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A STUDY OF ESTROGEN AND PROGESTERONE CYTOSOL RECEPTOR CONCENTRATION IN BENIGN AND MALIGNANT OVARIAN TUMORS AND A REVIEW OF MALIGNANT OVARIAN TUMORS TREATED WITH MEDROXY-PROGESTERONE ACETATE

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Abstract. ER and PR were assayed in 13 malignant and 20 benign ovarian tumors of different histologic types. ER was detectable in 67% and PR in 40% of the malignant tumors, compared with 35 and 45% in the benign tumors, respectively. The ER concentration was somewhat higher in the malignant lesions but there was no difference in the PR level.

A retrospective review of 39 primary ovarian tumors of different pathologic stages and classes, treated postoperatively with medroxyprogesterone acetate (MPA, Depo-Provera, Upjohn) and in most cases Melphalan, showed a high Karnofsky performance index and good complete and partial response rates. The need for a prospective, controlled study of the role of MPA treatment of malignant ovarian tumors is evident.

The value of estrogen and progesterone receptor determinations in breast carcinoma is now generally recognized (23). Such determinations are useful in predicting which patients with advanced mammary carcinoma will respond favorably not only to endocrine (1) but also to cytotoxic therapy (18). They also appear to be useful indicators of the general aggressiveness of the tumor and hence of the prognosis (21). Steroid receptor assays are now gradually being introduced for the investigation of other tumor types (16, 28).

Endometrial tumors — which are normally profoundly influenced by steroid hormones — appear to react in the same way as breast carcinoma (2) and, when disseminated, often respond favorably to treatment with hormones, especially gestagens (5, 24).

Gestagen treatment of patients with ovarian carcinoma has been tried, but without any uniform registration of objective responses, patients' ages, type of tumor, hormone preparation and dose used, and without analysis of the responses for any correlation with these factors. Hormone treatment was usually started when all other treatment modes, such as chemotherapy, surgery, and radiotherapy, had failed, but even so some patients seem to have responded (13, 20, 30, 31). It is also more or less generally agreed that gestagen therapy enhances the sense of well-being, alleviates discomfort and pain and improves the quality of the patient's life (30).

The positive results of gestagen treatment of ovarian tumors reported leads to an assumption that certain ovarian tumors contain receptors for steroids. Investigations in this field are still scanty. Table I lists published investigations, which have reported widely varying results. Friedman (9) found the highest PR content in premenopausal women and in well differentiated lesions, while those from postmenopausal women and poorly differentiated tumors had the highest ER titers. Clear cell cancer and mucinous cancer had few receptors. Endometroid cancer and serous tumors were ER-rich and endometroid tumors contained high PR concentrations.

Alkylating agents have been utilized more extensively than any other class of chemotherapeutic agent in patients with advanced ovarian cancer. Melphalan has often been accepted as the standard chemotherapeutic agent with which new drugs and drug regimens should be compared. Response rates have been reported as varying between 35 and 65% (25).

The aim of this investigation was to study the ER and PR content in benign and malignant ovarian tumors. Furthermore, the records from 39 patients with primary ovarian tumors treated with Depo-Provera (MPA) alone or in combination with Melphalan have been reviewed.

Table I. *Previous publications on estrogen (ER) and progesterone (PR) receptors in malignant and benign ovarian tumors.*

Authors	Year	Tumor type	No.	ER + (%)	PR + (%)
Taylor, R. W. *et al.*	1973	Adenocarcinoma	8	0	–
Kiang, D. T. & Kennedy, B. J.	1977	Malignant ovarian	5	40	–
Friberg, L. G. *et al.*	1978	Malignant ovarian	8	25	50
Holt, J. A. *et al.*	1979	Malignant epithelial ovarian	16	50	19
Hähnel, R. *et al.*	1979	Malignant ovarian	4	25	–
Bibro, M. C. *et al.*	1979	Malignant epithelial ovarian	29	100	0
Dapunt, O. *et al.*	1979	Malignant ovarian carcinoma	57	50	32
Friedman, M. *et al.*	1979	Malignant ovarian	34	100	100
		Benign ovarian	2	100	100
Grilli, S. *et al.*	1979	Malignant ovarian	10	60	60
		Benign ovarian	4	25	25
Jänne, O. *et al.*	1980	Malignant epithelial ovarian	21	71	38
		Benign epithelial ovarian	29	83	86
Stedman, K. E. *et al.*	1980	Ovarian carcinoma	11	45	25

MATERIAL AND METHODS

I. Steroid receptors. Primary ovarian tumors, 13 malignant and 20 benign, in 33 women not receiving hormone therapy, were assayed for cytosol estrogen (ER) and progesterone (PR) receptors. Tumor grading was based on the International Federations on Obstetrics and Gynecology (FIGO) criteria of 1965 (17).

The tumor samples were taken without delay and laid on ice after removal of the tumor mass at laparotomy. Non-tumorous tissue was removed and the sample was, when necessary, rinsed in ice-cold saline solution to remove blood and mucus. The tumor sample was divided and part of it was classified for histopathology according to the WHO recommendation of 1973 (26). The samples for receptor studies were put in a dry plastic box and immediately placed on dry ice and stored at -70°C. Receptor assay was performed within 3 months in accordance with methods described elsewhere (3).

Plasma samples for 17-β-estradiol, progesterone, follicle stimulating hormone and luteinizing hormone assays were obtained within 24 hours of the operation.

Table II. *Histological classification (according to the WHO recommendation of 1973) of 13 malignant and 20 benign primary ovarian tumors assayed for estrogen and progesterone receptors.*

	Number of samples	
Tumor type	Malignant	Benign
Epithelial tumors		
Serous	5	12
Mucinous	3	3
Clear cell	2	–
Brenner	–	1
Undiff. carcinoma	1	–
Sex cord stromal tumors		
Granulosa-stromal cell tumor	2	3
Germ cell tumor		
Teratoma	–	1

II. Medroxyprogesterone acetate treatment. A review was made of records of 39 patients, aged 32 to 77 (mean 58) years, with primary malignant ovarian tumors, who were operated on at the Department of Obstetrics and Gynecology, Malmö General Hospital, between December 1975 and January 1980. Histopathologic classification was according to the WHO recommendation of 1973 (26) and tumor grading was based on the FIGO recommendation of 1965 (17).

In most instances bilateral salpingo-oophorectomy was performed, in about half of the cases combined with hysterectomy and/or resection of the greater omentum. In 41% the operation was deemed macroscopically radical.

Radiotherapy was given postoperatively to 12 patients with different types and stages of tumors.

Postoperatively all patients were given 300 mg Depo-Provera (MPA) i.m. every 4th – 6th week, in 33 cases combined with Melphalan, usually 40 – 60 mg i.v., at the same intervals.

In the absence of any sign of recurrence after 2 years, chemotherapy was stopped, but in every case MPA injections were prescribed in perpetuity.

Table III. ***Estrogen (ER) and progesterone (PR) receptor concentrations***[1] *in 13 malignant primary ovarian tumors of different histologic types and tumor grades.*

Histopathological diagnosis	Tumor grade	ER (fmol/mg protein)	PR (fmol/mg protein)
Serous epithelial tumors			
Cystocarcinoma	III	5	568
Papillary cystadenocarcinoma, high diff.	I	ND	ND
Papillary cystadenocarcinoma	IV	5	ND
Papillary adenocarcinoma	IV	49	– [2]
Papillary adenocarcinoma, low diff.	III	64	239
Mucinous epithelial tumors			
Cystadenoma (possible malignant)	I	ND	ND
Cystocarcinoma	I	18	– [2]
Cystadenocarcinoma	I	3	597
Other epithelial tumors			
Undifferentiated epithelial	IV	11	– [2]
Clear cell	I	12	ND
Clear cell	III	ND	ND
Sex cord stromal tumor			
Granulosa theca cell	I	– [2]	330
Unclassified sex cord	II	ND	ND

1) Detection limits: ER ≥ 1 fmol/mg cytosol protein, PR ≥ 50 fmol/mg cytosol protein
2) Tissue volume insufficient for receptor assay

After the operation the women were examined clinically and hematologically every 4th – 6th week and at 3 – 6 month intervals also with ultrasound, computerized tomography, chest X-ray, liver scintigraphy, isotope renography and in some cases with three-dimensional X-ray.

Acceptance of a response as being objective required at least a 50% reduction in the size of a palpable tumor mass, or a 50% reduction in lesion size as demonstrated by radiograph, ultrasound or liver scintigraphy — and then only if the response had lasted for at least 6 months.

Subjective response was evaluated according to Karnofsky (15).

RESULTS

I. Steroid receptors. Mean age in the malignant group was 64 years (range 49 – 77) and in the benign, 61 years (range 36 – 84). There was no significant difference in age distribution between the two groups. Twenty-eight (86%) of the patients were postmenopausal. Seven malignant tumors were in clinical stages I – II and 6 in stages III – IV. The distribution of the histological tumor types is given in Table II.

Tables III and IV show the ER and PR concentrations in the malignant and the benign tumors, respectively. ER were present in 67% and PR in 40% of the malignant ovarian tumors, compared with 35 and 45% respectively, in the benign ones. Among serous tumors, 4 of 5 malignant and 5 of 13 benign lesions were ER+. The frequency of PR+ tumors was the same in the malignant as in the benign serous tumors. In 6 of the 7 ER+ benign tumors, the receptor level was ≥ 10 fmol/mg protein. Only 3 of 8 ER+ malignant tumors had R levels under that limit.

In 5 cases different parts of the tumor were assayed, mostly from solid or fibrous regions, from cyst walls, or from papillary excrescences in cysts. In none of the tumors studied did the concentration of receptors vary significantly from one part to another.

In 3 cases the metastases in the omentum and/or peritoneum were assayed. All the metastases studied contained ER and one, PR as well (Table V).

No correlation was found between receptor positivity, on the one hand, and the patient's age, the clinical stage, or the plasma hormone level, on the other.

II. MPA treatment. Table VI gives the distribution of the histologic groups. Forty-four per cent of the tumors were in clinical stages I – II and 56% in stages III – IV.

In the 33 patients treated with Melphalan and MPA, four serous papillary adenocarcinomas and one low differentiated granulosa theca cell tumor, all in stages III – IV, progressed after explorative laparotomy. The remaining 28 women (85%) responded to treatment. Most of them had Karnofsky performance index 100 until the late stages of tumor progression.

Table IV. *Estrogen (ER) and progesterone (PR) receptor concentrations[1] in 20 benign ovarian tumors of different histologic types.*

Histopathologic diagnosis	ER (fmol/mg protein)	PR (fmol/mg protein)
Serous epithelial tumors		
Cystoma	ND	ND
Cystoma	37	ND
Cystoma	8	200
Cystoma	ND	– [2]
Cystoma	ND	ND
Cystoma	ND	269
Papillary cystadenoma (borderline)	4	ND
Papillary cystadenoma	10	– [2]
Cystadenofibroma	ND	1 129
Cystadenofibroma	ND	556
Cystadenofibroma	ND	466
Cystadenofibroma	ND	– [2]
Mucinous epithelial tumors		
Cystadenoma	ND	ND
Cystoma	ND	ND
Cystoma	ND	– [2]
Other epithelial tumor		
Brenner	6	1 940
Sex cord stromal tumors		
Thecofibroma	ND	361
Thecofibroma	5	2 878
Fibroma	ND	– [2]
Germ cell tumor		
Dermoid cyst	ND	– [2]

1) Detection limits: ER ≥ 1 fmol/mg cytosol protein, PR ≥ 50 fmol/mg cytosol protein.
2) Tissue volume insufficient for PR assay.

In the 6 women treated with MPA alone, one stage IV serous tumor progressed after explorative laparotomy and the patient died 4 months later. Response was seen in the remaining 5 patients, of whom 2 had mucinous cystocarcinomas in stage III with pseudomyxoma peritonei, two mucinous tumors in stage I and IV, respectively, and one with a serous tumor, stage I.

Life table analysis did not reveal any statistical difference in overall survival in the two groups.

DISCUSSION

The results of steroid receptor assays in different studies are not readily comparable, probably due to the different assay methods used, different tumor

Table V. *Estrogen (ER) and progesterone (PR) receptor concentration (fmol/mg cytosol protein)[1] in primary malignant ovarian tumors and their metastases in peritoneal surface and omentum.*

Histopathologic diagnosis	Primary tumor		Metastases in peritoneal surface		Metastases in omentum	
	ER	PR	ER	PR	ER	PR
Serous papillary cystadenocarcinoma	5	ND	4	ND	7	ND
Papillary adenocarcinoma	49	–	10	ND	6	–
Low differentiated papillary serous ovarian tumor						
(right ovary)	17	ND				
			35	ND	11	294
(left ovary)	64	239				

1) Detection limits: ER + ≥ 1 fmol/mg cytosol protein, PR + ≥ 50 fmol/mg cytosol protein.

Table VI. *Histological classification (according to the WHO recommendation of 1973) of 40 malignant primary ovarian tumors treated with MPA, in most cases combined with Melphalan.*

Tumor type	No. of samples
Epithelial tumors	
Serous	23
Mucinous	6
Endometroid	1
Clear cell	2
Undifferentiated carcinoma	2
Sex cord stromal tumors	
Granulosa-theca cell	2
Unclassified	1
Germ cell tumors	
Embryonal carcinomas	2

types, but mostly because of the few cases in each study.

The ER and PR concentrations found in this study are in line with the largest studies (6, 9, 11, 14) in which the frequency of ER+ malignant ovarian tumors was found to be about 40−80%, and the frequency of PR+ malignant tumors somewhat lower.

The beneficial effect of treatment of mucinous malignant ovarian tumors with MPA alone should be regarded with some caution as this type of ovarian tumor often shows a low malignant growth. Our material is too sparse to draw any conclusions about the PR concentration. Friedman *et al.* (9) found a low PR concentration in mucinous tumors. MacLaughlin (19) has shown that MPA binds to both progesterone and testosterone receptors. No studies on the occurrence of the latter type of receptor in mucinous malignant ovarian tumors have been published.

An important question is whether clinical receptor determinations can improve the prediction of the response of ovarian carcinoma to gestagen or any other form of endocrine therapy and also to non-endocrine cytotoxics. Some predictive value is claimed by Bibro *et al.* (4), who examined 29 ovarian epithelial tumors, mainly serous. The tumors contained varying amounts of ER and preliminary results suggested a correlation between the receptor concentration and the response to chemotherapy and the length of survival, but no correlation with histopathology, grade, or stage. A quantitative relationship between the amount of ER in mammary tumors and the response rate to endocrine therapy is reported by some authors (1, 22).

Malignant tumors are heterogeneous cell populations which contain hormone-dependent, receptor-positive cells and hormone-independent, receptor-negative cells in varying proportions. In metastases or recurrent ovarian carcinoma it has only been possible to demonstrate the occurrence of receptors when the primary tumor was receptor-positive, though metastases from a receptor-positive primary tumor often contain only hormone receptor negative cell clones (11). It is probable that endocrine therapy can inhibit growth of the receptor-containing cells, but not of receptor-negative cells (1).

However, until convincing clinical correlation can be demonstrated between receptor concentrations and the response to therapy, it would seem premature to base the treatment of ovarian tumors on receptor assays alone.

By combining hormonal and cytotoxic chemotherapy, preferably in an early stage when the number of autonomous cells is still small, it should be possible to achieve better results. However, it is important to know first whether the cytotoxics used have any undesirable effect on the hormone receptors. Allegra *et al.* (1) found that prior chemotherapy did not significantly alter the incidence of ER positivity or the mean ER value.

Experimental studies with Doxorubicin and Methotrexate showed a dose-dependent effect on the binding capacity of estradiol-17-β to its receptor (7). Other expermental studies on cyclophosphamide treatment (27) have so far failed to reveal any such effect.

Few studies have been reported on the effect of radiological treatment on steroid receptors. Valenstein *et al.*(32) showed that prior radiotherapy did not appear to give false-negative ER assay results in breast cancer, but further studies are needed on the effect of radiotherapy on steroid receptors.

The "anti-estrogenic" effect of MPA can be partly ascribed to a reduction of estrogen receptor concentration. MPA does not bind to ER, but gestagens reduce the production of steroid receptors, thereby diminishing the sensitivity of the tumor to estrogen stimulation. Another consequence of gestagen therapy is a direct effect on gene transcription, which causes a diminished nucleic acid synthesis and mitotic activity.

Thus, many problems are waiting to be solved in the field of hormone receptors and hormone therapy of ovarian carcinoma and other malignant diseases. It is important to perform well designed clinical multi-

center trials and experimental studies as well as biochemical studies on steroid and gonadotropin receptors.

If it can be shown that malignant ovarian tumors respond objectively to gestagen therapy, it would be a great benefit to the patient, since such treatment often produces a valuable subjective effect. This is in great contrast to the profound, deleterious side effects of cytotoxic therapy.

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