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The Changes in the Binding Capacity of Testosterone-oestradiol Binding Globulin (TeBG) Following Castration and DES-D Administration in Patients with Prostatic Carcinoma

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Summary. The levels of plasma testosterone, testosterone-oestradiol binding globulin (TeBG) and total serum acid phosphatase (TSAP) following antiandrogenic hormone therapy were investigated in 17 patients with prostatic carcinoma. The low levels of plasma total and free testosterone induced by castration decreased further after diethylsitlboestrol diphosphate (DES-D) administration. Plasma TeBG binding capacity after castration was 118.9% of the pre-treatment level and increased to 193.9%, 204.0% and 212.7% at 1, 2 and 3 weeks after DES-D dosing. The in vitro binding of ^{3}H-testosterone to TeBG was not influenced in the presence of DES-D or stilboestrol. Clinical response following the DES-D therapy was associated with a decrease in the levels of TSAP. A significantly inversed correlation was found between the decrease in TSAP and increase in TeBG at completion of DES-D therapy. These results suggest that the high binding capacity of TeBG lowers the biologically active fraction of testosterone and thus may produce clinical effects.

Key words: TeBG, Free testosterone, Acid phosphatase, Prostatic carcinoma, Castration and DES-D therapy.

Introduction

Antiandrogenic hormone therapy has been widely used for the treatment of prostatic carcinoma. Many reports are available concerning the hormonal milieu of this disease [1, 5, 9]; however, few reports have been presented on TeBG, because of the difficulties of measuring TeBG.

Herein, we have measured the binding capacity of TeBG after castration and DES-D treatment in patients with prostatic carcinoma, using steady state polyacrylamide gel electrophoresis (SS-PAGE) developed by Ritzen et al. [8], and have calculated the free testosterone concentration in plasma. In addition, the effects of DES-D and stilboestrol on the binding of ^{3}H-testosterone to TeBG following DES-D therapy were studied. Furthermore, to study the role of TeBG in regulating the concentrations of unbound sex steroids during the treatments of prostatic carcinoma, the relationship between TSAP levels, the marker of the clinical effectiveness and the changes in TeBG following castration and DES-D therapy was surveyed.

Materials and Methods

Seventeen patients with histologically diagnosed advanced prostatic carcinoma were studied. All patients were castrated and 1 week after the operation they were given 500 mg of DES-D intravenously for 20 days. Blood samples were taken before and 1 week after castration, and also 1, 2 and 3 weeks after DES-D administration. After separation, the plasma samples were stored at −20 °C.

Plasma concentrations of testosterone and the binding capacity of TeBG were determined as follows; plasma was extracted twice with ethyl ether, the extract was taken to dryness under air jet, and then the residue was applied to thin layer plate (Kiesel gel 60 F_{254}, Merck). The plate was developed in solvent system, chloroform:ethyl acetate:petroleum ether = 50:45:5 (V/V/V). After the development the silica of testosterone area was taken and testosterone was measured by specific radioimmunoassay of Nieschlag and Loriaux [6]. To measure the binding capacity of TeBG the endogenous unbound steroids were removed first by treatment with 0.5% charcoal for 15 min at 4 °C. The stripped plasma was incubated with 20,000–60,000 cpm of ^{3}H-testosterone (S.A. = 93 Ci/mmol, The Radiochemical Centre Amersham Co., England) for 1 h at 4 °C in the absence or presence of various concentrations of testosterone, DES-D and stilboestrol, and then subjected to SS-PAGE (7.5% T, 5% C) at 4 °C, pH = 10.0. The electrophoresis was run for 3 h at 2 mA per gel containing 20,000 cpm of ^{3}H-testosterone. Following electrophoresis, the gels were sliced transversely (3 mm), each slice placed into counting vials containing 5 ml of Bray's solution (dioxane 880 ml, methanol 100 ml, ethylenglycol 20 ml, naphthalin 60 g, PPO 4 g, POPOP 0.4 g) and radioactivity was determined in a liquid scintilation spectrometer.

On the calculation of binding capacity of TeBG, Kd was determined. Aliquots (5 µl) of plasma were run in polyacrylamide gel containing different amounts of ^{3}H-testosterone (21 to 170×10^{-12} M). After electrophoresis, the amount of bound testosterone was calculated from the area under the TeBG peak, subtracting the free radioactivity. Knowing BPtot from Scatchard plot (Fig. 1) and

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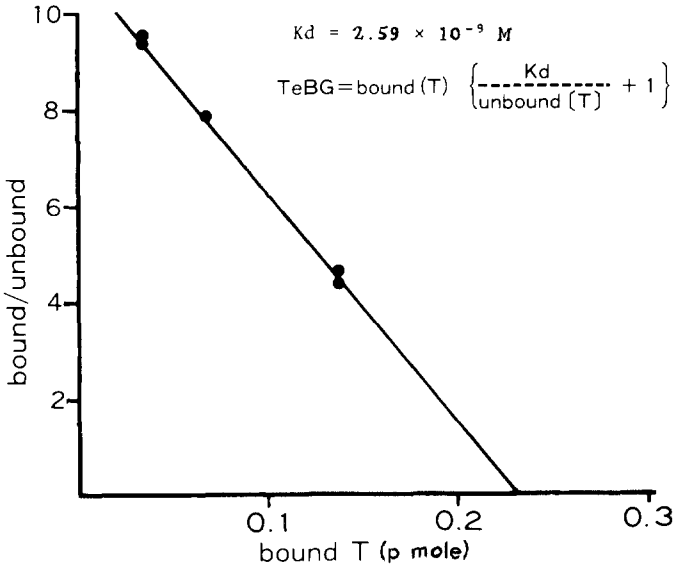

Fig. 1. Scatchard plot for determining the binding capacity and Kd of TeBG

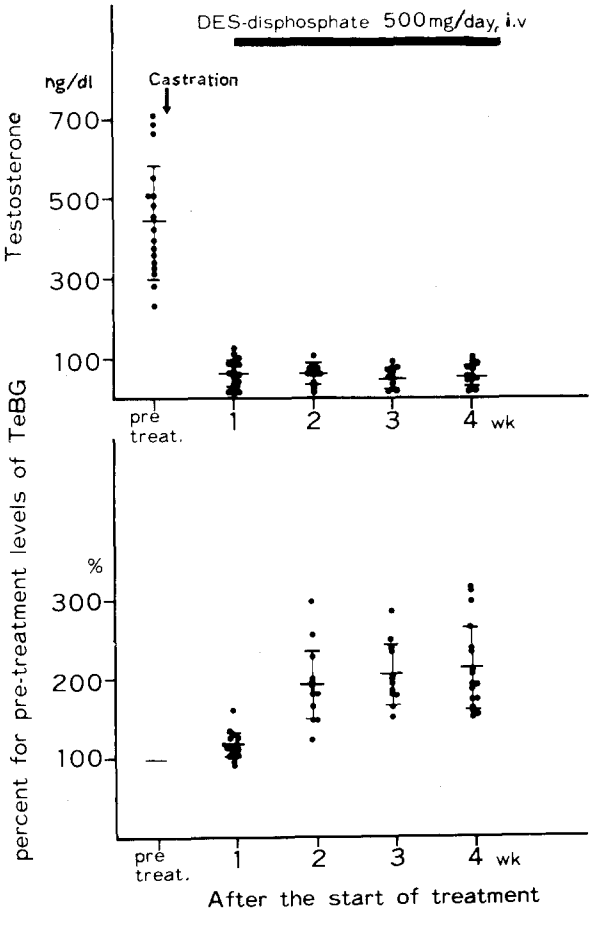

Fig. 2. Changes of plasma testosterone and TeBG levels after the treatment in patients with prostatic carcinoma

measuring [Su] and Sb in the gel, Kd can be calculated from the following equation [8].

$$BPtot = Sb\left(\frac{Kd}{[Su]} + 1\right)$$

where Kd = equilibrium constant of dissociation, Sb = bound steroid, Su = unbound steroid. Calculated Kd of normal human plasma was 2.59×10^{-9}M.

Calculation of the free testosterone concentration in plasma was based on the following equation employed by Pearlman [7].

$$Sb/Su = K_1(n_1p_1 - S) + n_2p_2k_2.$$

where Sb and Su represent the molar concentration of bound and unbound testosterone; k_1 equilibrium association constant for testosterone binding to TeBG, 3.86×10^8 M^{-1}, n_1p_1, the molar concentration of TeBG binding sites in plasma; S, the molar conentration of endogenous testosterone in plasma determined by radioimmunoassay; K_1, the equilibrium association constant for the binding of testosterone to albumin, 3.86×10^4 M^{-1}; n_2p_2, the molar concentration of albumin binding sites, 5.0×10^{-4} M. Since S = Sb + Su, it follows that the free testosterone concentration,

$$Su = \left[\frac{1}{1 + \frac{Sb}{Su}}\right] S$$

TSAP was assayed before and at 3 weeks after DES-D therapy using the method of Kind-King [3].

Results

Plasma testosterone levels and the binding capacity of TeBG during treatment in patients with prostatic carcinoma are shown in Fig. 2. Plasma testosterone level was approximately 10% of the pre-treatment level in the majority of the patients 1 week after castration. Following DES-D treatment plasma testosterone level decreased slightly, and was not detectable in three patients. In contrast, plasma TeBG level increased to 118.9% of the pre-treatment level at 1 week after castration. The administration of DES-D for 1 week raised TeBG levels to 193.9% compared to pre-treatment levels, and the levels remained high throughout DES-D therapy (204.0%–212.7% of the pre-treatment level).

Effects of testosterone, DES-D and stilboestrol on the binding of ^{3}H-testosterone to TeBG are shown in Figs. 3 and 4. Ten to 100 ng of unlabelled testosterone completely displaced ^{3}H-testosterone binding to TeBG, but 100 pg of testosterone was not enough to inhibit the binding. Increasing concentrations of DES-D from 100 pg to 1,000 ng did not cause any decrease in the binding of ^{3}H-testosterone to either TeBG or albumin. Stilboestrol, the active form of DES-D, did not cause any decrease in the binding of ^{3}H-testosterone to TeBG and albumin.

Plasma-free testosterone concentrations in patients with prostatic carcinoma after treatment are shown in Fig. 5. Plasma free testosterone concentration was 146.4 ± 56.5 (SD) pg/ml before treatment and decreased significantly to 15.9 ± 8.0 (SD) pg/ml at 1 week after castration. Following

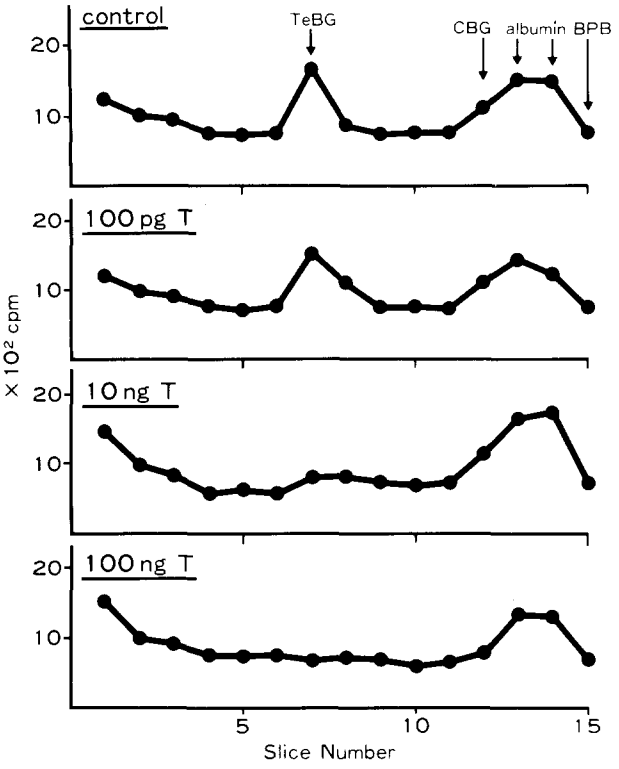

Fig. 3. Effect of testosterone on binding of ^{3}H-testosterone to TeBG, CBG and albumin

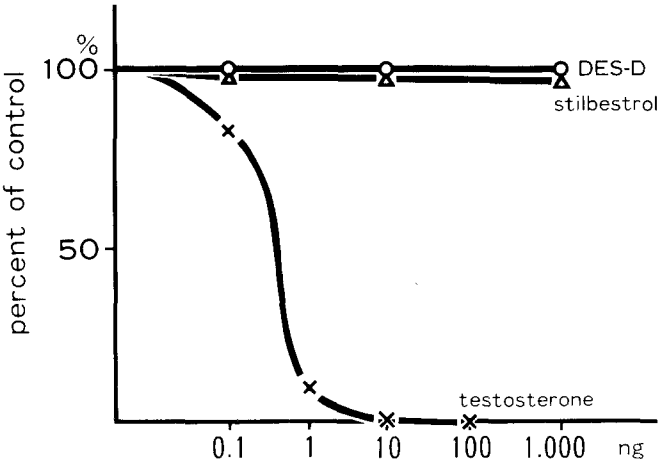

Fig. 4. Effects of testosterone, DES-D and stilboestrol on binding of ^{3}H-testosterone to TeBG

DES-D therapy plasma-free testosterone concentration decreased furthermore. At completion of DES-D therapy, plasma-free testosterone concentration decreased to approximately 9% of the pre-treatment level.

The levels of TSAP before treatment were high in 13 out of 17 cases. Both percent decrease of TSAP and percent increase of TeBG compared to the pre-treatment level at completion of DES-D therapy are plotted in Fig. 6. A significant inverse correlation ($Y = 173.4–0.501\ X, p < 0.01$) was observed between these parameters of TSAP and TeBG.

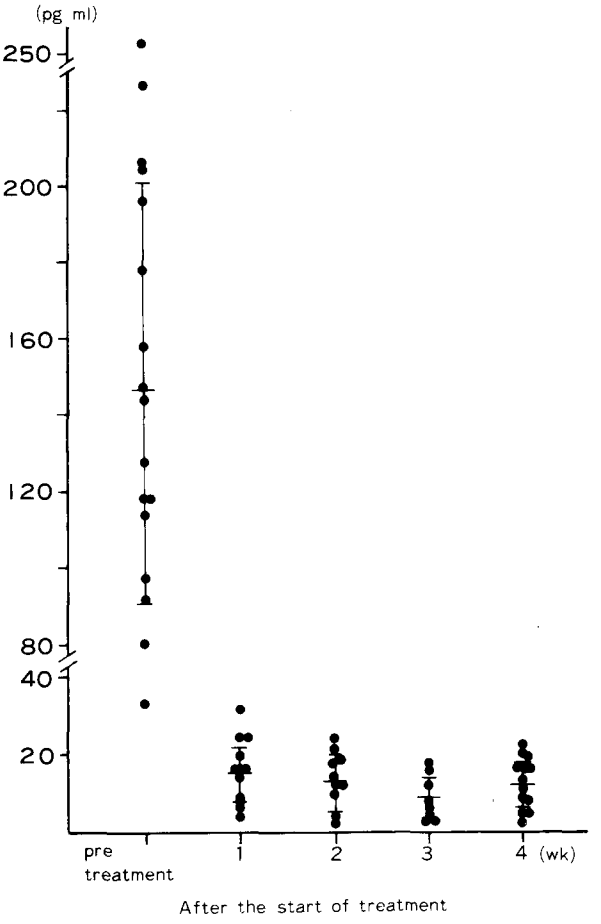

Fig. 5. Changes of plasma free testosterone levels after the treatment in patients with prostatic carcinoma

Discussion

The function of TeBG is presumed to prevent rapid fluctuations of biologically active sex steroids. TeBG was shown to be altered under various conditions such as pregnancy, ageing and oestrogen administration, when remarkable changes in endocrinological milieu occur. As shown in the present study, castration and DES-D administration caused a significant increase in TeBG level and decrease in plasma testosterone level confirming the previous resports [1, 9].

For the measurement of TeBG level, the ammonium sulphate precipitation and charcoal absorption methods were widely used; however, these methods did not provide quantitative levels accurately because of the effect of plasma albumin. The increase in plasma albumin with low affinity but high capacity to testosterone was about 7% after castration with oestrogen administration [5]. For this disadvantage, SS-PAGE has the advantage of high resolution of gel electrophoresis, since several components such as corticosteroid binding globulin (CBG) and albumin can be studied simultaneously in the same gel. The present estimate of plasma TeBG level by means of SS-PAGE at the termination of 10 g of DES-D administration was about 2.1 times

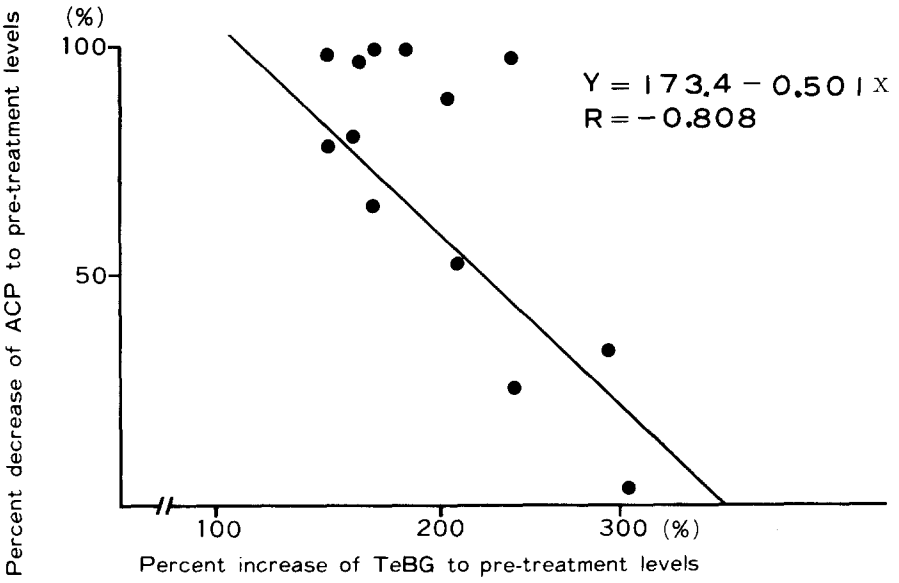

Fig. 6. Correlation between TSAP and TeBG following treatment in patients with prostatic carcinoma

that of pre-treatment level. The marked increase in ^{3}H-testosterone binding to CBG and albumin was also observed in the same gels (data not shown). The result of measurement of TeBG by ammonium sulphate precipitation or charcoal absorption showed that the TeBG level following oestrogen administration increased to 409% [1] and 480% [2] of the pre-treatment level. This difference of the TeBG levels might be due to the methods used or the kinds and dose of oestrogenic compounds. The results with higher levels obtained by ammonium sulphate precipitation and charcoal absorption methods might be partly due to the influence of increased albumin and CBG.

The increase in TeBG with the decrease in testosterone after DES-D administration lowers the active form of testosterone further. These changes may produce a hormonal milieu which is preferable to the suppression of the growth of prostatic carcinoma.

The mechanism of the increase of TeBG by DES-D administration was unclear, but it could be supported that the production of TeBG in the liver was stimulated by DES-D or the metabolism of TeBG was decreased by DES-D. The present study showed that DES-D and stilboestrol did not influence the binding of ^{3}H-testosterone to TeBG in vitro, suggesting no direct effects of DES-D on the binding capacity of TeBG.

In patients with prostatic carcinoma, the high TSAP levels decline in accord with the response of the anti-androgenic treatment, and so the determination of TSAP has been reported to be a useful tool in the assessment of the therapeutic response with regard to the survival rate [4]. The percent increase in TeBG after DES-D treatment correlated significantly with the percent decrease in TSAP. Thus the increase in TeBG level might produce a therapeutic response by decreasing free testosterone in patients with prostatic carcinoma.

In conclusion, DES-D did not influence the in vitro binding of ^{3}H-testosterone to TeBG and the increase in TeBG after DES-D treatment reduced the active form of plasma testosterone and thus led to the clinical effects with a decrease in TSAP level in prostatic carcinoma.

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