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Serum Androgen Bioactivity During 5α-Dihydrotestosterone Treatment in Elderly Men

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ABSTRACT: The androgens used in the treatment of age-related androgen decline have different bioactivities that cannot be evaluated with conventional detection methods for serum steroids. We have recently developed a recombinant cell bioassay for the determination of androgen bioactivity in human serum that is based on androgen-specific interaction between the ligand-binding domain (LBD) and the N-terminal region of the androgen receptor (AR). In this work, we examined the effect of topically applied 5α-dihydrotestosterone (DHT; 7.5–10 g of 2.5% DHT gel daily for 6 months) on circulating androgen bioactivity in 14 men (age range, 51–63 years) with symptoms of andropause and pretreatment serum testosterone less than 15 nM, or serum sex hormone-binding globulin concentration greater than 30 nM, or both. The mean (±SEM) pretreatment androgen bioactivity was 3.3 ± 0.3 nM testosterone equivalents, and the levels correlated with serum testosterone concentration ($r = .55$, $P < .05$). DHT gel treatment induced a sixfold increase (from 1.5 ± 0.1 nM to 9.0 ± 0.7 nM) in mean serum DHT level, whereas endogenous testosterone and estradiol levels measured with radioimmunoassays were suppressed by approximately 70% and approximately 50%, respectively ($P < .0001$). Concomitantly, serum androgen bioactivity increased by sevenfold (from 3.3 ± 0.3 to 23.6 ± 2.8 nM testosterone equivalents; $P < .0001$). We conclude that DHT gel therapy in elderly men significantly increases their circulating androgen bioactivity as measured with a mammalian cell bioassay. An androgen-specific bioassay such as ours may enable investigation of other androgens with different bioactivities, such as selective AR modulators.

Key words: Androgen decline, bioassay, DHT.
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In men, serum testosterone levels decline and serum sex hormone–binding globulin (SHBG) concentrations increase with aging; these reciprocal changes lead to even faster decline in bioavailable (non-SHBG bound) testosterone than that observed in total serum testosterone concentration alone (Basaria and Dobs, 2001; Snyder, 2001; Vermeulen, 2001). The symptoms of age-related androgen deficiency may include decreased muscle mass, osteoporosis, increased central body fat, and subjective symptoms such as decrease in libido, loss of memory, insomnia, and a lesser sense of well-being (Snyder, 2001; Vermeulen, 2001). It is possible to treat age-related androgen decline with oral testosterone derivatives, injections of testosterone esters, transdermal/transscrotal testosterone patches, or with transdermal 5α-dihydrotestosterone (DHT).

Because androgens used in substitution therapy differ in their biological activities and may interfere with the endogenous androgen-estrogen balance, the effect of steroid treatment on the circulating androgen milieu has been difficult to evaluate on the basis of biochemical measures. To this end, we have recently developed a mammalian cell bioassay that is based on androgen-dependent interaction between the ligand-binding domain (LBD) and the N-terminal region of the androgen receptor (AR; Raivio et al, 2001). The assay is androgen-specific, accounts for differences in androgen bioactivities, and enables detection of androgen bioactivity in human serum (Raivio et al, 2001). The aim of the present study was to investigate the effect of DHT gel treatment on serum androgen bioactivity in elderly men (Kunelius et al, 2002) who used a transdermal steroid application for 6 months. We have previously observed that this treatment significantly alters serum DHT-testosterone balance, but the net effect on circulating androgen bioactivity is equivocal.

Materials and Methods

Subjects

Fourteen men (age range 51–63 years, mean 55 years) from a previous study (Kunelius et al, 2002) treated with transdermal DHT gel were investigated. In short, the men should have had rarefaction of nocturnal penile tumescence and at least one of

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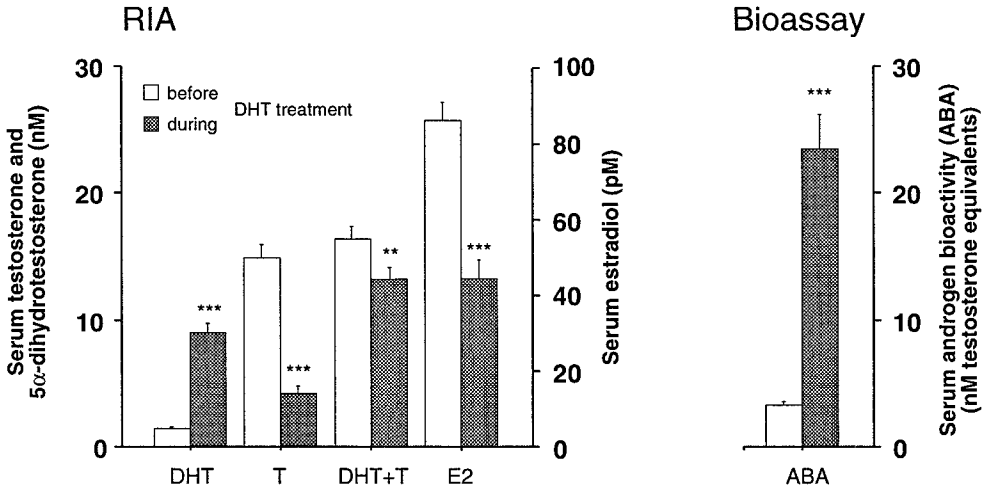

Serum DHT, testosterone, the sum of 2 androgens, and serum estradiol levels measured with RIAs (left panel), and serum androgen bioactivity (ABA) measured with the bioassay (right panel) in 14 elderly men before and after 6 months of daily transdermal DHT treatment (7.5–10 g of 2.5% DHT gel applied to upper arms/shoulders or to the abdomen daily) as described in ''Materials and Methods.'' Mean values with SEM are shown. ***$P < .0001$. **$P < .01$.

the following andropause symptoms: decreased libido, erectile dysfunction, urinary disorders, asthenia, or depressive mood together with either serum testosterone level less than 15 nM or serum SHBG concentration greater than 30 nM, or both. The men applied 2.5% DHT gel (Laboratories Besins Iscovesco, Paris, France) 7.5–10 g daily to upper arms/shoulders or to the abdomen. The dose of the gel was adjusted on the basis of serum DHT concentrations measured by radioimmunoassay (RIA) after 3 weeks of treatment (Kunelius et al, 2002). The study was approved by the ethical committee of the University of Oulu.

Androgen Bioassay

All plasmid constructs together with cell culture and transfection procedures of the androgen bioassay have been described elsewhere (Raivio et al, 2001). In short, COS-1 cells were transiently transfected with plasmids encoding the LBD and the N-terminal region of the AR. In the presence of androgens, these proteins interact, which is amplified by the ARIP3 coactivator expressed ectopically in the same cells. The reporter gene (luciferase) activity in cell lysates is derived from the androgen bioactivity in human serum added directly to the culture medium. Serum from each subject was sterile-filtered and stored at −70°C until used in the bioassay. Circulating androgen bioactivity was measured before and at 6 months of DHT treatment. The values are expressed in nanomolar testosterone equivalents.

Immunoassays

Hormone levels before and during DHT treatment obtained with immunoassays have been described elsewhere (Kunelius et al, 2002). In short, testosterone levels were determined using an ACS:180 chemiluminescence system with an ACS:180 analyzer (Chiron Corp, Emeryville, Calif) with intraassay and interassay coefficients of variation (CVs) of 4.0% and 5.6%, respectively. Serum DHT concentrations were measured by RIA after organic extraction and hydrophobic chromatography (Apter et al, 1976); the intra-assay and interassay CVs were 9.1% and 6.6%, respectively. Serum SHBG, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prostate-specific antigen (PSA) concentrations were measured by fluoroimmunometric methods with kits obtained from Perkin Elmer Life Sciences (Turku, Finland) using a 1235 AutoDELFIA automatic immunoassay system. The intra-assay and interassay CVs were as follows: SHBG, 1.3% and 5.1%; LH, 4.9% and 6.5%; FSH, 3.8% and 4.3%; and PSA, 1.2% and 3.8%. Serum estradiol levels were measured by RIA (Orion Diagnostica, Turku, Finland) with intra-assay and interassay CVs of 5.7% and 6.4%, respectively.

Data Analysis

The relationships between different variables were assessed with Spearman rank correlation analysis. A paired *t* test was used to investigate the treatment-induced changes in paired variables. Results were considered statistically significant when $P < .05$.

Results

Circulating androgen bioactivity (ABA) was investigated in 14 elderly men (Kunelius et al, 2002) using a recently validated androgen bioassay (Raivio et al, 2001). Mean (±SEM) serum hormone levels for these men at baseline are shown in the Figure. Serum ABA levels (mean 3.3 ± 0.3 nM, range 1.4–4.6 nM testosterone equivalents) correlated positively with serum testosterone levels ($r = .55$, $n = 14$, $P < .05$), but not significantly with age, serum gonadotropin, estradiol, DHT, or PSA levels.

Treatment with DHT gel for 6 months brought about clear changes in serum sex steroid levels measured with RIAs: mean DHT concentration increased sixfold (from 1.5 ± 0.1 nM to 9.0 ± 0.7 nM $P < .0001$), testosterone concentration was suppressed (from 14.7 ± 1.0 nM to 4.1 ± 0.5 nM $P < .0001$), and serum estradiol concentration

decreased (from 86 ± 5 to 44 ± 5 pM $P < .0001$). In addition, the sum of DHT and testosterone concentrations measured with RIAs decreased (from 16.2 ± 1.0 nM to 13.1 ± 0.9 nM during the treatment $P < .01$). Simultaneously with these changes, serum ABA displayed a substantial increase (from 3.3 ± 0.3 to 23.6 ± 2.8 nM testosterone equivalents; $P < .0001$). These changes are illustrated in the Figure. DHT treatment also suppressed mean LH levels (from 3.9 ± 0.4 IU/L to 2.2 ± 0.5 IU/L; $P < .001$), and FSH levels (from 4.7 ± 0.6 to 2.7 ± 0.4 IU/L; $P < .001$; n = 13). Mean serum SHBG at 0 months was 43 ± 3 nM, and at 6 months it was 38 ± 2 nM ($P = .051$); serum PSA did not change significantly during the study. At the end of the study, serum ABA and DHT levels were positively correlated ($r = 0.67$; n = 14, $P < .05$).

Discussion

The biological potencies of androgens available for substitution therapy differ, which cannot be evaluated by conventional detection methods for serum steroids. We have therefore introduced a novel means for quantifying circulating androgen bioactivity in elderly men by using a recombinant cell bioassay based on the androgen-specific interaction between two fragments of AR. The strengths of this assay are the specificity for androgens and the capability to account for differences in androgen biopotencies (Raivio et al, 2001).

At the onset of the study, serum testosterone and androgen bioactivity levels were positively correlated, suggesting that testosterone determines the circulating androgen milieu in elderly men. We have previously observed a similar relationship in prepubertal and early pubertal boys (Raivio et al, 2001). Serum androgen bioactivity levels in men after 6 months of DHT treatment were higher than in boys or men without treatments affecting the hypothalamic-pituitary-testicular axis (unpublished observations). DHT has higher affinity for the AR (Quigley et al, 1995) and it is a more potent androgen than testosterone. In agreement with these findings, serum androgen bioactivity increased during the course of this study, despite the decline in circulating testosterone levels. This suggests that our bioassay can be applied to investigate potencies of androgens used in substitution therapy. For example, relatively little is known about the androgenic properties of 7α-methyl-19-nortestosterone (MENT), a selective androgen receptor modulator already employed in androgen replacement therapy for men with hypogonadism (Anderson et al, 1999).

Currently used assays for evaluating responses to exogenous androgens in humans are not necessarily androgen-specific. For example, serum gonadotropin and SHBG levels are both affected by the actions of androgens and estrogens (van Look and Frölich, 1981; Sinnecker and Köhler, 1989; Finkelstein et al, 1991). Exogenous androgens used in the substitution therapy may be converted to estrogens by the aromatase enzyme, or androgens may alter the circulating androgen-estrogen balance by interfering with the function of the hypothalamic-pituitary-testicular axis, or both may occur. In keeping with the latter mechanism, topically applied DHT decreased serum estradiol levels in subjects in the present study. By contrast, the bioassay employed in this work is specific for androgens and should therefore be well suited for monitoring serum androgen bioactivity induced by the substitution therapy.

In conclusion, we have measured serum androgen bioactivity levels in elderly men before and during transdermal DHT therapy by using a recently developed androgen bioassay. Our results show that transdermal DHT significantly increases serum androgen bioactivity, despite a significant decrease in serum testosterone measured by RIA. A bioassay such as ours offers a novel means for monitoring bioactivities of drugs acting through the androgen receptor.

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