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Effects of Long Term GnRH Analogue Treatment on Hormone Levels and Spermatogenesis in Patients with Carcinoma of the Prostate

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Summary. The effects of long term GnRH treatment with the biodegradable depot formulation of ICI 118.630 on hormone levels and spermatogenesis were investigated in 18 males with advanced prostate cancer. Plasma levels of FSH, LH, testosterone, DHEA-S and SHBG were monitored at regular intervals. The drug suppressed FSH, LH and testosterone significantly and did not affect DHEA-S and SHBG plasma levels. Tissue specimens for histologic assessment and quantitative analysis of germinal cell types were obtained at secondary orchidectomy in 16 patients immediately following GnRH analogue treatment. Germinal cell maturation was arrested at the spermatogonial stage. In two patients discontinuing treatment histologic assessment of secundary orchidectomy specimens 9 and 10 months after the last GnRH analogue depot injection resulted in germinal cell maturation to late spermatides in part of the tubule cross sections. These results indicate that long term administration of the GnRH analoge fails to produce complete testicular sclerosis and spermatogenic arrest might be reversible.

Key words: Long term GnRH analogue treatment – Hormone profiles – Spermatogenesis

Introduction

There is conclusive evidence that GnRH agonistic analogues inhibit testicular androgen production by pituitary receptor down regulation [6, 13, 14, 16, 18–20]. Continued administration has been recommended to replace orchidectomy in the management of advanced stages of carcinoma of the prostate. Histological investigations of the effect on testicular morphology were mainly carried out on tissue specimens obtained after secondary orchidectomy. The studies resulted in impairment of spermatogenesis, atrophy of the Leydig cells and testicular fibrosis following daily subcutaneous injections of leuprolide or several daily intranasal sprays of buserelin [12, 15, 19].

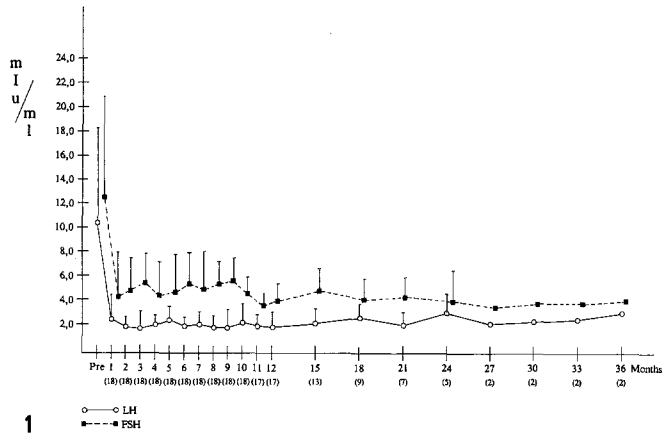
D-ser-$(Bu^t)^6$-azgly^{10}-LHRH (ICI 118.630) is another highly active GnRH agonistic analogue which has been incorporated into a d,l-lactide glycolide copolymer to develop a monthly biodegradable depot formulation providing a constant release of the drug [1, 13]. Previous studies on testicular morphology showed atrophy of the Leydig cells [17] and damage to germinal epithelium [9] as a consequence of decrease of gonadotrophins after short term treatment.

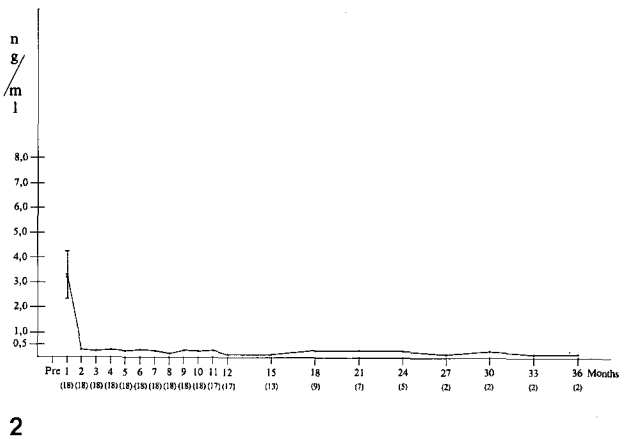
The effects of long term suppression of pituitary gonadotrophins and testosterone by GnRH analogues slowly released from depot formulations on spermatogenesis have not been clearly elaborated yet. It is of interest, whether prolonged administration of the drug produces irreversible changes in terms of complete atrophy of germinal cells or reversible inhibition of germinal cell maturation. The object of this study was to elucidate this problem.

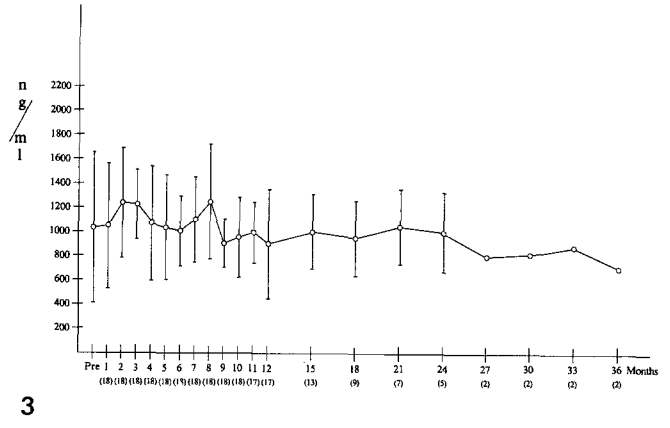
Patients and Methods

A total of 49 patients with either locally advanced or metastatic prostate cancer entered a continuing trial to assess the endocrine effects and clinical efficacy of the GnRH analogue ICI 118.630. Treatment was carried out with the biodegradable depot preparation consisting of a 50:50 d,l lactide glycolide copolymer which erodes releasing the drug and producing lactic and glycolic acids. The depot contained 3.6 mg of the drug and was injected subcutaneously into the anterior abdominal wall by a specific disposable applicator at monthly intervals. The average release of the drug was found to be 120 μg per day [13].

Plasma concentrations of FSH, LH, testosterone, DHEA-S and SHBG were measured monthly during the first year of treatment and every three months thereafter. Blood samples were collected between 8.00 and 12.00 a.m. FSH, LH and testosterone were measured with kits from CIS Sorin, Biomedica, Sallugia, Italy. Gonadotrophin assays were incubated over night at room temperature. Testosterone was extracted with diethyl-ether before the radioimmunoassays were carried out. Results were corrected for extraction yields and recovery was evaluated with serum samples spiked with 3-H-testosterone in parallel extractions.







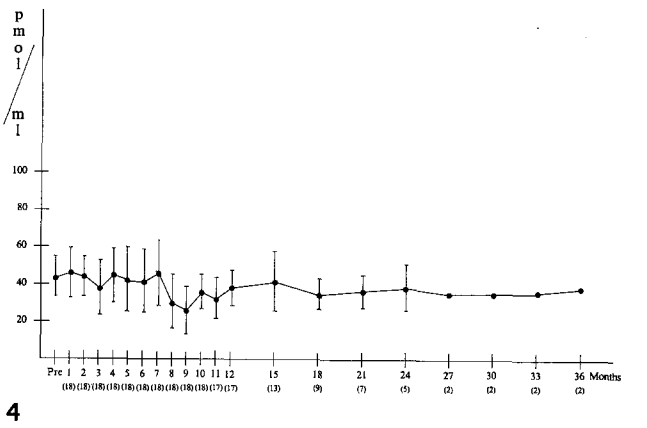


Fig. 1. FSH and LH: mean and standard deviation

Fig. 2. Testosterone (mean)

Fig. 3. DHEA-S: mean and standard deviation

Fig. 4. SHBG: mean and standard deviation

DHEA-S was analysed with kits from Cambridge Medical Diagnostics, Inc., Billerica, USA. SHBG was measured by saturation of binding sites with 3-H-5 alpha-dihydrotestosterone (Serono, Biodata, Italy). The between and within assay variability was monitored with pooled sera and with control sera, respectively (NMS-Biorad Laboratories, Richmond, USA).

The patients were monitored for objective tumor response by rectal digital examination, transrectal ultrasound, PAP, X-rays and bone scans at three month intervals. In 16 patients (age: 62–81 years) disease progression occured between 10 and 36 months after the institution of treatment. They underwent bilateral subcapsular orchidectomy and received cytotoxic therapy.

Patient P. St. (59 years) and patient M. G. (71 years) rejected further GnRH injections and visits to control the efficacy of treatment in the absence of tumor progress 13 and 26 months after the onset of therapy. The reason underlying the decision to refuse treatment was the desire to regain sexual activity in the younger individual and the other patient believed himself to be disease free and that no further treatment is necessary.

Both patients were readmitted to the hospital 9 and 10 months after the last GnRH depot injection presenting with objective tumor progression. They finally agreed on further treatment which consisted of secondary orchidectomy and cytotoxic agents. Plasma level of FHS, LH and testosterone were determined prior to orchidectomy.

Tissue specimens for histologic assessment were obtained from the equatorial area shortly after exposure of the testicles and immediately fixed in 2% glutaraldehyde and 1% osmic acid, both fixatives in 0.1 M sodium cacodylate buffer at pH 7.4. After dehydration, all specimens were embedded in Epon 812. Multiple semithin sections cut at 0.5 to 1.0 μm were stained with toluidine blue [10].

Tubular diameters were determined and analysis of germinal cell types was performed according to the technique previously described by De Kretser et al. [4]. The germinal cells were identified according to the criteria described by Clermont [3]. The cell types were classified into Sertoli cells, spermatogonia (type A, dark and pale, and typ B), primary spermatozytes, early spermatids (Sa, Sb1 and Sb2) and late spermatids (Sc, Sd1 and Sd2), 50 different cross sections cut at right angles to the longitudinal axis of the tubules were scored.

Results

Endocrinology

The endocrine data of patients obtained between the onset of therapy and the last visit prior to orchidectomy or refusal of further GnRH analogue treatment are documented in Figs. 1–4. Monthly injections of 3.6 mg of the GnRH analogue depot suppressed FSH and LH significantly. Testosterone concentrations were found to be at castrate levels (< 0,5 ng/ml) in all patients one month after the institution of treatment. Reelevation of the hormone levels did

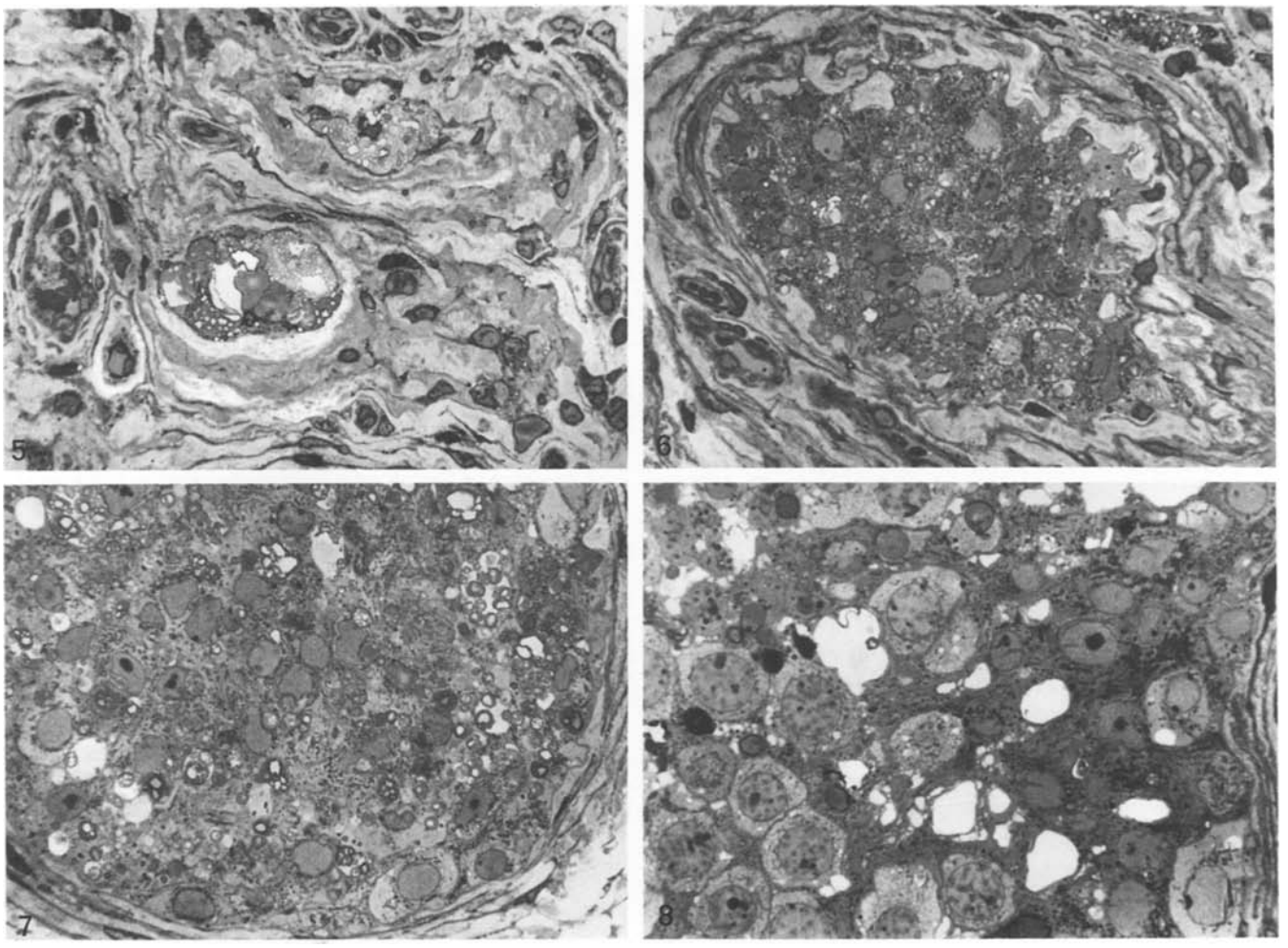


Fig. 5. Tubular atrophy and fibrotic degeneration with complete cell loss (x2300)

Fig. 6. Tubule cross section with Sertoli cells and complete absence of germinal cells (x2300)

Fig. 7. Tubule cross section presenting with areas of complete germinal cell loss besides sectors containing spermatogonia (x2300)

Fig. 8. Maturation of spermatogenesis to the late spermatid stages 10 months after cessation of GnRH analogue treatment (x2300)

Table 1. Hormone levels prior to secondary orchidectomy in patients discontinuing GnRH analogue treatment

	P. ST. (59 years)	M. G. (71 years)
FSH (mIU/ml)	14.3	18.9
LH (mIU/ml)	6.7	11.9
Testosterone (ng/ml)	4.9	2.9

not occur thereafter. There were no significant alterations of the plasma levels of DHEA-S and SHBG.

Plasma levels of FSH, LH and testosterone obtained prior to orchidectomy in the patients discontinuing GnRH analogue treatment are presented in Table 1.

Spermatogenesis

In testis tissue specimens removed immediately following GnRH analogue depot administration all tubules presented with moderate peritubular fibrosis and occasionally hyalinization and tubular atrophy were visible (Fig. 5). The mean tubular diameter ranged between 65–154 μm. Severe impairment of spermatogenesis was observed. Tubules presenting with absence of germinal cells (Fig. 6) were found besides those with germinal cells arrested at the spermatogonial stage. Within the same tubule cross section areas with complete germ cell loss were observed besides sectors with spermatogonia (Fig. 7). Spermatogenesis never developed beyond the spermatogonial stage.

On quantitative analysis of germinal cell types per tubule cross section the number of Sertoli cells was found to range between 0–19.3 ($\bar{X}$ = 13.4) and the number of spermatogonia between 0–8.9 ($\bar{X}$ = 4.1).

Table 2. Tubular diameter and cell types per tubule cross sections in orchidectomy specimens of patients discontinuing GnRH analogue treatment (x and range)

	P. ST. (59 years)	M. G. (71 years)
Tubular diameter (μm)	132.2 65–189	126.5 79–199
Sertoli cells	12.1 0–19.2	13.4 0–20.1
Spermatogonia	15.4 0–25.4	11.2 0–19.5
Primary Spermatozytes	18.4 0–37.4	16.9 0–41.2
Early Spermatids	9.1 0–47.8	11.5 0–59.2
Late Spermatids	1.6 0–15.2	0.6 0–12.1

In orchidectomy specimens of patients who discontinued GnRH analogue treatment testis morphology appeared heterogeneous. Tubules with maturation of germinal cells to late spermatids were observed (Fig. 8) among other tubules with absence of germinal cells and spermatogenic arrest at the spermatogonial and spermatozyte stages.

The results of detailed analysis of germinal cells are shown in Table 2.

Discussion

Our data confirm the inhibitory effect of the biodegradable depot formulation of ICI 118.630 on the pituitary-gonadal axis. FSH and LH were constantly suppressed and testosterone which was found to be at castrate level by day 29 after the first injections did not show any increase up to 36 months after the onset of therapy.

The plasma concentrations of SHBG which influence the levels of free androgen were not affected by the drug. These results suggest that the concentrations of free androgen will decrease in parallel with the measured total androgen. Monitoring of DHEA-S plasma levels demonstrated that the GnRH agonist did not interfere with the secretion of adrenal androgens.

The depot provides a constant release of the GnRH agonist [1, 13] and avoids transient surges of LH as observed following daily intermittent injections [7]. Profiles of testosterone obtained by serial sampling over the day were found to be identical to those observed after orchidectomy [7] indicating maximal suppression of androgen biosynthesis in the Leydig cells by the GnRH analogue depot.

During the initial phases of clinical trials in the management of prostate cancer GnRH analogues were administered by daily subcutaneous injections or several daily intranasal sprays. Due to the poor oral activity and the short half life of the drugs these regimens were necessary to achieve permanent suppression of circulating testosterone. Subsequently, most of the data available describing effects on testis morphology were obtained following daily intermittent administrations. The results appear somewhat controversial. Waxman et al. [19] described complete absence of spermatogenesis 6 months after the onset of daily intranasal buserelin. Rajfer et al. [12] found occasional germinal cells in the tubule sections after daily subcutaneous administration of leuprolide over 12 months, and arrest of spermatogenesis with only spermatogonia in the majority of the tubules was reported by Smith and Urry [15]; only a few tubules containing more highly differentiated germinal cells.

Tubules with complete absence of germinal cells, but with the presence of Sertoli cells and tubules with maturation arrest of spermatogenesis at the spermatogonial stage were observed in the testicles after prolonged administration of the biodegradable depot formulation of ICI 118. 630. Detailed quantitative analysis of germinal cell types resulted in a decrease of the basal germinal cell population. The mean number of spermatogonia per tubule cross section was found to be extremely reduced as compared to values obtained from the testicles of patients with obstructive azoospermia or oligozoospermia using the same technique of quantitative assessment of germinal cell types [4]. Di Silverio et al. [5] reported on degeneration of the nuclear membrane of spermatogonia. However, on light microscopy of semithin sections no abnormalities of the nuclei of the spermatogonia were visible.

Postpuberal hypogonadotrophic dysfunction of the pituitary gonadal axis produces maturation arrest of spermatogenesis with progressive loss of germ cells, diminished diameter of the seminiferous tubules and thickening of the tunica propria [2, 11]. Morphologic assessment of testicular tissue obtained at secondary orchidectomy 10 to 36 months after the institution of GnRH analogue treatment resulted in severe impairment of spermatogenesis, decrease of tubular diameter and thickening of tubular walls. However, in all testicles investigated tubule cross sections with spermatogonia were present. The drug failed to produce complete testicular sclerosis. Atrophic tubules with complete cell loss were only found occasionally, but focal areas of tubular degeneration have been described to be age dependent lesions of the testicles of elderly individuals independent from any treatment [8].

The morphological findings in the testicles after prolonged suppression of pituitary gonadotrophin release and androgen biosynthesis in the Leydig cells support the assumption that maturation arrest of spermatogenesis might be reversible after withdrawal of the drug. However, the opportunity to study hormone levels and testis morphology in patients discontinuing long term GnRH agonist treatment is rare. Two patients who rejected further GnRH agonist administration after prolonged treatment provided an opportunity to investigate the hormone levels and spermatogenesis after cessation of therapy. Secondary orchidecto-

my was carried out 9 and 10 months after the last GnRH agonist injection and analysis of FSH, LH and testosterone was performed prior to the removal of testicular tissue. Both gonadotrophins and testosterone plasma levels were found to be significantly elevated as compared to values observed during GnRH analogue treatment. These findings indicate that the suppressive action of prolonged GnRH agonist administration on the pituitary gonadal axis is reversible even in elderly individuals. There is conclusive evidence that active spermatogenesis in terms of germinal cell maturation to early spermatids in some tubules is associated with a reactivation of the pituitary gonadotrophin release and androgen biosynthesis in the Leydig cells. Maturation of germinal cells beyond the spermatogonial stage was never observed in testicular tissue immediately following continued GnRH analogue treatment. However, the morphological pattern of the testicles was not found to be normal and tubules presenting with damage to the seminiferous epithelium in terms of absence of germinal cells and maturation arrest were visible. However, testis morphology cannot be expected to return to normal in males over 70 years of age since impairment of spermatogenesis and tubular cell loss were described to be characteristic lesions of the testicle of the aging male [8].

The data presented in this study furnish evidence that maturation arrest of spermatogenesis associated with pituitary gonadal down regulation by long term GnRH analogue treatment is reversible. These findings could be of significance for further assessment of possible impacts of GnRH analogues on male contraception.

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