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EFFECTS OF ORCHIDECTOMY AND DIFFERENT MODES OF HIGH DOSE ESTROGEN TREATMENT ON CIRCULATING "FREE" AND TOTAL 1,25-DIHYDROXYVITAMIN D IN PATIENTS WITH PROSTATIC CANCER

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Summary—Serum levels of total 1,25-dihydroxyvitamin D (1,25$(OH)_2$D), vitamin D binding protein (DBP), sex hormone binding globulin (SHBG), testosterone, estradiol 17β (E_2) and the "free" 1,25$(OH)_2$D index were measured before and during treatment in prostatic cancer patients treated by orchidectomy ($n = 15$), with combined i.m. polyestradiol phosphate (PEP) + oral ethinyl estradiol (EE) ($n = 10$) and with i.m. PEP only for 3 months, followed by addition of oral EE ($n = 9$). Total concentrations of 1,25$(OH)_2$D and DBP were unaffected by orchidectomy and treatment with i.m. PEP only, but were significantly elevated during treatment including oral EE. SHBG levels were unaffected by orchidectomy, slightly increased by i.m. PEP only and greatly increased by oral EE. The free 1,25$(OH)_2$D index was slightly elevated by treatment including oral EE. Evidence was obtained that the increase in 1,25$(OH)_2$D levels observed during oral estrogen treatment was secondary to the estrogen-augmented increase in DBP and not a result of an estrogen-stimulated synthesis of 1,25$(OH)_2$D. Furthermore, the stimulatory effect of estrogen on DBP concentrations seemed to be dependent on the route of administration of the hormone.

INTRODUCTION

Serum levels of 1,25-dihydroxyvitamin D (1,25$(OH)_2$D), the biologically most active vitamin D metabolite, have been reported to increase during estrogen treatment and during conditions with high endogenous estrogen levels [1–3]. The mechanism for this increase is not known. It has been suggested that it is due to an estrogen stimulated increase in vitamin D binding protein (DBP) synthesis [1]. In women treated with estrogens, the increase in the binding protein resulted in elevated total levels of 1,25$(OH)_2$D, whereas the free, non-protein bound fraction was unchanged [1–4] or slightly elevated [5]. This increase in DBP occurred only when the estrogens were administered orally. Transdermal estrogen treatment had no effect on serum DBP concentrations [6, 7]. Similar observations have been reported for other "steroid sensitive" proteins synthesized by the liver e.g. sex hormone binding globulin (SHBG) and pregnancy-associated α_2-macroglobulin (α_2-PAG) [8].

The possible role of testosterone (T) in the vitamin D metabolism is unclear. Animal studies have shown that T has a stimulatory effect on the renal 25-hydroxyvitamin D_3-1α-hydroxylase activity which is the key enzyme in the production of 1,25$(OH)_2$D [9]. Long standing deficiency of T, such as in Klinefelter's syndrome, has been shown to be accompanied by low 1,25$(OH)_2$D levels and osteoporosis. T substitution in these patients increased circulating levels of both total and free 1,25$(OH)_2$D [10]. In male puberty, both increased and unchanged levels of 1,25$(OH)_2$D have been reported [11, 12].

Orchidectomy and high dose estrogen therapy are two commonly used treatments of prostatic cancer. In order to further elucidate possible associations between estrogen/androgen balance and vitamin D homeostasis in the human, we have measured serum concentrations of total and free 1,25$(OH)_2$D, DBP, SHBG, T and estradiol 17β (E_2) in prostatic cancer patients before and during treatment with parenteral and oral estrogens and by orchidectomy.

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MATERIAL AND METHODS

Patients

Thirty-four patients, aged 56–82 years [mean age 72.4 ± 1.0 (SEM) years], with cytologically and/or histologically confirmed, locally advanced, untreated prostate cancer (T_{3-4}; G_{2-3}; M_0) were consecutively included in the study. None of the patients had any clinical or laboratory signs of hepatic, biliary, intestinal or renal malfunction or any pituitary, gonadal or adrenocortical abnormalities. All patients were euthyroid. Apart from the estrogen treatment no medication was given that could interfere with the serum levels of $1,25(OH)_2D$ or DBP. The following treatment regimens were studied:

1. Fifteen patients underwent bilateral orchidectomy as the sole treatment. Blood samples were taken before therapy and 6 months after surgery.
2. Ten patients received 0.15 mg oral ethinyl estradiol (EE), (Etivex, Leo AB, Helsingborg, Sweden) daily and polyestradiol phosphate (PEP), (Estradurin, Leo AB, Helsingborg, Sweden) at a dose of 160 mg i.m. every 4th week. Blood samples were taken before and after 3 months of estrogen therapy.
3. Nine patients received PEP 160 mg i.m. every 4th week. After 3 months, EE at a dose of 0.15 mg per oz/daily was added and PEP dosage was reduced to 80 mg i.m. every 4th week. Blood samples were taken before and after 3 and 6 months of treatment.

Serum was separated after centrifugation and stored protected against light at −70°C until analyzed. The study was approved by the ethical committee of Huddinge University Hospital and an informed consent was obtained from each patient.

ANALYTICAL METHODS

All assays were carried out in duplicate. Total serum levels of $1,25(OH)_2D$ were determined using a commercial radioreceptor kit from INC STAR (Stillwater, MN, U.S.A.). Serum levels of DBP were measured by quantitative immunoelectrophoresis [13] using rabbit immunoglobulin to human Gc-globulin (DAKO Immunoglobulins A/S, Copenhagen, Denmark). Serum concentrations of T, E_2 and SHBG were determined by radioimmunological or immunoradiometric (SHBG) methods using commercial kits from Diagnostic Products Corp. (Los Angeles, CA, U.S.A.) (T and E_2) and Farmos Diagnostic OY Turku (Finland) (SHBG), respectively. Details and characteristics of the methods have been given in previous communications [8, 13].

The non-protein bound fraction of $1,25(OH)_2D$ was calculated as "the free $1,25(OH)_2D$ index", that is the molar ratio of $1,25(OH)_2D$ and vitamin D binding protein, as described by Bouillon *et al.* [1]. This "free $1,25(OH)_2D$ index" is a simple model used to describe the small free $1,25(OH)_2D$ fraction, which we assume, in analogy with other steroid and thyroid hormones, exerts the biological effects.

Statistical evaluation

Serum concentrations during treatment are given as percent changes from pretreatment values (100%). The variables were normally distributed and two-tailed *t*-tests for paired observations and linear regressions were used for statistical evaluation. Data are given as means ± SEM.

RESULTS

Pretreatment values for sex hormones, binding proteins and total and free $1,25(OH)_2D$ are given in Table 1. There were no significant correlations between pretreatment values of total or free $1,25(OH)_2D$ on the one hand and binding proteins or sex steroids on the other or between DBP levels and sex steroids. Percent

Table 1. Pretreatment serum concentrations of sex steroids, binding proteins and total and free $1,25(OH)_2D$ in patients with prostatic cancer

	Castration $n = 15$	Combined estrogens[a] $n = 10$	Sequential estrogens[b] $n = 9$
Testosterone (nmol/l)	18.1 ± 1.0	21.0 ± 2.1	25.6 ± 2.5
Estradiol (pmol/l)	117 ± 14	ND[c]	131 ± 10
SHBG (nmol/l)	35 ± 3	37 ± 3	45 ± 4
DBP (μmol/l)	4.5 ± 0.3	5.7 ± 0.2	5.2 ± 0.5
$1,25(OH)_2D$ (pmol/l)	60 ± 5	58 ± 5	70 ± 7
Free $1,25(OH)_2D$ index $\times 10^5$	1.42 ± 0.15	1.04 ± 0.09	1.52 ± 0.29

Values are given as means ± SEM; [a]0.15 mg oral EE daily + 160 mg PEP i.m. every 4th week; [b]160 mg PEP i.m. every 4th week during the first 3 months; 80 mg PEP i.m. every 4th week + 0.15 mg oral EE daily during the following 3 months; [c]ND, not determined.

changes during different treatment regimens are given in Fig. 1. Single treatment with PEP did not suppress T to orchidectomy levels; however, such an effect was obtained by addition of oral treatment. Orchidectomy reduced circulating E_2 levels by about 50% while single PEP treatment caused a tremendous increase in E_2. Serum SHBG and DBP were unchanged by orchidectomy. During single drug PEP treatment, a slight but significant increase was observed in SHBG but not in DBP. Addition of oral estrogens caused pronounced elevations in both binding proteins, especially SHBG. Circulating $1,25(OH)_2D$ was unaffected by orchidectomy or parenteral estrogens, but increased significantly during oral estrogen treatment. The free $1,25(OH)_2D$ index remained about constant by the different treatments. A small but significant increase was seen in the group who received combined estrogen treatment.

When data from all patients receiving oral EE were pooled (group 2 and 6-month values from group 3) and Δ-values = differences between values during treatment and pretreatment value were calculated, significant positive correlations were obtained between $\Delta 1,25(OH)_2D$ and ΔDBP ($r = 0.53$, $P < 0.05$), between $\Delta 1,25(OH)_2D$ and ΔSHBG ($r = 0.55$, $P < 0.05$) and between ΔDBP and ΔSHBG ($r = 0.48$, $P < 0.05$).

DISCUSSION

In the present study, orchidectomy had no significant effects on $1,25(OH)_2D$ and DBP

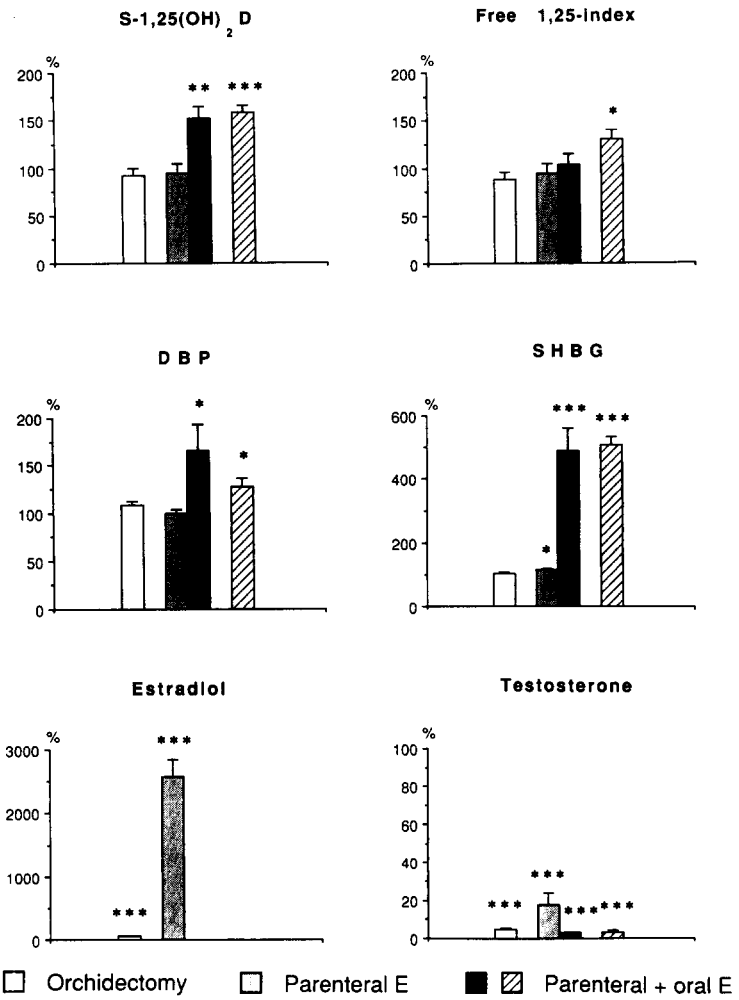

Fig. 1. Serum concentrations of $1,25(OH)_2D$, DBP, SHBG, E_2 and T and the free $1,25(OH)_2D$ index in patients with prostatic cancer, treated by orchidectomy, with parenteral estrogens or with parenteral + oral estrogens, respectively. Values are expressed as percent of pretreatment values; mean and SEM. Significances of differences between treatment and pretreatment values: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. □: Orchidectomy. □: Single parenteral estrogens during first 3 of 6 months sequential estrogen treatment; ■: Parenteral + oral estrogens during last 3 months of sequential estrogen treatment; ▨: Parenteral + oral estrogens during 6 months of combined estrogen treatment.

levels. This supports the view of Krabbe *et al.* [12] that androgens are without effects on vitamin D homeostasis in men. In contrast Francis *et al.* [10], as well as ourselves (Hagenfeldt *et al.*, unpublished work) found increased $1,25(OH)_2D$ levels following androgen treatment in hypogonadal men. In animal studies, T has been shown to stimulate the 25-hydroxyvitamin D_3-1α-hydroxylase, the key enzyme in the production of $1,25(OH)_2D$ [9]. The mechanism for the stimulatory effect of testosterone administration on the $1,25(OH)_2D$ production has yet to be established. However, an unequivocal comparison of the relation between androgen status and $1,25(OH)_2D$ in the present investigation and in previous studies [10, 12] is not possible. The present study included elderly men whereas the study by Krabbe *et al.* [12] was performed on boys during puberty. In the studies on hypogonadal men referred to above, all subjects had been hypogonadal for more than 10 years and many of the patients had not passed through a normal puberty.

Our results concerning effects on DBP and SHBG levels are in accordance with previous investigations [6, 8, 14, 15]. Thus, DBP seems to share the properties of other "steroid sensitive" proteins: no response to orchidectomy, nonexistent or minor response to parenteral estrogens but significant response to oral estrogen administration. The far more pronounced response following the latter mode of administration may be explained by high local concentrations of the drug in the liver, resulting from the first liver pass [16]. However, compared to some other "steroid sensitive" proteins (SHBG,α_2-PAG), DBP is an insensitive indicator of the effects of oral estrogens upon the liver [17, 18]. There is little reason to believe that the estrogen/androgen balance is the major physiological regulator of DBP and other "steroid sensitive" proteins [16].

Our results concerning the relations between $1,25(OH)_2D$ and DBP are in accordance with some previous studies on postmenopausal women treated with oral or parenteral estrogens [1, 4–7]. The insensitivity of $1,25(OH)_2D$ to parenteral estrogens, even at doses giving very high serum levels of E_2, and the significant correlations between $1,25(OH)_2D$, DBP and SHBG during oral estrogen therapy indicate that the increase in $1,25(OH)_2D$ during the latter treatment is secondary to the estrogen-augmented increase in DBP. However, in two studies on menstruating women a covariation of circulating $1,25(OH)_2D$ and estrogens was demonstrated, without any changes in DBP or other vitamin D metabolites [2, 3]. It was speculated that this covariation was due to a promoting effect of estrogens upon the 1α-hydroxylation of 25-hydroxyvitamin D and an inhibitory effect upon the synthesis of 24,25-dihydroxyvitamin D [3]. However, several other explanations are possible, and it is evident that the regulation of circulating $1,25(OH)_2D$ is complex and multifactorial.

A slight but significant increase in the free $1,25(OH)_2D$ index was observed in our patients treated with combined parenteral and oral estrogens. This is in accordance with the findings of Cheema *et al.* [5] but not with the results of Bouillon *et al.* [1] and Selby *et al.* [15]. The reason for this discrepancy is not known. It should be noted that a similar "paradoxical" increase in free cortisol has been observed, when transcortin and, concomitantly, cortisol levels are increased by oral estrogen treatment [19].

To conclude, the effect of oral estrogens on circulating $1,25(OH)_2D$ found here is probably only secondary to the effect on DBP levels. The results do not support a role of the gonadal steroids as important regulators of circulating $1,25(OH)_2D$ and DBP in elderly men.

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