

Dramatic Suppression of Plasma and Urinary Prostate Specific Antigen and Human Glandular Kallikrein by Antiandrogens in Male-to-Female Transsexuals

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Abstract

Purpose: Prostate specific antigen (PSA) and human glandular kallikrein (hK2) are mainly produced by the prostate and their genes are regulated by androgens through the androgen receptor. We determine whether PSA and hK2 change significantly in plasma and urine after antiandrogen treatment in male-to-female transsexuals.

Materials and Methods: Plasma and urine PSA and hK2 were measured with highly sensitive immunofluorometric procedures capable of detecting within 1 or 6 ng./l. PSA or hK2, respectively. Study groups consisted of 10 men treated with cyproterone acetate only (group 1), 15 transdermal estradiol plus cyproterone acetate (group 2) and 31 ethinyl estradiol plus cyproterone acetate (group 3). Plasma and urine samples were collected before initiation of treatment as well as after 4 months of hormonal therapy. For a subset of group 3 patients blood and urine samples were also obtained after 12 months of treatment.

Results: Cyproterone acetate, a steroidal antiandrogen, alone or with estradiol was able to suppress greater than 90% of plasma and urinary PSA and hK2 concentration after 4 or 12 months of therapy.

Conclusions: Cyproterone acetate therapy causes dramatic suppression of plasma and urinary PSA and

hK2 in men without prostate cancer. Since cyproterone acetate is used for prostate cancer treatment, suppression of PSA after hormonal therapy may not accurately reflect therapy success in reducing tumor burden.

Prostate specific antigen (PSA) and human glandular kallikrein (hK2) are closely related serine proteases which are readily detectable in male serum or plasma and urine with sensitive immunofluorometric procedures. In males the primary source of PSA and hK2 in blood are the epithelial cells of the prostate gland whereas in urine most PSA has been shown to originate from the periurethral glands.^{1,2} However, PSA and hK2 expression is not limited to the male urogenital system. Both kallikreins are expressed in female tissues, such as the epithelial cells of the normal breast, and exist at relatively high levels in female nipple aspirate fluid, breast cyst fluid and milk of lactating women.^{3,4}

Androgen and estrogen receptors are central to hormonal regulation of the prostate.⁵ Previous studies have demonstrated that PSA and hK2 are primarily up regulated by androgens and progestins.⁶ While estrogens and androgens exert their effect independently, a close relationship exists between them, as determined in tissue culture studies when maximal prostate cell stimulation occurred in the presence of estrogen and androgen. There is some

indication that estrogens have the ability to exert their effect by modulating the sensitivity of the prostate to androgens.⁵

The mechanism of activation of the androgen receptor has partially been elucidated. With androgenic stimulation the C terminal portion of the androgen receptor forms a complex with its ligand, and the complex is then translocated to the nucleus where it can transactivate PSA, hK2 and other genes.⁷

Transactivation occurs through the interaction of the N terminal portion of the androgen receptor with the PSA promoter and enhancer which contain 3 distinct androgen response elements.⁸⁻¹¹ A similar process is thought to occur in extraprostatic tissues.^{4,12} In LNCaP prostatic carcinoma cells prolonged androgenic stimulation resulted in self-limited transactivation of androgen sensitive promoters due to the existing negative feedback of the androgen receptor on its own production.¹³

While androgens stimulate, antiandrogens have the ability to block up regulation of androgen regulated genes. One such antiandrogen is cyproterone acetate, which has antiandrogenic and progestational properties.¹⁴ This synthetic steroid can also affect growth hormone, free cortisol and pituitary luteinizing hormone (LH) levels.¹⁴ Cyproterone acetate acts as a classic antiandrogen by counteracting transcription from the androgen receptor gene in LNCaP cells. In vitro it has been shown that cyproterone acetate acts as an androgen agonist on kallikrein gene transcription, that is it fails to antagonize androgenic induction.¹⁵ However, this finding may have been due to an insufficient amount of antiandrogen, since cyproterone acetate has a relatively low affinity for the androgen receptor, thus requiring several hundred-fold excess in addition to the amount of androgen needed to block androgenic action effectively.¹⁶ In vivo cyproterone acetate has a more potent effect with coadministration of estrogen¹⁴ because the latter negatively regulates LH pulse, thus affecting androgenic stimulation.¹⁷ In effect with this combination estrogen has a protective action on cyproterone acetate via hypothalamus pituitary axial hormone regulation.¹⁷

Advanced prostatic carcinomas are androgen dependent. Treatment strategies involve the administration of antiandrogens to starve tumor cells, thus inducing growth arrest or apoptosis.^{18,19} Because of its antiandrogenic nature and suppressant effect on LH release and, thus, on testosterone, cyproterone

acetate is used to treat prostatic carcinoma.^{20,21}

However, prolonged androgenic suppression may result in cessation of androgen induced differentiation of tumorigenic cells which, in turn, may lead to selection for androgen independent tumor cells.¹⁸ For this reason androgen withdrawal therapy with treatment interruptions has been suggested as a better alternative for tumors, such as Shionogi carcinoma, to prevent loss of androgen dependence.¹⁸

Successful treatment of prostatic adenocarcinoma relies heavily on PSA as the monitoring tumor marker. The clinical value of PSA is somewhat limited by the fact that it is also elevated in patients with benign prostatic hyperplasia and by the realization that PSA may change during hormonal manipulations without necessarily reflecting tumor volume. We evaluated the effect of administration of antiandrogens on plasma and urine PSA and hK2 in 56 male-to-female transsexuals who were undergoing long-term antiandrogen therapy. Our study may give insight as to how much these biomarkers change during long-term therapy in patients who do not have cancer and how effective this antiandrogen is in blocking androgen dependent gene expression in the normal prostate gland.

Materials and Methods

Drug treatment and sample collection. Treatment consisted of 100 µg. oral ethinyl estradiol daily with 100 mg. cyproterone acetate daily for 12 months in 31 transsexual males with a median age of 30 years (range 18 to 43, group 1) transdermal 17-[beta]-estradiol administered twice weekly with 100 mg. daily cyproterone acetate for 12 months in 15 transsexual males with a median age of 31 years (range 20 to 44, group 2) and 100 mg. daily cyproterone acetate alone for 12 months in 10 males with a median age of 34 years (range 19 to 50, group 3). Venous blood samples were collected between 9 and 11 a.m. after an overnight fast at baseline and after 4 months of hormonal administration for all patients, and also after 12 months of hormonal administration for groups 1 and 2. Fasting 2-hour morning urine samples were collected at baseline and after 4 months of hormonal treatment for all patients, and also after 12 months of hormonal treatment for groups 1 and 2. Blood samples were separated within 1 hour of sample collection, and plasma, serum and urine samples were stored at -20C until immunological analyses were performed.

Immunological assays. PSA was measured in undiluted plasma and urine initially diluted 1:100 in a 60 gm./l. bovine serum albumin solution, pH 7.40, using a 1-step time resolved immunofluorometric assay performed as described elsewhere.²² For urine samples with results below the detection limit, that is less than 1 ng./l., measurement was repeated without prior sample dilution. hK2 was measured in undiluted plasma and urine diluted 1:5 as described previously for PSA using a 2-step time resolved immunofluorometric assay developed by us, which was performed as described elsewhere.²³ When assay results of the diluted urine samples were below the detection limit, that is less than 6 ng./l., assays were rerun without sample dilutions. PSA and hK2 results were reported in ng./l. after adjustments were made for dilutions when applicable. Commercially available immunoassay kits were used to determine other parameters in serum and urine, including serum free thyroxine, serum testosterone (nmol./l.), serum 5[alpha]-dihydrotestosterone after oxidation and extraction, serum 17-[beta]-estradiol, serum androstenedione after extraction, serum dehydroepiandrosterone sulfate, serum thyrotropin and 24-hour urinary free-cortisol after extraction. Chemiluminescence immunoassays were used to assess serum LH and follicle-stimulating hormone (FSH). In addition, commercially available immunoradiometric assays were used to measure serum sex hormone-binding globulin, prolactin, insulin and growth hormone.

Statistical analysis. Because the distributions of all measured variables were nonGaussian, statistical

analysis was performed using nonparametric tests. If values were below the lower limit of detection, the value of that limit was used for statistical analysis (LH 0.3 IU/l. and FSH 0.5 IU/l.). Wilcoxon's signed ranks test was used to analyze the effects of hormonal administration at baseline versus 4-month values and, when applicable, versus 12-month values. Association of PSA and hK2 in plasma and urine with other measured parameters was examined before treatment, 4 months after treatment and, when applicable, 12 months after treatment using Spearman's correlation coefficients.

Results

Plasma and urine PSA and hK2 before, and after 4 and 12 months of steroidal antiandrogen treatment are shown in tables 1 and 2. In general there was a dramatic suppression of plasma and urinary PSA and hK2 with all 3 drug regimens. After 4 months of treatment the most effective suppression of PSA and hK2 in plasma was seen with ethinyl estradiol plus cyproterone acetate treatment while in urine treatment with ethinyl estradiol or transdermal estradiol was more effective than cyproterone acetate alone. Although the differences between pretreatment and posttreatment PSA or hK2 in plasma or urine were highly significant (Wilcoxon's signed ranks test p <0.001), those among the various treatments were not statistically significant. For urine and plasma PSA continuation of treatment beyond 4 months resulted in further suppression, while for hK2 this effect was not observed (tables 1 and 2).

TABLE 1. Plasma and urinary PSA before and after treatment of male-to-female transsexuals

Treatment	No. Pts.	Median Plasma PSA (ng./l.)		Median Urine PSA (ng./l.)	
		Pretreatment	4 Mos.	Pretreatment	4 Mos.
Cyproterone acetate	10	333	35	10,064	1124
Estradiol patch + cyproterone acetate	15	272	41	19,885	92
Ethinyl estradiol + cyproterone acetate	31	296	25	30,138	438

At 12 months median plasma PSA was 10 ng./l. and urine PSA was 251 ng./l. in 17 samples available for analysis from group 3.

Table 1. Plasma and urinary PSA before and after treatment of male-to-female transsexualsAt 12 months median plasma PSA was 10 ng./l. and urine PSA was 251 ng./l. in 17 samples available for analysis from group 3.

TABLE 2. Plasma and urinary hK2 before and after treatment of male-to-female transsexuals

Treatment	No. Pts.	Median Plasma hK2 (ng./l.)		Median Urine hK2 (ng./l.)	
		Pretreatment	4 Mos.	Pretreatment	4 Mos.
Cyproterone acetate	10	216	28	1258	104
Estradiol patch + cyproterone acetate	15	201	16	1016	39
Ethinyl estradiol + cyproterone acetate	31	193	6.3	1180	43

At 12 months median plasma hK2 was 9.0 ng./l. and urine hK2 was 46 ng./l. in 17 samples available for analysis from group 3.

Table 2. Plasma and urinary hK2 before and after treatment of male-to-female transsexualsAt 12 months median plasma hK2 was 9.0 ng./l. and urine hK2 was 46 ng./l. in 17 samples available for analysis from group 3.

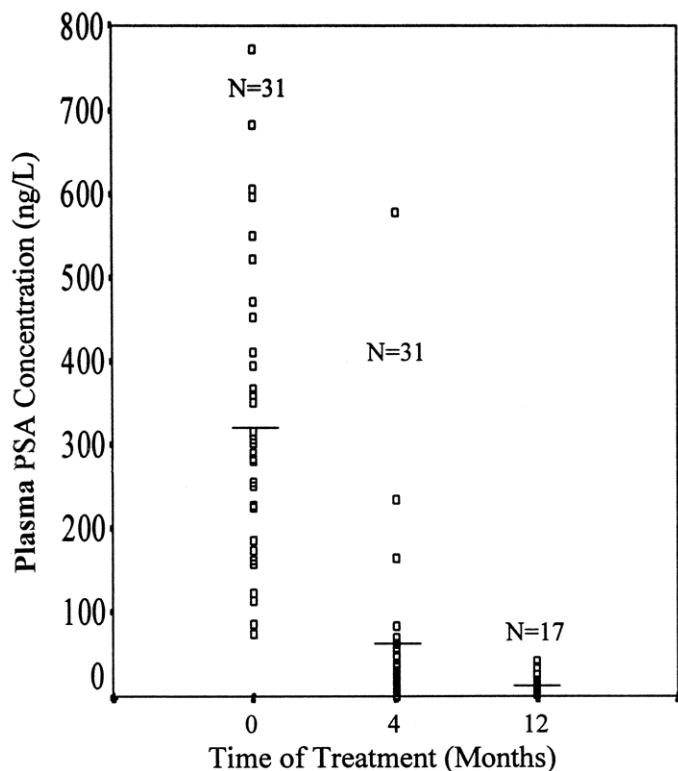


Fig. 1. Plasma PSA of male patients undergoing ethinyl estradiol plus cyproterone acetate therapy at baseline (0), and after 4 and 12 months of treatment. Solid horizontal lines indicate mean PSA before treatment (327 ng./l.), and after 4 (58 ng./l.) and 12 (15 ng./l.) months of treatment. Differences between PSA before treatment and after 4 and 12 months of treatment were statistically significant (Wilcoxon's signed ranks test $p < 0.001$).

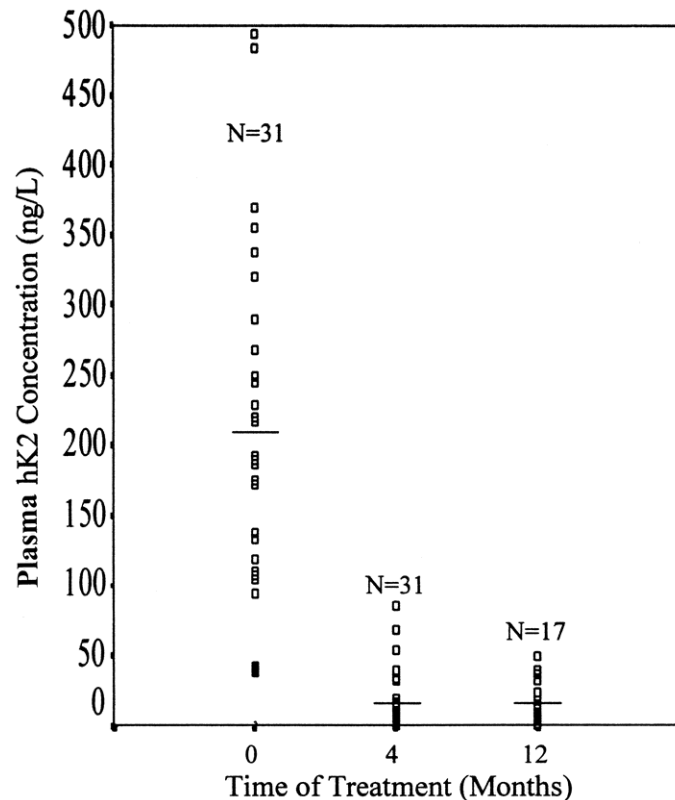


Fig. 2. Plasma hK2 of male patients undergoing ethinyl estradiol plus cyproterone acetate therapy at baseline (0), and after 4 and 12 months of treatment. Solid horizontal lines indicate mean hK2 before treatment (208 ng./l.), and after 4 (14 ng./l.) and 12 (16 ng./l.) months of treatment. Differences between pretreatment levels and those after 4 as well as 12 months after treatment were statistically significant (Wilcoxon's signed ranks test $p < 0.001$).

Plasma PSA and hK2 before, and after 4 and 12 months of treatment for patients receiving ethinyl estradiol plus cyproterone acetate are shown in figures 1 and 2. We found a statistically significant correlation between plasma PSA and plasma hK2 (56 patients, pretreatment $r = 0.53$ and $p = 0.002$ and 4-month treatment period $r = 0.60$ and $p < 0.0001$). In addition, good correlation was noted between plasma hK2 and body mass index after 12 months of treatment in 17 patients ($r = 0.635$, $p = 0.006$). There were no other significant correlations between PSA or hK2 and the other biochemical parameters measured (data not shown).

Discussion

Androgen blocking therapy is a common treatment for androgen dependent prostatic carcinomas which leads to histological changes of the prostate as well as dramatic changes in PSA.^{18,21,24} Cyproterone acetate with or without estradiol was given to male transsexuals to aid in the assumption of secondary

female sexual characteristics. Because transsexuals are free of any extra, circulatory sex steroids and estrogen feedback on LH does not differ from nontranssexual controls, any observed changes in PSA and hK2 due to antiandrogen treatment should be expected to be similar for nontranssexual males with a similar drug regimen.²⁵ There was extensive down regulation of PSA and hK2 in plasma and urine with all 3 treatments. Although this finding will not alter the interpretation of our data hK2 levels were generally about 10-fold higher in our study than those measured by other methods. This difference is likely due to differences in standardization as also indicated by others.²⁶ Albeit slightly less effective than the other 2 treatment modes, therapy based solely on cyproterone acetate resulted in significant PSA and hK2 down regulation in vivo, as estimated by the corresponding median or mean values in plasma and urine. This finding attests to the fact that cyproterone acetate can successfully outperform testosterone in vivo, although it only has 10% of the affinity of testosterone for the androgen receptor.²⁷ Therefore it

is clear that during prostatic adenocarcinoma treatment, when cyproterone acetate is used to suppress tumor growth for extended periods, a decrease in plasma PSA or hK2 may not be an accurate indicator of tumor volume reduction. Instead, it may reflect cyproterone acetate induced PSA and hK2 suppression in normal as well as cancerous prostatic tissue.

Conclusions

Cyproterone acetate alone or with ethinyl estradiol was able to suppress plasma and urinary PSA and hK2 dramatically in male-to-female transsexuals. Our study suggests that for prostate cancer patients treated with cyproterone acetate PSA or hK2 suppression on cyproterone acetate therapy may not accurately reflect treatment success. Instead, it may be a marker of down regulation of PSA and hK2 genes in normal and likely in cancerous prostatic tissue.

Footnotes

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References

1. Breul, J., Pickl, U. and Hartung, R.: Prostate-specific antigen in urine. *Eur Urol*, 26:18, 1994.
2. Iwakiri, J., Grandbois, K., Wehner, N. et al: An analysis of urinary prostate specific antigen before and after radical prostatectomy: evidence for secretion of prostate specific antigen by periurethral glands. *J Urol*, 149:783, 1993.
3. Diamandis, E. P. and Yu, H.: New biological functions of prostate-specific antigen? *J Clin Endocrinol Metab*, 80:1515, 1995.
4. Diamandis, E. P. and Yu, H.: Nonprostatic sources of prostate-specific antigen. *Urol Clin North Am*, 24:275, 1997.
5. Collins, A. T., Zhiming, B., Gilmore, K. et al: Androgen and oestrogen responsiveness of stromal cells derived from the human hyperplastic prostate: oestrogen regulation of the androgen receptor. *J Endocrinol*, 143:269, 1994.
6. Zarghami, N., Grass, L. and Diamandis, E. P.: Steroid hormone regulation of prostate-specific antigen gene expression in breast cancer. *Br J Cancer*, 75:579, 1997.
7. Horwitz, K. B., Jackson, T. A., Bain, D. L. et al: Nuclear receptor coactivators and corepressors. *Mol Endocrinol*, 10:1167, 1996.
8. Schuur, E. R., Henderson, G. A., Kmetec, L. A. et al: Prostate-specific antigen expression is regulated by an upstream enhancer. *J Biol Chem*, 271:7043, 1996.
9. Cleutjens, K. B., van der Korput, H. A. and Ehren-van Eekelen, C. C.: A 6-kb promoter fragment mimics in transgenic mice the prostate-specific and androgen-regulated expression of the endogenous prostate specific-antigen gene in humans. *Mol Endocrinol*, 11:1256, 1997.
10. Cleutjens, K. B., van der Korput, H. A., Ehren-van Eekelen, C. C. et al: An androgen response element in a far upstream enhancer region is essential for high, androgen-regulated activity of the prostate-specific antigen promoter. *Mol Endocrinol*, 11:148, 1997.
11. Cleutjens, K. B., van Eekelen, C. C., van der Korput, H. A. et al: Two androgen response regions cooperate in steroid hormone regulated activity of the prostate-specific antigen promoter. *J Biol Chem*, 271:6379, 1996.
12. Breul, J., Pickl, U. and Schaff, J.: Extraprostatic production of prostate specific antigen is under hormonal control. *J Urol*, 157:212, 1997.
13. Wolf, D. A., Herzinger, T., Hermeking, H. et al: Transcriptional and posttranscriptional regulation of human androgen receptor expression by androgen. *Mol Endocrinol*, 7:924, 1993.
14. de Vries, C. P., Gooren, L. J. and van der Veen, E. A.: The effect of cyproterone acetate alone and in combination with ethinylestradiol on the hypothalamic pituitary adrenal axis, prolactin and growth hormone release in male-to-female transsexuals. *Horm Metab Res*, 18:203, 1986.
15. Wolf, D. A., Schulz, P., Fittler, F.: Transcriptional regulation of prostate kallikrein-like genes by androgen. *Mol Endocrinol*, 6:753, 1992.
16. Wakeling, A. E., Furr, B. J., Glen, A. T. et al: Receptor binding and biological activity of steroidal and nonsteroidal antiandrogens. *J Steroid Biochem*, 15:355, 1981.
17. Gooren, L.: Androgens and estrogens in their negative feedback action in the hypothalamo-pituitary-testis axis: site of action and evidence of their interaction. *J Steroid Biochem*, 33:757, 1989.
18. Akakura, K., Bruchovsky, N., Goldenberg, S. L. et al: Effects of intermittent androgen suppression on androgen-dependent tumors. Apoptosis and serum prostate-specific antigen. *Cancer*, 71:2782, 1993.
19. Diamond, T., Campbell, J., Bryant, C. et al: The effect of combined androgen blockade on bone turnover and bone mineral densities in men treated for prostate carcinoma: longitudinal evaluation and response to intermittent cyclic etidronate therapy. *Cancer*, 83:1561, 1998.
20. Goldenberg, S. L., Bruchovsky, N., Rennie, P. S. et al: The combination of cyproterone acetate and low dose diethylstilbestrol in the treatment of advanced prostatic carcinoma. *J Urol*, 140:1460, 1988.
21. Stepanov, V. N., Goriunov, V. G., Chechenin, M. G. et al: Cyproterone acetate (Androcur-depo) in the treatment of inoperable prostatic cancer. *Urol Nefrol (Mosk)*, 6: 33, 1995.
22. Ferguson, R. A., Yu, H., Kalyvas, M. et al: Ultrasensitive detection of prostate-specific antigen by a time-resolved immunofluorometric assay and the Immulite Immunochemiluminescent third-generation assay: potential applications in prostate and breast cancers. *Clin Chem*, 42:675, 1996.
23. Black, M. H., Magklara, A., Obiezu, C. V. et al: Development of an ultrasensitive immunoassay for human glandular kallikrein with no cross-reactivity from prostate-specific antigen. *Clin Chem*, 45:790, 1999.
24. de Voogt, H. J., Rao, B. R., Geldof, A. A. et al: Androgen action blockade does not result in reduction in size but changes histology of the normal human prostate. *Prostate*, 11:305, 1987.
25. Gooren, L.: The endocrinology of transsexualism: a review and commentary. *Psychoneuroendocrinology*, 15: 3, 1990.
26. Stenman, U. H.: New ultrasensitive assays facilitate studies on the role of human glandular kallikrein (hK2) as a marker for prostatic disease. *Clin Chem*, 45:753, 1999.
27. Pinsky, L., Kaufman, M., Gil-Esteban, C. et al: Regulation of the androgen receptor in human genital skin fibroblasts, with a review of sex steroid receptor regulation by homologous and heterologous steroids. *Can J Biochem Cell Biol*, 61:770, 1983.