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Treatment with Luteinizing Hormone-Releasing Hormone Antagonists: is Serum Testosterone Reduction the Only Mechanism?

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Abstract

Background: Androgen deprivation therapy (ADT) by surgical or medical castration is recommended for advanced or metastatic prostate cancer. Recent literature suggests that medical castration by luteinizing hormone receptor hormone (LHRH) antagonists might have advantages over treatment with LHRH agonists in patients with metastatic prostate cancer when prostate specific antigen (PSA) progression free survival and overall survival are concerned. Using a state-of-the-art method to assess levels of testosterone, we investigated whether a potential difference in clinical outcome between different forms of ADT might be related to differences in serum testosterone concentrations. We further searched for evidence in literature for other biochemical pathways explaining a potential benefit of LHRH antagonists over LHRH agonists.

Methods: Patients underwent surgical castration (n=34) or received an LHRH antagonist (n=25). Serum samples were obtained more that 3 months after initiation of ADT. Testosterone levels were determined using isotope dilution-liquid chromatography tandem mass spectrometry. Dehydroepiandrosterone sulphate (DHEAS), androstenedione, sex hormone-binding globulin (SHBG) and inhibin B levels were determined.

Results: All surgically castrated subjects and all but one subject in the LHRH antagonist group had serum testosterone values less than 50 ng/dL. No difference was found between groups in serum testosterone, DHEAS, androstenedione and SHBG. Patients who underwent surgical castration had significantly lower levels of inhibin B compared to patients treated with degarelix

Conclusion: Using a highly sensitive and specific technique of testosterone determination, no difference was found between patients after surgical castration and patients on LHRH antagonists. Thus, differences in clinical outcome between different forms of ADT are accounted for by testosterone independent pathways or mechanisms.

Abbreviations: ADT: Androgen Deprivation Therapy; CV: Coefficient of Variation; DHEAS: Dehydroepiandrosterone Sulphate; EGF: Epidermal Growth Factor; EGFR: Epidermal Growth Factor Receptor; FSH: Follicle Stimulating Hormone; ID-LC-MS/MS: Isotope Dilution-Liquid Chromatography-Tandem Mass Spectrometry; LH: Luteinizing Hormone; LHRH: Luteinizing Hormone-Releasing Hormone; LHRH-R: Luteinizing Hormone-Releasing Hormone Receptor; LOQ: Limit Of Quantification; LUTS: Lower Urinary Tract Symptoms; PSA: Prostate-Specific Antigen; RIA: Radio Immuno Assay; s.c.: Subcutaneously; SHBG: Sex Hormone-Binding Globulin.

Introduction

Androgen deprivation therapy (ADT) by either bilateral orchiectomy (surgical castration) or medical castration (luteinizing hormone-releasing hormone (LHRH) agonists, LHRH antagonists or estrogens) is recommended for advanced or metastatic prostate cancer [1]. The aim of ADT is to reduce serum testosterone concentrations to a castrate level which is currently defined as <50 ng/dL, although recent developments advocate for lowering this threshold to <20 ng/dL [2,3].

LHRH agonist therapy results in an initial increase in serum testosterone concentration, also known as flare or flare-up. Anti-androgens can be administered to counteract the symptoms of this initial rise in serum testosterone, but at present, there is a lack of solid evidence for its clinical necessity [4]. The LHRH antagonist degarelix (Firmagon®) has shown to be non-inferior to LHRH agonist treatment at maintaining low testosterone levels in patients with metastatic prostate cancer [5]. A recent study has pooled the results of five randomized trials comparing LHRH antagonists with LHRH agonists. The authors concluded that degarelix was associated with prostate-specific antigen (PSA) progression-free and overall survival compared with LHRH agonists [6].

Other studies showed that treatment with degarelix leads to greater reductions in serum alkaline phosphatase levels in patients with metastatic prostate cancer compared to leuprolide over a 1-year treatment period [7]. Also, it was shown that degarelix might improve lower urinary tract symptoms (LUTS) and achieves a greater reduction in prostate volume in prostate cancer patients compared to goserelin combined with bicalutamide [8-10]. In men with preexisting cardiovascular disease, LHRH antagonists appear to reduce the number of cardiac events during the first year of treatment compared to LHRH agonists [11].

Recent evidence suggests that an association is present between levels of serum testosterone in men on ADT and clinical outcome. Progression-free survival and cancer–specific survival are reported to be higher in those on ADT with sustained low testosterone levels compared to those on ADT who experience testosterone breakthroughs of 32-50 ng/dL [12,13]. In five different studies that compared the activity of degarelix to a LHRH agonist (i.e. leuprolide or goserelin) in patients with metastatic prostate cancer, those receiving degarelix showed a significant lower risk of PSA progression or death in the first year of treatment [6]. For now, the exact

explanation for this difference is unknown, but it might well is that more thorough and sustained suppression of serum testosterone levels might be one of underlying mechanisms [7].

In previous comparative studies, measurements of serum testosterone levels were done by poorly performing immunoassays, making definite conclusions on the timing to achieve a castrate level of serum testosterone and the levels of serum testosterone themselves hardly possible [14]. In this study, we describe the results of serum testosterone measurements in patients with advanced or metastatic prostate cancer on LHRH antagonist therapy using a highly sensitive and specific isotope dilution-liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS) method [15]. We compared the testosterone concentrations in patients on degarelix to those in surgically castrated men. Using a state-of-the-art method to assess levels of testosterone, we investigated whether a potential difference in clinical outcome between different forms of ADT might be related to differences in serum testosterone concentrations. We further searched for evidence in literature for other biochemical pathways and mechanisms explaining a potential difference between LHRH antagonists and LHRH agonists.

Materials and Methods

Study population

In this retrospective study, a total of 59 subjects were included. Thirty-four patients underwent surgical castration, 24 because of advanced or metastatic prostate cancer and 10 patients as part of a gender transition. There were 25 patients who received degarelix for metastatic prostate cancer at a starting dose of 240 mg subcutaneously (s.c) for 1 month, followed by s.c. maintenance doses of 80 mg monthly. None of the patients received other hormonal therapies such as anti-androgens, abiraterone, enzalutamide, ketoconazol or any other medication that could interfere with the gonadal axis. All patients were treated for at least three months before blood samples were drawn.

Serum testosterone determination

Venous blood was collected at a random time during the day from each subject. The day of venous blood sampling was at least one week after the last degarelix injection and at least one week before the next scheduled LHRH antagonist injection. Serum was aliquoted and stored at -20° Celcius until assayed.

Serum total testosterone was measured using the ID-LC-MS/MS as described in detail before [15]. The lower limit of quantification (LOQ) was 0.1 nmol/L (or 2.9 ng/dL), intra-assay and inter-assay coefficient of variation (CV) at levels less than 1,0 nmol/L were less than 5% and less than 13%, respectively.

Other parameters

Androstenedione was measured by a radio immuno assay (RIA) (DSL, Webster, Texas) which featured a LOQ of 0.5 nmol/l. Intra-assay and inter-assay CV for levels greater than 6 nmol/L were 6% and 9%, respectively, and for levels less than 6 nmol/L were 8% and 12%, respectively. RIA was also used for dehydroepiandrosterone sulphate (DHEAS). The LOQ was 0.2 µmol/L. Intra-assay and inter-assay variation at 3 µmol/l was 6% and 10%, respectively, and at 10 µmol/l was 4% and 9%, respectively. An immunometric assay on an Immulite® 2500 (Siemens Diagnostics) was used to determine the sex hormone-binding globulin (SHBG) concentration. The LOQ for SHBG was 2 nmol/l, and the intra-assay and inter-assay CV for the whole range was less than 3% and 4%, respectively. Inhibin B was measured using a immunoassay (Beckman Coulter). The LOQ was 15 ng/L. Intra-assay and inter-assay CV for the whole range was less than 3% and 4%, respectively.

Statistics

Statistical analysis was done using SPSS® 20.0. Statistical analysis of differences between groups was performed using the Mann-Whitney U test. The median value and 95% confidence intervals for testosterone, androstenedione, DHEAS and SHBG were calculated.

Results

Patient characteristics are displayed in Table 1. There were no significant differences between groups in clinical and tumor characteristics. Also, no differences were found between groups in PSA levels and hormonal status (i.e. castration naïve or castration resistant prostate cancer).

All evaluated patients had serum testosterone levels less than 50 ng/dl, and 31 (91%) and 23 (96%) had levels less than 20 ng/dL in the surgical castration and degarelix group respectively. Serum castrate levels of testosterone levels were not statistically significant between those treated with degarelix and those surgically castrated (Figure 1).

There were no significant differences between the groups in levels of SHBG, DHEAS and androstenedione. Patients who underwent surgical castration had inhibin B levels below the limit of quantification, which is significantly lower compared to the levels of inhibin B in patients treated with degarelix.

Discussion

Bilateral orchidectomy, LHRH agonist and LHRH antagonist therapy aim at lowering serum testosterone to a castrate level. With this, prostate cancer growth and progression cease, signs and symptoms of advanced or disseminated disease diminish and the lives of prostate cancer patients may be extended. Bilateral orchiectomy achieves these goals by surgical removal of the testes which are the primary testosterone producing organs. LHRH antagonists primarily have their action by binding LHRH receptors (LHRH-R) in the pituitary gland, thereby blocking the downstream sequelae of hormone production in the hypothalamo-pitituary-gonadal axis. Eventually, this leads to cessation of testosterone production by the Leydig cells in the testes.

	Bilateral orchiectomy	Degarelix	p-value*
Subjects (*n*)	34	25	
Mean age (years)[range]	71.6 [58.0-86.8]	74.1 [59.0-81.3]	ns
Hormone naive cancer (%)*	10/24 (41.7%)	10/25 (40.0%)	ns
CRPC*	14/24 (58.3%)	15/25 (60.0%)	ns
Metastatic disease (%)*	4/24 (16.7%)	7/25 (28.0%)	ns
Median PSA-level (ng/mL) [range]	6.4 (0.6-67)	22 (0.1-988)	ns
Median serum testosterone level (ng/dL); (ID-LC-MS/MS) [range]	8.1 [2.6–25.1]	7.8 [3.5–242.5]	0.664
Median SHBG level (nmol/L)	43.6 [17.7–121.2]	44.9 [16.0–89.6]	0.903
Median androstenedion level (nmol/L) [range]	2.1 [0.6–8.4]	2.9 [1.2–8.5]	0.218
Median DHEAS level (µmol/L) [range]	1.1 [0.3–5.5]	1.8 [0.3–6.7]	0.429
Median Inhibin B level (ng/L) [range]	<15 [<15]	37.5 [<15–123]	<0.001

Table 1: Patient characteristics and serum hormone levels after treatment with LHRH agonist therapy
*cancer specific characteristics in the bilateral orchiectomy group only apply to 24 subject who underwent castration because of prostate cancer
ns: not significant; LHRH: Luteinizing Hormone-Releasing Hormone; BMI: Body Mass Index; CRPC: Castration Resistant Prostate Cancer; ID-LC-MS/MS: Isotope Dilution-Liquid Chromatography-Tandem Mass Spectrometry; SHBG: Sex Hormone-Binding Globulin; DHEAS: Dehydroepiandrosterone Sulfate.

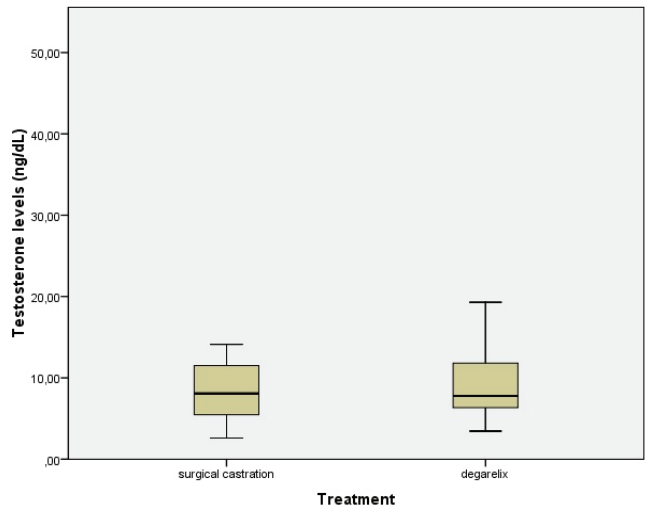

Figure 1: Box plot showing serum testosterone levels in patients after orchiectomy and after LHRH antagonist therapy (degarelix) using ID-LC-MS/MS. Upper and lower quartiles are represented by rectangle and maximum and minimum observed values are represented by whiskers (outlier not shown). Median value for surgical castration was 8.1 ng/dL and median value for degarelix therapy was 7.8 ng/dL (p=0.664)

In the present study, we measured testosterone levels in men on ADT after bilateral orchiectomy or LHRH antagonist therapy. Our data showed that all men in the surgical castration group and all but one man in the degarelix group achieved castrate levels of testosterone (i.e. below 50 ng/dL). No significant difference with respect to serum testosterone level was found between surgically castrated men and men on LHRH antagonist therapy. This indicates that in the end, treatment with degarelix might be as effective as surgical castration in achieving a castration level of serum testosterone. Data on the time to achieve castration level of serum testosterone could not be retrieved from this study. Also, no difference was found between the two groups in levels of SHBG, androstenedion and DHEAS. Serum levels of inhibin B were below the limit of quantification in the surgical castration group, which implicates that the surgical castration was complete and remaining presence of Leydig cells is unlikely.

In an earlier study from our group, it was shown that men on LHRH agonist therapy have significant lower concentrations of serum testosterone than men after surgical castration [3]. It might well be extrapolated that men on LHRH agonist therapy have lower serum castrate levels of testosterone than those on LHRH antagonist therapy.

In literature, there is some evidence that patients with advanced prostate cancer have improved disease control with degarelix versus LHRH agonists and that PSA progression free survival and overall survival increase. Urinary tract events and joint and musculoskeletal events decrease with degarelix compared with LHRH agonists [6]. Primary endpoints of these trials were change in testosterone level, change in International Prostate Symptom Score or prostate volume instead of survival. Also, these studies have a short follow-up of only one year, while the median survival of patients with newly diagnosed metastases of prostate cancer is 42 months, which makes it difficult to draw conclusions about survival [1]. The results of our current study suggest that these effects, if present, might not be explained by differences in testosterone levels or by the suppression of testosterone levels.

The differences in disease-related outcome in patients with advanced disease treated with LHRH antagonists and LHRH agonists may be explained by the distinct modes of action of both treatments. Most evident, LHRH agonists stimulate the LHRH-R and LHRH antagonist block the LHRH-R. Besides presence in the pituitary gland, the LHRH-R is relatively highly expressed in in (benign) basal epithelial cells as well in luminal cells of the prostate but not in the prostate stroma cells [16]. The expression is also high in breast, kidney, thymus and in lymphocytes [16,17]. The LHRH-R can also be found in lower concentrations in the hippocampus, the olfactory system, cerebral cortex, cerebellum, heart, adrenal glands and the bladder [18].

In prostate cancer, the LHRH-R has been identified and the LHRH-R expression persists despite prolonged exposure to LHRH agonists. These receptors were also moderately to highly express in lymph node metastases of prostate cancer [19]. Also, LHRH-Rs are expressed with high prevalence in hormone naïve prostate cancer cells as well as in castration resistant prostate cancer cells [19]. Other studies have shown that prostate cancer cells have a higher expression of LHRH-Rs compared to normal prostatic tissue [17]. The exact downstream sequelae of the stimulation of the LHRH-R are not completely understood, but both for LHRH antagonists and LHRH agonists it has been described in *in vitro* studies that they exert a direct antiproliferative effect on human prostate cancer cells [20-22]. It has even been suggested that the presence of LHRH-Rs in prostate cancer leads to better clinical status and outcome of the disease [23]. These findings imply that there could be an effect of LHRH-R targeted therapy on prostate cancer besides the castrating effect.

Patients who underwent surgical castration had lower inhibin B levels compared to the levels of inhibin B in patients treated with degarelix. In an *in vivo* model, it was shown that inhibin suppresses prostate cancer growth rate by almost 3-fold [24]. The role of inhibin in prostate cancer pathogenesis and its effect on the course of the disease remain to be clarified, but inhibin may act as a tumor suppressor in prostate cancer [25].

Besides suppression of luteinizing hormone (LH), LHRH agonists and LHRH antagonists also suppress follicle stimulating hormone (FSH) levels [5]. The FSH receptor is expressed in normal human prostatic tissue and in benign prostatic hyperplasia. Interestingly, it has been shown that the FSH receptor is expressed more intensely in prostate cancer tissue, particularly in metastatic disease [26,27]. In tumor blood vessels, FSH receptors are present whereas FSH receptor expression was not found in the blood vessels of non-malignant tissues. Suppression of the levels of FSH may thus be associated with tumor growth and tumor cell proliferation [28]. Indeed, in an *in vitro* model, FSH was found to increase proliferation in the human castration resistant prostate cancer cell lines PC3 and Du145 [29]. Different studies showed that LHRH antagonists suppress FSH levels more profoundly than LHRH agonist [5,30]. Klotz et al. [5] showed that FSH concentrations decreased with 89% after administration of degarelix compared to 54.8% patients receiving leuprolide. A more robust suppression of the FSH mediated proliferative pathway by LHRH antagonists as compared to LHRH agonists might potentially be an alternative mechanism by which LHRH antagonists interfere in the tumor cell biology, thereby improving disease outcome. However, the exact molecular mechanisms and the clinical relevance of more robust FSH suppression by LHRH antagonists have not been fully elucidated.

There is evidence of a possible link between the LHRH-R and the epidermal growth factor pathway (EGF). EGF is a growth factor which is known to stimulate cell growth, proliferation and differentiation by binding the epidermal growth factor receptor (EGFR). This binding initiates a variety of biochemical changes in the cell (increased glycolysis and protein synthesis amongst other things) which ultimately leads to increased DNA synthesis and cell proliferation. Over expression of the EGFR is associated with disease progression and poor prognosis in prostate cancer and it has also been linked to the transition of prostate cancer to castration resistant prostate cancer [31-33]. In other studies, it was shown that therapy targeting the EGFR leads to inhibition of human prostate cancer growth, possibly due to anti-angiogenic activity [34,36].

In an *in vivo* model, it was shown that treatment with a LHRH antagonist decreased the level and mRNA expression of EGFR in prostate cancer [36]. Therefore, LHRH antagonist therapy could also decelerate prostate cancer progression through the EGFR pathway.

As mentioned before, in a large, pooled patient population comparing degarelix with LHRH agonists, patients on degarelix had a lower risk of death after adjusting for prognostic factors [6]. As the number of prostate cancer deaths in this study was relatively small, differences in disease outcome might probably be explained by a lower incidence of cardiovascular events in the degarelix group [11]. Patients with preexisting cardiovascular disease who were treated with degarelix had a lower risk of experiencing a cardiovascular event (or even death) compared to patients receiving LHRH agonist treatment with an absolute risk reduction of 8,2% in the first year of treatment [11]. Mechanisms other than the mere suppression of serum testosterone might well be responsible for this difference in disease outcome between LHRH antagonists and LHRH agonists. This is particularly as the LHRH-R is expressed in the human heart [37]. Treatment with LHRH agonists causes the lean body mass to fall 3% with a rise in fat mass of 10% causing a 2% increase in body weight. This change in body composition could probably alter the risk of cardiovascular events as is the observed rise in triglycerides level (26%) total cholesterol level (approximately 10%), and the lower body insulin sensitivity [38]. Though, the stimulation of these LHRH-R by LHRH agonists or otherwise, the blockage of this receptor by LHRH antagonists has yet unknown effects on heart condition, cardiac vascularity, and the occurrence of atherovascular disease.

Prostate cancer is considered to be a form of cancer which is highly heterogeneic, which provides a challenging problem for clinical disease management. Improved and detailed understanding of all genetic alterations and variations in prostate cancer might also lead to better understanding of clinical effects of different forms of androgen deprivation therapy [39]. One could hypothesize that due to tumor heterogeneity, different pathways other than the ones including androgens could determine disease outcome. This would correspond with the findings of this current study that the possible difference in outcome between patients treated with LHRH agonists and LHRH antagonists cannot be explained by a difference in serum testosterone concentrations only.

Conclusion

By using a state-of-the-art method of determination, serum testosterone concentrations are equally reduced by treatment with a LHRH antagonist compared to surgical castration. As there are suggestions that disease outcome of men treated by LHRH antagonists improves as compared to other forms of ADT such as LHRH agonists, our study showed that mere suppression of serum testosterone level does not seems to be the biochemical explanation for this difference. LHRH antagonists might interfere in other hormonal and molecular pathways or otherwise directly suppress the downstream sequelae of ligand to LHRH-R binding.

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