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Steroid receptors and response of ovarian cancer to hormones *in vitro*

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Summary. Oestrogen receptor (ER) and progesterone receptor (PR) content and the response *in vitro* to tamoxifen (T), medroxyprogesterone acetate (MPA) and to a combination of the two hormones were determined in 21 epithelial ovarian carcinomas. The response was assessed by the level of adenosinetriphosphate in the cells. ER and PR were detected in 62% and 57%, respectively, with significant variations between the different histopathological cancer types. ER and PR predicted the response *in vitro* in 62% of the tumours exposed to the combined hormones, and in 38% and 33% of those exposed to T and MPA, respectively. The value of steroid-receptor determinations in selecting the proper hormonal treatment in ovarian cancer is significantly reduced because of the high proportion of incorrect predictions.

The value of oestrogen receptors (ER) and progesterone receptors (PR) in predicting the clinical response of breast cancer to hormonal therapy is generally recognized (McGuire *et al.* 1975). But even in breast cancer some receptor-positive patients do not respond to hormonal therapy and 5–8% of the ER-negative tumours respond to various hormonal treatments (McGuire *et al.* 1975). Although steroid-receptor assays are gradually being introduced for the investigation of other tumour types, the relation of receptor content to the clinical response of these tumours to endocrine and cytotoxic treatment is still too uncertain to be of any practical value.

There have been few studies on steroid receptors in ovarian cystadenocarcinomas and the reported precentages of ER- and PR-positive tumours vary widely (Holt *et al.* 1979; Jänne *et al.* 1980; Bergqvist *et al.* 1981; Quinn *et al.* 1982). Friedman *et al.* (1979) found high concentrations of both ER and PR in endometrioid tumours, but steroid receptors have also been detected in serous, mucinous and mesonephroid cancers which traditionally have not been regarded as hormone-dependent. According to Quinn *et al.* (1982) the mean ER content of serous tumours is even higher than that of endometroid tumours.

Clinical response to various hormones has usually been investigated in advanced ovarian cancer, when other treatments such as surgery, radiotherapy and chemotherapy have failed (Varga & Henriksen 1964; Ward 1972; Malkasian *et al.* 1977). However, no controlled trials of hormonal therapy have been reported, and there is an evident need for prospective studies on the role of various hormones in the treatment of malignant ovarian tumours. It is also uncertain whether the presence or absence of steroid receptors in malignant ovarian tumours correlate with the response to hormones.

In this study, we have determined the cytoplasmic ER and PR contents in a series of ovarian epithelial cystadenocarcinomas and correlated these with the repsonse to tamoxifen (T), medroxyprogesterone acetate (MPA) and a combination of both hormones using a new in-vitro method for assessing the sensitivity of the tumour cells (Kangas *et al.* 1984).

Materials and methods

The tumour samples were obtained at primary gynaecological laparotomy before any other treatment in 21 patients. Several biopsies from the areas of solid and active cancer tissue were taken for histology, receptor determinations and tumour cell assay *in vitro*.

Histopathologically, the tumours were classified according to the WHO recommendation (Serov *et al.* 1973); 20 were primary ovarian cystadenocarcinomas and one was a metastatic breast cancer to the ovary. Table 1 details some clinical characteristics of the 21 patients and their tumours; 17 of them were postmenopausal, mean age 59 years (SD 6) and four were of reproductive age, mean age 39 years (SD 5).

Samples for tumour-cell assay were immediately transferred to sterile test-tubes containing RPMI 1640 medium and minced with a scalpel within 1 h of excision. If a sufficient number of living cells was obtained by simple scraping, the red blood cells present in the sample were lysed with NH_4Cl. Living cells were collected by centrifugation and used for culture. If necessary, the tumour cells were detached from the matrix by incubating overnight with collagenase before lysing the red cells. RPMI 1640 medium with 10% fetal calf serum, L-glutamine (292 mg/l), penicillin (100 i.u./ml) and streptomycin (100 μg/ml) was used as cell growth medium. The cells were incubated at 37°C in 5% CO_2 for 1–5 days in microtiter plates (100 μl/sample) or in plastic tubes (1–2 ml sample). The drug effects were assessed by bioluminescence, i.e. by measuring the levels of adenosinetriphosphate (ATP), the basic energy source of living cells (Kangas *et al.* 1984). Briefly, a typical procedure was as follows. Separate plates for each incubation period were prepared. Drugs and cells (100 μl) were added and incubated under the stated conditions. The plate to be measured was removed from the incubator, 100 μl of 1% cold trichloracetic acid was added to the plate—at which point the mixture becomes yellow—and mixed about 15 times with a Finnpipette. A 100-μl fraction was transferred to tubes containing 400 μl buffered (Tris-EDTA 0·25 M, pH 7·75) ATP monitoring reagent (LKB-Wallac, Turku, Finland), and after a short vortexing, the ATP level was read in a luminometer (LKB Luminometer 1250, LKB-Wallac). External ATP standard was added if necessary. ATP levels were

Table 1. Clinical details of the 21 patients and their tumours

Patient no.	Age (years)	Time from menopause (years)	Clinical stage	Histopathological type	Differentiation grade
1	64	15	IV	Serous	3
2	58	11	IV	Serous	2
3	59	8	Ic	Serous	3
4	53	3	III	Serous	3
5	59	6	IV	Serous	2
6	64	12	Ib	Serous	2
7	42	—	Ia	Serous	2
8	70	20	IV	Serous	2
9	58	7	IV	Serous	3
10	55	6	Ia	Mucinous	Borderline
11	32	—	Ia	Mucinous	Borderline
12	57	7	Ia	Mucinous	Borderline
13	52	6	III	Endometrioid	3
14	67	15	IIb	Mesonephroid	2
15	55	2	IV	Mesonephroid	2
16	53	4	IV	Mesonephroid	2
17	41	—	Ic	Mesonephroid	2
18	64	13	III	Undifferentiated	3
19	40	—	Ia	Undifferentiated	3
20	67	20	III	Undifferentiated	3
21	47	2	IV	Metastatic breast cancer in ovary	3

significantly correlated with cell number, viability, ^{3}H-thymidine incorporation and stem-cell assay (Kangas *et al.* 1984).

The hormonal drugs tested were: medroxyprogesterone acetate (MPA; Lutopolar, Medipolar, Oulu, Finland) and tamoxifen (T; synthesized by Medipolar, Oulu, Finland). T and MPA were dissolved in ethanol, which was evaporated to dryness before addition of cells in suspension. The final concentrations were 5×10^{-6} (T) and 5×10^{-5} (MPA), which are similar to IC-50 values for MCF-7 cell line (mammary adenocarcinoma of human origin).

To assess the sensitivity of the tumour cells to the hormones *in vitro*, the cells were cultured for 2 days after which ATP levels were measured. If the ATP level was $\leqslant$50% of the corresponding value in the control culture without hormone, the tumour cells were regarded as having responded to the hormone.

To determine the ER and PR content, the tissue samples were frozen immediately after excision. The tissues were homogenized in ice-cold TEDG-buffer (0·04 M Tris-HCl, 0·002 M EDTA, 0·001 M dithiothreitol and 10% w/v glycerol, pH 7·4) with an Ultra-Turrax 18/10 shaft 10 N. The cytosols were obtained after centrifugation at 40 000 ***g*** at 0–4°C for 1 h. Aliquots of cytosol (0·1 ml) were incubated for 16–20 h at 0°C with six different concentrations 0·16–5 nM ^{3}H-2,4,6,7-oestradiol (115·0 Ci/mmol, New England Nuclear) or 0·32–10 nM (17 α-methyl-^{3}H)-promegestone (R-5020; 87·0 Ci/mmol, New England Nuclear) in 0·1 ml of TEDG-buffer with or without 200-fold excess of unlabelled diethylstilboesterol or R-5020. Bound and free hormones were separated by incubation with 0·5 ml of a mixture of 0·5% charcoal and 0·05% dextran in TEDG-buffer containing 1 mg/ml gelatin for 30 min. After centrifugation, 0·5 ml of the supernatant was used for measurement of the radioactivity. The number of binding sites and the dissociation constants were determined for each sample according to Scatchard (1949). Protein content of the cytosols was assayed according to the method of Bradford (1976).

Results

ER and PR contents

ER ($\geqslant$ 3 fmol/mg cytosol protein) was detected in 13 of the 21 tumours (62%) and PR ($\geqslant$ 10 fmol/mg cytosol protein) in 12 (57%). The highest ER and PR contents were found in the primary serous and endometroid tumours and in the metastatic breast cancer of the ovary (Table 2). The mean ER content was greater in the group of conventionally hormone-dependent tumours, endometroid cancer and metastatic breast cancer, than in the serous ovarian cancers ($P<0·02$). On the other hand, the ER content in serous tumours appeared to be greater than that in the other three epithelial ovarian cancers, and compared with mesonephroid tumours, this difference almost reached statistical significance ($P<0·05$). All of the mucinous tumours had low levels of ER. None of the mesonephroid or undifferentiated tumours contained ER.

The mean PR content of the conventionally hormone-dependent tumour types and serous cancers seemed to be greater than that of the other epithelial tumour types, but the only significant difference was found between the hormone-dependent tumours and the mesonephroid cancer ($P<0·05$). PR was detected in two of the three mucinous cancers but in none of the mesonephroid or undifferentiated tumours.

No correlation was found between receptor content and the patient's age, the clinical stage or the grade of differentiation.

Response to hormones

Of the 21 tumours studied, 7 (33%) responded to T, 8 (38%) to MPA and 15 (71%) to the combination of T and MPA (Table 2). The response rate to combined treatment was statistically significantly greater than that to T alone ($P<0·05$). The mean percentage of living cells after exposure to the combined hormones was 38·5 (SEM 7·4), which was lower than after exposure to T ($P<0·05$; Table 4). The responses to combined hormones were also significantly better than those to T or to MPA alone both in the ER-positive tumours ($P<0·01$ and $P<0·05$, respectively) and in the PR-positive tumours ($P<0·02$ and $P<0·05$, respectively).

The response to a single hormone was weakest in the serous tumours, but six out of nine tumours responded to the combined hormones, the mean percentage of living cells in the in-vitro assay being 44·3 (SEM 6·9). This was significantly better than the response either to T (mean 68·3%, SEM 3·8) or to MPA (mean 73·3%, SEM 5·0) ($P<0·01$ and $P<0·05$, respectively). The responses of mucinous tumours were surprisingly good, both to the single and the combined

Table 2. Steroid receptor content and tumour cell response *in vitro* to hormone exposure in 21 ovarian tumours

Patient no.	Histopathological type	Receptor content (fmol/mg cytosol protein)		Percentage of cells surviving exposure to hormones[a]		
		ER	PR	T	MPA	T+MPA
1	Serous	6	13	61	79	77
2	Serous	28	10	72	51	32
3	Serous	50	16	88	55	4
4	Serous	25	29	53	69	38
5	Serous	43	13	83	78	59
6	Serous	38	464	65	81	58
7	Serous	1	0	99	76	50
8	Serous	31	16	79	58	36
9	Serous	93	42	60	68	45
10	Mucinous	7	44	100	20	16
11	Mucinous	13	16	33	21	9
12	Mucinous	6	0	31	16	7
13	Endometrioid	216	54	26	42	3
14	Mesonephroid	2	0	2	26	1
15	Mesonephroid	0	8	27	28	4
16	Mesonephroid	0	0	83	115	101
17	Mesonephroid	0	0	59	38	21
18	Undifferentiated	1	6	16	2	1
19	Undifferentiated	0	5	98	104	86
20	Undifferentiated	2	7	75	62	111
21	Metastatic breast cancer to ovary	60	222	47	85	49

[a]Cell survival is estimated by the amount of ATP as a per cent of the value in the corresponding control culture; $\leqslant$ 50% indicates a positive response (see text).
ER, Oestrogen receptor; PR, progesterone receptor; T, tamoxifen; MPA, medroxyprogesterone acetate.

hormones. The same was true of the endometrioid tumours and of three of the four mesonephroid cancers. The mucinous and mesonephroid tumours responded better to the combined hormones than to a single hormone, but the difference did not reach statistical significance. One of the three undifferentiated tumours responded to hormone exposure. The metastatic breast cancer in the ovary seemed to be moderately sensitive both to T and to combined hormones.

Correlation between ER and PR contents and hormonal response

The ability of the receptor contents to predict correctly the appropriate response to the different hormones is shown in Table 3. In the 21 tumours, both ER and PR were able to predict correctly the response to T in eight (38%), to MPA in seven (33%) and to the combined hormones in 13 (62%). Thus, the correlation was best for combined drug exposure, although not statistically significant.

Table 4 presents the mean percentages of surviving cells after single and combined hormone treatment in the in-vitro assay in relation to the receptor status of the tumours. There were no significant differences in the mean percentages of surviving cells between the receptor-positive and negative tumours after exposure to a single hormone. After exposure to the combined hormones the mean responses appeared to be higher in the ER-positive than in ER-negative cancers, but the difference did not reach statistical significance. In both the ER-positive and PR-positive cancers, the mean percentages of surviving cells were significantly lower after exposure to the combined hormones than those after exposure to a single hormone.

Discussion

In this study, ER was present in 62% and PR in

Table 3. Correlation between receptor content and tumour cell response to hormone exposure *in vitro*

Patient no.	Histopathological type	Ability of receptor content to predict the appropriate response to hormones[a]					
		ER/T	ER/MPA	ER/T+MPA	PR/T	PR/MPA	PR/T+MPA
1	Serous	–	–	–	–	–	–
2	Serous	–	–	+	–	–	+
3	Serous	–	–	+	–	–	+
4	Serous	–	+	+	–	+	+
5	Serous	–	–	–	–	–	–
6	Serous	–	–	–	–	–	–
7	Serous	+	+	+	+	+	+
8	Serous	–	–	+	–	–	+
9	Serous	–	–	+	–	–	+
10	Mucinous	–	+	+	–	+	+
11	Mucinous	+	+	+	+	+	+
12	Mucinous	+	+	+	–	–	–
13	Endometrioid	+	–	+	+	–	+
14	Mesonephroid	–	–	–	–	–	–
15	Mesonephroid	–	–	–	–	–	–
16	Mesonephroid	+	+	+	+	+	+
17	Mesonephroid	+	–	–	+	–	–
18	Undifferentiated	–	–	–	–	–	–
19	Undifferentiated	+	+	+	+	+	+
20	Undifferentiated	–	–	–	+	+	+
21	Metastatic ovarian cancer (breast ca)	+	–	+	+	–	+
	Positive correlation	8/21 (38%)	7/21 (33%)	13/21 (62%)	8/21 (38%)	7/21 (33%)	13/21 (62%)

[a]Results are expressed as positive (+) or negative (–) correlations; ER and PR are considered negative at levels of < 3 and < 10 fmol/mg cytosol protein, respectively; cell response *in vitro* was considered positive if $\leqslant 50\%$ of cells survived after hormone exposure.

T, Tamoxifen; MPA, medroxyprogesterone acetate; ER, oestrogen receptor; PR, progesterone receptor.

Table 4. Effect of steroid receptor content on tumour cell response to hormone exposure *in vitro*

Steroid receptor content[a]	No. of patients	Percentage of cells surviving exposure to hormones[b]		
		T	MPA	T+MPA
ER-positive	13	61·4 (6·4)*	55·6 (6·8)**	33·3 (6·7)
ER-negative	8	57·4 (13·4)	56·4 (14·1)	46·9 (16·5)
PR-positive	12	63·9 (6·4)***	58·9 (6·4)**	35·5 (6·9)
PR-negative	9	54·4 (12·2)	51·9 (13·2)	42·4 (15·2)

[a]ER and PR are considered negative at levels of < 3 and < 10 fmol/mg cytosol protein, respectively.

[b]Results are shown as the mean (SEM) percentage of surviving cells determined by the amount of ATP as a per cent of the value in the corresponding control culture.

Significance of differences compared with combined hormone (T+MPA) exposure: $^{*}P < 0{\cdot}01$; $^{**}P < 0{\cdot}05$; and $^{***}P < 0{\cdot}02$.

ER, Oestrogen receptor; PR, progesterone receptor; T, tamoxifen; MPA, medroxyprogesterone acetate.

57% of 21 epithelial ovarian carcinomas. The results concur with some other recent studies (Jänne *et al.* 1980; Bergqvist *et al.* 1981; Quinn *et al.* 1982) but disagree with some others (Bibro *et al.* 1979; Holt *et al.* 1979). The lower limit of actual receptor content in ovarian carcinomas is not known and therefore the values reported in the literature differ considerably. In our series, ER content <3 fmol/mg protein and PR content <10 fmol/mg protein were considered negative. These values also represent the lower limits of acceptable receptor contents in mammary carcinomas (Vihko *et al.* 1980).

The highest ER and PR contents were found in the serous and endometroid ovarian cancers and in the breast cancer metastases in the ovary. Receptor contents in the mucinous tumours were low and none of the mesonephroid or undifferentiated cancers was ER or PR positive. Similarly, Friedman *et al.* (1979) and Quinn *et al.* (1982) found only a few receptors in mucinous and mesonephroid cancers, while serous and endometrioid tumours were receptor-rich. Thus, the receptor contents vary considerably according to cancer type and individual variations within each tumour type also occur.

Usually a tumour is most sensitive to the therapy given first (Price *et al.* 1981). In the assessment of adjuvant treatments for each patient, two objectives generally exist; first to distinguish unresponsive from responsive tumours and thereby to avoid ineffective chemotherapy and second, in responsive tumours, to choose the most effective drugs. Studies directed towards the development of human tumour models in nude mice have not been very encouraging (Bjondahl *et al.* 1980; Grönroos & Kangas 1979). However, Bogden *et al.* (1978) and Mäenpää *et al.* (1984) have reported that the subrenal capsule assay has a great predictive value in the assessment of tumour sensitivity to single and combination chemotherapy. On the other hand, a variety of methods have been developed to assess the response of the cultured cells, including morphological assessment of the cultures, inhibition of radioactive precursor incorporation, stem cell assay and, more recently, ATP measurement (Kangas *et al.* 1984). In the present study, the latter rapid and sensitive method was chosen to measure the response to hormones.

The response rate to a combination of T and MPA was 71%, which is rather high. Theoretically, this combination should be the most efficient by acting through the two different steroid receptor mechanisms. On the other hand, the response rates to T and MPA individually were as low as 33 and 38%.

Even if some recent studies have shown that many of the ovarian epithelial cancer types possess steroid receptors, there has been no convincing evidence that these receptors can predict responsiveness of the individual tumours. From our results, it is evident that the receptor contents predict best the response to the combined hormones; both ER and PR had a positive correlation in 62%. This finding is in line with the predictive results in breast cancer, where the receptors have a positive correlation with the clinical response to hormonal treatments in 60–80% of the cases (McGuire 1975).

In conclusion, the steroid receptors could predict correctly the response of the ovarian carcinomas *in vitro* to T or MPA in only 33–38% of tumours, while the predictive value to the combination of these hormones was 62%. Thus, the usefulness of steroid receptors alone in selecting the hormonal therapy in ovarian carcinoma is significantly reduced due to the high proportion of incorrect predictions. By contrast the combined use of steroid receptor determinations and the kind of in-vitro test used in this study, could be valuable in selecting ovarian carcinoma patients for hormonal treatments. These two test systems could actually show the presence of the receptor proteins and, at the same time, indicate the steroid effect within the cells from the receptor levels to the overall biological response.

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