Long-term endocrine effects of administration of either a non-steroidal antiandrogen or a luteinizing hormone-releasing hormone agonist in men with prostate cancer

Andrea Decensi¹, Rosalba Torrisi¹, Vincenzo Fontana², Paola Marroni³, Paola Padovani², Domenico Guarneri¹, Francesco Minuto⁴ and Francesco Boccardo¹

Departments of Medical Oncology, II², Biostatistics² and Clinical Pathology², National Institute for Cancer Research, Genoa, Italy:
University Department of Endocrinology and Metabolism⁴, Genoa, Italy


The claimed ability of non-steroidal antiandrogens to preserve libido and sexual potency is sought as a potential improvement in the palliative management of prostate cancer. A critical issue for the clinical use of these compounds is, however, the reported evidence in the rat that an increase in testosterone concentrations as a consequence of the androgen negative feedback interruption. On the other hand, the recovery of testicular function after long-term inhibition by luteinizing hormone-releasing hormone (LHRH) analogs is also an important concern in view of the proposed use of these compounds for the treatment of several non-malignant conditions. We addressed these issues by studying the long-term endocrine effects induced by the administration of either the non-steroidal antiandrogen nilutamide or the depot preparation of D-Trp⁶-LHRH in men with prostate cancer. Treatment with the antiandrogen induced a marked increase in gonadotropin levels. LH concentrations rising from a mean (SEM) of 17.5 ± 1.6 to a maximum of 56.6 ± 6.9 kU/l (p < 0.001), while mean testosterone and 17β estradiol- concentrations rose only by about 50% and 70% over pretreatment values, testosterone levels reaching a plateau after 1 month of treatment. In the subjects treated with the LHRH agonist, 6 months after discontinuation of long-term administration the mean (±SEM) LH had risen to 36.9 ± 6.8 IU/l while mean testosterone levels were still as low as 1.7 ± 0.7 and rose only to a maximum of 4.2 ± 1 nmol/l after high-dose human chorionic gonadotropin loadings. We conclude that in elderly men with prostate cancer: (i) stimulation of the entire axis by non-steroidal antiandrogens induces only a mild testosterone increase, the tests being the site of the reduced response; (ii) prolonged inhibition of the pituitary–gonadal axis induced by LHRH agonists may not be reversible at the testicular level.

Andrea Decensi, Istituto Nazionale per la Ricerca sul Cancro, Viale Benedetto XV, 10-16132 Génova, Italy

Orchiectomy or LHRH agonists represent the standard endocrine treatment of advanced prostate cancer. An important drawback in palliative management of sexually active patients, however, is the loss of libido and sexual potency that accompanies testosterone suppression. In early preliminary studies, it was shown that the administration of the non-steroidal antiandrogen flutamide was able to interfere purely with the androgen negative feedback, resulting in a paradoxical state of hypergonadotropic hypergonadism (1) that allowed the preservation of sexual potency in some patients (2). Nevertheless, the use of this class of antiandrogens as single therapeutic agents of prostate cancer has been very limited so far, mainly because extrapolations from animal studies suggested that the stimulatory effect on the hypothalamic–pituitary–gonadal axis could be a pronounced and progressively increasing process that might ultimately overcome the antiandrogen activity of the drug (3). Recently, however, we have observed in a phase II (activity) study that the administration of nilutamide, a second-generation antiandrogen (3), could achieve results comparable with other standard hormonal therapies and maintain libido and sexual potency in about half of the patients (4). Moreover, unaltered nocturnal erections have been documented objectively in prostate cancer patients treated with the antiandrogen casodex (5).

In the present work, the endocrine effects of long-term administration of nilutamide in prostate cancer patients have been studied in parallel with those induced by prolonged treatment with D-Trp⁶-LHRH. In fact, the study of these antiandrogenic strategies that produce opposite endocrine environments (hypergonadotropic hypergonadism versus hypogonadotropic hypogonadism) may provide complementary information on the pathophysiology of the hypothalamic–pituitary–
gonadal axis in elderly men. For instance, stimulation of the entire axis induced by the antiandrogen or, on the contrary, its recovery after prolonged inhibition of the LHRH agonist may provide insight into the reduction site(s) of the endocrine gonadal function involved in the ageing process (6). In particular, we focused on two aspects with important therapeutic implications:

(i) the entity and the course of the stimulation induced by the antiandrogen on the testis, which may result in an inadequate long-term control of circulating androgens;

(ii) the reversibility of testicular function after long-term LHRH agonist suppression, the relevance of which is underscored by the proposed use of these compounds in several non-malignant conditions, including treatment of age-related disorders such as benign prostatic hyperplasia (7) or protection of gonadal damage induced by chemotherapy (8).

Materials and methods

Subjects and treatments

The study population consisted of a series of 21 men in the GnRH agonist group (median age 72 years, range 58–78 years) and 12 men in the antiandrogen group (median age 71 years, range 57–78 years) seen at the authors’ institution as part of two consecutive phase II clinical trials performed by the investigators of the Italian Prostatic Cancer Project (4, 9). Patients were affected with locally advanced (stage C) or metastatic (stage D) prostate cancer and had received no prior treatment. Inclusion criteria for the study were good performance status and a life expectancy of greater than 6 months. Informed consent was obtained from each patient after the trials had been approved by the Institutional Review Board of the National Institute of Genoa. Treatment consisted of either the sustained-release (depot) d-Trp6-LHRH (Decapeptyl, Ipsen Biotech, Milano, Italy), administered as a 3.75-mg 4-weekly intramuscular dose, or the antiandrogen nilutamide (Anandron, Roussel Pharma, Milano, Italy), taken at the daily oral dose of 300 mg (two 50-mg tablets every 8 h). Treatments continued until disease progression.

Study protocol

Blood samples for hormone and sex hormone binding globulin (SHBG) measurement were taken between 08.00 and 09.00 h at the following times: before treatments, at week 1 in the LHRH agonist group only (to evaluate the initial LH rise), at months 1 and 3 and subsequently at 3-month intervals up to 1 and 2 years in the antiandrogen and LHRH agonist group, respectively. The sera were separated by centrifugation and stored at −80°C until assayed.

In a subset of 14 patients in the agonist group (median age 72 years, range 60–78 years) and seven patients in the antiandrogen group (median age 72 years, range 61–76 years), a stimulation test with 100 µg of GnRH IV (Serono) was carried out after a median treatment time of 9 and 8 months, respectively. Responses of LH and FSH were measured at 0, 30, 45, 60 and 90 min after GnRH administration and compared with those observed in an age-matched group of seven healthy men (median age 72 years, range 64–75 years) who served as controls.

Testicular responsiveness was evaluated in 15 patients (median age 71 years, range 58–76 years) being treated with ß-Trp6-LHRH for a median time of 21 months (range 18–27 months) and compared with five eunuchoidal subjects, 67–79 years old (median 73 years), referred to hospitalization for disorders other than prostate cancer. The response of testosterone to a 5000 IU i.m injection of human chorionic gonadotropin (hCG; Profasi, Serono, Roma, Italy) was evaluated before and then 48 and 72 h after the stimulation. The testosterone response to the hCG test was re-evaluated later in six patients who had been off treatment for various intervals, having achieved a complete objective tumor response that lasted until the time of the test. In detail, two patients received 5000 IU i.m after treatment had been discontinued for 3 months and the other four patients received 1000 IU of hCG every other day for three doses after treatment had been suspended for 6, 6, 9 and 9 months, respectively.

Measurement of hormones and SHBG

All hormone determinations were performed in duplicate by RIA using commercially available kits purchased from Diagnostic Product Corp., Los Angeles, CA. Serum LH, FSH and PRL concentrations were determined by liquid-phase RIAs. The intra-assay coefficient of variation (CV) was 5% for LH, 7% for FSH and 6% for PRL, and the respective interassay CV values were 11%, 11% and 10%. The reference range for serum LH and FSH in normal men was 5–20 IU/l, and that for serum PRL was 0–15 µg/l. Serum progesterone, 17-hydroxyprogesterone (17-OHP), androstenedione, testosterone, 17β-estradiol (E2) and dehydroepiandrosterone (DHEA) concentrations were measured by liquid-phase RIAs. Prior to the assay, 17-OHP, androstenedione and DHEA were extracted with diethyl ether. The intra-assay CV was 5.2% for progesterone, 6.5% for 17-OHP, 4.5% for androstenedione, 7% for testosterone, 6% for E2 and 5.5% for DHEA; the respective interassay CVs were 7.1%, 9%, 6.3%, 10%, 11% and 7%. The reference range for normal men was: progesterone, 0.1–3 nmol/l; 17-OHP, 0.6–5.4 nmol/l; androstenedione, 1.7–14.3 nmol/l; testosterone, 12.5–34.4 nmol/l; E2, 22–161 pmol/l; DHEA, 0.7–34 nmol/l. Serum SHBG concentrations were measured using a non-competitive liquid-phase IRMA provided by Farnos Diagnostica (Oulunsalo, Finland). Intra- and interassay CVs were 3.2% and 5%, respectively. The reference range in normal men was
Fig. 1. Effects of treatment with nilutamide (—, solid circles) and D-Trp^6^-LHRH (—– —, open circles) on mean (±SEM) LH, FSH and PRL levels. **p<0.01 and ***p<0.001 (ANOVA). "p<0.01 vs basal level (t-test) and **°p<0.001 vs month 1 (ANOVA).

10–70 nmol/l. Analysis of product/substrate ratios in serum concentrations was used to infer relative activities of testicular steroidogenic enzymes.

Statistical analyses

All results are given as the mean±SEM. Computed analysis of data was done using SPSS/PC+ statistical software (SPSS Inc., Chicago, 1990). Within each treatment group, comparison of hormone values before and after treatment was performed by non-parametric two-way analysis of variance (Friedman ANOVA). Comparison of stimulation tests between treated and control groups was performed using multivariate analysis of variance for repeated measures (MANOVA). Factors of comparison were drug (drug vs no treatment), time and interaction (treatment x time). Because of significant departure from normal distribution, these data were log-transformed.

Results

Pituitary hormones

Figure 1 shows the behaviour of pituitary hormones during treatment with either D-Trp^6^-LHRH or the antiandrogen nilutamide. At baseline, no significant difference in hormone concentrations between the two treatment groups was observed. Treatment with the agonist during the first week induced a significant elevation of LH levels and a decrease of FSH levels but without a corresponding increase of testosterone concentrations (from a basal level of 17.5±1.4 down to 16.2±2.4 nmol/l). Thereafter, while the LH level settled, FSH levels showed a significant progressive return to pretreatment values. In the antiandrogen group, the levels of gonadotropins showed a highly significant rise. Prolactin concentrations were not affected by the
antiandrogen but they were inhibited significantly by the LHRH analog.

The stimulation test with exogenous GnRH is shown in Fig. 2. As expected, gonadotropin response was absent during treatment with the agonist, while LH responsiveness was significantly more enhanced in patients treated with nilutamide than in controls. No difference was observed for FSH responsiveness.

**Testicular steroids**

The behaviour of testicular steroids and their ratios during both treatments is illustrated in Figs. 3 and 4. Before treatment, there was no significant difference in hormone levels or their ratios between the two treatment groups, except for progesterone, 17-OHP and the androstenedione/17-OHP ratio (data not shown). Treatment with the agonist induced a highly significant decrease of 17-OHP, testosterone and E2 levels and a marginally significant decline of androstenedione concentrations. No significant variation of SHBG levels was observed (data not shown). The concentrations of progesterone showed a non-significant increase up to the ninth month of treatment (from a basal level of 1.74 ± 0.19 up to 2.28 ± 0.28 nmol/l), while a significant drop was evident thereafter (1.14 ± 0.21, 0.48 ± 0.06 and 0.38 ± 0.05 nmol/l at the 12th, 18th and 24th month, respectively; p < 0.05). The ratio of 17-OHP/progesterone decreased significantly compared with baseline values, whereas the androstenedione/17-OHP ratio increased. As the decline of DHEA concentrations paralleled those of androstenedione, the androstenedione/DHEA ratio remained unchanged.

As a result of antiandrogen administration, there was a highly significant increase in the concentrations of progesterone and 17-OHP and a marginally significant increase in the concentrations of testosterone (from 12.8 ± 1.7 to a maximum of 19.8 ± 1.3 nmol/l) and E2 (from 109.2 ± 15.2 to a maximum of 189.1 ± 24.1 pmol/l). The values of the 17-OHP/progesterone ratio increased, while those of androstenedione/17-OHP decreased. The other hormone ratios, including E2/testosterone, were not affected.

In 15 patients treated long-term with the agonist, mean testosterone levels rose from a basal level of 0.55 ± 0.5 to a maximum of 3.2 ± 0.7 nmol/l after stimulation with 5000 IU of hCG, whereas it rose from 17.4 ± 3.1 to 33.3 ± 8.3 nmol/l in age-matched controls. After a median of 6 months off LHRH agonist administration, the mean LH level had increased to a mean value of 36.9 ± 6.8 IU/l but the mean basal testosterone level was still as low as 1.7 ± 0.7 and rose to a maximum of 4.2 ± 1 nmol/l following high-dose hCG stimulation(s).

**Discussion**

The claimed ability of non-steroidal antiandrogens to preserve sexual activity in elderly men is sought as a potential improvement in the palliative management of
advanced prostatic cancer (4, 10, 11). As with the use of LHRH agonists in non-malignant conditions such as benign prostatic hyperplasia (7), the endocrine effects of either prolonged stimulation or inhibition of the pituitary–gonadal axis, which might result in either an excessive androgen increase or an irreversible inhibition of gonadal function, respectively, are of crucial importance in elderly men. Our results show that treatment with the antiandrogen nilutamide induces a highly significant rise of basal gonadotropin concentrations and an enhanced LH response to exogenous GnRH. Despite the progressive and substantial elevation in the basal and stimulated LH response, the increase of testosterone concentrations was only marginally significant (about 50%) and reached a plateau after the first month. The occurrence of such mild and self-limiting stimulation of the testis emphasizes the difference with the 17-fold increase observed in the intact adult rat (3) and, more importantly, tends to reduce the potential risk of loss of control on circulating androgens by the receptor antagonist. In contrast to our findings, administration of nilutamide at the same dose up to 8 weeks in young eugonadal men was shown to induce a much higher increment (two- to threefold) of T and E2 levels and a much lower increase in LH levels (12). While the difference in basal and stimulated LH concentrations may reflect both the age-related increase in the gonadotropin-suppressive effect of androgens (with consequent increased responsiveness to its interruption) (13) and the inhibitory action of E2, the effect of an inadequate neutralization of androgens by the receptor antagonist in young men cannot be ruled out. Nevertheless, the lower testosterone increase observed in elderly compared to young men, despite higher concentrations of immunoreactive LH and lower concentrations of E2, points to a primary Leydig cell impairment as the main site of the age-related gonadal function decline, which in addition may be aggravated by illness (14).

As regards the pattern of gonadal effects, the sustained LH elevation induced by the antiandrogen elicited a selective blockade of 17, 20-desmolase, as inferred by the decreased androstenedione/17-OHP ratio. This defect of the “late” steroidogenic pathway, which distinguishes the process of gonadal desensitization, is attributed to an estrogen-mediated action (15). In our study, an indirect role of estrogens may be advocated in light of the observed E2 increase, which also may have reduced testosterone biosynthesis by attenuating LH bioactivity (16). In addition, gonadal desensitization might be secondary to a direct enzymatic inhibition exerted by the
antiandrogen, as shown in the rat (17). Unlike flutamide (18), the observation of an unaltered E2/testosterone ratio seems, however, to exclude an important influence of nilutamide on estrogen metabolism at the hepatic level.

As far as antagonadal effects of the LHRH agonist are concerned, the decrease of the 17-OHP/progesterone ratio and the slight accumulation of progesterone indicate that the main site of steroidogenic inhibition, resulting from the loss of LH bioactivity, is selectively localized at the level of 17α-hydroxylase (19). In addition, our data indicate that the testicular secretory response following prolonged LHRH agonist administration remains suppressed in most patients even after several months of LH recovery from treatment suspension and repeated hCG loadings. This observation is corroborated further by morphological studies showing the occurrence of severe pathological damage of both germ and Leydig cells after prolonged LHRH agonist administration (20, 21). Furthermore, the marked lowering of progesterone levels that we observed after 9–12 months of LHRH agonist suppression suggests the onset of a defect in the “early” steroidogenic pathway, such as a massive receptor loss (15), as an additional, time-dependent lesion of the steroid biosynthetic pathway. A therapeutic implication of the observed testicular impairment in neoplastic disorders could be either a reduced agonist dosage or a prolonged interval between two injections after an initial period. A more important, ethically relevant question, however, could arise when treating long-term age-related, non-malignant conditions in which the reversibility of gonadal function is required.

Interestingly, the sharp increase in LH concentrations following the first depot injection of the agonist was not accompanied by an increase in testosterone levels. This conflicts with data reported in similar patients with the use of daily agonist injections, which were shown to induce up to a 50% elevation of testosterone levels (22). This difference, which has important clinical implications in terms of “flare-up” risk in endocrine-dependent tumors, might be related to the 5–15-fold reduction of the daily dose released by the depot preparation (approximately 100 μg).

Finally, the dissociation of gonadotropin response induced by D-Trp⁶-LHRH during the first week seems indicative of a different regulation of gonadotropin synthesis by GnRH and its analogs, as confirmed by the recent observation that the pattern of GnRH pulsatility can selectively regulate gonadotropin subunit gene expression (23). The subsequent progressive rise of FSH levels, already seen by other authors (24), may suggest the occurrence of additional peripheral events, such as the decrease of inhibin production by GnRH analogs, similarly to what is observed in vitro with the native hormone (25), or the increased effect of activin (24). As to the marked suppression of PRL concentrations observed during treatment with the agonist, although this immediately reflects the decrease of sex hormones, a direct inhibitory effect of the LHRH agonist on PRL synthesis and secretion also has been proposed (26–28).

In conclusion, our results have shown that in elderly men with prostate cancer:

(i) stimulation of the entire axis by non-steroidal antiandrogens induces only a mild testosterone increase, the testis being the site of the reduced response;

(ii) prolonged inhibition of the pituitary–gonadal axis induced by LHRH agonists may not be reversible at the testicular level.

Acknowledgments. The authors wish to acknowledge the technical contribution of Mr B Costantini, Miss A Fossati and Mr R T Willey.

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ACTA ENDOCRINOLOGICA 1993. 129


Received March 1st, 1993
Accepted May 25th, 1993