0022-5347/91/1463-0790$03.00/0
THE JOURNAL OF UROLOGY
Vol. 146, 790-793, September 1991
Printed in U.S.A.

EFFECT OF THE ADDITION OF ESTROGEN TO MEDICAL CASTRATION ON PROSTATIC SIZE, SYMPTOMS, HISTOLOGY AND SERUM PROSTATE SPECIFIC ANTIGEN IN 4 MEN WITH BENIGN PROSTATIC HYPERTROPHY

ALICE C. LEVINE, ALEXANDER KIRSCHENBAUM, MICHAEL DROLLER AND J. LESTER GABRILOVE

From the Division of Endocrinology, Bronfman Department of Medicine, and Petrie Department of Urology, Mount Sinai Medical Center, New York, New York

ABSTRACT

A total of 4 men with benign prostatic hypertrophy who underwent medical castration therapy with a long-acting gonadotropin-releasing hormone agonist (leuprolide) for more than 6 months elected to add an estrogen transdermal patch (0.05 mg. to the skin biweekly) to the leuprolide regimen. The average prostatic size (transrectal ultrasound), serum prostate specific antigen (PSA) levels and symptoms of prostatism were dramatically decreased with leuprolide alone. The addition of estrogen for 6 months did not result in any change in prostate size, symptoms or serum PSA levels over that seen with leuprolide alone. The development of squamous metaplasia was noted in 1 man with leuprolide alone and in 1 man after the addition of estrogen. Immunohistochemical staining with anticytokeratin 903 antibodies reveals that squamous metaplasia appears to arise from prostatic basal cells. We postulate that the target cell for estrogen action in the prostate is the prostatic basal cell. In the absence of androgen the only direct effect of estrogens is the induction of squamous metaplasia.

KEY WORDS: prostate, estrogens, tumor antigens, prostatic hypertrophy

Benign prostatic hypertrophy, an almost universal disorder of elderly men, is an androgen-related disease. We previously reported that treatment with a long-acting gonadotropin-releasing hormone (GnRH) analogue, D-Leu^6-GnRH proethylamide (leuprolide*) decreased the size and symptoms of prostatism, as well as serum prostate specific antigen (PSA) levels in elderly men with benign prostatic hypertrophy.[1-3] The serum PSA levels and histological sampling after androgen deprivation indicated that medical castration results in atrophy of prostatic epithelial cells with less obvious effects on prostatic stroma.[3, 4] Since benign prostatic hypertrophy is believed to be primarily a stromal driven process, it is important to investigate the factors that affect stromal growth.

The role of estrogens in the development and maintenance of benign prostatic hypertrophy is controversial. Studies in the dog model (which differs from human benign prostatic hypertrophy in that it is primarily a glandular form of hypertrophy) indicate that estrogens enhance prostatic growth and increase the number of androgen receptors in the prostate.[5, 6] The administration of estrogen or aromatizable substrates to monkeys does not result in increased prostatic weight.[7, 8] These same studies yielded conflicting results as to whether estrogens stimulate the growth of prostatic stroma. In the human, treatment of normal men with antiandrogen alone or antiandrogen in conjunction with oral estrogen did not alter prostate size although the estrogen treatment resulted in an increase in the percentage of stromal cells.[9]

We observed (unpublished data) that the addition of the antiestrogen tamoxifen to leuprolide treatment in 3 men with benign prostatic hypertrophy caused a worsening of hot flushes and some enlargement in prostatic size. Accordingly, men with benign prostatic hypertrophy receiving leuprolide treatment who reported severe hot flushes were offered the addition of estrogen to the regimen. To our knowledge this is the first report in humans of the effect of estrogens on the prostate of functionally castrated men with benign prostatic hypertrophy.

MATERIALS AND METHODS

A total of 4 elderly men with benign prostatic hypertrophy (average patient age 84 ± 4 years) who were receiving leuprolide acetate (7.5 mg. intramuscularly monthly) for more than 6 months (average 24 ± 5 months) elected to add an estrogen transdermal patch† (0.05 mg. to the skin biweekly) for the relief of hot flushes induced by leuprolide. Informed consent was obtained from all men before treatment. The men were examined, and blood samples were collected before and monthly during treatment. Urinary symptom scores were determined for 2 major categories, obstructive and irritative, from patient interviews as previously described.[1, 2]

Serum testosterone and estradiol were measured by radioimmunoassay after chromatographic separation using Sephadex LH-20.[10, 11] Serum dihydrotestosterone was measured by radioimmunoassay after selective inactivation of testosterone with potassium permanganate.[12] All assays were performed in duplicate. Serum prostate specific antigen (PSA) was measured using a solid-phase, 2-site immunoradiometric assay (Hybritech).[13] The intra-assay and interassay coefficients of variation for each assay were less than 10%.

Prostatic volume was measured at bimonthly intervals by transrectal ultrasonography using an Aloka chair-mounted radial scanner with a 3.5 mHz. probe. Prostatic volume was calculated by planimetry. The day-to-day reproducibility of this method is 95%.[14]

Transrectal prostatic needle biopsies were performed under ultrasonic guidance using an 18 gauge Bard Monopty needle.‡ On the average, 6 core biopsies were obtained from different zones of the prostate.[15]

Immunohistochemical staining for anticytokeratin 903 was

Accepted for publication January 24, 1991.
Supported in part by Grant M01-RR-0071 from the National Institutes of Health, National Center for Research Resources.
* Tap Pharmaceuticals, Inc., Deerfield, Illinois.
† Estraderm, CIBA Pharmaceutical Co., Summit, New Jersey.
‡ Bard Urological Division, Covington, Georgia.

done with a monoclonal antibody against 903 with an avidin-biotin-complex method.

RESULTS

Serum testosterone and dihydrotestosterone levels, which were maintained at castrate levels on leuprolide alone, did not increase with the addition of estrogen. Serum estradiol levels increased significantly with estrogen treatment (fig. 1). In addition, all 4 men had some gynecomastia while receiving estrogen, an indication that the estrogen levels were biologically significant.

There was a significant decrease in prostatic volume with medical castration alone (fig. 2). Prostatic volume was unchanged with the addition of estrogen. The decrease in the obstructive symptoms of prostatism seen with leuprolide therapy also was maintained with the addition of estrogen. Two of 4 men had urinary catheters before any medical treatment. The catheters were removed and normal voiding returned with leuprolide treatment. Estrogen therapy did not alter these restored normal voiding patterns. Hot flushes were greatly decreased with the addition of estrogen. No side effects, other than the appearance of gynecomastia, were observed in the 4 men treated with estrogen.

Serum PSA levels, a measure of prostatic epithelial cell function, were decreased significantly with leuprolide alone as previously reported.[3] The addition of estrogen had no effect on serum PSA levels (fig. 3).

Of 4 patients in this study 2 underwent prostatic needle biopsy. In 1 man on combination treatment biopsy showed atrophy of the glands. Prostatic needle biopsy in the other man before and after estrogen treatment demonstrated that squamous metaplasia developed after estrogen was added to the leuprolide treatment (fig. 4).

We also noted the development of squamous metaplasia in 2 of 2 men (not included in this estrogen study) who underwent transurethral prostatectomy for benign prostatic hypertrophy after 6 months on leuprolide treatment alone. Immunhistochemical staining of prostatic sections with anticytokeratin 903, which selectively stains prostatic basal cells, revealed that the squamous metaplasia from 1 of these patients appears to arise from prostatic basal cells (fig. 5).

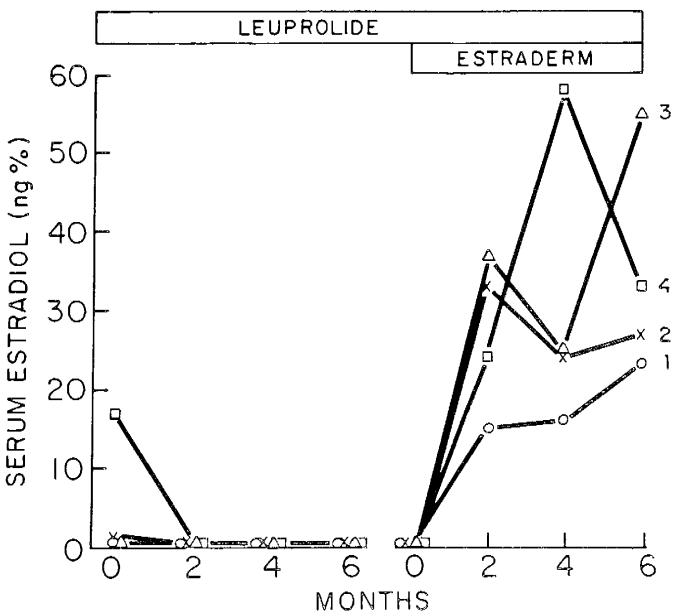

FIG. 1. Serum estradiol levels in 4 men with benign prostatic hypertrophy receiving leuprolide alone followed by leuprolide combined with estrogen for 6 months.

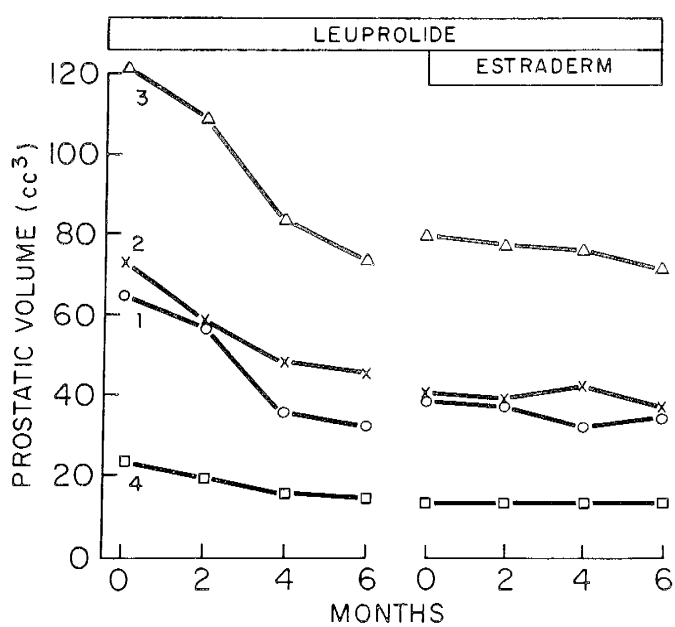

FIG. 2. Prostatic volume measured with transrectal ultrasonography in 4 men with benign prostatic hypertrophy receiving leuprolide alone followed by leuprolide combined with transdermal estrogen for 6 months.

DISCUSSION

Although early histological changes characteristic of benign prostatic hypertrophy have been reported in men in their twenties, a dramatic increase in prostatic size is noted, from autopsy studies, beginning around age 60 years.[16, 17] It has been noted that this increase in prostatic size occurs at a time when there is a change in serum hormone levels in men with an increase in the estrogen-to-androgen ratio.[18, 19] For this reason, the search has been on to define the role of estrogens in the development and maintenance of benign prostatic hypertrophy. The results of previous human studies using estrogens have been difficult to interpret because the observed effects may all have been secondary to the lowering of androgens seen with estrogen therapy.

Reports of estrogen receptor content in human prostates, as measured by biochemical assays, have been variable.[20-24] Early reports using immunohistochemistry were unable to demonstrate significant estrogen receptor levels in the human pros-

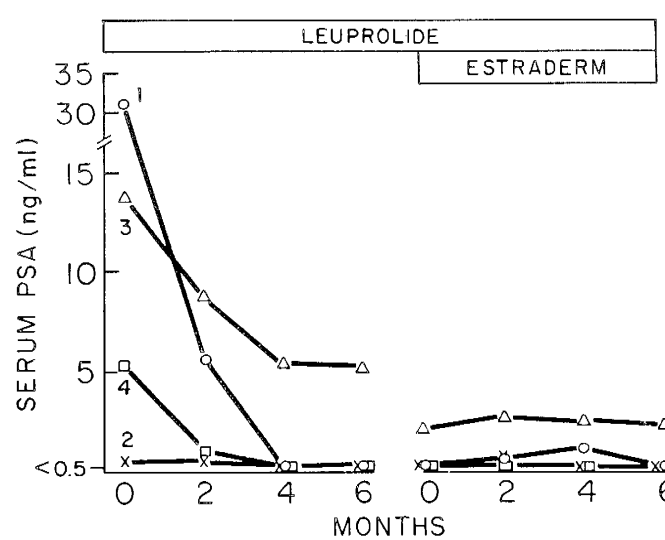

FIG. 3. Serum PSA levels in 4 men with benign prostatic hypertrophy receiving leuprolide alone followed by leuprolide combined with transdermal estrogen for 6 months.

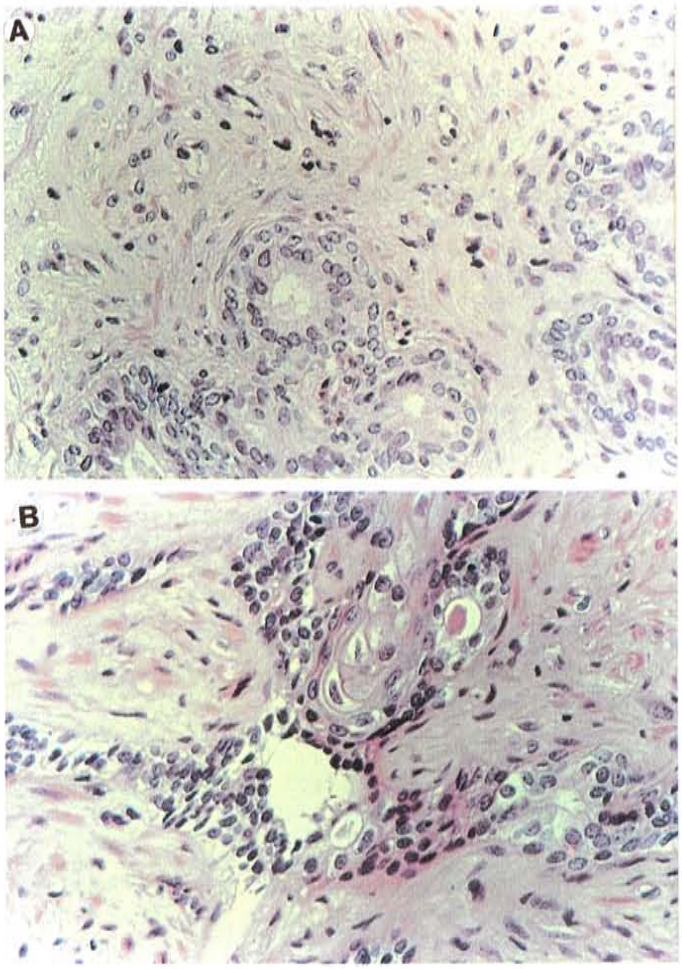

FIG. 4. *A*, prostatic needle biopsy specimen from 1 man with benign prostatic hypertrophy after receiving leuprolide alone. *B*, prostatic needle biopsy specimen from same man performed after combination treatment with leuprolide plus estrogen. Note development of squamous metaplasia after addition of estrogen. H & E, reduced from ×20.

FIG. 5. Immunohistochemical staining with anticytokeratin 903 antibodies of prostate specimen from 1 man with benign prostatic hypertrophy who received leuprolide therapy alone. Reduced from ×40.

tate.[24-26] Refined methods, involving longer or repeated incubations with primary and secondary antibodies, have shown that nuclear estrogen receptors are present in the periglandular prostatic stroma of some prostates. Notably, these studies have revealed that there is intense staining for nuclear estrogen receptors in normal prostatic basal cells and especially in basal cell hyperplasia.[27, 28]

We demonstrate that leuprolide alone (which lowers androgens to a much greater extent than serum estrogens, thereby increasing the serum estrogen-to-androgen ratio) can induce squamous metaplasia. The addition of estrogen to leuprolide causes a further induction of squamous metaplasia. Indeed, the association of estrogens with squamous metaplasia of the prostate was first noted more than 50 years ago.[29] Immunohistochemical staining with anticytokeratin 903 antibodies demonstrates that squamous metaplasia appears to arise from prostatic basal cells.

We propose that the target cell for estrogen action in the prostate is the prostatic basal cell. We speculate that in the presence of androgens, elevations in the serum estrogen-to-androgen ratio may stimulate prostatic basal cells to secrete growth factors. Growth factors secreted from basal cells in the presence of estrogens and androgens may stimulate growth of neighboring acinar and stromal cells in a paracrine fashion. In the absence of androgen, estrogens induce squamous metaplasia of prostatic basal cells. We do not find any evidence of a direct stimulatory effect of estrogens on prostatic stroma or prostatic acinar cells in medically castrated men with established benign prostatic hypertrophy.

Ms. Meiyue Ren provided technical assistance with immunocytochemistry.

REFERENCES

1. Gabrilove, J. L., Levine, A. C., Kirschenbaum, A. and Droller, M.: Effect of a GnRH analogue (leuprolide) on benign prostatic hypertrophy. J. Clin. Endocr. Metab., **64:** 1331, 1987.
2. Gabrilove, J. L., Levine, A. C., Kirschenbaum, A. and Droller, M.: Effect of long-acting gonadotropin-releasing hormone analog (leuprolide) therapy on prostatic size and symptoms in 15 men with benign prostatic hypertrophy. J. Clin. Endocr. Metab., **69:** 629, 1989.
3. Levine, A. C., Kirschenbaum, A., Kaplan, P., Droller, M. J. and Gabrilove, J. L.: Serum prostate-antigen levels in patients with benign prostatic hypertrophy treated with leuprolide. Urology, **34:** 10, 1989.
4. Peters, C. A. and Walsh, P. C.: The effect of nafarelin acetate, a luteinizing-hormone-releasing hormone agonist, on benign prostatic hyperplasia. New Engl. J. Med., **317:** 599, 1987.
5. Walsh, P. C. and Wilson, J. D.: The induction of prostatic hypertrophy in the dog with androstanediol. J. Clin. Invest., **57:** 1093, 1976.
6. Trachtenberg, J., Hicks, L. L. and Walsh, P. C.: Androgen- and estrogen-receptor content in spontaneous and experimentally induced canine prostatic hyperplasia. J. Clin. Invest., **65:** 1051, 1980.
7. West, N. B., Roselli, C. E., Resko, J. A., Greene, G. L. and Brenner, R. M.: Estrogen and progestin receptors and aromatase activity in rhesus monkey prostate. Endocrinology, **123:** 2312, 1988.
8. Habenicht, U. F., Schwarz, K., Neumann, F. and el Etreby, M. F.: Induction of estrogen-related hyperplastic changes in the prostate of the cynomolgus monkey (Macaca fascicularis) by androstenedione and its antagonization by the aromatase inhibitor 1-methyl-androsta-1,4-diene-3,17-dione. Prostate, **11:** 313, 1987.
9. de Voogt, H. J., Rao, B. R., Geldof, A. A., Gooren, L. J. and Bouman, F. G.: Androgen action blockade does not result in reduction in size but changes histology of the normal human prostate. Prostate, **11:** 305, 1987.
10. Abraham, G. E.: Handbook of Radioimmunoassay. New York: M. Dekker, vol. 5, p. 591, 1977.
11. Carr, B. R., Mikhail, G. and Flickinger, G. L.: Column chromatography of steroids on sephadex LH-20. J. Clin. Endocr. Metab., **33:** 358, 1971.
12. Puri, V., Puri, C. P. and Anand Kumar, T. C.: Serum levels of dihydrotestosterone in male rhesus monkeys estimated by a non-chromatographic radioimmunoassay method. J. Steroid Biochem., **14:** 877, 1981.
13. Liedtke, R. J. and Batjer, J. D.: Measurement of prostate-specific antigen by radioimmunoassay. Clin. Chem., **30:** 649, 1984.
14. Walz, P. H., Wenderoth, U. and Jacobi, G. H.: Suprapubic transves-

ical sonography of the prostate: determination of prostatic size. Eur. Urol., **9:** 148, 1983.
15. Hodge, K. K., McNeal, J. E., Terris, M. K. and Stamey, T. A.: Random systematic versus directed ultrasound guided transrectal core biopsies of the prostate. J. Urol., **142:** 71, 1989.
16. Berry, S. J., Coffey, D. S., Walsh, P. C. and Ewing, L. L.: The development of human benign prostatic hyperplasia with age. J. Urol., **132:** 474, 1984.
17. Swyer, G. I. M.: Post-natal growth changes in the human prostate. J. Anat., **78:** 130, 1944.
18. Haug, E., Aakvaag, A., Sand, T. and Torjesen, P.: The gonadotrophin response to synthetic gonadotrophin-releasing hormone in males in relation to age, dose, and basal serum levels of testosterone, oestradiol-17beta and gonadotrophins. Acta Endocr., **77:** 625, 1974.
19. Zumoff, B., Strain, G. W., Kream, J., O'Connor, J., Rosenfeld, R. S., Levin, J. and Fukushima, D. K.: Age variation of the 24-hour mean plasma concentrations of androgens, estrogens, and gonadotropins in normal adult men. J. Clin. Endocr. Metab., **54:** 534, 1982.
20. Murphy, J. B., Emmott, R. C., Hicks, L. L. and Walsh, P. C.: Estrogen receptors in the human prostate, seminal vesicle, epididymis, testis, and genital skin: a marker for estrogen-responsive tissues? J. Clin. Endocr. Metab., **50:** 938, 1980.
21. Tilley, W. D., Keightley, D. D. and Marshall, V. R.: Oestrogen and progesterone receptors in benign prostatic hyperplasia in humans. J. Steroid Biochem., **13:** 395, 1980.
22. Wolf, R. M., Schneider, S. L., Pontes, J. E., Englander, L., Karr, J. P., Murphy, G. P. and Sandberg, A. A.: Estrogen and progestin receptors in human prostatic carcinoma. Cancer, **55:** 2477, 1985.
23. Robel, P., Eychenne, B., Blondeau, J.-P., Baulieu, E.-E. and Hechter, O.: Sex steroid receptors in normal and hyperplastic human prostate. Prostate, **6:** 255, 1985.
24. Ekman, P., Barrack, E. R., Greene, G. L., Jensen, E. V. and Walsh, P. C.: Estrogen receptors in human prostate: evidence for multiple binding sites. J. Clin. Endocr. Metab., **57:** 166, 1983.
25. Pertschuk, L. P., Eisenberg, K. B., Macchia, R. J. and Feldmann, J. G.: Heterogeneity of steroid binding sites in prostatic carcinoma: morphological demonstration and clinical implications. Prostate, **6:** 35, 1985.
26. Harper, M. E., Sibley, P. E., Francis, A. B., Nicholson, R. I. and Griffiths, K.: Immunocytochemical assay for estrogen receptors applied to human prostatic tumors. Cancer Res., suppl. 8, **46:** 4288s, 1986.
27. Wernert, N., Gerdes, J., Loy, V., Seitz, G., Scherr, O. and Dhom, G.: Investigations of the estrogen (ER-ICA-test) and the progesterone receptor in the prostate and prostatic carcinoma on immunohistochemical basis. Virchows Arch. [A], **412:** 387, 1988.
28. Schulze, H. and Claus, S.: Histological localization of estrogen receptors in normal and diseased human prostates by immunocytochemistry. Prostate, **16:** 331, 1990.
29. Moore, R. A. and McLellan, A. M.: A histological study of the effect of the sex hormones on the human prostate. J. Urol., **40:** 641, 1938.