Eur J Cancer Clin Oncol, Vol. 22, No. 1, pp. 105–110, 1986.
Printed in Great Britain.

0277-5379/86$3.00+0.00

Effect of Tamoxifen on Steroid Hormone Receptors and Creatine Kinase Activity in Human Endometrial Carcinoma

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Abstract—*Estrogen receptor (ER), progesterone receptor (PR) and creatine kinase (CK) were measured in cancerous tissue from 29 post-menopausal patients with endometrial carcinoma under basal conditions and after a short course of tamoxifen treatment. ER and PR were detected in nearly all tumors. CK was detected in all of the tumors examined. After tamoxifen, PR and CK increased simultaneously in 26% of cases, while they were either enhanced, decreased or unmodified in the remainder. No correlation could be found between increase of PR and tumor differentiation. CK, however, was enhanced only in the more differentiated cancers. These results indicate that only a percentage of endometrial cancers are responsive to tamoxifen. It is hypothesized that patients bearing these tumors are those likely to benefit from endocrine therapy.*

INTRODUCTION

CLINICAL, epidemiological and experimental data suggest that some human endometrial carcinomas are estrogen-dependent (for a review see [1]). It is also well-recognized that progestins induce objective remission in about one of three patients with advanced or metastatic disease [2–5]. However, on the basis of clinical criteria alone, it is not possible to select those patients likely to respond to progestin therapy [4–7]. A few studies have shown that the measurement of progesterone receptors (PR) — an end-product of estrogen action (8–10) — in endometrial carcinoma may be of help in the selection of patients for endocrine therapy [11, 12]. On the other hand, since PR has been found in the large majority (60–80%) of endometrial cancers, the receptor itself does not seem to be a valuable indicator of estrogen-dependent cell proliferation. By analogy with breast cancer [14, 15], it is possible that certain receptor-positive endometrial tumors do not regress with endocrine manipulations because of a defect in the receptor response pathway distal to the initial binding of steroid to receptor.

Recently, Baulieu and co-workers proposed a 'hormonal challenge test' [16–18] to verify the integrity of the entire metabolic sequence that should be triggered by the binding of hormone to receptor. The test is based on the increase of PR in cancer cells provoked by antiestrogen molecules such as tamoxifen (TAM). In the present study we have extended this hormonal challenge test to include the measurement of creatine kinase (CK) which has been reported to be a very sensitive marker of estrogen action in rodent uterus [19–21] as well as in human breast cancer [22, 23].

Accepted 19 July 1985.
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MATERIALS AND METHODS

Reagents and chemicals

[2,4,6,7-17-beta-^{3}H]-estradiol (99 Ci/mmol), [17-alpha-methyl-^{3}H]promegestone (87 Ci/mmol) were purchased from New England Nuclear. Non-radioactive 17-alpha-promegestone was a gift from Roussel-Uclaf; all reagents and chemicals used were of analytical purity grade.

Patients and tissues

This study included 29 patients with histologically confirmed endometrial carcinoma. All patients had menopaused spontaneously at least 3 yr previously. Uterine curettages were performed on day 0. After the first biopsy, patients received 40 mg of TAM (Nolvadex)® p.o. daily for 7–10 days until the second bioptic specimen was obtained. In most cases this second endometrial sampling was carried out at the time of hysterectomy. A minimum of 100 mg of tissue was obtained, part of

which was submitted to pathology for histologic confirmation. The remainder was rapidly washed with isotonic saline solution and stored in liquid nitrogen until processed (for a period of not longer than 4 weeks). Tumor differentiation was evaluated according to the F.I.G.O. criteria.

Homogenization and cytosol preparation

All operations were carried out at 0–4°C. Tissues were rapidly thawed at 4°C, minced with scissors and homogenized in 6–8 vol of 10 mM Tris–HCl, 1.5 mM EDTA, 0.02% NaN_3, 10% glycerol and 12 mM monothioglycerol, pH 7.4 (TENMG), using a glass–glass homogenizer. The homogenates were centrifuged at 105,000 ***g*** for 45 min to obtain cytosol.

Receptor assay

Because of the limited amount of tissue, a single steroid saturating point assay was used to measure both ER and PR. Cytosol (100 μl) was incubated with either [^{3}H]estradiol (5 nM) or [^{3}H]promegestone (20 nM) for 16–18 hr at 4°C. Non-specific binding was estimated from parallel sets of tubes containing a 200-fold molar excess of non-radioactive diethylstilbestrol (for ER) or promegestone (for PR). After incubation the tubes were placed in an ice-water bath and 400 μl of a suspension of dextran-coated charcoal (0.5–0.05%) in TENMG buffer were added to each tube. After 15 min the tubes were centrifuged at 3000 ***g*** for 6 min and radioactivity in the supernatant counted. Results were expressed as fmol hormone bound/mg cytosol protein. Receptors were considered absent if below 5 fmol/mg protein and 10 fmol/mg protein for ER and PR, respectively.

CK assay

CK activity was measured spectrophotometrically according to the method of Oliver [24]. Results were expressed as units/mg cytosol protein.

An at least two-fold increase (or decrease) with respect to the control (i.e. before TAM) value in either receptor level or CK activity was arbitrarily chosen as indicative of the effect of TAM on tumor cells.

Protein determination

The protein content of the cytosol samples was estimated by the method of Bradford [25], using bovine serum albumin as standard.

RESULTS

Sixteen carcinomas were classified as well-differentiated (WD), seven as moderately differentiated (MD) and six as poorly differentiated (PD). All biopsies, both before and after TAM, were composed to a large extent of carcinomatous tissue and were almost totally devoid of normal or hyperplastic glandular epithelium. TAM treatment did not cause any relevant and reproducible histologic modification. In only two specimens (case Nos 18 and 24, Table 1) was an increase in tumor differentiation from the first to the second biopsy observed.

Effect of TAM on ER and PR concentration

The majority of tumor specimens contained both ER and PR. Overall, 23/26 (88%) had measurable ER (median 35 fmol/mg protein, range 5–540) and 25/27 (93%) PR (median 78 fmol/mg protein, range 10–1099). The distribution of receptors as related to tumor differentiation is illustrated in Fig. 1. ER and PR content progressively decreased from WD to PD tumors.

The concentrations of ER and PR under basal conditions and after TAM administration are shown in Table 1. In three tumors ER were undetectable both before and after TAM. A more than two-fold increase of PR was observed in 11/28 (39%) tumors exposed to TAM. The increase was independent of tumor differentiation (6/16 WD, 3/6 MD, 2/6 PD). One specimen which was initially PR-negative became PR-positive at the second biopsy (case No. 1, Table 1). The mean increase was 192 fmol/mg protein for WD carcinomas, 121 fmol/mg protein for MD carcinomas and 24 fmol/mg protein for PD carcinomas. Following TAM, ER decreased in the large majority of tumors examined.

Effect of TAM on CK activity

CK activity was present in all of the specimens examined (median 0.43, range 0.031–5.185). Enzyme levels did not correlate with tumor differentiation (Fig. 2). After TAM, CK increased in 14/29 (48%) tumors. The increase was more frequently observed in WD than in MD tumors (12/16 vs 2/7). No effect of TAM was noted in PD tumors.

Correlation between receptors and CK activity

No correlation could be found between CK levels and ER or PR content, either under basal conditions or after TAM administration (data not shown). A simultaneous increase of PR and CK was observed in 7/27 (26%) carcinomas. Eleven tumors showed an increase of PR or CK alone while no modification of PR or CK was observed in the remaining 11 cases.

DISCUSSION

In agreement with the results of previous investigators [10, 13], we found that a high percentage of endometrial carcinomas contained both ER and

Table 1. *Effect of tamoxifen on ER, PR and CK in human endometrial carcinoma*

Case	ER(fmoles/mg protein)		PR(fmoles/mg protein)		CK(U/mg protein)	
Well-differentiated	Before TAM	After TAM	Before TAM	After TAM	Before TAM	After TAM
1	0	0	0	16*	0.097	0.640*
2	5	0	0	0	0.170	0.081
3	8	6	47	100*	0.065	0.161*
4	31	23	86	330*	0.337	1.052*
5	35	0	190	120	1.096	0.978
6	41	N.D.	78	149	0.748	1.009
7	65	14	41	108*	1.340	4.393*
8	85	5	23	24	0.261	1.041*
9	98	41	260	1102*	0.162	2.569*
10	151	44	43	54	0.431	1.147*
11	172	34	471	600	2.020	5.091*
12	211	0	645	614	0.211	2.707*
13	414	58	556	549	0.383	2.587*
14	540	99	1099	1218	1.645	2.741
15	N.D.	14	561	1112	1.464	4.035*
16	N.D.	N.D.	537	1763*	1.760	4.576*
Moderately differentiated						
17	5	0	38	34	0.343	0.358
18	5	0	46	26	0.425	0.141
19	23	14	13	172*	0.035	0.020
20	31	10	171	758*	5.185	3.096
21	105	10	37	15	1.440	2.140
22	466	8	21	45*	3.897	8.544*
23	N.D.	N.D.	N.D.	N.D.	0.663	5.673*
Poorly differentiated						
24	0	0	0	0	0.031	0.026
25	0	0	27	0	0.334	0.129
26	5	0	10	35*	1.230	0.994
27	11	11	22	148*	0.405	0.117
28	14	5	151	151	0.739	0.427
29	17	0	57	54	4.240	3.672

*An increase of PR or CK level above two.
N.D., not determined.

PR. Similarly, receptor levels were found to be related to tumor differentiation, higher values being observed in more differentiated cancers [13, 26]. On the other hand, CK activity was not related to tumor differentiation. This is in contrast with the results of a previous study [27] in which the enzyme activity was found to be higher in WD than in PD endometrial carcinomas. However, in that study only a small number of samples were investigated and the brain type isozyme instead of whole CK activity was measured. Nor was any relation found between CK levels and steroid hormone receptor content. A similar lack of correlation between receptors, CK activity and several hormone-dependent enzymes has been observed in human breast cancer [23, 28, 29].

The administration of TAM for a few days caused marked effects both on receptors and on CK activity. As expected, PR concentration was increased after TAM administration and, in one case, induction of receptor was observed. A striking effect of TAM on ER concentration was also observed, this decreasing markedly in almost all tumors. This latter phenomenon, which has been observed by others [30], is probably due to retention of the antiestrogen–receptor complex in the nucleus after its translocation with failure of replenishment of the receptor in the cytoplasm [31, 32]. The proportion of TAM-responding tumors in terms of PR increase found in the present study (40%) is lower than that reported by Mortel *et al.* [10], who observed an increase in receptor levels in 73% cases, and Spona *et al.* [30], who found an increase in 2/3 carcinomas. This discrepancy could be ascribed to the fact that in our study we considered as TAM-responsive only those tumors

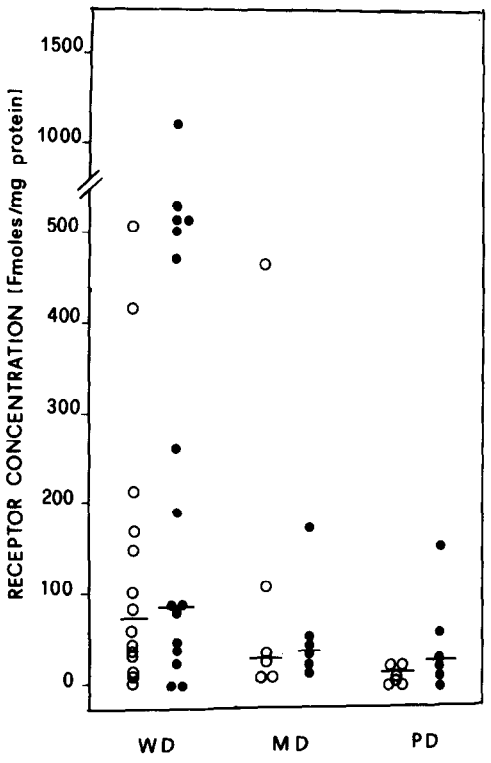

Fig. 1.

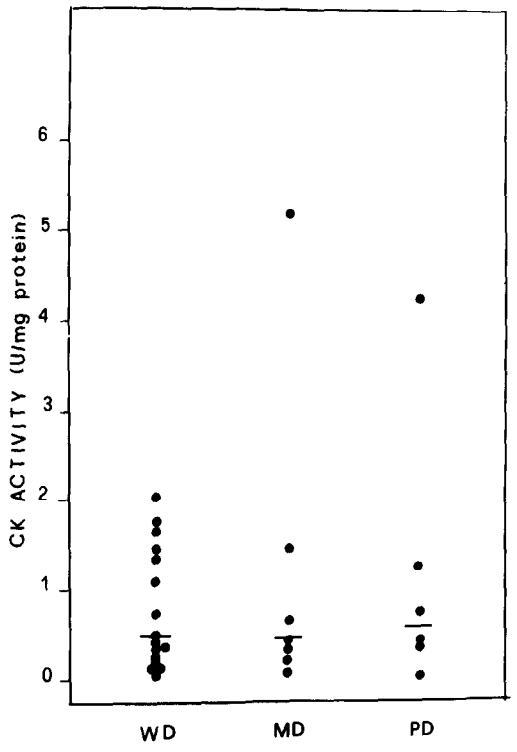

Fig. 2.

showing an increase in receptor levels greater than two over the pre-treatment values, while in the above-mentioned studies no cut-off was employed to evaluate TAM responsiveness.

CK activity was enhanced by TAM administration in about half of the tissue samples analyzed. This increase of CK by TAM is difficult to explain, primarily since not much is known yet on the hormonal regulation of this enzyme in humans. In human endometrium CK activity progressively increases during the follicular phase of the cycle, with maximum levels occurring during the luteal phase [27, 33, 34]. Amroch *et al.* have recently furnished evidence that enzyme activity is estrogen-responsive in human breast cancer maintained in short-term organ culture [23]. These data could indicate that CK activity is estrogen-responsive in humans and that the increased levels noted after TAM are due to the well-known estrogen-like activity of the antiestrogen [31, 32, 35–37]. Further *in vitro* studies utilizing estrogen-responsive human cell lines should clarify this point.

A simultaneous increase of PR and CK due to TAM was noted in 7/27 (26%) tumors. The reason why only a limited percentage of endometrial carcinomas respond to TAM in terms of PR and CK is not clear. It could be ascribed to the different cellular distribution of these two proteins. Endometrial cancer, as well as normal endometrium, is composed of both stromal and glandular epithelial cells, in different proportions. While PR (and ER) is located in both these two cell types, biochemical and immunohistochemical evidence suggests that CK is preferentially or almost exclusively located in the glandulo-epithelial cells [34, 38]. Thus relative proportions of neoplastic epithelial and normal stromal elements from one tumor to another or within the same tumor mass could account for the differential response of PR and CK to TAM. However, careful histologic evaluation confirmed that all the specimens assayed here were composed to a large extent of malignant cells. Alternatively, by virtue of the genetic alteration in cancer, only some endometrial carcinomas could have maintained a full responsiveness to the hormonal (antiestrogenic) signal. One intriguing possibility to be verified is that these carcinomas could be those readily responding to endocrine therapy.

In conclusion, from the biochemical analyses performed in this study, it appears that human endometrial carcinoma is a heterogeneous neoplasia. This is also reflected clinically by the variable response to endocrine therapy. Hopefully, the combined measurement of PR and CK in the form of the dynamic challenge test as used in the present study may provide a reliable means of selecting those patients likely to benefit from hormonal therapy. Finally, since TAM increases PR, thus rendering the cells more responsive to progestins, without having a growth-promoting effect on cancer cells, it is conceivable that the sequential administration of TAM and progestins may produce better results (in terms of duration of response and survival) than a single drug treatment schedule.

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