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PHARMACOLOGICAL AND CLINICAL STUDIES OF THE ANTIANDROGEN ANANDRON

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Summary—This paper summarizes the animal and human studies with Anandron available at the time of the meeting. The following was demonstrated in the rat and confirmed in man: interaction of Anandron with the prostatic androgen receptor, antiandrogen activity against testosterone (in particular against the early transient rise induced by LHRH analogs) and adrenal androgens. Thus, as shown in 4 different double blind studies performed in stage D_2 prostrate cancer patients, the combination of Anandron with surgical or chemical castration enhanced the beneficial effects of castration alone and thus seems a step forward in the hormonal treatment of prostatic carcinoma.

INTRODUCTION

Androgens of testicular and adrenal origin are the principal growth factors for both normal or cancerous prostatic tissue. The non-steroid antiandrogen, Anandron^R (Nilutamide = RU 23 908), is being developed at Roussel Uclaf [1–5] for the treatment of prostatic cancer patients whose testosterone is suppressed by chemical or surgical castration, to counteract the trophic effect of adrenal androgens which remain after castration [6–8] and to inhibit the "flare-up" effect due to the early transient rise in testosterone when castration is performed by treatment with LHRH analogs [9–12]. This paper summarizes the animal and human studies currently available with Anandron.

ANIMAL STUDIES

Anandron was selected on three *pharmacological criteria*:

(1) Anandron interacted with the cytosol androgen receptor (AR) of the castrated rat prostate with a weak relative binding affinity (RBA), decreasing with the time of incubation (RBA = 4.5% and 0.5% of that of testosterone after 30 min and 24 h of incubation at 0°C), indicating that the complex formed between Anandron and the receptor was less stable than that formed with testosterone [13]. This was related to an antiandrogen activity of Anandron against exogenous androgens, at doses between 1 and 50 mg/kg on the castrated rat prostate without agonist activity even at high doses [2, 3, 14].

The RBA of Anandron was higher for the AR of the prostatic tissue of hamster, dog, and human than for the rat prostate AR and this was related to a more potent antiandrogen activity in the hamster and dog than in the rat [14].

(2) Anandron did not interact with the receptors of other steroid hormones and thus was devoid of other hormonal or antihormonal activities, by contrast to steroid antiandrogens [3, 5, 14–20].

(3) Anandron was active by itself, by contrast to flutamide which needs to be converted into hydroxyflutamide in order to act [21–23].

The *pharmacokinetics* of Anandron in the rat and dog [4, 24] revealed a good absorption by oral route and a long plasma half-life (around 7 h in the rat and 11 h in the dog). A high (in the μM order) and sustained plasma level of Anandron was obtained after a single oral administration of 10 mg/kg to castrated rats. This could explain why, in spite of its low RBA and its rapid dissociation from the AR *in vitro*, a high and sustained interaction with AR was observed *in vivo* [4, 5].

Anandron was thus selected to treat prostatic carcinoma in men, and the *rationale of combining antiandrogen treatment with surgical or chemical castration* was demonstrated in experimental models designed to mimic the therapeutic context [5, 14]. Three points were to be confirmed:

(1) Anandron was able to inhibit the trophic effect of adrenal androgens on the prostate of castrated rats. Adrenal androgens are very weak androgens by themselves but can be converted in the prostatic cell into testosterone and dihydrotestosterone (DHT) [6–8, 25]. Plasma assays in castrated men [8] indicated that while testosterone and DHT concentrations were less than 10% of those measured before castration, the concentration of 3α androstanediol glucuronide, which is the metabolite of DHT excreted in the plasma and thus reflects tissular DHT concentration, remains ~30% of pre-castration levels, presumably due to the transformation of adrenal androgens into DHT. However, laboratory animals, and especially the rat, secrete very low

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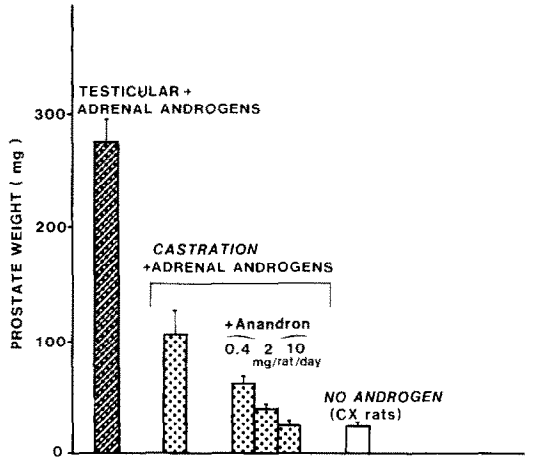

Fig. 1. Inhibition by Anandron of the trophic effect of adrenal androgens on the "castrated" rat prostate. Group of 5 adult male rats were continuously administered adrenal androgens by minipumps for 15 days. One group was left intact, while the other had their testosterone secretion suppressed by orchiectomy from the first day of adrenal androgen administration. Each castrated group was treated simultaneously with solvent or increasing doses of Anandron. One group of rats was orchiectomized but had no adrenal androgen supplementation. Prostate weights were determined on the 16th day.

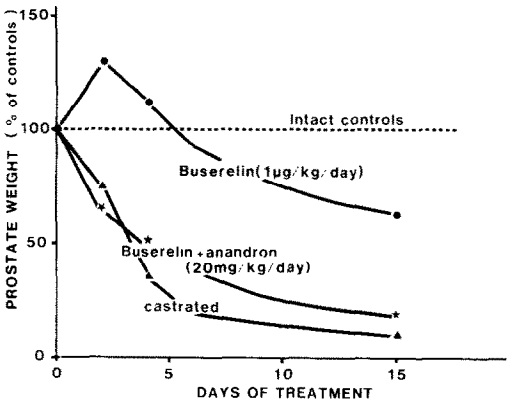

Fig. 2. Inhibition of the effect of the initial rise in testosterone induced by buserelin treatment in the rat. Groups of 5 adult male rats received daily for 15 days a s.c. injection of buserelin (1 µg/kg per day) alone or combined with an oral administration of Anandron (20 mg/kg per day). Control intact rats and a group of castrated rats received solvent only. The rats were killed after 2, 4 or 15 days of treatment or 2, 4 or 15 days after castration and prostates were weighed.

amounts of adrenal androgens in comparison to men. In order to clarify the role of adrenal androgens, we introduced the four principal adrenal androgens (Δ4 androstenedione, 11β-hydroxy androstenedione, DHEA and DHEA sulfate) to rats by minipumps for 15 days (Fig. 1). This resulted in plasma levels equivalent to those observed in humans (Δ4 = 0.7 ± 0.1, 11β OH Δ4 = 1.3 ± 0.2, DHEA = 3.7 ± 0.6, DHEA sulfate = 684 ± 55 ng/ml). Under these conditions when testosterone secretion was suppressed by orchiectomy, the prostate weight was 30% of that of non-castrated controls, the remaining tissue probably stimulated by the trophic effect of the adrenal androgens. When Anandron was given to rats from the day of castration, the trophic effect of adrenal androgens was completely inhibited and the weight of the prostate was reduced to that of totally androgen deprived rats (Fig. 1). Similar results were obtained in rats receiving adrenal androgens, but whose testosterone secretion was suppressed by diethylstilboestrol or LHRH analog (buserelin) [5, 14].

(2) Anandron was also shown to inhibit the "flare-up" effect of LHRH analogs in the rat (Fig. 2) When intact rats were given a daily administration of buserelin, there was a transient increase in prostate weight due to the transient rise in testosterone. The castrating effect of buserelin started only after a few days in contrast to the immediate atrophic effect of orchiectomy. The combination of Anandron with buserelin completely inhibited this transient trophic activity of the LHRH analog and the castrating effect of the combination was very similar to that of orchiectomy.

(3) Finally, the antiandrogen effect of Anandron was measured in an experimental androgen-dependent tumor model, the Shionogi carcinoma model [26]. The combination of Anandron with castration was able not only to delay the appearance of tumors in mice inoculated with carcinoma cells, but also to decrease the incidence of tumors, although in the few remaining tumors androgen-independent cells were able to grow under the total androgen blockade treatment [14].

STUDIES IN MAN

Studies were performed in man, to assess the kinetic profile of Anandron, to confirm its antiandrogenic activity and to evaluate its tolerance and efficacy in patients with prostate cancer.

(1) *Kinetics*: The compound was almost completely absorbed from the gastrointestinal tract; a high concentration of non-metabolized compound was observed during the first hours following administration of a single dose and the half-life of elimination was about 48 h, which permits a once-a-day administration [23].

During chronic intake, the steady-state concentration was attained after about 2 weeks, and was proportional to the dose [23, 27].

(2) *The anti-androgenic activity* of Anandron was studied in six healthy volunteers who received the compound at the dose of 300 mg/day. After 4 and 8 weeks of treatment, the plasma concentrations of LH, testosterone and estradiol, and the LH response to LHRH stimulation had markedly increased, a enhanced the beneficial effects of castration alone on

consequence of the inhibition by Anandron of the negative feedback exerted by androgens on the secretion of LH by the pituitary [28].

(3) *Studies in prostate cancer*: A non-comparative study of the combination of Anandron with either orchiectomy or buserelin in patients with advanced cancer was reported by Labrie *et al.*, to give very good results, especially in patients who had no previous hormonal treatment, with very few adverse effects [29].

Four multicenter double-blind clinical trials in prostate cancer were therefore initiated in order to compare castration alone and the combination of castration with the antiandrogen Anandron: all studies included patients with advanced prostate cancer who had not received previous hormonal treatment, with a life expectancy of at least 3 months and no other life-threatening disease. Follow-up examinations were performed after 1, 3, 6, 12, 18 and 24 months of treatment. Individual criteria, such as pain, performance status, primary tumor size, metastases on bone scan and chest X-ray and prostatic acid phosphatase, were evaluated and the NPCP (National Prostatic Cancer Project) criteria [30] were used to determine the objective response at each of the 6-monthly follow-ups.

The designs of the four studies varied slightly in that: Study I [31] was a dose–response study and three groups were compared: orchiectomy + Anandron 300 mg/day; orchiectomy + Anandron 150 mg/day; and orchiectomy + placebo; results were reported for 160 patients followed for 6–36 months.

In study II [32], buserelin 500 μg/day + placebo was compared to buserelin + Anandron 300 mg/day; results on 49 patients followed for 6–36 months were reported.

Study III [33] compared orchiectomy + Anandron 300 mg/day and orchiectomy + placebo; (170 patients followed for 3–24 months).

In Study IV [34] patients with Stage C as well as stage D prostate cancer were included (100 patients) and were treated either with orchiectomy + Anandron 300 mg/day or with orchiectomy + placebo.

For patients with metastatic disease, there were several differences between the Anandron-treated groups and the groups treated with orchiectomy or buserelin alone:

More patients improved subjectively in the active drug groups, as evidenced by the higher percentages of patients who reported decreased bone pain and whose performance status was improved at months 1, 3, and 6 of treatment (significantly different from placebo in two studies). When Anandron was given together with buserelin (study II) it prevented the increase in bone pain reported during the first month of treatment by several patients treated with buserelin alone.

The efficacy was also apparent on objective criteria since the percentages of normalized prostatic acid phosphatase levels were higher in the Anandron-treated groups, as well as the numbers of patients with a decreased number of metastases on bone scan.

When subjective and objective criteria were pooled as proposed by the NPCP to evaluate the percentages of patients who had progression of their disease within the first 6 months of treatment the differences between the Anandron and placebo groups were of a similar magnitude in the 4 studies, with 24–40% of patients in progression at 6 months in the placebo groups compared to only 9–23% in the Anandron groups (Table 1).

In two studies, there was a suggestion that the addition of Anandron may prolong survival time.

Frequently reported undesirable symptoms were hot flushes and gastro-intestinal symptoms, of similar frequency in both Anandron and placebo groups; impaired visual adaptation to darkness (24% with Anandron, 3% with placebo); and intolerance to alcohol (15% with Anandron, 0% with placebo). Only few patients had to stop treatment because of adverse events.

DISCUSSION

The antiandrogenic activity of Anandron has been demonstrated in animals and in man.

In four different double blind studies performed in patients with metastatic prostate cancer who had not received previous hormonal treatment, the combination of Anandron with orchiectomy or buserelin

Table 1. Objective progressions (NPCP criteria) in the first 6 months of treatment in patients evaluable for efficacy

			% progression		
				Anandron (mg/day)	
Investigator	Castration	*n*	Placebo	150	300
Brisset *et al.*	Orchiectomy	121	34	20	19
Navratil *et al.*	Buserelin	33	40		23
Béland *et al.*	Orchiectomy	100	38		22
Namer *et al.*	Orchiectomy	55	24		9

quality of life and objective tumor response. This added efficacy was probably due to the inhibition by this antiandrogen of the adverse effects on tumor growth of adrenal androgens which are not suppressed by castration alone: as demonstrated in rats, adrenal androgens in plasma concentrations equivalent to those found in men were above to maintain prostatic cell growth of castrated animals while Anandron counteracted this stimulatory effect.

Moreover, when castration was achieved by the LHRH analog buserelin, the evolution of bone pain during the first month of treatment suggests that Anandron may prevent the LHRH analog-induced flare-up of the disease. On the rat prostate, Anandron treatment was able to inhibit the trophic effect due to the early increase in testosterone induced by buserelin.

The combination of Anandron with castration seems therefore to be a step forward in the hormonal treatment of metastatic prostate cancer, with possible prolonged survival in two studies. However, although adding Anandron to castration will improve symptoms and objective tumor regression rate by preventing growth of androgen-dependent tumor cells, such treatment has no impact on hormone-independent tumor cells. These, as shown in the experimental tumor model, probably continue growing and eventually cause a relapse of the disease. An additional non-endocrine therapy might further improve the results obtained with the association of castration and Anandron.

REFERENCES

1. Raynaud J. P., Azadian-Boulanger G., Bonne C., Perronnet J. and Sakiz E.: Present trends in antiandrogen research. In *Androgens and Antiandrogens* (Edited by L. Martini and M. Motta) Raven Press, New York (1977) pp. 281–293.
2. Raynaud J. P., Bonne C., Bouton M. M., Lagacé L. and Labrie F.: Action of a non steroid anti-androgen, RU 23 908 in peripheral and central tissues. *J. steroid Biochem.* **11** (1979) 93–99.
3. Raynaud J. P., Bonne C., Moguilewsky M., Lefebvre F. A., Bélanger A. and Labrie F.: The pure antiandrogen RU 23 908 (Anandron*), a candidate of choice for the combined antihormonal treatment of prostatic cancer: a review. *Prostate* **5** (1984) 299–311.
4. Raynaud J. P., Moguilewsky M., Tournemine C., Coussedière D., Salmon J., Husson J. M., Bertagna C., Tremblay D., Pendyala L., Brisset J. M., Vallencien G., Serment G., Navratil H., Dupont A. and Labrie F.: Pharmacology and clinical studies with Anandron. In EORTC Genito-urinary Group Monograph 2, Part A: *Therapeutic Principles in Metastatic Prostatic Cancer* (Edited by F. M. Schroeder and B. Richards). Alan R. Liss, New York (1985) pp. 99–120.
5. Moguilewsky M., Fiet J., Tournemine C. and Raynaud J. P.: Pharmacology of an anti-androgen, Anandron, used as an adjuvant therapy in the treatment of prostate cancer. *J. steroid Biochem.* **24** (1986) 139–146.
6. Geller J.: Rationale for blockade of adrenal as well as testicular androgens in the treatment of advanced prostate cancer. *Semin. Oncol.* Suppl. 1, **12** (1985) 28–35.
7. Geller J., Albert J. and Loza D.: Steroid levels in cancer of the prostate—markers of tumour differentiation and adequacy of anti androgen therapy. *J. steroid Biochem.* **11** (1979) 631–636.
8. Fiet J., Villette M., Bergagna C., De Géry A., Hucher M., Husson J. M. and Raynaud J. P.: Plasma hormone levels before and after orchiectomy in prostate cancer patients. In *Proc. 2nd Int. Symp. Cancer of the Prostate* (Edited by G. Murphy and S. Khoury). Alan R. Liss, New York (1987).
9. Donnelly R. J.: Continuous subcutaneous administration of "Zoladex" (ICI 118, 630 an LHRH analogue) to patients with advanced prostatic cancer. *J. steroid Biochem.* **20** (1984) 1375.
10. Kerle D., Williams G., Ware H. and Bloom S. R.: Failure of long-term luteinising hormone releasing hormone treatment for prostatic cancer to suppress serum luteinising hormone and testosterone. *Br. med. J.* **289** (1984) 468–469.
11. Smith J. A., Glode L. M., Wettlaufer J. N., Stein B. S., Glass A. G., Max D. T., Anbar D., Jagst C. L., and Murphy G. P.: Clinical effects of gonadotropin releasing hormone analogue in metastatic carcinoma of the prostate. *Urology* **XXV** (1985) 106–114.
12. Waxman J., Man A., Hendry W. F., Whitfield H. N., Besser G. M., Tiptaft R. C., Paris A. M. I. and Oliver R. T. D.: Importance of early tumor exacerbation in patients treated with long acting analogues of gonadotrophin releasing hormone for advanced prostatic cancer. *Br. med. J.* **291** (1985) 1387–1388.
13. Raynaud J. P., Bouton M. M. and Ojasoo T.: The use of interaction kinetics to distinguish potential antagonists from agonists. *Tips* **1** (1980) 324–327.
14. Moguilewsky M., Cotard M., Proulx L., Tournemine C. and Raynaud J. P.: What is an antiandrogen and what is the physiological and pharmacological rationale for combined "Castration + antiandrogen" therapy. In *Proc. 2nd Int. Symp. Cancer of the Prostate* (Edited by G. Murphy and S. Khoury). Alan R. Liss, New York (1987).
15. Duncan M. R. and Duncan G. R.: An *in vivo* study of the action of antiglucocorticoids on thymus weight ratio, antibody titre and the adrenal pituitary–hypothalamus axis. *J. steroid Biochem.* **10** (1979) 245–259.
16. Guthrie G. P. and John W. J.: The *in-vivo* glucocorticoid and antiglucocorticoid actions of medroxyprogesterone acetate. *Endocrinology* **13** (1980) 1393–1396.
17. Neumann F.: Pharmacology and potential use of cyproterone acetate. *Horm. Metab. Res.* **9** (1977) 1–13.
18. Neumann F.: Experimental basis for the clinical use of antiandrogens. In *Regulation of Androgen Action* (Edited by N. Bruchovsky, A. Chapdelaine and F. Neumann). Congressdmak R. Bruckner, Berlin (1984) pp. 43–45.
19. Tausk M. and De Visser J.: Pharmacology of orally active progestational compounds animals studies. In *Pharmacology of the Endocrine System and Related Drugs: Progesterone, Progestational Drugs and Antifertility Agents* (Edited by M. Tausk). Pergamon Press, Oxford (1972) p. 35.
20. Tisell L. E., Salander H.: Androgenic properties and adrenal depressant activity of megestrol acetate observed in castrated male rats. *Acta endrocr., Copenh.* **78** (1975) 316–324.
21. Katchen B., Buxbaum S.: Disposition of a new nonsteroid antiandrogen, trifluoro-2-methyl-4′-nitro-*m*-propionotoluidide (flutamide), in men following a single oral 200 mg dose. *J. clin. Endocr. Metab.* **41** (1975) 373–379.
22. Koch H.: Flutamide, a new non-steroidal antiandrogen. *Drugs Today* 20 (1984) 561–574.
23. Tremblay D., Dupront A., Meyer B. M. and Pottier J.:

The kinetics of antiandrogens in humans. In *Proc. 2nd Int. Symp. Cancer of the Prostate* (Edited by G. Murphy and S. Khoury). Alan R. Liss, New York (1987).
24. Pettier J., Coussedière D. and Raynaud J. P.: Pharmacokinetics of a non-steroid anti-androgen (RU 23 908) in rats and dogs. In 67th Ann. Mtg Endocr. Soc. Baltimore (1985) Abstr. 954, p. 239.
25. Harper M. E., Pike A., Peeling W. B. and Griffiths K.: Steroids of adrenal origin metabolized by human prostatic tissue both *in vivo* and *in vitro*. *J. Endocr.* **60** (1984) 117–125.
26. Minesita T. and Yamaguchi K.: An androgen-dependent tumor derived from a hormone-independent spontaneous tumor of a female mouse. *Steroids* **4** (1964) 815–830.
27. Pendyala L., Creaven P., Huben R., Tremblay D., Mouren M. and Bertagna C.: Clinical pharmacokinetics of a new antiandrogen Anandron (RU 23 908). In *Proc. 2nd Int. Symp. Cancer of the Prostate* (Edited by G. Murphy and S. Khoury). Alan R. Liss, New York (1987).
28. Gooren L. J. G. and Asschenman H.: Effects of the new antiandrogen RU 23 908 on the gonadotropin secretion in the human male. In 3rd Int. Forum of Andrology, Paris (1985) Abstr., p. 31.
29. Labrie F., Dupont A., Bélanger A., Labrie C., Lacourcière Y., Raynaud J. P. Husson J. M., Emond J., Houle J. G., Girard J. G., Monfette G., Paquet J. P., Vallières A., Bosse C. and Delisle R.: Combined antihormonal treatment in prostate cancer, a new approach using an LHRH agonist or castration and an antiandrogen. In *Hormones and Cancer: Progress in Cancer Research and Therapy* (Edited by F. Bresciani, R. J. B. King, M. E. Lippman, M. Namer and J. P. Raynaud). Raven Press, New York, Vol. 2 (1984) pp. 533–543.
30. Schmidt J. D., Johnson D. E., Scott W. W., Gibbons R. P., Prout G. R. and Murphy G. P.: Chemotherapy of advanced cancer. Evaluation of response parameters. *Urology* **7** (1976) 602–610.
31. Brisset J. M., Boccon-Gibod L., Botto H., Camey M., Cariou G., Duclos J. M., Duval F., Gontiès D., Jorest R., Lamy L., Le Duc A., Mouton A., Petit M., Prawerman A., Richard F., Savatovsky I. and Vallancien G.: Anandron (RU 23 908) associated to surgical castration in previously untreated stage D. prostate cancer: a multicenter comparative study of two doses of the drug and of a placebo. In *Proc. 2nd Int. Symp. Cancer of the Prostate* (Edited by G. Murphy and S. Khoury). Alan R. Liss, New York (1987).
32. Navratil H.: Double-blind study of Anandron versus placebo in stage D2 prostate cancer patients receiving buserelin. In *Proc. 2nd Int. Symp. Cancer of the Prostate* (Edited by G. Murphy and S. Khoury). Alan R. Liss, New York (1987).
33. Beland G., Elhilali M., Fradet Y., Laroche B., Ramsey E. W., Venner B. N. and Tewari H. D.: Total androgen blockade versus orchiectomy in stage D2 prostate cancer. In *Proc. 2nd Int. Symp. Cancer of the Prostate* (Edited by G. Murphy and S. Khoury). Alan R. Liss, New York (1987).
34. Namer M.: Clinical applications of antiandrogens. Satellite Symp. Antisteroids of VIIth Int. Congr. Hormonal Steroids, Marbella (1986).