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Stronger Suppression of Serum Testosterone and FSH Levels by a Synthetic Estrogen than by Castration or an LH-RH Agonist

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Abstract. Serum levels of LH, FSH and testosterone (T) were measured by radioimmunoassays in 36 patients with advanced prostate cancer before and during androgen ablation therapies. Both leuprolide acetate (LH-RH agonist: LHRH-A) and diethylstilbestrol diphosphate (DES-DP) administration decreased serum LH significantly to an undetectable level (LHRH-A: $P<0.01$, DES-DP: $P<0.05$). LHRH-A and DES-DP diminished serum FSH to 20% of the pre-treatment level ($P<0.005$) and to an undetectable level ($P<0.001$), respectively. LHRH-A and DES-DP decreased serum T to the castration level and an undetectable level, respectively ($P<0.001$). Serum levels of the same 3 hormones before and after DES-DP administration were measured in 8 patients who received DES-DP after LHRH-A treatment or castration because of relapse of the disease. DES-DP lowered serum FSH further than LHRH-A to an undetectable level ($P<0.005$) and diminished T further than previous treatments to an undetectable level ($P<0.05$ *vs.* LHRH-A, $P<0.01$ *vs.* castration). These results suggest that 1) DES-DP is able to reduce T production from extra-testicular site(s), and achieve the minimal serum T level, and 2) this DES-DP action appears to be one of the mechanisms of the effectiveness of estrogen on refractory prostate cancer after castration or LHRH-A. In addition, basal (independent of LH-RH) FSH secretion in elderly men is about 20% of total FSH secretion and DES-DP inhibits the basal FSH secretion at the level of the pituitary.

Key words: Prostate cancer, Synthetic estrogen, Testosterone, FSH, LH-RH agonist, Castration
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APPROXIMATELY a half of all patients with prostate cancer are found at the advanced stage. Huggins *et al.* proved the effectiveness of ablation of endogenous testicular androgen in advanced prostate cancer about 50 years ago [1]. Since that time, the main stream of treatment of advanced prostate cancer has been from castration to synthetic estrogens (SEs), to gestagens and to LH-RH agonists (LHRH-As) [2]. Recently, non-steroidal androgen receptor blocker became an alternative. All of the therapies are aimed at blocking androgen action on prostate cancer cells [3], but the effect is temporary and relapse of the disease is within a few years inevitable.

Interestingly, SEs are often effective for a while on prostate cancer relapse after treatment with castration or LHRH-As [4, 5]. A direct cytotoxic effect of estrogens on prostate cancer cells has been proposed to explain the mechanism of the above phenomenon [6–8]. Other assumptions have been also proposed, including the effects of SEs on testosterone binding globulin [9] and adrenal androgen synthesis [10].

The effects of endocrine therapies for prostate cancers on serum hormone levels have been

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described in many reports [1–3, 10]. Each hormone therapy has been reported to induce a specific hormonal environment in elderly men, but differences in the hormonal environment according to the treatments have not been well analyzed. The present study was performed retrospectively to evaluate the responses of serum LH, FSH and testosterone (T) to treatments with SE, LHRH-A and castration in the elderly with advanced prostate cancer.

Materials and Methods

Blood samples were collected from patients with stage D1 or D2 prostate cancer without treatment (14 patients) as controls, 1 to 44 months (mean: 11.2) after starting leuprolide acetate administration (3.75 mg/4 week, 16 patients) as an LHRH-A group, 1 to 51 months (mean: 13.6) after starting diethylstilbestrol diphosphate (DES-DP) administration (300 mg/day: 13 patients) as a DES-DP group, and 1 to 60 months (mean: 13.1) after castration (12 patients) as a castration group.

In our clinic, a patient showing relapse of prostate cancer with LHRH-A or after castration generally receives DES-DP. Blood samples were collected from 4 patients during LHRH-A treatment and during DES-DP administration after relapse of the disease. Blood samples were collected from another 4 patients after castration and during DES-DP administration after the relapsing.

Serum LH and FSH were measured by radioimmunoassays (RIAs) with WHO standards of pituitary gonadotropins. Serum testosterone was measured by RIA with the coated tube method. Lower limits of the present assays for LH, FSH and testosterone are 0.5 mIU/m*l*, 0.5 mIU/m*l* and 5 ng/d*l*, respectively. Serum LH and FSH were not measured in the castration group.

The statistical methods used in the present study were a multiple-comparison technique (Tukey-Kramer HSD comparisons) and paired *t*-test with JMP (software for statistics from SAS Institute Inc.). For convenience in statistical analysis, values for undetectable levels of serum LH, FSH and testosterone were considered to be 0.5 mIU/m*l*, 0.5 mIU/m*l*, and 5 ng/d*l*, respectively.

Results

Patients' characteristics are shown in Table 1. The patients' age ranged from 59 to 93 years with a median of 73.7. Patients in the DES-DP group were younger than those in the control ($P<0.01$) and castration ($P<0.01$) groups. Patients in the LHRH-A group were younger than those in the castration group ($P<0.05$). There were no differences among the treatment groups in either the performance status of patients or the length of treatment.

Serum LH levels in both DES-DP and LHRH-A groups were undetectable excepting 1 patient in LHRH-A group as shown in Fig. 1A. Serum FSH in the LHRH-A group was approximately 20% of the control group ($P<0.001$) and serum FSH levels of all patients in the DES-DP group were undetectable ($P<0.001$) as shown in Fig.1B.

As shown in Fig. 2, serum T in the LHRH-A group was on the same level as the castration group which was approximately 5% of controls ($P<0.001$). Serum T levels in the DES-DP group were undetectable in 9 out of 13 patients ($P<0.001$), but no difference among the DES-DP, LHRH-A and castration groups in serum FSH or T was confirmed

Table 1. Patients' characteristics according to the treatment for advanced prostate cancer

Treatment	n	age (years*)		treatment length (months*)
Control (not treatment)	14	81 (66–85)		~
LH-RH agonist**	17	73 (59–85)	b	11.2 (1–44)
DES-DP***	13	69 (59–86)		13.6 (1–55)
Castration	12	83 (65–93)		13.1 (1–60)

a a

*: mean (range), **: leuprolide acetate, ***: diethylstilbestrol diphosphate, a: $P<0.01$, b: $P<0.05$.

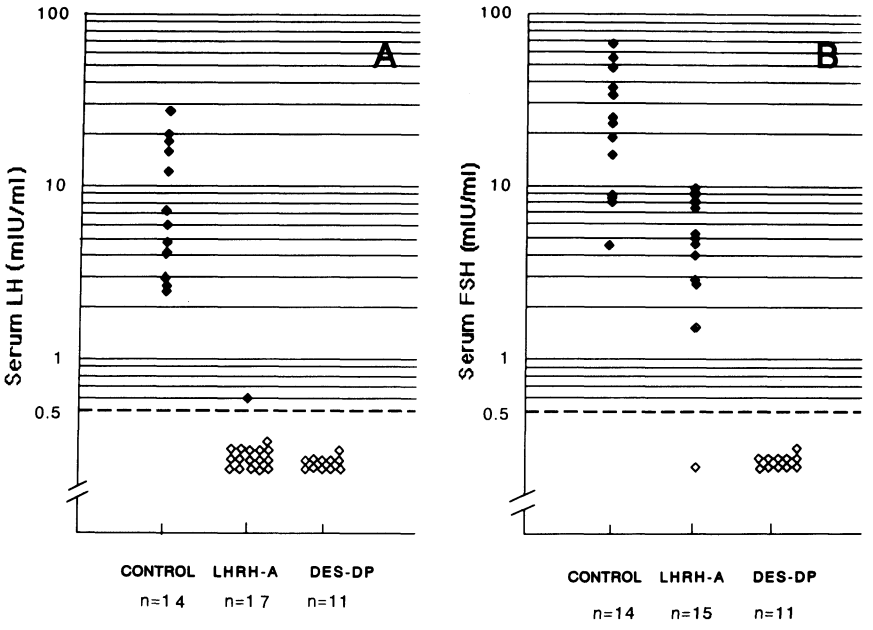

Fig. 1. A: Suppression of serum LH levels by LH-RH agonist (LHRH-A) or diethylstilbestrol diphosphate (DES-DP) in patients with advanced prostate cancer. LH levels of patients after administration of both LHRH-A and DES-DP were similarly suppressed ($P<0.01$ in LHRH-A *vs.* no treatment (CONTROL) and $P<0.05$ in DES-DS *vs.* CONTROL). ◆ denotes each case and ◇ denotes each case whose level is undetectable, ----: lower limit of level detectable by RIA. B: Suppression of serum FSH levels by LHRH-A or DES-DP in the same groups as shown in Fig. 1A. Serum FSH levels of patients treated with LHRH-A decreased to approximately 20% of controls ($P<0.001$ in LHRH-A *vs.* CONTROL). DES-DP diminished serum FSH to undetectable levels ($P<0.001$ in DES-DP *vs.* CONTROL). ◆ denotes each case and ◇ denotes each case whose level is undetectable, ----: lower limit of level detectable by RIA.

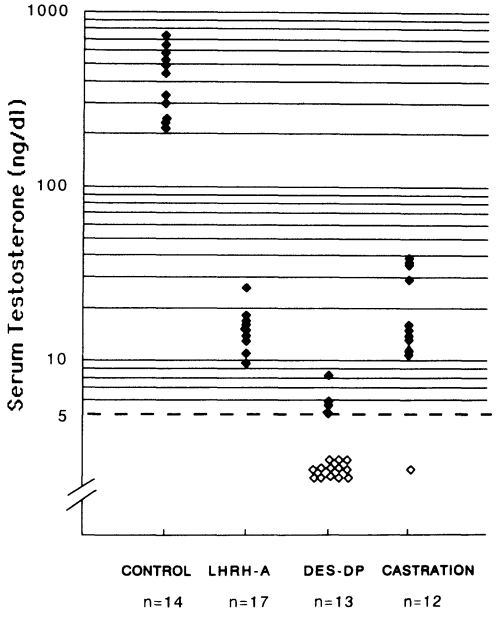

Fig. 2. Decline in serum testosterone (T) levels by castration, LHRH-A or DES-DP in the same groups as shown in Fig. 1. Serum T levels of patients after castration or LHRH-A administration were still significant and were approximately 5% of control levels ($P<0.001$ *vs.* CONTROL). Serum T after DES-DP administration was lowered to an undetectable level except in a few cases ($P<0.001$). No statistical difference is confirmed among the 3 treatments. ◆ denotes each case and ◇ denotes each case whose level is undetectable, ----: lower limit of level detectable by RIA.

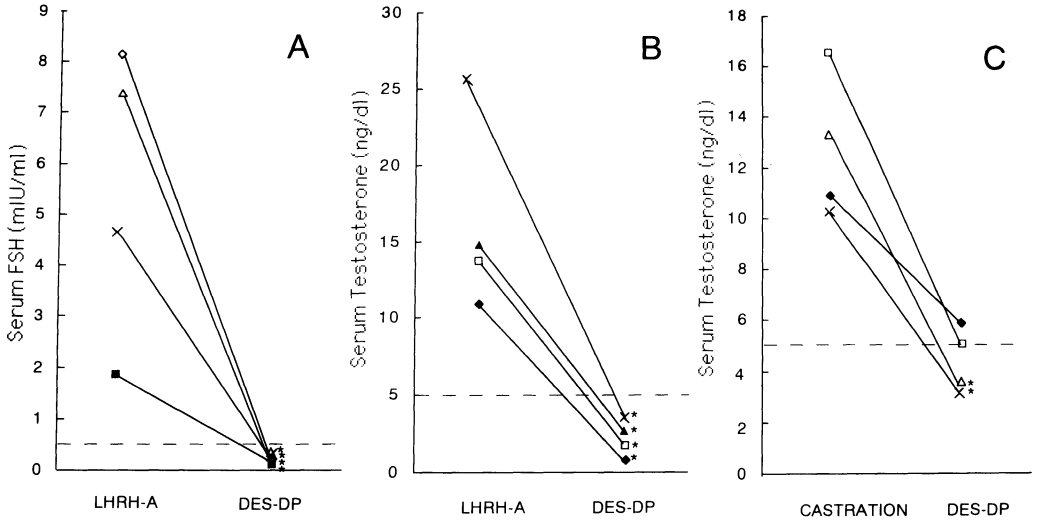

Fig. 3. A: Suppression of serum FSH by DES-DP administration in patients (n=4) treated with LHRH-A. DES-DP lowered serum FSH to undetectable level ($P<0.05$). *: undetectable case, ----: lower limit of level detectable by RIA. B: Suppression of serum testosterone (T) by DES-DP administration in patients treated with LHRH-A. DES-DP diminished serum T significantly ($P<0.005$). *: undetectable case, ----: lower limit of level detectable by RIA. C: Suppression of serum T by DES-DP administration in castrated patients (n=4). DES-DP decreased serum T remarkably ($P<0.01$). *: undetectable case, ----: lower limit of level detectable by RIA.

statistically (by Tukey-Kramer HSD comparisons).

In 4 patients showing relapse with LHRH-A, DES-DP administration decreased serum FSH ($P<0.05$) and T ($P<0.005$) significantly (Figs. 3A and 3B). In 4 other patients who received castration, DES-DP lowered serum T ($P<0.01$) remarkably (Fig. 3C).

Discussion

Since serum T levels decline with age in senescence and patients in the present study were younger in the DES-DP group than in the castration and LHRH-A groups, it appears unlikely that lower serum testosterone levels after DES-DP administration in patients castrated or with LHRH-A were due to the effect of aging. Further, the effect of DES-DP seems not to result from negative feed-back of DES-DP to the pituitary-adrenal axis, namely a reduction in adrenal androgens caused by decrease in serum ACTH, because estrogens have been known to stimulate the adrenal cortex in women by increasing serum ACTH as the result of enhancement of cortisol binding globulin production [9]. In addition, estradiol-17β has been reported to stimulate the production of CRF by the isolated hypothalamus *in vitro* [11] and also to increase serum pregnenolone, cortisol and dehydroepiandrosterone sulfate (DHEA-S) in postmenopausal women [12].

On the other hand, estrogen has been reported to eliminate serum DHEA-S in men [13]. Estrogens inhibit porcine testicular microsomal cytochrome P-450 (17α-hydroxylase/C-17,20-lyase) *in vitro* [14] and maternal treatment with estrogens induces reduced expression of the messenger RNA and protein for cytochrome P-450 (17α-hydroxylase/C-17,20-lyase) in fetal Leydig cells of the rat [15]. Taken together, potent inhibition of the adrenal enzyme(s) by estrogens might be one of the mechanisms of DES-DP's effect on serum T levels.

The present results indicate that DES-DP administrations block T supply from the extra-testicular site(s) which is still present after castration, but do not show that the treatment decreases adrenal androgen synthesis or secretion. Approximately 5% of circulating testosterone in human males has been demonstrated to be supplied by peripheral bio-conversion of adrenal androgens [16]. After surgical or medical castration, nonetheless, approximately 40% of the dehydrotestosterone (DHT) concentration remains in the prostate comparing with intact men [17],

and concentrations of circulating androgen metabolites are 40 to 50% of those of intact men [18]. Actually, levels of plasma DHEA-S in adult men are 100 to 500 fold higher than those of testosterone. Further, estrogens are thought to inhibit activities of the enzymes, such as 17β-hydroxysteroid dehydrogenase, which involve T formation from adrenal androgens in peripheral sites [19]. Consequently, the reduction of serum T to an undetectable level by DES-DP might be, at least to some extent, attributable to the action of peripheral bio-conversion.

Not a few reports [3, 20, 21] have emphasized the importance of adrenal androgens in the proliferation of prostate cancer cells and the therapeutic advantage of maximal androgen blockade. Amplification of the androgen receptor gene in hormone-resistant prostate cancer cells has also been reported [20], which suggests that a low concentration of T after castration is still meaningful in the growth of hormone refractory prostate cancer cells.

The effect of DES-DP administration on serum T levels provides evidence that therapeutic doses of SEs are able to achieve stronger androgen ablation than castration or LHRH-A administration, which offers an insight into mechanisms of the effect of SEs on prostate cancer relapsing after castration or LHRH-A treatment. Nevertheless, the aforementioned 2 putative mechanisms need to be elucidated.

The current findings on the effect of LHRH-A on serum FSH are consistent with those in previous reports [2] and suggest that basal FSH secretion independent of LH-RH in aged males is present as observed in human females [22]. LH-RH-independent FSH synthesis and secretion have been recognized by using monolayer cell cultures and perifusion of the rat pituitary [23]. Estradiol-17β has been unable to suppress the basal FSH secretion from cultured pituitary cells obtained from intact and castrated male rats at the physiological concentration of 0.1 nM [24], but the therapeutic dose of DES-DP in the current study suppressed the basal FSH secretion in aged males, possibly by a direct action on the pituitary. Consistent with the present findings, estradiol-17β has been shown to inhibit transcription of genes encoding the subunits of ovine FSH [25].

In conclusion, the therapeutic dose of DES-DP is able to suppress basal FSH secretion by direct action on the pituitary, and circulating T by inhibiting both LH secretion and extragonadal production of T in aged men, which offers a clue to the mechanisms of the effect of SEs on prostate cancer relapsing after castration or LHRH-A administration.

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