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THE EFFECTS OF MEDROXYPROGESTERONE ACETATE ON ENZYME ACTIVITIES IN HUMAN ENDOMETRIAL CARCINOMA

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Summary—Creatine kinase (CK), isocitrate (ICDH) and glucose-6-phosphate dehydrogenase (G-6-PD) activities and cytosol oestrogen (RE_c) and progestin (RP_c) receptors have been measured in twenty post-menopausal subjects with endometrial carcinoma. Diagnostic curettage and hysterectomy specimens were obtained from each patient with intervening medroxyprogesterone acetate therapy (MPA). In RP_c rich tumours the activity of creatine kinase was significantly increased following MPA and was attributed to an increase in the BB-isoform. There was a similar though less significant increase in ICDH activity. Neither CK nor ICDH were significantly increased following MPA therapy in progesterone receptor poor specimens. There was a small but significant increase in G-6-PD activity following MPA regardless of RP_c content. RE_c was decreased in some but not all RP_c rich tumours following MPA and the trend was significant. The highly significant increase in CK activity confirms the apparent response of this enzyme to progesterone in normal human endometrium during the menstrual cycle.

INTRODUCTION

The effects of progestins on the uterus are thought to be mediated at least in part, by intracellular progestin receptors [1]. Available evidence suggests that the presence of such receptors in human endometrial carcinoma cells may provide a reliable index of their responsiveness to progestin therapy [2, 3]. However, the presence of progestin receptor does not guarantee cellular response as there may be biochemical defects distal to the step of steroid–receptor interaction. A biochemical end-point of progestin action would provide a more accurate assessment of sensitivity and in this respect oestradiol-dehydrogenase, isocitrate dehydrogenase (ICDH)- and glucose-6-phosphate dehydrogenase (G-6-PD) have been shown to be progestin sensitive in human endometrium [4–6]. Recently creatine kinase (CK) which is rapidly induced in response to oestradiol in the rat uterus [7] has been described as progesterone sensitive in human endometrium [8, 9].

In the present study, therefore, the effects of medroxyprogesterone acetate on CK, ICDH and G-6-PD activities have been measured in human endometrial carcinomatous tissue. Curettage and hysterectomy specimens were obtained from each patient who received intervening MPA treatment thus allowing direct comparison of paired samples. The effects of MPA on enzyme activites were compared with the effects on oestrogen and progestin receptor.

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EXPERIMENTAL

Endometrial samples from 20 postmenopausal women were obtained at the time of diagnostic curettage and endometrial carcinoma was diagnosed histologically. Patients were then treated with medroxyprogesterone acetate (MPA) at a dose of 200 mg twice daily for at least 4 days prior to hysterectomy when the matched hysterectomy sample was obtained. Receptor status was measured in all subjects whereas enzyme activity was measured in 16 patients only.

Materials

[2,4,6,7-^{3}H] oestradiol (90 Ci/mmol) and [1,2,6,7-^{3}H] progesterone (80 Ci/mmol) were obtained from the Radiochemical Centre, Amersham, Buckinghamshire, U.K. All other chemicals were purchased from Sigma Chemical Company, Poole, Dorset.

Methods

The samples were stored in liquid nitrogen prior to assay. The tissue was washed and then homogenised in 9 vol of buffer [sodium phosphate (10 mM), dithiothreitol (1 mM), glycerol (10% v/v) pH 7.4] by 10 hand-operated strokes of a Teflon pestle in a glass homogeniser at 4°C. An aliquot of the homogenate was removed for DNA estimation by the method of Burton[10]. The homogenate was centrifuged at 800 *g* for 10 min and the nuclear oestrogen receptor measured in the resulting pellet by the method described by King *et al.*[11]. The supernatant was removed and centrifuged at 200,000 *g* for 1 h to provide the cytosol

in which REC, RPC and the enzyme activities were assayed. A portion of the supernatant was also retained for protein determination by the method of Lowry[12].

RE_c and RP_c were measured as previously described [13]. The enzyme activities were all measured spectrophotometrically at 340 nM in an assay volume of 3 ml at 25°C. CK activity was measured by a coupled enzyme assay in a mixture containing imidazole acetate (50 mM) pH 6.7, phosphocreatine (9 mM), glucose (5 mM), ADP (2 mM), magnesium acetate (20 mM), $NADP^+$ (0.5 mM), diadenosine pentaphosphate (40 μm), dithiothreitol (5 mM), hexokinase (IU), glucose-6-phosphate dehydrogenase (0.5U). Isoenzymes of CK were separated on a DEAE-cellulose A50 ion exchange column which had been equilibrated with buffer [NaCl (20 mM); Tris–HCl (100 mM) pH 7.9]. The MM isoenzyme was not adsorbed, the MB form was eluted with 40 mM NaCl, 100 mM Tris–HCl pH 6.4, 5 mM $MgSO_4$ + 0.4 mM EDTA and the BB isoenzyme was eluted with 350 mM NaCl in the same buffer. The activities of each isoenzyme were then measured spectrophotometrically. G6PD activity was measured in an assay mixture containing Tris–HCl (50 mM) pH 7.5, glucose 6 phosphate (1 mM), $NADP^+$ (0.4 mM) and magnesium chloride (6 mM).

ICDH activity was assayed in a mixture containing Tris–HCl (50 mM) pH 7.5, isocitrate (4 mM), $NADP^+$ (0.3 mM) and manganese chloride (4 mM). One unit of enzyme activity was defined as that which produced 1 μmol of reduced cofactor per minute at 25°C.

RESULTS AND DISCUSSION

Creatine kinase activity

Total creatine kinase activity was measured in 16 patients, and the isoenzyme composition analysed in 14 of these, before and after treatment with medroxyprogesterone acetate (Table 1). The data has been grouped according to RP_c status using a cut-off value of 20 fmol/mg protein to distinguish between receptor rich and poor specimens. There was no significant difference in total creatine kinase activity in the curettage samples of RP_c rich and poor specimens (Table 1), in which, with the exception of two RP_c poor specimens, CK-BB was the predominant isoenzyme. No significant difference was observed in the activity of corresponding isoenzymes in RP_c rich and poor curettage specimens. Following treatment with MPA there was a highly significant increase in total creatine kinase activity in the RP_c rich group only; this increase in enzyme activity was predominantly due to an increase in the BB-isoform (Table 1). There was no correlation between the concentration of RP_c in the D&C specimens and the change in CK-BB activity in either the RP_c rich ($r = 0.318$, $n = 7$) or RP_c poor ($r = 0.182$, $n = 7$) group. The data in Table 1 is summarized graphically in Fig. 1 which illustrates the predominance of the BB-isoform of creatine kinase and its increase following treatment with MPA.

In order to further evaluate the effect of MPA on CK-BB activity in each patient the change in activity was considered relative to RP_c concentration (Fig. 2). The arbitary cut-off value of 20 fmol/mg protein RP_c distinguished six out of seven patients with significantly elevated values of CK-BB activity following MPA; the remaining patient had an RP_c value of 14 fmol/mg protein. However, setting a lower cut-off value to exclude an RP_c of 14 fmol/mg protein would also include two samples that showed little or not change. It may be more appropriate to consider a value of CK-BB in hysterectomy specimens follow-

Table 1. Activity of CK and its isoforms before and after MPA treatment in RP_c rich and poor specimens

	Total CK		CK-MM		CK-MB		CK-BB	
	D&C	Hyst	D&C	Hyst	D&C	Hyst	D&C	Hyst
RPC rich	(a)		(b)		(c)		(d)	
1	2.08	15.26	0.19	0.58	0.04	0.15	1.84	14.53
2	0.74	14.77	0.19	0.84	0.16	0.87	0.39	13.03
3	6.86	17.72	0.82	1.79	0.03	0.11	6.01	15.82
4	1.94	14.66	0.64	0.38	0.11	0.13	1.19	14.14
5	1.17	8.00	0.38	0.31	0.02	0.06	0.76	7.64
6	0.66	6.31	0.30	0.40	0.05	0	0.35	5.91
7	3.48	3.64	0.85	0.73	0.03	0.06	2.59	2.85
8	0.02	2.57	—	—	—	—	—	—
RPC poor	(e)		(f)		(g)		(h)	
1	0.83	1.45	0.54	0.96	0.01	0	0.28	0.49
2	2.19	0.50	0.32	0.23	0	0.17	1.87	0.09
3	1.36	2.53	0.18	0.07	0	0.06	1.17	2.41
4	1.05	3.17	0.33	0.36	0.19	0.58	0.53	2.23
5	1.02	1.30	0.19	0.23	0.13	0.17	0.70	0.90
6	0.47	11.51	0.32	0.90	0.09	0.09	0.07	10.52
7	1.02	2.32	0.25	0.17	0.18	0.10	0.59	2.05
8	2.40	1.70	—	—	—	—	—	—

Total creatine kinase activity (U/mg DNA) and that of the MM, MB and BB isoenzymes, were measured in curettage (D&C) and hysterectomy (hyst) specimens with intervening MPA therapy. RP_c rich and poor specimens were distinguished using a cut-off value of 20 fmol/mg protein. The significance of the difference between D&C and hyst values in each patient was determined by the Wilcoxon's signed ranked test for paired observations. Significance of the difference between D&C and hyst: (a) $P < 0.005$; (b) $P = 0.353$; (c) $P = 0.108$; (d) $P < 0.01$; (e) $P = 0.234$; (f) $P = 0.8$; (g) $P = 0.295$; (h) $P = 0.205$.

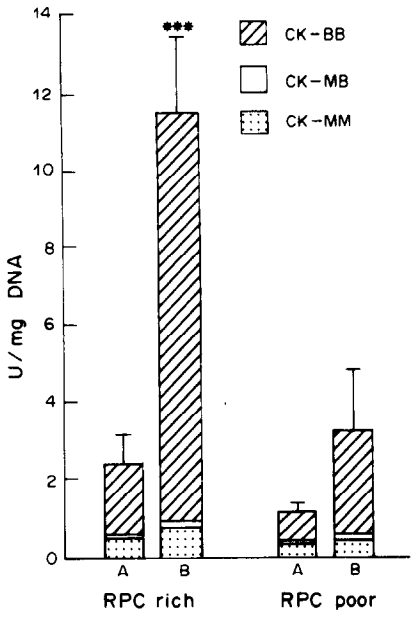

Fig. 1. Creatine kinase activity in human endometrial carcinoma before (A) and after (B) treatment with MPA. Curettage and hysterectomy specimens were obtained from each individual patient, with intervening MPA treatment. Total enzyme activity (U/mg DNA) is presented as mean ± SEM for RP_c rich and poor tumours with 8 paired observations in each group. The contribution of each of the isoforms of CK is indicated within each histogram. B > A by Wilcoxon signed rank test for paired observations. $***P < 0.005$.

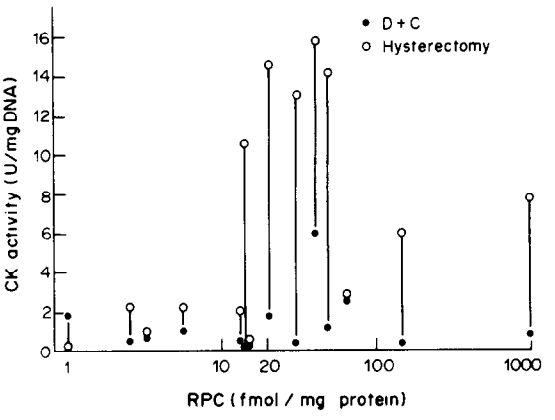

Fig. 2. The activity of CK-BB before and after progestin treatment related to the pretreatment progesterone receptor content of each sample. The activities before (●) and after (○) treatment in each patient are connected by solid lines.

ing MPA as an index of response regardless of RP_c; accepting the limitations of the number of observations all significantly elevated CK-BB values were greater than 4 U/mg DNA. However further studies involving a larger number of patients and incorporating clinical data which at present is too early for evaluation, will be necessary to establish such a relationship.

The observation that CK-BB constitutes the majority of total CK activity is in agreement with preliminary experiments on normal, hyperplastic and neoplastic endometrium [9]. In normal human endometrium CK-BB is elevated during the secretory phase of the menstrual cycle suggesting that the enzyme is progesterone sensitive [8, 9]. These observations contrast with those reported in the rat uterus where CK-BB was found to be oestrogen sensitive [7] and may be a further example of species variation in enzyme sensitivity to hormonal stimulation [14]. However, CK activity has recently been shown to be sensitive to Vitamin D and its metabolites in rat tissues [15] and in certain human breast tumours it is sensitive to oestradiol stimulation [16, 17]. These observations therefore suggest the possibility that rather than being sensitive to a specific steroid hormone CK activity may be responsive to a family of such steroids. Given the role of CK in buffering cellular ATP levels [18] and the central role of ATP in cellular function CK activity may be increased to accommodate the metabolic changes associated with the cellular actions of several steroid hormones.

Isocitrate dehydrogenase

There was no significant difference in ICDH activities between progesterone receptor rich and poor curettage specimens (Fig. 3). The activity of ICDH was significantly increased following MPA treatment in receptor rich specimens only, but there was no correlation between the concentration of RP_c in the pre-treatment sample and the change in ICDH activity in either the RP_c rich ($r = 0.062$, $n = 8$) or the RP_c poor ($r = 0.551$, $n = 8$) group. The increase was detectable by comparing specimens obtained from individual patients but was not apparent by comparing the means of each treatment group. The extent of stimulation of ICDH activity was less than that of

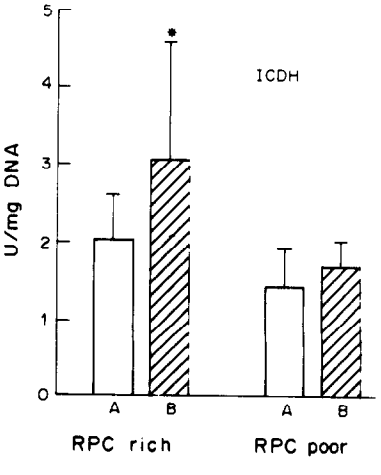

Fig. 3. Isocitrate dehydrogenase activity in human endometrial carcinoma before (A) and after (B) MPA treatment in RP_c rich and poor specimens. B > A by Wilcoxon signed rank test for paired observations $*P < 0.05$.

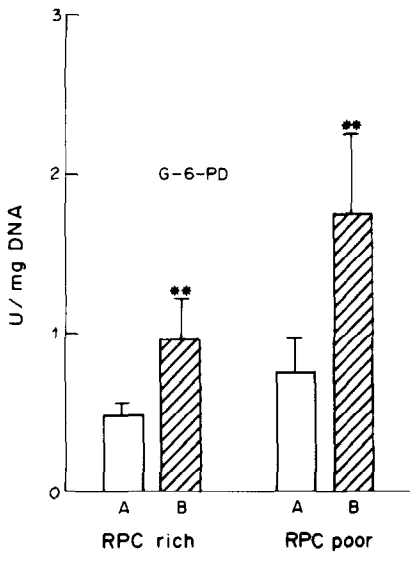

Fig. 4. Glucose-6-phosphate dehydrogenase activity in samples described in legend to Fig. 3. B > A by Wilcoxon's signed rank test for paired observations $^{**}P < 0.01$.

CK (compare Figs 1 and 3) suggesting that measurement of changes in CK activity may be a more sensitive index of a tissue effect of MPA. In the postmenopausal endometrium ICDH activity is progestin sensitive and inversely correlated with epithelial cell labelling index suggesting its usefulness as a marker of progestin inhibition of cell growth [19]. ICDH activity has also been demonstrated to be sensitive to progestin stimulation in endometrial carcinoma [19] although in contrast to the present study an association with progestin receptor status was not found. This discrepancy is probably related to the effect of MPA on RP_c and hence whether receptor status is based on levels measured before or after treatment (see below).

Glucose-6-phosphate dehydrogenase

No significant difference in G-6-PD activity was observed between curettage samples which were RP_c rich or poor (Fig. 4). The increase in G-6-PD activity that was observed following MPA treatment was apparently independent of RP_c status in contrast to CK and ICDH. The regulation of G-6-PD is known to be relatively complex involving transcriptional and translational control with sensitivity to both oestradiol and progesterone [20]. The effects of MPA on G-6-PD observed in the present study could therefore be the result of a common mechanism which is not receptor mediated or may represent different effects in RP_c rich and poor tumours.

Oestrogen and progestin receptors

The effects of MPA on RE_c are shown in Fig. 5 and indicate a statistically significant decrease in oestrogen receptor content in RP_c rich tumours only. Previous studies have indicated that RE_c is decreased following progestin treatment in endometrial carcinoma [21, 22] although the relationship with RP_c status was not reported. The mechanism of progestin regulation of oestrogen receptor in endometrial carcinoma is unknown but may involve modulation of RE replenishment [23, 24] and/or modification of the oestradiol stimulus via induction of oestradiol dehydrogenase [4]. An increase in oestradiol dehydrogenase activity following MPA treatment has been demonstrated in endometrial carcinoma [4].

A highly significant decrease in RP_c was observed following MPA treatment (Fig. 6) consistent with other reports [22]. Translocation into the nucleus and occupation by MPA may account in part for the decrease in RP_c although it is unlikely that this totally explains the observation [22]. A mechanism involving limitation of the oestrogenic stimulus as outlined above would also result in a decrease of the oestrogen induced progestin receptor. Nuclear oestrogen recep-

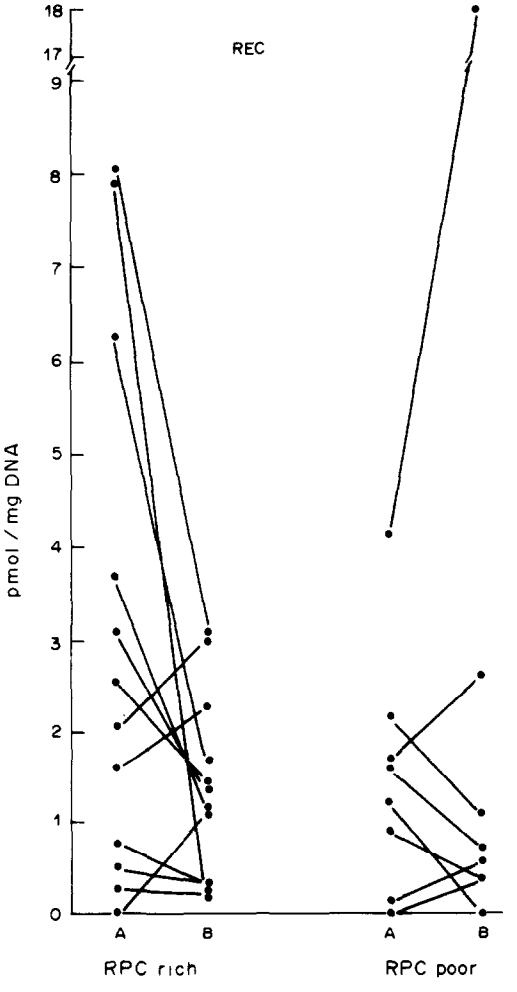

Fig. 5. Cytosol oestrogen receptor content of endometrial carcinoma specimens before (A) and after (B) treatment with MPA; solid lines connect observations made in the same patient. B > A Wilcoxon signed rank test for paired comparisons $^{*}P < 0.05$ (RP_c rich).

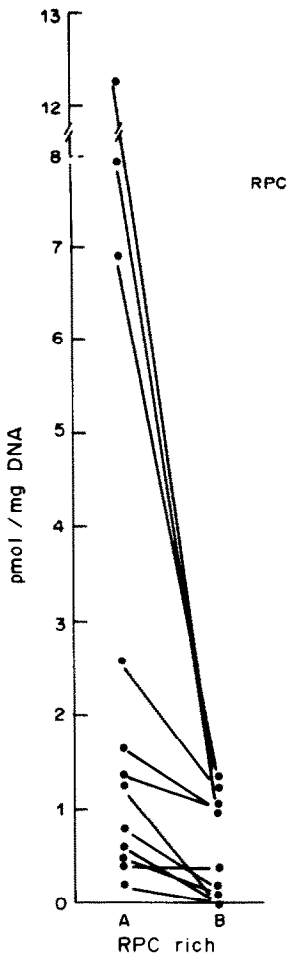

Fig. 6. Cytosol progestin receptor content of endometrial carcinoma specimens before (A) and after (B) treatment with MPA in RP_c rich specimens. Solid lines connect observations made in the same patient. B < A by Wilcoxon's signed rank test for paired comparisons $P < 0.005$.

tor levels were found to be relatively low compared with RE_c and were unaffected by MPA treatment (data not shown).

CONCLUSION

Creatine kinase is progestin sensitive in the majority (6/7) of endometrial carcinomas that contain cytosol progestin receptors while only 1/7 RP_c poor samples demonstrated a change of a similar magnitude. An increase in CK-BB activity accounts for the increase in total CK activity and the progestin sensitivity of this isoform in endometrial carcinoma is consistent therefore with its apparent progesterone sensitivity in normal human endometrium. The progestin effect on CK-BB was more marked than the effects on ICDH activity, RE_c or RP_c which were consistent with those previously reported for endometrial carcinoma. CK-BB is apparently therefore a suitable end-point of progestin sensitivity in endometrial carcinoma although further studies are required to ascertain its potential value as a screening test for determining tumours most susceptible to progestin therapy.

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