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Estrogen and Progestogen Therapy in Advanced Ovarian Cancer: Preliminary Report

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Eleven women with advanced ovarian cancer were treated with a sequential and combined hormonal regimen designed to induce and bind tumor progesterone receptors. Two partial responses were seen, and two patients with a recent history of rapid tumor progression achieved disease stabilization. One patient experienced a transient ischemic cerebrovascular episode while on therapy, and a second patient discontinued therapy because of nausea. The regimen was able to induce progesterone receptors *in vivo*. One patient had no progesterone receptor in a pretreatment tumor biopsy, but did have a high titer of receptors after her first cycle of treatment.

INTRODUCTION

Ovarian cancer represents a solid tumor that has demonstrated favorable response rates to multiple treatment modalities including surgery [1], radiotherapy [2], and cytotoxic chemotherapy [3]. Unfortunately, only 10–15% of patients are cured in advanced stages of the disease. Stanhope *et al.* [4] reviewed the efficacy of second-line chemotherapy after previous treatment including alkylating agents. They found complete or partial response in only 6.1% of patients. During the past 5 years, *cis*-platinum has emerged as a most effective chemotherapeutic agent in ovarian cancer; however, it has significant dose-limiting renal and neurologic toxicity. The successful treatment of ovarian cancer patients, therefore, remains a major challenge in cancer therapy, and new modalities must be explored.

Hormones interact with tissues by binding to specific receptors. It is, therefore, important to establish the presence of receptor activity in tumors to determine the potential for antitumor effect [5]. It is also necessary to determine if hormone receptor binding is capable of generating other biologic effects [6,7]. Such effects may be seen at the cellular level by demonstrating the emergence of characteristic

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proteins. One example is the induction of progesterone receptor in tumor cytosol in response to an estrogen mileu. Ideally, for the purposes of cancer treatment, hormone effect may be demonstrated by decreased malignant tumor growth or by actual tumor tissue regression.

Metastatic breast and endometrial carcinomas have well-established hormonal sensitivity and response to hormonal therapy [8,9]. It has been demonstrated that less than 40% of all ovarian cancer tissues possess a detectable level of specific low affinity, but have high capacitance receptors for progestogen [7,10–16]. In addition, several studies have shown a low response of ovarian cancer to even high doses of progestogen [12,16–19]. If increased progesterone receptor could be generated by a specific treatment modality [20], perhaps the efficacy of progestogen therapy could be enhanced.

Common epithelial ovarian tumors were removed from a group of patients undergoing tumor reductive surgery. Estrogen and progesterone cytosol receptors were measured. A second group of patients was studied to determine if the response rate to progestogen treatment can be enhanced by the induction of increased quantities of tumor progesterone receptor. In one patient, tumor hormone receptor values were measured before and during hormonal therapy.

MATERIALS AND METHODS

A. Clinical study. All postmenopausal patients who were treated at the University of Texas M. D. Anderson Hospital for ovarian cancer and disease progression while receiving an alkylating agent and *cis*-platinum chemotherapy and with an estimated survival of at least 3 months were considered eligible for protocol inclusion. An attempt was made to obtain a biopsy specimen of any accessible tumor for receptor analysis prior to therapy. This was possible in only one patient. Measurable disease was required for entry into the study. This included masses measurable by clinical examination or sonography. Peritoneal or pleural effusions were not considered evaluable. Patients who had undergone recent hormonal therapy or who had unrelieved bowel obstruction were excluded. Patients gave informed consent based on a protocol approved by the Human Investigation Surveillance Committee. Disease status was evaluated at monthly intervals. Attempts were made to repeat tumor biopsy during treatment when possible. Ethinylestradiol, 0.1 mg, was given daily for 25 days, and medroxyprogesterone acetate, 100 mg, was given daily from the 8th to the 25th day of estradiol treatment. No medication was given for 5 days between treatment cycles to allow for regeneration of tumor estrogen receptor. All medications were given orally. Patients and their families were questioned to determine performance status and side effects while on treatment. Performance status was scored on a modification of the Karnovsky scale [21] where 0 corresponded to Karnovsky 90–100, 1 to 70–80, 2 to 50–60, 3 to 30–40, and 4 to 10–20. Standard clinical criteria [22] were used in the classification of treatment results. Evaluable patients received at least two cycles of therapy. Treatment was repeated until disease progression or drug intolerance occurred.

B. Hormone receptor study. Biopsy specimens for receptor analysis were frozen in liquid nitrogen immediately after collection, and a portion of this tissue

was subsequently thawed and processed in the routine manner for histologic examination to confirm that no benign tissue was included. Frozen tissue was powdered in a Thermovac stainless-steel autopulverizer. The powder was then weighed and added to cold 0.1 *M* Tris–Hcl/0.0015 *M* EDTA buffer (pH 7.4 at room temperature) containing 0.5 m*M* dithiothreitol (TED) with 10% glycerol (TEDG). The pulverized tissue was homogenized two times for 10 sec each with 1-min cooling periods using a Polytron tissue homogenizer with a rheostat setting of five. Fresh tissue was homogenized in TEDG buffer with an all-glass Kontes homogenizer with a motor-driven pestle.

The homogenates were centrifuged at 800*g* for 10 min at 4°C. The pellets were washed twice with TEDG buffer, and the supernates from each were added to the 800*g* supernatant. The crude cytosol was then centrifuged at 50,000 rpm for 1 hr. TEDG buffer was added until the final concentration of the cytosols was equivalent to 100 mg tissue/ml.

Determination of specific binding to cytoplasmic estrogen receptors was primarily performed by a gel filtration assay. The assay was performed by adding [^{3}H]estradiol dissolved in ethanol along with 0.01 ml propylene glycol to 12 × 75-mm disposable test tubes that had been heated at 500°F for 3–4 hr. In addition, 100-fold excess of unlabeled dehydrotestosterone was added to block the binding of [^{3}H]estradiol to sex hormone binding globulin. The ethanol was removed under a stream of nitrogen. Cytosol (0.2 ml) was added and the tubes were incubated at 4°C for at least 2 hr. After incubation, an aliquot (0.02 ml) was removed to determine the concentration of ^{3}H-labeled ligand present in each tube. The amount of bound ligand was determined by applying 100 μl to individual Sephadex G-25 columns (5-in. diPSO pipets). After 1 min of 0.004 ml TED buffer wash was applied. Then 0.3 ml of TED buffer was added and the eluant was discarded. The bound ligand was eluted with 0.7 ml of TED buffer directly into 5-ml minivials. Scintillation fluid (3½ ml) was then added for counting.

Quantitative measurements of progesterone receptors were performed by a modification of the hydroxylapatite (HAP) batch assay previously described [23–26]. The HPA assay was performed by adding cytosol (0.2 ml) to tubes that contain hydroxylapatite (0.125 ml) and gently stirred for 30 min. The mixture was then washed with TED buffer (2 ml), containing 5 m*M* KH_2PO_4 (TEDP), and centrifuged at 800*g* for 2 min. The supernatant was discarded and the pellets were incubated with increasing quantities of labeled ligand in 0.3 ml of 0.05 *M* TED containing 200-fold excess of unlabeled cortisol to block binding to cortisol globulin. Following incubation at 4°C for 2 hr, the concentration of free hormone was determined by centrifuging the tubes and removing an aliquot (0.03 ml) of the supernatant for counting. After washing the pellet three times with 0.01 *M* TEDP buffer, the bound hormone was determined by adding scintillation counting solution and then counting.

Analysis of data. Binding data were analyzed according to the method of Scatchard [27] as modified by Rosenthal [28]. Corrections for nonspecific binding were obtained from the horizontal portion of the Scatchard plots as previously described [29]. For single-point assays, specific binding was calculated as the difference in bound radioactivity between samples incubated with and without unlabeled progesterone.

RESULTS

A. Clinical Study (Table 1)

Using the WHO criteria, two partial responses were seen. Two patients with a recent history of rapid disease progression immediately prior to hormonal therapy achieved stabilization of measurable lesions without actual disease regression, and this was accompanied with a subjective decrease in painful symptoms related to malignant masses. No objective response to therapy was seen in six patients, and progression of disease was noted in one. No correlation of response to therapy with performance status was seen. One patient sustained a vertebrobasilar system cerebrovascular accident while receiving her first course of hormonal therapy. A review of her clinical records revealed no predisposing features. She experienced sustained neurologic improvement after medication was withdrawn. Treatment was stopped because of progressive disease in six cases. One patient who achieved partial response on treatment discontinued further therapy after two cycles because of severe nausea, although no increase in nausea was seen in other patients in this cohort.

The patient who underwent tumor cytosol hormone receptor determinations both before and during hormonal therapy (case F) had a histologically confirmed malignant nodule at her vaginal apex that was easily accessible to biopsy. After one cycle of hormonal therapy, her estrogen receptor titer decreased by one-half. Progesterone receptor was not detectable prior to hormonal therapy; however, high levels of progesterone receptor were found after treatments. Hormonal treatment was discontinued after three cycles because no objective response was seen.

B. Hormone Receptor Study

Receptor data on 13 patients with epithelial ovarian carcinoma are presented in Table 2. Two patients had progesterone receptor values in excess of 6000 fmole/g wet tissue, but 8 of 13 had values less than 250. These data include the patient who subsequently demonstrated an increase after estrogen and progesterone therapy. Estrogen receptor values were measured in a wide range without correlation with progesterone receptor values.

DISCUSSION

Patients with ovarian cancer and disease progression on chemotherapy consisting of *cis*-platinum and an alkylating agent have a poor prognosis. This prognosis was reflected in the high mortality from disease progression seen in patients in this study. Any antitumor response seen in such patients is, therefore, of interest. Low rates of response to progestogen therapy in ovarian cancer have been observed in previous studies. Hormonal therapy, therefore, should be considered in these patients. Progestogens may decrease the rate of growth of certain malignant tumors by a transfer of tumor cell population to a G_0 phase of the cell cycle. This may arrest the progression of disease without satisfying criteria for objective partial response. In patients with advanced disease, this may be of significant benefit. There is relatively little information on receptor value in ovarian carcinoma.

TABLE 1
CLINICAL PROFILE

Case	Age	Histology	Stage	Grade	Cycles given	Performance status	Survival[a] (Months)	Response	Comments
A	73	Adeno	III	3	3	3	4	None	Subcutaneous mass unchanged by therapy
B	52	Mesonephroid	III	3	1	2	5	None	Transient ischemic cerebrovascular episode
C	61	Serous	III	3	2	2	2	None	Vaginal nodules unchanged by therapy
D	56	Endometrioid and serous	III	3	3		Alive	Stabilization of a pelvic mass for 3 months that had a recent history of rapid growth	Less pain
E	43	Serous	III	3	2	3	3	None	Bowel obstruction developed on therapy
F	51	Serous	III		2	3	Alive	None	Treatment stopped for no response—vaginal mass biopsy
G	84	Mucinous	III	1	1½	3	2	Rapid progression on therapy	Subcutaneous mass
H	56	Serous	III	2	2	1	Alive	Partial response in a supraclavicular mass for 2 months	Stopped for severe nausea
I	54	Endometrioid	III	2	4	1	6	Pelvic mass decreased for 3 months—confirmed on sonography	Disease progressed after 33 months of therapy
J	54	Adeno	III	3	3	2	Alive	Still on therapy	Stabilization of disease for 3 months after history of rapid growth on cytotoxic chemotherapy
K	44	Serous	III	3	3	3	3	No response	Died—cachexia

[a] Duration of survival since hormonal therapy began.

TABLE 2
HORMONE RECEPTOR PROFILE

Case	Age	Stage	Histology	Receptors (fmole/g wet tissue)		
				Progesterone		Estrogen
1	65	III	Mucinous	<250		535
2	68	III	Endometrioid	6,128		2074
3	46	I	Mucinois	398		2622
4	55	III	Mesonephroid	39,400		530
5	67	III	Endometrioid	<250		466
6	69	IV	Serous	8,746		<25
7	69	III	Serous	1,324		951
8	31	III	Serous	<250		2484
9	57	III	Serous and endometrioid	<250		277
10	63	III	Serous	<250		<25
11	66	III	Mesonephroid	<250		549
12 (Case F)	52	III	Serous	<250	pretreatment	180
				1,816	post-treatment	84
13	46	III	Mesonephroid	1,444		147

It is difficult to determine which values are elevated at this time. We have submitted estrogen and progesterone receptor information as preliminary data and hope to correlate levels ultimately with known prognostic factors. The response rate to even high-dose progestogen therapy could be explained on the basis of low progesterone receptor levels as see in 3 of 13 patients in Table 2. Successful attempts to increase these values could be associated with greater responsiveness to progestogen therapy.

Ovarian tumors with endometrioid histology may have enhanced levels of hormone receptors (10,11) compared with other histologic types. They also may have enhanced response to hormonal manipulation. Two patients in this study had a biologic response, and one patient with endometrioid histology had substantial albeit short-lived response to hormonal treatment.

The presence of vaginal metastases provided an opportunity for monitoring the effect of hormonal therapy on tumor cytosol hormone receptor *in vivo* in one patient studied. Unfortunately, we were not able to correlate this with clinical tumor regression.

Based on these findings, we conclude that hormonal therapy may have a role in the treatment of advanced ovarian cancer. Attempts should be made to monitor receptor assays to increase our understanding of the effect of such treatment on the tumor.

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