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INFLUENCE OF PROGESTINS ON SERUM HORMONE LEVELS IN POSTMENOPAUSAL WOMEN WITH ADVANCED BREAST CANCER—I. GENERAL FINDINGS

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Summary—The influence of oral high dose progestin (medroxyprogesterone acetate, MPA and megestrol acetate, MA) treatment on serum hormone levels was studied in ten postmenopausal women with advanced breast cancer. The gonadotropins and ACTH were significantly reduced by >50 and 23%, respectively. Serum cortisol, DHEAS, androstenedione and testosterone were all significantly reduced (mean reduction between 64 and 76%), while serum estrone, estradiol and estrone sulfate were significantly reduced by 20–30%. Sex hormone binding globulin (SHBG) and corticosteroid binding globulin (CGB) were reduced by 68 and 25%, respectively.

Although the dose of MA used (160 mg/day) was only 1/6 of the MPA dose (1000 mg/day), the mean serum level of MA was 2-fold higher than the mean serum level of MPA. MPA treatment gave a more pronounced suppression of SHBG than MA treatment, while estrone sulfate levels were more suppressed by MA.

These finding suggest a differential effect of MPA and MA on certain plasma hormones, possibly of importance for understanding the mechanism of action of the two drugs. The reduction of estrone sulfate may be beneficial for the action of MA against breast cancer.

INTRODUCTION

About one-third of unselected patients with advanced breast cancer will respond to endocrine treatment. Oral high doses of synthetic progestins such as medroxyprogestrone acetate (MPA) or megestrol acetate (MA) cause response rates comparable to those seen with tamoxifen (TAM) treatment [1, 2].

The exact mechanism(s) by which progestins cause regression of breast cancer is not clear. Suppression of adrenal steroid synthesis [3, 4], alterations in tumor hormone metabolism [5, 6] or estradiol receptor (ER) status [7], accelerated hepatic metabolism of steroids [8] as well as a possible cytotoxic effect on tumor cells [9] have all been suggested.

Several studies have shown a dose-related reduction in serum androgen levels during progestin treatment [3, 4, 10–12]. Suppression of serum levels of estrone (E_1), estradiol (E_2) and sex hormone binding globulin (SHBG) [3, 10] have been reported, but progestin effects on serum estrone sulfate (E_1S) have not been reported. There is evidfence that E_1S may be metabolized to E_2 and thus act as an important source for active estrogen in breast cancer cells [13, 14].

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The importance of alterations in serum hormones during progestin treatment is unclear. Previous results from our group have suggested that alterations in E_1S disposition could be of importance in the mechanism of action of endocrine therapy for breast cancer [15]. Thus, we wanted to conduct a more extensive study on serum hormones with particular attention to E_1S in patients receiving treatment with MA and MPA to explore possible important alterations in serum hormone levels.

EXPERIMENTAL

Patient characteristics

Ten postmenopausal women with advanced breast cancer who were to receive progestin therapy for progessing disease were enrolled in the study. Median age was 71 years (range: 51–78 yr), and median body weight was 76 kg (range: 52–87 kg) (Table 1). All patients gave their verbal informed consent to participate in the study. Previous chemotherapy or endocrine therapy was terminated at least three weeks (range: 3–52 weeks) before entering the study. No concomitant endocrine or cytotoxic therapy was allowed during the period of investigation. Other drugs were kept unchanged. Drugs known to be hepatic enzyme inducers or inhibitors were not used. None of the patients were smokers or alcohol abusers. Serum

Table 1. Patient characteristics of ten postmenopausal advanced breast cancer patients treated with progestins (MPA[a] and MA[b])

Patient No.	Age (years)	Body wt[1] (kg)	Stage	DFI[2] (months)	Time 1st recurrence[3] to MPA/MA therapy (months)	Previous therapy[4]	Response
1[a]	51	87	IV	0	14	T	PD
2[a]	76	52	III	30	2	T	PD
3[a]	69	71	II	75	129	O, CF, T, AG	SD
4[a]	78	65	II	18	2	T	PR
5[a]	77	81	IV	0	52	P, T	PR
6[b]	71	83	II	24	55	O, C, F, T, P, MPA, AG, A	PD
7[b]	72	66	IV	0	15	T	PD
8[b]	57	83	II	12	57	T, CMFP	PR
9[b]	75	65	II	50	137	P, T	SD
10[b]	62	83	IV	0	34	CMF, T	PD

[1]Body weight, no change during the study time. [2]Disease free interval.
[3]For stage IV patients: time from primary diagnosis to start of MPA/MA therapy.
[4]Abbreviations used: A = adriamycin, AG = aminoglutethimide, C = cyclophosphamide, F = 5-fluoro-uracil, M = methotrexate, MA = megestrol acetate, MPA = medroxyprogesterone acetate, O = oophorectomy, P = prednisone, PD = progressive disease, PR = partial response, SD = stable disease, T = tamoxifen.

creatinine, BUN, albumin, coagulating factors and bilirubin were normal in all patients.

Drug schedules

Five patients (Nos 1–5) were given MPA (Farlutal®, Farmitalia Carlo Erba AB) 500 mg b.i.d. (one tablet of 500 mg at 8 a.m. and 8 p.m.) and five patients (Nos 6–10) MA (Megestat®, Bristol Myers) 160 mg o.d. (4 tablets of 40 mg at 8 a.m.). Treatment was continued until objective evidence of disease progression was obtained.

Blood samples

Blood samples were always drawn at 2 p.m. Sampling was performed on three consecutive days, (a) before progestin treatment, (b) on day 8 of treatment, and (c) on three consecutive days after three weeks on progestin treatment. The samples were allowed to coagulate for 1 h, serum was separated by centrifugation, and stored at −20°C until analysis.

Measurement of serum progestin levels

Serum levels of MPA and MA were quantitated by a radioimmunoassay (RIA) method as described by Ortiz[16] with modifications as previously described [17]. Briefly, the progestins were quantitated by RIA method after hexane extraction, using an antiserum raised against MPA-3-CMO-BSA. All samples from each patient were done in duplicate in the same run. The detection limit was 5 ng/ml for both progestins. Mean intra- and interassay coefficient of variation (C_V) were 7 and 14% and 6 and 19% for MPA and MA respectively.

Other analyses

Serum levels of follicle stimulating hormone (FSH), luteinising hormone (LH), adrenocorticotropic hormone (ACTH), prolactin (PRL), cortisol, SHBG, dehydroepiandrosteron sulfate (DHEAS), androstenedione (A), testosterone (T), progesterone (P) and corticosteroid binding globulin (CBG) were all measured by commercially available RIA kits routinely used in our laboratory. Detailed information about each test is given in Table 2.

Serum levels of E_1, E_2 and E_1S were measured as described previously [18,19]. Sensitivity, intra- and interassay C_V for the different methods are given in Table 3.

In five patients (Nos 1, 2, 4, 7, 10) who had stopped TAM treatment less than 5 weeks before the start of

Table 2. Normal range, sensitivity and coefficient of variation (C_V) of different tests used in the study

Test	Normal range	Sensitivity	Intraassay C_V(%)	Interassay C_V(%)
FSH (IU/l)	12–80	1.4	5.8	6.4
LH (IU/l)	19–80	1.4	6.2	9.9
ACTH (ng/l)	<100	5	6.8	12.1
Prolactin (MU/l)	<350	65	7.6	10.8
Cortisol (nmol/l) 8 a.m.	200–650	10	6.6	9.0
8 p.m.	55–250			
Progesterone (nmol/l)	<1.5	0.2	4.1	7.1
DHEAS (μmol/l)	0.30–2.70	0.3	3.2	7.0
Androstenedione (nmol/l)	2.1–6.4	0.2	4.6	14.5
Testosterone (nmol/l)	0.35–2.8	0.3	6	8.9
SHGB (nmol/l)	35–100	5.0	3.9	13.6
CBG (μg/ml)	31–53	0.25	4.9	5.0

Abbreviations used: FSH = follicle stimulating hormone, LH = luteinising hormone, ACTH = adrenocorticotropic hormone, DHEAS = dehydroepiandrosteron sulfate, SHBG = sex hormone binding globulin, CBG = corticosteroid binding globulin.

Table 3. Sensitivity and coefficients of variation (C_V) in the assays of the different estrogens

Test	Sensitivity (pmol/l)	Intraassay C_V(%)	Interassay C_V(%)
Estrone	20	8.3	11.8
Estradiol	20	10.1	13.7
Estrone sulfate	42	6.9	8.6

the study, serum levels of TAM were measured before start of progestin treatment by the method of Lien *et al.*[20].

Statistical methods

Statistical differences between hormone levels before and after 3 weeks of treatment were tested by the Wilcoxon Matched Pair Sign Rank Test. *P*-values are reported as two-tailed.

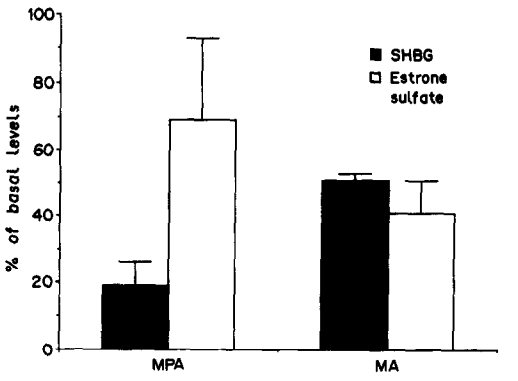

Fig. 1. Mean of individual % of basal levels (±SD) of estrone sulfate and sex hormone binding globulin (SHBG) during MPA ($n = 5$) and MA ($n = 5$) therapy (after 3 weeks).

RESULTS

Hormone levels before and after 1 and 3 weeks of progestin therapy are shown in Table 4. The findings after 3 weeks of progestin therapy may be summarized as follows:

(1) The mean serum levels of both ACTH and cortisol were significantly reduced; 23% ($P = 0.03$) and 76% ($P = 0.003$) respectively. This large fall in serum cortisol could be partly explained by a 25% decrease in CBG ($P = 0.006$).
(2) The mean serum levels of androgens DHEAS, A and T were reduced by approximately 65% ($P = 0.009$, $P = 0.004$ and $P = 0.003$ respectively). The reduction in mean serum levels of estrogens was lower; E_1 34% ($P = 0.003$), E_2 21% ($P = 0.008$) and E_1S 30% ($P = 0.03$).
(3) There was no significant reduction in the level of serum progesterone (19% ($P = 0.06$)).
(4) Mean serum level of SHBG was reduced by 68% ($P = 0.003$).
(5) The mean serum levels of the gonadotropins FSH and LH were reduced by 51 and 67% respectively ($P = 0.003$ for both). Serum PRL remained unchanged.

The mean serum level of MPA was 106 ng/ml (range 32–181 ng/ml) after 1 week of treatment and 112 ng/ml (range 64.5–196.3 ng/ml) after 3 weeks of treatment For MA, the corresponding levels were 220 ng/ml (range 151–348 ng/ml) and 284 ng/ml (range 203.3–434 ng/ml) respectively.

The mean serum level of TAM in the 5 patients examined were 23.8 ng/ml, which is less than 10% of the usual therapeutic serum level of tamoxifen when doses of 30 mg o.d. are given (data not shown).

While most serum hormones were equally suppressed by MA and MPA treatment, possible different effects on SHBG and E_1S are shown in Fig. 1. Due to the small number of patients no statistical analyses has been done.

DISCUSSION

Pretreatment hormone levels were as previously reported for postmenopausal women [3, 10, 26]. An estrogenic effect of TAM on CBG and SHBG [21]

Table 4. Absolute levels (mean ± SD) and % reduction of basal levels of endocrine parameters in ten postmenopausal advanced breast cancer patients before and during progestin treatment (MPA and MA)

Hormone	Before	1 week	(%)	3 weeks	(%)	*P*
FSH (IU/l)	30.7 ± 10.8	22.3 ± 10.2	27.4	15.0 ± 8.3	51.1	**
LH (IU/l)	26.1 ± 12.9	20.4 ± 14.9	21.8	8.7 ± 5.2	66.7	**
ACTH (ng/l)	27.8 ± 18.7	19.5 ± 15.1	29.9	21.3 ± 13.1	23.4	*
Prolactin (MU/l)	391.7 ± 334.4	361.0 ± 307.8	7.8	395.8 ± 206.7	+1.1	NS
Cortisol (nmol/l)	311.0 ± 125.0	144.1 ± 92.7	53.7	75.3 ± 50.1	75.8	**
Progesterone (nmol/l)	0.65 ± 0.33	0.58 ± 0.40	10.8	0.53 ± 0.34	18.5	NS
DHEA-S (μmol/l)	1.09 ± 1.00	0.65 ± 0.74	40.4	0.38 ± 0.43	65.5	**
Androstenedione (nmol/l)	1.4 ± 1.5	0.5 ± 0.3	64.3	0.5 ± 0.3	64.3	**
Testosterone (nmol/l)	0.45 ± 0.23	0.23 ± 0.16	48.9	0.16 ± 0.14	64.4	**
Estrone (pmol)	103.6 ± 67.3	74.6 ± 50.1	28.0	68.8 ± 43.4	33.6	**
Estradiol (pmol/l)	47.1 ± 14.9	39.3 ± 13.4	16.6	37.1 ± 15.4	21.2	**
E_1S (pmol/l)	1125.4 ± 1042.6	745.1 ± 534.5	33.8	784.6 ± 803.4	30.3	*
SHGB (nmol/l)	71.4 ± 42.5	49.6 ± 36.1	30.5	23.0 ± 19.6	67.8	**
CBG (μg/ml)	49.0 ± 11.2	44.3 ± 9.3	9.6	36.5 ± 5.0	25.1	**

Abbreviations used: FSH = follicle stimulating hormone, LH = luteinising hormone, ACTH = adrenocorticotropic hormone, DHEAS = dehydroepiandrosteron sulfate, SHBG = sex hormone binding globulin, CBG = corticosteroid binding globulin, E_1S = estrone sulfate.
P = comparison between before level and 3 weeks level: $^{**}P < 0.01$, $^{*}P < 0.05$.
NS = not significant.

and LH [22] in the 5 patients who had terminated TAM less than 5 weeks before enrollment to the study cannot be excluded [24], but is unlikely due to the low serum levels of TAM. No effect of TAM on PRL has been reported [23].

Serum ACTH levels fell by an average of 20% after 3 weeks of progestin treatment. Others have reported suppression of ACTH to undetectable levels [25] or no change in ACTH at all [4]. Low serum cortisol with no elevation of ACTH is consistent with an inhibitory effect of progestins (or some of their metabolites) on the adrenal–hypothalamic–pituitary axis.

Progestin suppression of gonadotropin serum levels could be due to the action of progestin itself or one of its metabolites on the hypothalamic–pituitary level [3, 26]. This may be of importance, as the postmenopausal ovarian androgen secretion seems to be under gonadotropin control [27]. 50% of serum T and 30% of serum A in postmenopausal women have been reported to be of ovarian origin [28]. An LH-RH agonist has been reported to suppress serum androgens [27] and cause response in a small number of postmenopausal women suffering from breast cancer [29]. However, this response rate is inferior to that of high-dose progestins, and although it may be beneficial, it is unlikely that this mechanism plays an important role in the action of progestin treatment in postmenopausal women.

During progestin treatment the serum levels of androgens (A, T and DHEAS) were suppressed by 65%. In contrast, serum E_1 and E_2 levels were suppressed by only 20–30%.

Estrogens are mainly produced by peripheral aromatization of androgens in postmenopausal women, and one might have expected an estrogen suppression of similar magnitude as for androgens. However, analogous findings have been reported for postmenopausal patients treated with LH-RH-analogues [27], and there is no direct evidence suggesting a stimulatory effect of progestins on the aromatase enzyme.

The hepatic synthesis of SHBG is regulated by different serum hormones. Androgens and glucocorticoids decrease whereas estrogens increase the levels of both SHBG and CBG [21, 30, 31]. The serum level of SHBG is also increased by thyroid hormones [21]. In our study MA and MPA treatment suppressed the serum level of both of these steroid binding globulins. Serum androgens were suppressed by 65% and estrogens by only 28%, and Pannuti *et al.*[32] reported decrease in thyroid hormones. The effect of progestin on SHBG and CBG is probably not mediated through alterations in serum steroids. These changes may rather be caused by an androgenic (or glucocorticoid) effect of the progestins themselves on hepatic protein synthesis. A reduced serum level of SHBG may be of biological importance, since this would increase the free fraction of both T and E_2 available to the tumour cells.

Conflicting results have been published on changes in serum PRL levels [3, 11, 12, 25, 32] during progestin treatment. In this study we found no change in serum PRL level.

Millington *et al.* reported that the intratumoral concentration of E_1 and E_2 are 10-fold the serum levels [33], suggesting a local estrogen production [34] or an active uptake mechanism. It is not known whether alterations in serum levels of E_2 and E_1 cause similar alterations inside the tumour cells. The moderate suppression of serum E_1 and E_2 during progestin treatment is probably of minor importance in causing regression of breast cancer. However, serum levels of E_1S were significantly suppressed, particularly during MA treatment. It has been suggested that serum level of E_1S is an important source of intratumoral E_1 and E_2 concentrations [34–37]. Therefore, suppressing serum E_1S may be an important part of the mechanism of action of progestins in breast cancer treatment. Evidence suggests that E_1S is produced by conjugation of circulating E_1 and E_2 [38, 39]. Whether progestins reduces E_1S by reducing its production rate or by stimulating its metabolism remains uncertain. A stimulated metabolism of E_1S has been reported for aminoglutethimide [40], a known liver enzyme inducer, also used in breast cancer treatment. A similar stimulating effect of oral progestin has not been found [41]. Further investigations are needed to find the mechanism of E_1S alteration.

The effect of estrogen on tumour cells is thought to be mediated by ER. No ER binding of progestins has been reported. A down-regulation of ER production mediated by the binding of progestin to progesterone receptor (PgR) has been reported [7]. Other mechanisms of action seem to exist, as reported by our group of an additive effect of MPA to chemotherapy treatment in ER− breast cancer patients [42]. An effect of MPA mediated through PgR in these patients is less probable since ER-PgR+ tumours are infrequently observed [43]. Androgen receptor (AR) mediated action may be more likely since ER-AR+ has been found in 20% of metastatic tumors [39], and progestin binding to AR has been reported [44]. Also, progestin action through glucocorticoid receptor [44], or a direct effect, independent of any receptor [9], cannot be excluded.

The mechanism(s) of action of progestins is complex. Our study shows suppression of the gonadotropins but not on PRL, and suggests an androgenic (or glucocorticoid) effect on SHBG and CBG, and a glucocorticoid action on ACTH. Although considerable changes in serum levels of different hormones are found, the reducton of E_1S appears to be of particular interest for the biological mechanism of progestins.

The hypothesis that the two progestins may have differential effects on serum levels of E_1S and SHBG is currently being tested in further studies.

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