Pergamon

PII: S0960-0760(97)00157-X

J. Steroid Biochem. Molec. Biol. Vol. 64, No. 3-4, pp. 193–198, 1998

Printed in Great Britain
0960-0760/98 $19.00 + 0.00

Effect of the Dual 5α-Reductase Inhibitor Pnu 157706 on the Growth of Dunning R3327 Prostatic Carcinoma in the Rat

T. Zaccheo*, D. Giudici and E. di Salle

Experimental Endocrinology, Research/Oncology, Pharmacia and Upjohn, Viale Pasteur 10, 20014 Nerviano (MI), Italy

PNU 157706 [*N*-(1,1,1,3,3,3-hexafluorophenylpropyl)-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide] is a novel, potent and selective dual 5α-reductase inhibitor. We have investigated its effect on tumor growth, endocrine organ weights and prostatic dihydrotestosterone (DHT) content in rats bearing the androgen dependent Dunning R3327 prostatic carcinoma. Animals with tumor diameters of about 1 cm were treated orally for 9 weeks with PNU 157706 (2 and 10 mg/kg/day, 6 days a week) or they were castrated, to check the hormone responsiveness of the tumor. PNU 157706 was effective at both doses tested in reducing tumor growth (53 and 51% inhibition at 2 and 10 mg/kg/day, respectively), while castration caused higher inhibition (82%) of tumor growth. A marked reduction of ventral prostate weight occurred in rats treated with both doses of PNU 157706 (75 and 78%) or castrated (91%). Seminal vesicle weight was also reduced by PNU 157706 administration (56 and 61% inhibition), whereas testes, adrenal, thymus and pituitary weights were not affected. Prostatic DHT content was markedly suppressed (85 and 91%) in PNU 157706 treated rats, compared to 95% suppression caused by castration. These data support a possible role of dual 5α-reductase inhibitors in the hormonal therapy of prostatic cancer. © **1998 Elsevier Science Ltd. All rights reserved.**

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INTRODUCTION

Androgens are necessary for growth, maintenance and functional activity of the prostate gland. Androgens are also involved in prostatic cancer development and modification of hormonal stimulation could be useful in preventing or inhibiting tumor growth [1]. In the normal, hyperplastic or cancerous prostatic tissue testosterone (T) is irreversibly metabolized via the 5α-reductase enzyme to 5α-dihydrotestosterone (DHT), which appears to be the major intracellular androgen; in fact, in the prostate gland DHT concentration is higher than that of T and it is known that the androgen receptor has a higher affinity for DHT than for T [2–4]. Therefore reduction of DHT synthesis through inhibition of 5α-reductase activity can represent an interesting approach to androgen deprivation for prostatic tumor control.

PNU 157706 [*N*-(1,1,1,3,3,3-hexafluorophenylpropyl)-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide] is a novel dual inhibitor of 5α-reductase [5]. The compound was highly potent in inhibiting human recombinant 5α-reductase type I and type II isozymes, showing IC_{50} values of 3.9 and 1.8 nM and therefore it was several folds more potent than finasteride (IC_{50} values of 313 and 11.3 nM), particularly on type I isozyme. Tested on a crude preparation of rat prostatic 5α-reductase, PNU 157706 caused enzyme inhibition with an IC_{50} value of 34 nM, compared to 58 nM shown by finasteride. In adult male rats a single oral dose of 10 mg/kg of PNU 157706 caused a marked and longer lasting reduction of prostatic DHT content than did finasteride (at 24 h inhibition by 89 and 47%, respectively). In prepubertal T- or DHT-implanted castrated rats, PNU 157706, at the oral dose of 10 mg/kg/day for 7 days, markedly reduced ventral prostate weight in T- but not in DHT-implanted animals, thus showing to be devoid of any anti-androgen activity. After repeated (28 days) oral dosing in rats, the compound was

*Correspondence to T. Zaccheo. Tel: 2 48383235; Fax: 2 48383987; e-mail: tiziana.zaccheo@eu.pnu.com.
Received 29 May 1997; accepted 13 Oct. 1997.

found to be 16-fold more potent than finasteride in reducing prostate weight.

The antitumor effect of PNU 157706 on the androgen dependent Dunning R3327 prostatic carcinoma [6] in the rat was therefore investigated in this study. Tumor growth rate during treatment, as well as endocrine organ weights and prostatic DHT content were evaluated.

MATERIALS AND METHODS

Animals

Male Copenhagen rats, weighing approximately 200 g, were supplied by Harlan Nossan (Correzzana, Italy). Animals were housed in temperature controlled rooms ($22 \pm 2°C$) on a circadian rhythm of 12 h of light (6 a.m. to 6 p.m.) and 12 h of darkness. They were fed a commercially available chow (Altromin MT, supplied by Rieper, Italy) and water was available *ad libitum*.

Prostatic tumor model

The Dunning R3327 prostatic carcinoma was kindly provided by Dr H. Altman, Papanicolau Cancer Research Center, University of Miami (FL, U.S.A.). The tumor was maintained by serial transplantation in Copenhagen rats. The tumor was passed by harvesting fresh tumor, dicing it with sterile scissors in sterile 0.9% NaCl solution and aseptically implanting a single tumor fragment (approximately 3–4 mm in size) subcutaneously in the flank of the recipient animal under mild diethyl-ether anesthesia.

Treatment and experimental set-up

Tumor bearing rats with tumor diameters of 0.5–1 cm were randomly assigned to the different treatment groups (7–8 animals/group). PNU 157706 (synthesized at the Chemistry Dept. of Pharmacia and Upjohn, Italy) was suspended in 0.5% Methocel (A-4C Premium, Dow Chemical, U.S.A.) containing 0.4% Tween 80 (Merck, U.S.A.) and given orally at doses of 2 and 10 mg/kg/day, 6 days a week for 9 weeks. Control animals received the vehicle (5 ml/kg). One group of animals was castrated, under diethyl-ether anesthesia, on the first experimental day (day 0) and treated with the vehicle. Tumor growth was followed by measuring the two perpendicular diameters with calipers and tumor weight was calculated according to the formula: $d^2 \times D/2$, were d is the minimum and D is the maximum diameter [7]. The weight of each tumor during the treatment period was expressed as the ratio of initial tumor weight, measured on day 0. The area under the ratio of tumor weight vs time curve (AUC) was calculated by the linear trapezoidal method up to 9 weeks. Animals were sacrificed at the end of the treatment (24 h after the last dose) and ventral prostate, seminal vesicles, testes, epididymides, adrenals, thymus, pituitary and liver were removed and weighed. Ventral prostates were immediately frozen on dry ice, then stored at −20°C for DHT assay.

DHT determination

The prostatic content of DHT was measured by radioimmunoassay (RIA), after sample extraction and high pressure liquid chromatography (HPLC) purification. Each prostate sample was thawed and homogenized in 4 ml of acetone:acetonitrile mixture (1:1) with a Polytron apparatus. Essicated extract was then loaded into a to C_{18} Sep Pack Plus cartridge (Waters, U.S.A.) and the fraction containing DHT was eluted with 4 ml of 60% acetonitrile in water. HPLC purification of the extracted sample was performed using a Nova Pack C_{18}-reversed phase column (Waters) in isocratic conditions, using 38% acetonitrile in water as the mobile phase (1 ml/min). The fraction containing DHT (2.7 ml, retention time $\approx$ 25 min) was collected and essicated. DHT concentration was measured using the [^{3}H]DHT RIA Kit supplied by ICN Biomedicals (Carson, CA, U.S.A.). The sensitivity of the assay was 0.07 ng/100 mg of tissue. The DHT content in the prostate of castrated animals was measured in pools of two glands each.

RESULTS

Effect on tumor growth

Figure 1 and Table 1 show the effect of PNU 157706 at the doses of 2 and 10 mg/kg/day, given orally 6 days a week for 9 weeks, on tumor growth. One group of animals was castrated to check the hormone responsiveness of the tumor. The mean tumor weight $\pm$ SE at the beginning of the experiment (day 0) was 0.52 ± 0.05 g in the control group, 0.54 ± 0.04 g in the group treated with PNU 157706

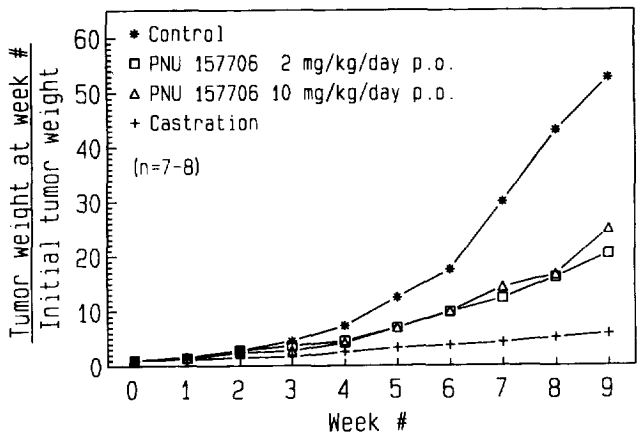

Fig. 1. Effect of 9-week oral treatment with 2 or 10 mg/kg/day of PNU 157706 on tumor growth in the Dunning R3327 prostatic tumor model in rats. Castration was performed the first treatment day.

Table 1. Effect of 9-week treatment with PNU 157706 on Dunning R3327 prostatic tumor growth in rats

Treatment group	Dose (mg/kg/day p.o.)	No. of rats	Tumor growth	
			AUC (0–9 week)[a] (ratio × week)	inhibition (%)
Control	–	8	146 ± 18	–
PNU 157706	2	8	68 ± 13	53**
PNU 157706	10	7	71 ± 18	51**
Castration	–	8	26 ± 3	82**

[a]AUC = Area under the ratio of initial tumor weight, calculated by the linear trapezoidal method from 0 to 9 weeks (mean ± SE).
**$p < 0.01$ vs control group (Dunnett's test).

2 mg/kg, 0.51 ± 0.06 g in the group treated with PNU 157706 10 mg/kg and 0.52 ± 0.05 in the castrated group. The weekly recorded tumor weights, expressed as a ratio of the initial weight, are reported in Fig. 1. The AUC (0–9 week) values, expressed as ratio × week, are reported in Table 1. During the 9-week observation period the Dunning R3327 tumor grew progressively in the control group (AUC, mean ± SE = 146 ± 18), whereas castration resulted in a markedly lower tumor growth (AUC, 26 ± 3), thus demonstrating the androgen responsiveness of the tumor. Treatment with the 5α-reductase inhibitor PNU 157706 significantly decreased tumor growth at both doses tested (AUC, 68 ± 13 and 71 ± 18).

Effect on endocrine organ weight

The endocrine organs of tumor bearing rats were excised and weighed at the 9-week sacrifice (Figs 2 and 3). Since PNU 157706 treatment and castration slightly reduced the body weight gain (Fig. 3), the relative weights of the organs are reported. Treatment with PNU 157706 at the doses of 2 and 10 mg/kg/day caused a decrease of ventral prostate weight of 75 and 78%, respectively (Fig. 2). Seminal vesicle weights were also reduced by 56 and 61%, whereas testis weights were not affected. Epididymides were slightly reduced at both doses. No effects on adrenal, thymus, pituitary and liver weights were observed. As expected, castration markedly reduced ventral prostate (91%) and seminal vesicle (73%) weights; thymus and pituitary weights were increased in castrated rats, whereas adrenal and liver weights were not affected.

Effect on prostate DHT content

Treatment for 9 weeks with PNU 157706 at 2 and 10 mg/kg/day resulted in a marked decrease (85 and 91%) in prostatic DHT content, measured 24 h after the last dose (Fig. 4). Castration caused 95% reduction of DHT content to a mean value of 0.24 ng/gland. The limit of detection of DHT assay was 0.07 ng/100 mg of tissue.

DISCUSSION

The Dunning R3327 rat prostatic carcinoma is a well characterized model system. It shares several characteristics with human prostatic cancer, including slow growth rate, responsiveness to hormonal therapy, histology similar to that of a well differentiated carcinoma [6]; moreover, it contains the enzyme 5α-reductase [8, 9]. Results presented in this paper show that the 5α-reductase inhibitor PNU 157706 was effective in inhibiting the Dunning tumor growth. In fact the compound, at the oral doses of 2 and 10 mg/kg/day given for 9 weeks, caused a reduction of tumor growth by 51–53%. The effect on tumor growth was associated with an even higher inhibitory effect on prostate weight (75–78% decrease). Castration caused a higher inhibition of tumor growth (82%) and a higher involution of prostate (91%) than did PNU 157706.

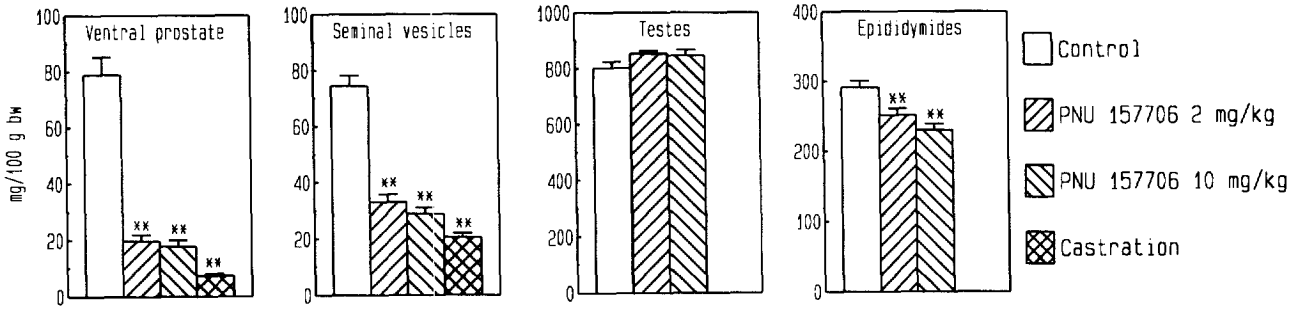

Fig. 2. Effect of 9-week oral treatment with 2 or 10 mg/kg/day of PNU 157706 on the relative organ weight of rats bearing the Dunning R3327 prostatic carcinoma. Bars represent mean ± SE (7–8 animals per group). **$P < 0.01$ vs controls by Dunnett's test.

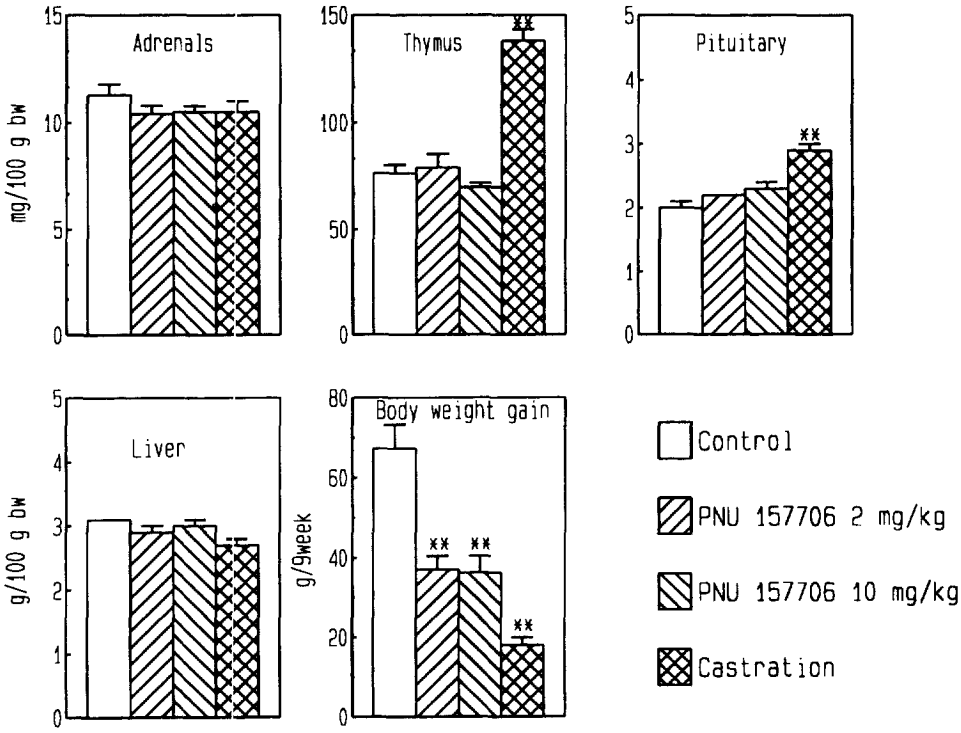

Fig. 3. Effect of 9-week oral treatment with 2 or 10 mg/kg/day of PNU 157706 on the relative organ weight of rats bearing the Dunning R33327 prostatic carcinoma. Bars represent mean ± SE (7–8 animals per group). **$P < 0.01$ vs controls by Dunnett's test.

Since the initial report of Huggins and Hodges [10], the hormonal therapy of advanced or metastatic prostate cancer has traditionally consisted of surgical or chemical castration in order to block testicular androgens and therefore to lower circulating T. More recently combined therapy involving surgical or medical castration plus an antiandrogen, in order to block the remaining adrenal androgens, has been tried in an attempt to produce total androgen blockade [11]. However, such a systemic reduction in the circulating T level has several side effects including sterility, impotence, loss of libido, hot flushes and decreased muscle mass [12, 13]. Inhibitors of 5α-reductase provide a novel and selective approach to androgen deprivation [14]. Treatment with such inhibitors results, both in experimental animals and in humans, in lowering prostatic DHT and consequently in involution of the prostate size. However, 5α-reductase inhibitor treatment does not result in a reduction of circulating T and therefore does not induce the side effects observed with castration or luteinizing hormone-releasing hormone (LH-RH) analogs.

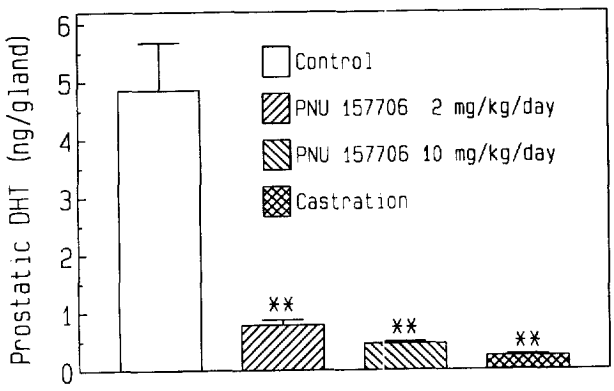

Fig. 4. Effect of 9-week oral treatment with 2 or 10 mg/kg/day of PNU 157706 on ventral prostate DHT content of rats bearing the Dunning R3327 prostatic carcinoma. The sensitivity of the DHT assay was 0.07 ng/100 mg of tissue. Bars represent mean ± SE (7–8 animals per group). **$P < 0.01$ vs controls by Dunnett's test.

Molecular cloning studies have identified two genes that encode the type I and type II isozymes of 5α-reductase [15]. These isozymes have different tissue localization: 5α-reductase type II is mainly located in human urogenital tissues, such as the prostate, whereas 5α-reductase type I is predominant in the human nongenital skin and in liver. As regards the tumor 5α-reductase enzyme, it has been reported that both type I and type II isozymes are expressed in human prostatic carcinoma [16] and type II isozyme seems to be more expressed in stromal than in the epithelial compartment [17]. LNCaP cells, an androgen sensitive human prostatic cancer cell line, were reported to contain type I 5α-reductase [18]. Moreover, the DU145 human prostatic cancer cell line, whose growth is androgen independent, also contains type I 5α-reductase [19]. No data are, to date, available on the characteristics of the enzyme in the Dunning prostatic tumor used in the experiment

reported here. However, both isoforms of 5α-reductase are expected to be expressed in this prostatic tumor tissue, as it has been reported for the normal rat prostate [20].

Finasteride, the first 5α-reductase inhibitor developed [21], has been subsequently discovered to be a selective inhibitor of type II human 5α-reductase [22]. The compound has recently been introduced into the market for the therapy of symptomatic benign prostatic hyperplasia [23, 24]. However, its clinical effectiveness was found to be less satisfactory than initially expected and could be probably accounted for by incomplete suppression of circulating DHT, due to the residual DHT synthesis through type I isozyme not inhibited by the compound [24]. Experimental data on the antitumor activity of finasteride on the Dunning R3327 prostatic carcinoma have shown that the compound was ineffective on tumor growth at 25 mg/kg/day, a dose which reduced by 64% the size of the prostate in the same animals [25]. Additional studies on the Dunning R3327 H tumor line have demonstrated no tumor growth inhibition by finasteride, at doses up to 100 mg/kg/day [9]. These results are likely due, in addition to incomplete DHT suppression, to the remaining T which stimulates tumor growth [25]. Further, the results from the first clinical study with finasteride in prostatic cancer patients indicated that this type of monotherapy is not adequate for the metastatic disease [26]. However, in a more recent study in which 120 men with detectable prostate specific antigen (PSA) levels following radical prostatectomy were treated with finasteride or placebo for one year, a significant delay in PSA rise was seen in one year, with continued suppression up to one year after treatment discontinuation [27].

A potent, dual inhibitor of both type 1 and II 5α-reductases could better suppress circulating DHT and provide more efficient treatment for prostate cancer than a type II inhibitor like finasteride. PNU 157706 represents an improvement in the field of 5α-reductase inhibitors on account of its *in vitro* potency on both human isozymes and of its extraordinary *in vivo* potency in the rat [5]. In fact, in a previous study the compound has been found to be 16-fold more potent than finasteride in reducing prostate weight in adult rats treated orally for 28 days [5]. Data presented in this paper indicate, as mentioned above, that potent inhibition of both 5α-reductase isoforms results in 90% suppression of prostatic DHT and in significant reduction of androgen-dependent tumor growth. It must be noted, however, that despite the comparable reduction of prostatic DHT content achieved with castration and PNU 157706 dosing, the antitumor effect of castration was higher than that observed with the 5α-reductase inhibitor. Unlike castration and similarly to other 5α-reductase inhibitors [28, 29], PNU 157706 does not suppress intraprostatic T concentration [5]. Reduction of the content of DHT but not of T is expected to occur also in the Dunning tumor tissue [25]. The remaining tumor T could be the cause for the lower antitumoral effect observed in PNU 157706-dosed compared to castrated animals. Therefore, a combination therapy with a 5α-reductase inhibitor and an androgen receptor antagonist, to neutralize the remaining T, could be a potential therapy for the treatment of prostatic cancer. In addition, such a type of combined therapy with finasteride and the antiandrogen flutamide in patients with prostatic carcinoma has been recently shown to have the advantage of maintaining the sexual function in most men [30].

Acknowledgements—The authors would like to thank R. Vivaldi for his excellent technical assistance.

REFERENCES

1. Ford L. G., Brawley O. W., Perlman J. A., Nayfield S. G., Johnson K. A. and Kramer B. S., The potential for hormonal prevention trials. *Cancer* **74** (1994) 2726–2733.
2. Hammond G. L., Endogenous steroid levels in the human prostate from birth to old age: A comparison of normal and disease tissues. *J. Endocr.* **78** (1978) 7–19.
3. Klein H., Bressel M., Kastendieck H. and Voigt K. D., Quantitative assessment of endogenous testicular and adrenal sex steroids and of steroid metabolizing enzymes in untreated human prostatic cancerous tissue. *J. Steroid Biochem.* **30** (1988) 119–130.
4. Liao S., Liang T. and Tymoczko J. L., Structural recognition in interactions of androgens and receptor proteins and in their association with nuclear components. *J. Steroid Biochem.* **3** (1972) 401–407.
5. di Salle, E., Giudici, D., Radice, A., Zaccheo, T., Ornati, G., Nesi, M., Panzeri, A., Délos, S. and Martin, P. M., PNU 157706, a novel dual type I and II 5α-reductase inhibitor. *J. Steroid Biochem. Molec. Biol.*, 1998, this issue.
6. Isaacs J. T. and Coffey D. S., Adaptation versus selection as the mechanism responsible for the relapse of prostatic cancer to androgen ablation therapy as studied in the Dunning R3327-H adenocarcinoma. *Cancer Res.* **41** (1981) 5070–5075.
7. Geran R. I., Greenberg N. H., Mc Donald M. M., Schumacher A. M. and Abbott B. J., Protocol for screening chemical agents and natural products against animal tumors and other biological systems. *Cancer Chemother. Rep.* **3** (1972) 1–103.
8. Isaacs J. T., Isaacs W. B., Feitz W. F. J. and Scheres J., Establishment and characterization of seven Dunning rat prostatic cancer cell lines and their use in developing methods for predicting metastatic abilities of prostatic cancer. *Prostate* **9** (1986) 261–281.
9. Lamb J. C., Levy M. A., Johnson R. K. and Isaacs J. T., Response of rat and human prostatic cancers to the novel 5α-reductase inhibitor, SKF 105657. *Prostate* **21** (1992) 15–34.
10. Huggins C. and Hodges C. V., Studies on prostatic cancer. I. The effect of castration, of estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate. *Cancer Res.* **1** (1941) 293–297.
11. Labrie F., Belanger A., Simard J., Labrie C. and Dupont A., Combination therapy for prostate cancer. *Cancer* **71** (1993) 1059–1067.
12. Vogelzang N. J. and Kennealey G. T., Recent development in endocrine treatment of prostate cancer. *Cancer* **70** (1992) 966–976.
13. Hsieh W.-S. and Simons J. W., Systemic therapy of prostate cancer. New concepts from prostate cancer tumor biology. *Cancer Treatment Rev.* **19** (1993) 229–260.
14. Schröder F. H., 5α-Reductase inhibitors and prostatic disease. *Clin. Endocrinol.* **41** (1994) 139–147.

15. Andersson S. and Russell D. W., Structural and biochemical properties of cloned and expressed human and rat steroid 5α-reductases. *Proc. Natl. Acad. Sci. USA* **87** (1990) 3640–3644.
16. Bonkhoff H., Stein U., AumÜller G. and Remberger K., Differential expression of 5α-reductase isoenzymes in the human prostate and prostatic carcinomas. *Prostate* **29** (1996) 261–267.
17. Silver R. I., Wiley E. L., Davis D. L., Thigpen A. E., Russell D. W. and McConnell J. D., Expression and regulation of steroid 5α-reductase 2 in prostate disease. *J. Urol.* **152** (1994) 433–437.
18. Sutkowski D. M., Audia J. E., Goode R. L., Hsiao K. C., Leibovitch I. Y., McNulty A. M. and Neubauer B. L., Responses of LNCaP prostatic adenocarcinoma cell cultures to LY 300502, a benzoquinolinone human type I 5α-reductase inhibitor. *Prostate* **6 (Suppl.)** (1996) 62–66.
19. Délos S., Iehlè C., Martin P. M. and Raynaud J. P., Inhibition of the activity of basic 5α-reductase (type 1) detected in DU 145 cells and expressed in insect cells. *J. Steroid Biochem. Molec. Biol.* **48** (1994) 347–352.
20. Normington K. and Russell D. W., Tissue distribution and kinetic characteristics of rat steroid 5α-reductase isozymes. Evidence for distinct physiological functions. *J. Biol. Chem.* **267** (1992) 19548–19554.
21. Brooks J. R., Berman C., Primka R. L., Reynolds G. F. and Rasmusson G. H., 5α-Reductase inhibitory and anti-androgenic activities of some 4-azasteroids in the rat. *Steroids* **47** (1986) 1–19.
22. Andersson S., Berman D. M., Jenkins E. P. and Russell D. W., Deletion of steroid 5α-reductase 2 gene in male pseudohermaphroditism. *Nature* **354** (1991) 159–161.
23. Gormley G. J., Stoner E., Bruskewitz R. C., Imperato-McGinley J., Walsh P. C., McConnell J. D., Andriole G. L., Geller J., Bracken B. R., Tenover J. S., Darracott Vaughan E., Pappas F., Taylor A., Binkowitz B. and Ng J., The effect of finasteride in men with benign prostatic hyperplasia. *N. Engl. J. Med.* **327** (1992) 1185–1191.
24. Peters D. H. and Sorkin E. M., Finasteride, a review of its potential in the treatment of benign prostatic hyperplasia. *Drugs* **46** (1993) 177–208.
25. Brooks J. R., Berman C., Nguyen H., Prahalada S., Primka R. L., Rasmusson G. H. and Slater E. E., Effect of castration, DES, flutamide, and the 5α-reductase inhibitor, MK-906, on the growth of the Dunning rat prostatic carcinoma, R3327. *Prostate* **18** (1991) 215–227.
26. Presti J. C., Fair W. R., Andriole G., Sogani P. C., Seidmon E. J., Ferguson D., Ng J. and Gormley G. J., Multicenter, randomized, double-blind, placebo-controlled study to investigate the effect of finasteride (MK-906) on stage D prostate cancer. *J. Urol.* **148** (1992) 1201–1204.
27. Andriole G., Lieber M., Smith J., Soloway M., Schroeder F., Kadmon D., DeKernion J., Rajfer J., Boake R., Crawford D., Ramsey E., Perreault J., Trachtenberg J., Fradet Y., Block N., Middleton R., Ng J., Ferguson D. and Gormley G., Treatment with finasteride following radical prostatectomy for prostate cancer. *Urology* **45** (1995) 491–497.
28. Rittmaster R. S., Magor K. E., Manning A. P., Norman R. W. and Lazler C. B., Differential effect of 5α-reductase inhibition and castration on androgen-regulated gene expression in rat prostate. *Molec. Endocrinol.* **5** (1991) 1023–1029.
29. Lamb J. C., English H., Levandoski P. L., Rhodes G. R., Johnson R. K. and Isaacs J. T., Prostatic involution in rats induced by a novel 5α-reductase inhibitor, SK and F 105657: Role for testosterone in the androgenic response. *Endocrinology* **130** (1992) 685–694.
30. Fleshner N. E. and Fair W. R., Anti-androgenic effects of combination finasteride plus flutamide in patients with prostatic carcinoma. *Br. J. Urol.* **78** (1996) 907–910.