SE of 8 tumors/group.

MPA administration, there was a significant diminution and cessation of tumor growth which lasted for about a 20 week period. Then the tumors began to regrow essentially at the same rate as those growing in the presence of tamoxifen, as was previously reported for EnCa-X. Histologic examination of tumors excised from control, tamoxifen, MPA or tamoxifen + MPA treated animals during the growth suppression and regrowth phases exhibited no remarkable alterations in the morphologic appearance.

Tumors from tamoxifen + MPA combination treatment group excised at week 16 (growth suppression) and 30 (regrowth) were serially transplanted in another group of castrated nude mice and the rate of tumor growth with and without sustained estradiol-17β was monitored. The tumor growth was accelerated in the presence of estrogen compared to controls. Further, the progesterone receptor status of tumors, determined by Western blot analysis, revealed its synthesis and reappearance upon estrogen administration. These results indicated lack of any alteration in the original properties of EnCa-101 upon combined treatment with tamoxifen and progestin.

The progesterone receptor status of tumors removed at various times during treatment from all the groups were also monitored by Western blot analysis. While tumors grown in the presence of tamoxifen alone revealed the characteristic progesterone receptor protein pattern of triplets at mol. wt 116,000 and singlet at mol. wt 81,000, the tamoxifen + MPA grown tumors were devoid of these proteins in both cytosolic and nuclear fractions at both growth suppression and regrowth phases. These studies identified that the downregulation and disappearance of progesterone receptor during continuous

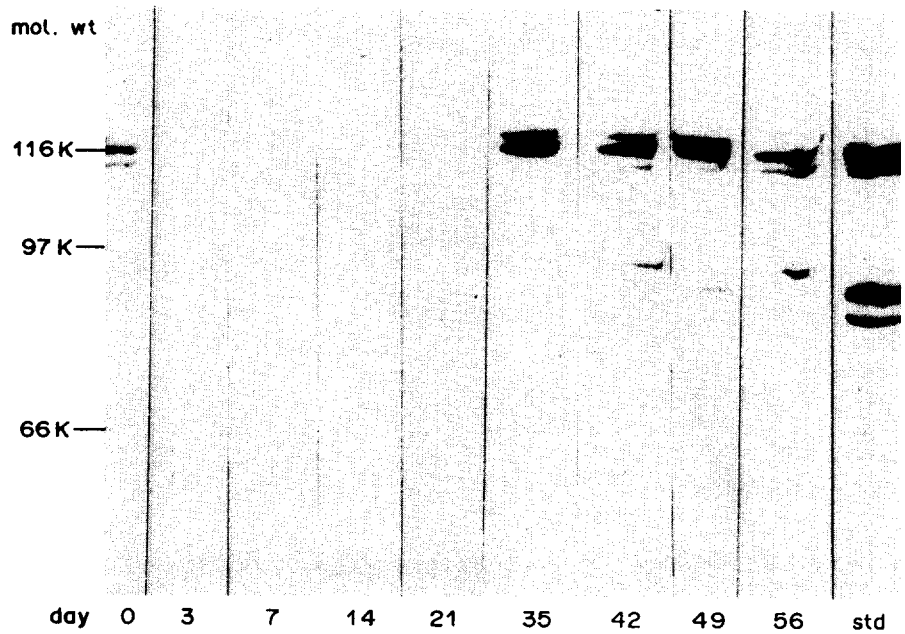

Fig. 3. Western blot analysis of PR in the cytosols of EnCa 101 grown in the presence of 200 pg/ml E_2 and after a single i.m. administration of 2 mg depo Provera. Lane 1—prior to MPA administration; lane 2—3 days; lane 3—7 days; lane 4—14 days; lane 5—21 days; lane 6—35 days; lane 7—42 days; lane 8—49 days; lane 9—56 days after MPA administration. Lane 10—standard PR preparation. Reprinted with permission from *Am. J. Obstet. Gynecol.*

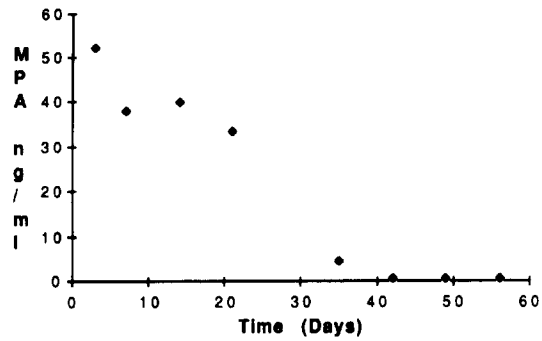

Fig. 4. MPA levels in serum of animals given a single administration of depo Provera. Serum MPA was determined by a specific RIA after 2 mg i.m. administration of depo Provera to EnCa 101 bearing nude mice. Reprinted with permission from *Am. J. Obstet. Gynec.*

progestin administration may render the tumor nonresponsive to progestin.

Since the presence of progesterone receptor is essential for eliciting response to progestin therapy, an intermittant progestin treatment regimen where the tumor cells are allowed to regenerate their complement of progesterone receptor may be expected to render them more responsive to progestin action and overcome the resistance phenomenon observed during continuous progestin therapy. Prior to testing this prediction, we examined the dynamics and modulation of tumor PR status after a single administration of various doses of depo Provera to nude mice bearing EnCa-101 grown in the presence of sustained estradiol-17β. The blood levels of MPA, tumor PR profiles and the tumor growth rates were monitored following a single i.m. administration of 1, 2, 5 and 10 mg depo Provera, using the multisite tumor transplantation system [9, 10].

While a 1 mg dose led to the decrease in the intensity of progesterone receptor bands on Western blot analysis, there was no disappearance of receptor under these conditions primarily due to the rapid clearance of MPA from the blood stream. Administration of a 10 mg dose, on the other hand, resulted in the increasing accumulation of serum MPA for as late as 4 weeks, and the progesterone receptors were absent throughout the experimental period of 28 days. The effects of 2 mg depo Provera revealed the downregulation and disappearance followed by the restoration of progesterone receptor proteins (Fig. 3). The immunostaining intensity of progesterone receptor on Western blots was dramatically reduced between 3 to 7 days (lanes 2, 3) followed by their disappearance between weeks 2 and 3 (lanes 4, 5). The receptor bands begin to reappear at week 4 and achieve essentially the original intensity of estrogen-treated tumors from week 5 and thereafter (lanes 6–9). Interestingly, serum MPA concentrations of 20 ng/ml appears to be a threshold level below which the receptor protein synthesis resumes (Fig. 4). A similar but delayed pattern of

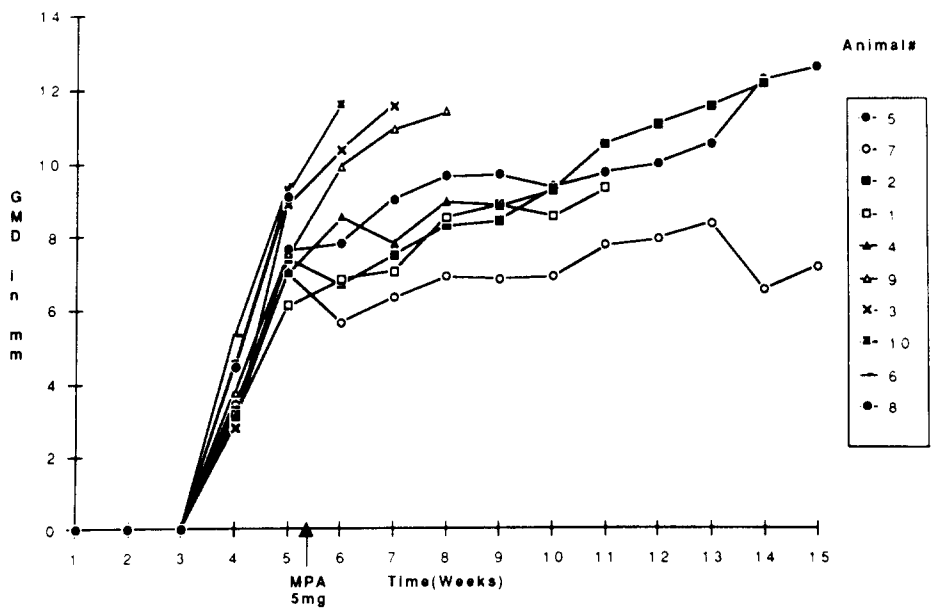

Fig. 5. EnCa 101 growth in nude mice in the presence of E_2 and after a single administration of 5 mg MPA. Tumor growth was monitored by vernier calipers and expressed as geometric mean diameter (GMD) in millimeters. The tumor measurements in individual animals ceased after their killing for tumor PR determinations. Similar tumor growth inhibition was observed with a single injection of 2 mg MPA. Reprinted with permission from *Am. J. Obstet. Gynec.*

reappearance of progesterone receptor was obtained with 5 mg depo Provera. The receptor levels comparable to the estrogen exposed tumors were reached by 13 weeks. Again, the resynthesis of progesterone receptor resumes when the serum levels of MPA were less than 20 ng/ml.

The tumor growth measurements in animals receiving 2 and 5 mg MPA indicated that the tumor growth suppression under the influence of progestin lasted for 6–10 weeks (Fig. 5).

These studies indicate that a 2 mg MPA at 6-weekly intervals would be ideal for testing the efficacy of intermittent progestin administration in the control of EnCa-101 growth. A comparison of the effectiveness of the intermittent vs the continuous progestin administration on the growth of EnCa-101 is underway.

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