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Pathological and morphometric assessment of testicular parameters in patients with metastatic prostate cancer following treatment with either the antiandrogen Casodex (ZM176,334) or bilateral orchidectomy

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Abstract Casodex is an orally active non-steroidal antiandrogen that is highly selective for androgen receptors in animals and man. It is indicated for the non-surgical treatment of advanced prostate cancer in man. The present open controlled study in 13 Casodex-treated and 21 orchidectomy-alone (control) patients addressed the hypothesis that chronic administration of antiandrogens will result in Leydig cell hyperplasia as a result of feedback inhibition of the pituitary resulting in increased luteinising hormone (LH) stimulation of Leydig cells. Although Casodex has been shown to produce a moderate rise in circulating plasma testosterone concentration on chronic treatment in prostate cancer patients, a controlled histopathological and morphometric assessment of the testis following orchidectomy in relapsed Casodex patients showed no effect on Leydig cell populations compared with an orchidectomy alone (control) group. No evidence for induction of Leydig cell hypertrophy or hyperplasia as a result of chronic oral administration of 50 mg Casodex daily was obtained in this study.

Key words Casodex (ZM176,334) · Orchidectomy · Prostate cancer · Testis · Histopathology · Morphometry

Prostate cancer has become the most frequently encountered cancer in men (currently representing about 22.4% of all cases of cancer) and is the second greatest cancer-related cause of death in males [7, 8]. Treatment of the disease has focused on its androgen dependence [6] by abolition of androgen stimulation through decreases in production of androgens or antagonism of androgen binding to receptors and signalling within tumour cells [13–15]. Endocrine manipulation is also achieved by the use of luteinising hormone releasing hormone (LHRH) analogs such as goserelin (Zoladex), which act centrally to effect a reduction in LH release and consequent reduction in LH-mediated stimulation of testicular Leydig cells [1].

It is now clear that pure antiandrogens constitute an effective treatment for prostate cancer. These compounds, such as flutamide, nilutamide and Casodex block androgen action by competing with dihydrotestosterone (DHT) binding at the androgen receptor [3]. Significant clinical benefits may also result from antiandrogen therapy in combination with elimination of testicular androgens by orchidectomy or medical castration (LHRH agonist) [1]. This approach can prevent the LH-mediated increase in testicular testosterone levels that are seen following monotherapy with peripherally non-selective antiandrogens. Clearly, an understanding of the influence of mono-antiandrogen or combined antiandrogen/LHRH agonist treatment on testicular function and structure is germane to the design of therapeutic strategies.

Administration of Casodex to rats was reported by Furr et al. [4] to possess a peripherally selective action, although later studies [10] in adult men generated a small increase in plasma LH comparable to that seen in patients receiving flutamide alone. The importance of this observation with regard to LH stimulation of Leydig cell function led to our consideration of the influence of chronic Casodex therapy on Leydig cell hypertrophy or hyperplasia. This report details our extensive investigation of the effects on Leydig cell and seminiferous tubule parameters in patients with metastatic prostate cancer treated with 50 mg Casodex daily, compared with similar patients having bilateral orchidectomy without prior antiandrogen therapy.

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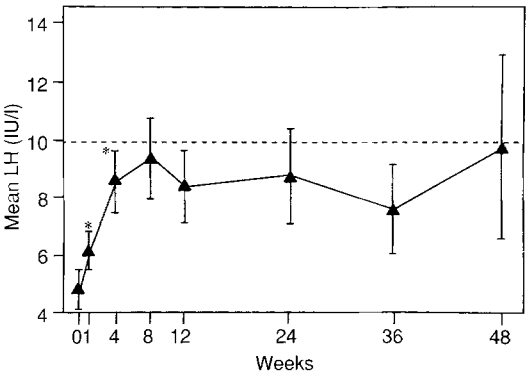

Fig. 1 Group mean serum LH levels (± SE) from a previous study in which 20 patients with advanced prostate cancer were given Casodex (50 mg) daily for a mean period of 42 weeks and extensive endocrine assessments made serially throughout. Note that at no time do the mean serum LH levels extend outside the upper limit of the normal range (dotted line). *Statistically significant change from baseline ($P < 0.001$)

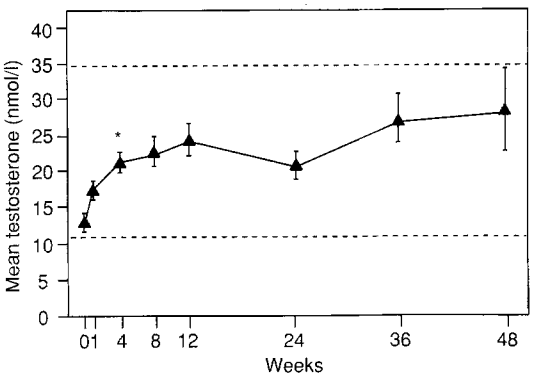

Fig. 2 Group mean serum testosterone levels (± SE) from the same study as outlined above showing slight increases with time but none which extend outside the normal range (upper and lower limits of normal range shown by dotted lines). *Statistically significant change from baseline ($P < 0.001$)

Materials and methods

Clinical trial design

This was an open, non-randomised study in which the effects on testicular morphology of oral antiandrogen therapy with Casodex (50 mg daily) were evaluated in previously untreated metastatic prostate cancer patients. Patients from previous studies who consented to participate in this study entered at the point of bilateral orchidectomy. No other systemic treatment for prostate cancer was given before orchidectomy.

Testicular samples were derived from patients who had undergone either a total or a subcapsular bilateral orchidectomy as part of their randomised therapy (control group, $n = 21$) or received Casodex initially, followed by a bilateral orchidectomy performed for clinical relapse (Casodex group, $n = 13$). Plasma testosterone concentrations at the time of entry into the study were 15.2 and 14.9 nmol/l in the control and Casodex-treated groups, respectively. All patients had confirmed metastatic disease, the majority being UICC tumour category T 3 or T 4 (91.7% control group and 86.7% Casodex group). The mean duration of Casodex therapy at the time of orchidectomy was 35.3 weeks (range 12–58 weeks). No samples were obtained from isolated biopsy material.

Hormone levels (testosterone and LH) were not monitored throughout this study as they were assessed in a previous investigation by Mahler et al. [12]. The study reported by these authors measured serum levels of LH, FSH, testosterone, DHT, prolactin, oestradiol, sex hormone binding globulin (SHBG), dehydroepiandrosterone (DHEA) and androstenedione in a group of 20 previously untreated patients with advanced prostate cancer with good prognostic factors. Each patient was given 50 mg Casodex daily for a mean period of 42 weeks (range 8–84 weeks). Statistically significant increases in mean LH and mean testosterone levels by comparison with baseline were noted, but these remained within the normal range and were not regarded as being clinically significant (Figs. 1, 2).

Tissue preparation

Samples of testicular tissue were immersed in a solution of 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2–7.4), sliced into small pieces (1 mm^3) and fixed for 1 h. Following a rinse in 0.1 M phosphate buffer, the tissue was postfixed in phosphate-buffered 2% osmium tetroxide (pH 7.4), dehydrated in ethanol and 1,2-epoxypropane and finally embedded in Epon resin. Semi-thin sections (1.5 μm thick) were stained with toluidine blue prior to microscopical examination. Testicular tissue from left and right sides was kept separate at all times during processing.

Histopathological assessment

Histological examination of tissue sections was undertaken without prior knowledge of treatment received before orchidectomy, and any abnormalities were recorded and graded as follows:

Tubular sclerosis – the total loss of seminiferous epithelium in a proportion of tubules with hyaline degeneration and tubular fibrosis. The percentage of tubules affected was scored on a point system of 0% (–), 1–20% (+), 21–40% (++), 41–60% (+++), 61–80% (++++) and 81–100% (+++++) as determined by visual approximation.

Nodular hyperplasia – clusters of Leydig cells exhibiting a discrete rounded outline and a size of 50% of the seminiferous tubule diameter or greater. Lesions were scored on a scale ranging from minimal (+) to moderate (+++) according to their extent.

Peritubular fibrosis – recorded when integrity of the seminiferous tubule was retained but peritubular basement membrane thickening and fibrosis was present. Severity was scored on a scale of minimal (+) to moderate (+++).

Focal chronic inflammatory cell infiltrate – a (multi)focal, interstitial mononuclear cell infiltrate present at minimal (+) to mild (++) severity.

Quantitative histological assessment

All tissue evaluations were performed without knowledge of treatment. Tissue sections were examined at an objective lens magnification of ×25 with a Leitz Ergolux light microscope interfaced via a JVC ky-F 30 camera to a Joyce Loebl Magiscan image analysis system (Applied Imaging, Sunderland, UK).

In accordance with established stereological procedures [16], determination of the numbers of seminiferous tubules was performed by counting all whole tubules within the field and those that intersected the bottom and left hand side edges. All other tubules were rejected. Leydig cell nuclei were counted in the Leydig cell (interstitial) tissue areas: all other nuclei, e.g. fibroblast, macrophage, were excluded. No adjustment for the cytoplasmic area of these other cell types in the Leydig cell detected area was made, as they represented only a very small fraction of the latter. Object areas (seminiferous tubules and Leydig cells) were delineated interactively

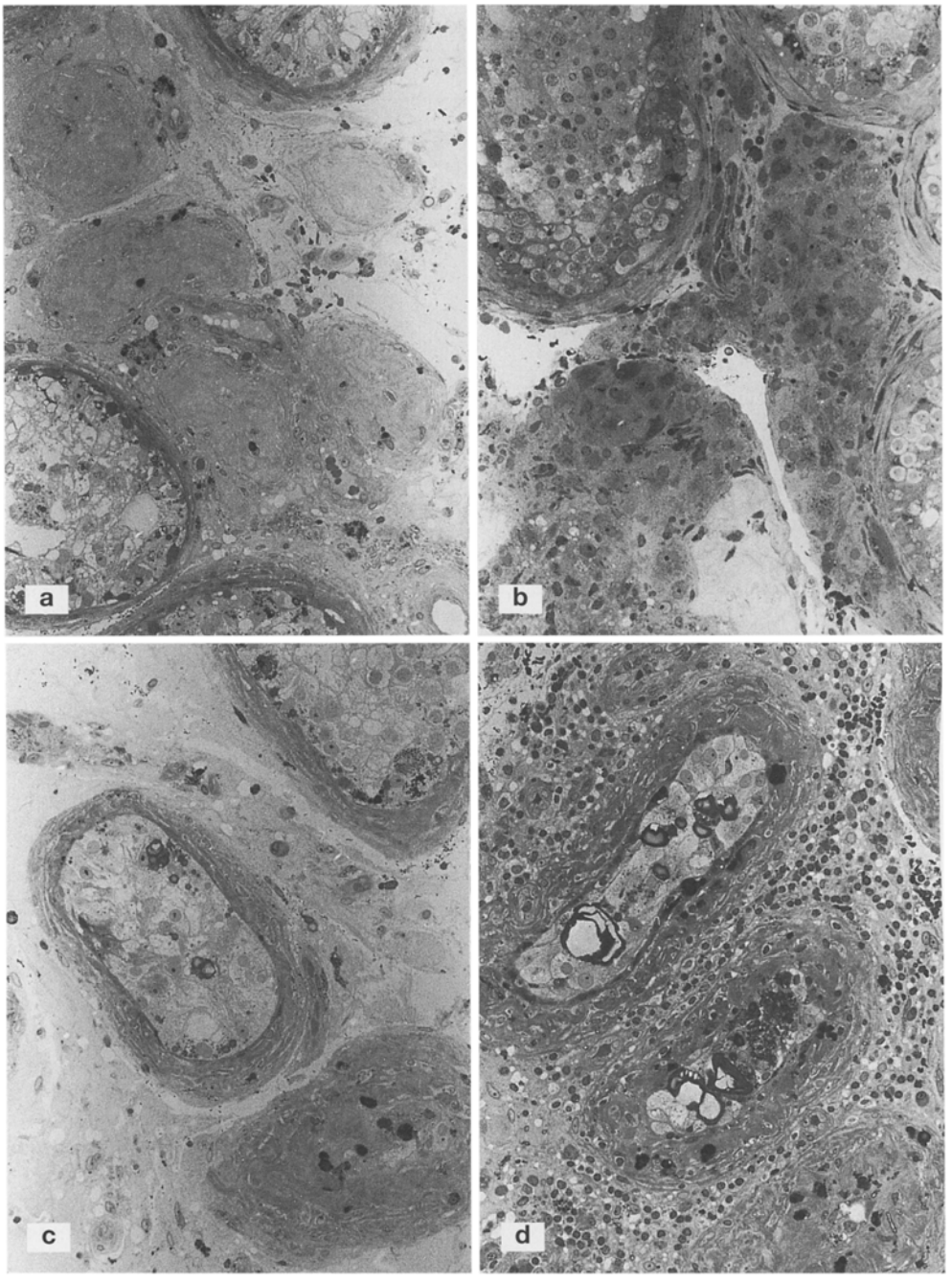

Fig. 3 Photomicrographs of testicular lesions. **a** severe tubular sclerosis, **b** Leydig cell nodular hyperplasia, **c** peritubular fibrosis **d** focal chronic inflammatory infiltrate. Toluidine blue-stained plastic sections. Original magnification ×100

with a light pen and the defined areas measured automatically. Areas were presented as square micrometres per standard frame area and represent actual values measured (as the system was calibrated weekly during the assessment phase of this study and variation in total detected areas was always less than 0.1%). The following parameters were measured in each field: seminiferous tubule area and number; Leydig cell area and nuclear numbers; field area (constant).

Preliminary study

In order that the optimum number of fields per section and numbers of sections would be determined, it was important to ascertain the major sources of variation of data derived from large and small field numbers; for example, it was necessary to determine whether mean and standard deviation values showed statistically significant differences between few and many fields, i.e. 5, 10, 15 and 20 fields. This analysis was performed on the assumption that the tissue sections were representative (in histological terms) of the entire testis from which they were taken. Calculations of the slide-to-slide and field-to-field variation were made and the optimum number of slides per patient and fields per slide estimated assuming a relative cost in terms of resources of 10:1 for preparation of a section through to evaluation of a field. The outcome of this evaluation was that 10 fields per section from each of the left and right testis should be assessed (20 fields per patient).

Table 1 Histopathological assessment of severity and incidence of lesions in left/right testes (*NAD* no abnormality detected)

Treatment group	NAD	Tubular sclerosis	Nodular hyperplasia	Peritubular fibrosis	Focal chronic inflammatory infiltrate
Control ($n=21$)	12	(+++/−) 2 (++/−) 1 (+/−) 2	(+++/−) 1 (++/−) 1 (+/−) 3		(++/−) 1 (+/−) 1
Total		5	5	0	2
Casodex 4 ($n=13$)		(+++++/−) 1 (+/−) 4	(++/++) 1 (++/+) 1 (+/+) 1 (++/−) 1 (+/−) 1	(+++/+++)1	
Total		5	5	1	0

Table 2 Quantitative histological evaluation of Leydig cell and seminiferous tubule parameters [means (SE)]

	Treatment group	
	Control ($n=21$)	Casodex ($n=13$)
Leydig cells		
Area (μm^2)/field	2405 (528)	2178 (457)
No. of cells/field	5.7 (1.1)	4.6 (1.0)
Cell size (μm^2)	388 (34)	442 (29)
Seminiferous tubules		
Area (μm^2)/field	46550 (2550)	45110 (2200)
No. of tubules/field	2.31 (0.16)	2.53 (0.14)
Size (μm^2)	20410 (750)	18060 (650)*

* $P<0.05$ for difference between groups

Main study

On completion of the data acquisition step, a weighted analysis was performed due to the differences noted in the numbers of fields assessed between patients. Weighted analysis of variance was used to fit treatment and study effects. Least-squares (ls) treatment means [5] are used to present the data in the tables.

Results

Histopathological assessment

The numbers of patients exhibiting lesions in one or both testes were comparable in the two groups, although a higher proportion of controls showed no histological abnormality (Fig. 3, Table 1). Lesions were frequently unilateral and, in the case of tubular sclerosis and peritubular fibrosis, were considered to be long standing and pre-existing. There were no significant effects of Casodextreatment on the incidence of nodular Leydig cell hyperplasia or chronic inflammatory cell infiltration, nor was the severity score of any of the lesions noted exacerbated by treatment.

Quantitative histological evaluation

Comparisons of Leydig cell and seminiferous tubule parameters are presented in Table 2. The mean number of Leydig cells and mean cell area per field in the control and Casodex-treated groups were 5.7 and 2405 μm^2, and 4.6 and 2178 μm^2, respectively. The mean number of seminiferous tubules and mean tubule area per field in the control and Casodex-treated groups were 2.31 and 46550 μm^2, and 2.53 and 45110 μm^2, respectively. A statistically significant difference ($P<0.05$) in seminiferous tubule size only was noted between control and Casodex-treated groups. The ls mean size was 20410 μm^2 and 18060 μm^2 for control and Casodex-treated samples respectively.

Discussion

This orchidectomy-controlled study demonstrated that the daily administration of 50 mg Casodex as a treatment for advanced prostate cancer (by comparison with an age-matched control group) did not cause Leydig cell hyperplasia or hypertrophy. Neither histopathological nor morphometric assessment of the two groups of testicular samples examined showed biologically significant differences. Leydig cell mean number and size (indicative of hyperplasia and hypertrophy, respectively) and seminiferous tubule number and area were unaffected by treatment with Casodex at this dose level (50 mg) for 12 weeks or longer. The statistically significant difference in seminiferous tubule size was due to the additive effect of a slightly smaller number of tubules having a slightly larger area in the control group. This may indicate a minor (< 10%) reduction in the size of the seminiferous tubules in the Casodex-treated group. However, histopathological assessment showed evidence of neither frank tubular atrophy nor of other associated pathology. The extent of tubular sclerosis recorded in patients in both treatment and control groups was comparable and would not contribute disproportionately to morphometric results of group mean seminiferous tubule area.

Elevated LH levels resulting from the blockade of hypothalamic or pituitary androgen receptors would be expected to induce Leydig cell hyperplasia: the magnitude of any observed effects being dependent on the nature and degree of the compound's pharmacological actions, i.e. whether it is active centrally as well as peripherally. Lunglmayr [10] has reported a slight, but statistically significant elevation in LH, testosterone and oestradiol levels in patients with prostate tumours during Casodex therapy with 10, 30 or 50 mg per day which were within the normal range in the majority of patients. Confirmation of a slight increase in mean serum testosterone levels associated with daily treatment with 50 mg Casodex for 1 year has been demonstrated by Lee et al. [9] while Mahler et al. [12] found an increase of about 80–100% above the initial levels in LH and testosterone concentrations. DHEA-S, SHBG and prolactin remained unaltered throughout therapy. Furthermore, Lunglmayr et al. [11], in an assessment of the effect of Casodex on the pituitary-gonadal axis and human testicular morphology, demonstrated moderate increases in serum testosterone and oestradiol levels. Light and electron microscopical examination revealed hyperactive Leydig cells with nucleonematous nuclei and dilated smooth endoplasmic reticulum. Paradoxically, changes in LH and FSH were not detectable in their study. The evidence provided by these studies suggests that small increases in LH levels (within the physiological range) may not be associated with detectable changes in Leydig cell morphology that are statistically and biologically significant.

In the present trial, testosterone levels were measured at the point of entry into the study only, so that no comparisons with the studies reporting time-related changes in hormonal concentrations were possible. Clearly, the previously observed elevations of testosterone levels [10–12] are indicative of increased activity of the Leydig cell. This accords with the slight rise in LH following administration of Casodex in man. However, it is noteworthy that the present study demonstrated that this does not consequently result in Leydig cell hypertrophy or hyperplasia.

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