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Rapid elimination kinetics of free PSA or human kallikrein-related peptidase 2 after initiation of gonadotropin-releasing hormone-antagonist treatment of prostate cancer: potential for rapid monitoring of treatment responses

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Abstract

Background: The utility of conventional prostate-specific antigen (PSA) measurements in blood for monitoring rapid responses to treatment for prostate cancer is limited because of its slow elimination rate. Prior studies have shown that free PSA (fPSA), intact PSA (iPSA) and human kallikrein-related peptidase 2 (hK2) are eliminated more rapidly after radical prostatectomy. In contrast, all three markers have similarly slow elimination rates after castration induced by gonadotropin-releasing hormone (GnRH) agonists, possibly due to the slow onset of castration. Therefore, we assessed elimination rates of tPSA, fPSA, iPSA and hK2 after rapid induction of castration with degarelix (Firmagon®), a novel GnRH antagonist.

Methods: This study included 24 patients treated with degarelix. Blood was taken at 1, 3, 7, 14, 21 and 28 days after injection of degarelix. Free and total PSA were measured with a commercial dual-label assay, and with inhouse research assays of intact PSA and hK2.

Results: Median (interquartile range, IQR) tPSA at baseline was 23.4 (15.8, 59.8). Twenty-two patients (92%) reached castrate levels of testosterone within 24 h of degarelix initiation, and all patients did so within 72 h. All kallikrein forms declined in an exponential fashion after degarelix administration. The median time to 50% reduction in biomarker level was 8–9 days for tPSA or complexed PSA vs. 2–4 days for hK2, iPSA and fPSA. The percentage eliminated at day 3 and day 7 was significantly higher for hK2, iPSA and fPSA than for tPSA (all $p<0.02$), while tPSA and complexed PSA were similar.

Conclusions: The rapid decline of fPSA, iPSA and hK2 after fast induction of castration with degarelix is similar to that reported after prostatectomy and offers a novel, informative method to monitor rapid onset of therapeutic action targeting signaling of the androgen receptor.

Keywords: androgen deprivation therapy; human kallikrein-related peptidase 2; prostate cancer; prostate-specific antigen; tumor markers.

Introduction

Prostate-specific antigen (PSA) and kallikrein-related peptidase 2 (hK2) are serine proteases that are produced primarily in the epithelial cells of the prostate gland (1, 2). Production of these biomarkers is controlled by dihydrotestosterone and testosterone. Release of PSA from the prostate results in concentrations in seminal fluid ranging from 0.2 to 5 mg/mL (3), while retrograde release results in PSA concentrations in blood only one millionth as high (4). In blood, PSA is primarily bound in a stable complex with α_1-antichymotrypsin (ACT), a form referred to as complexed PSA (cPSA). Free, non-bound PSA (fPSA) comprises several non-catalytic forms, e.g., single-chain (i.e., intact PSA) zymogen forms or multi-chain forms internally cleaved at Lys_{145} or Lys_{146} (i.e., nicked PSA), and a decreased ratio of nicked-to-free PSA is associated with higher risk of prostate cancer (5–7). Several lines of evidence [as reviewed in (8)] also suggest that fPSA and hK2 are more closely related to malignancy than cPSA or total PSA (tPSA).

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The mechanism of elimination of fPSA forms, including intact PSA (iPSA), and hK2 [mostly occurring in free, unbound forms in blood (9)], differs from that of cPSA. Due to their small size (roughly 30 kDa), fPSA, iPSA and hK2 can be eliminated via glomerular filtration. In contrast, cPSA's much larger size (90 kDa) largely precludes glomerular filtration as the major route of elimination. This idea is also supported by experimental data from patients with renal insufficiency undergoing renal transplantation; fPSA and hK2, but not cPSA, decreased rapidly after successful transplantation (10). Consistent with this, analysis of blood samples drawn from organ-specific veins has revealed that the concentration of fPSA, but not cPSA, decreased across the renal circulation, while cPSA was implicated to be lower in the suprahepatic vein than in the infrahepatic vein (11). This has also been used to speculate about whether or not cPSA is eliminated via specific receptor uptake in the liver, although the rate of clearance of cPSA (≤1.0 ng/mL/day) cast some doubts on the efficiency of this mechanism (12). The specific receptors involved in the removal of cPSA have not been established, but were hypothesized to be serpin receptors (13).

Although the benefits of PSA-based screening for prostate cancer are controversial (14), PSA is uniformly accepted as a marker for monitoring treatment (8, 15, 16). Following radical prostatectomy of organ-confined tumors, blood levels of PSA and hK2 become undetectable because the principal source of PSA and hK2 production has been removed. A post-surgery rise in any of these markers (i.e., biochemical recurrence) can thus be attributed to extracapsular or disseminated malignant growth (17, 18). Our prior reports showed that the rapid clearance of fPSA and hK2 was in sharp contrast to the much slower clearance of cPSA after radical prostatectomy studies where the elimination rates were determined by analyzing blood samples collected before and after surgery (12, 18). The levels of cPSA declined in a linear fashion at a rate of <1 ng/mL per day, whereas fPSA and hK2 were eliminated at a median half-life of 12–18 h (12, 18). Therefore, analysis of fPSA and hK2 would allow a more rapid and instructive assessment of treatment responses as their post-treatment elimination from blood is much faster than that of cPSA or tPSA. However, a study of patients with metastatic disease receiving medical castration therapy showed similar and much longer elimination rates for all PSA forms and hK2. After the initiation of the treatment with a GnRH agonist, a minor initial rise in PSA-levels was followed by a slow exponential decrease in the levels of all PSA forms and hK2 at a median 'half-life' of 12–14 days (19).

The reasons for the differences in elimination rates after radical prostatectomy vs. GnRH agonist treatment are unclear, but one explanation could stem from the fact that GnRH agonists are known to cause an initial 'flare' of testosterone levels before the onset of castration, and therefore the shutoff of PSA and hK2 production may be prolonged. Competitive GnRH antagonists are agents of a new class that immediately inhibit luteinizing hormone secretion, resulting in castration without any initial flare reaction (20). After subcutaneous administration of the GnRH antagonist degarelix, most patients reach medical castration within 48 h (21).

We hypothesized that the fast onset of action of GnRH antagonists may lead to elimination profiles of the various forms of PSA and hK2 that are similar to the profiles seen after radical prostatectomy. Our aim with this study was to investigate the elimination rates of PSA subfractions and hK2 after medical castration by a GnRH antagonist, using a subgroup selected from a multicenter, open-labeled phase II study of degarelix.

Materials and methods

Compound

Degarelix (Firmagon®) is a linear decapeptide amide containing seven unnatural amino acids (22) approved internationally to treat advanced-stage prostate cancer patients. It is a long-acting competitive GnRH antagonist with a high affinity and selectivity for GnRH receptors. It has high water solubility and low histamine-releasing properties. Degarelix is reconstituted in a mannitol solution prior to subcutaneous administration. It spontaneously forms a gel-like depot when it comes in contact with body fluids, which results in a sustained release of degarelix and prolonged testosterone suppression (23).

Subject enrollment

Independent Ethics Committees in each of the participating countries approved this study. The criteria for inclusion in the phase II study of degarelix were a histologically proven prostate adenocarcinoma, an ECOG score ≤2, a PSA level ≥2 ng/mL, a testosterone level >6 nmol/L, and a life expectancy ≥6 months. Patients considered candidates for curatively intended radiotherapy or prostatectomy were not included. Patients who had, within the prior 3 months, received testosterone-manipulating drugs or undergone surgical or other hormonal manipulation (i.e., GnRH-agonists/antagonists, antiandrogens, estrogens) were excluded. Also excluded were patients with pathological elevations of alanine aminotransferase and/or bilirubin. To avoid study discontinuation, we excluded patients with severe illnesses not related to prostate cancer, a known hypersensitivity to any component included in degarelix, or a medical history of anaphylactic reactions.

Study cohort

The main study included 177 patients, 24 of which were treated with a dose of 200 mg (40 mg/mL); these 24 constituted the cohort used to study elimination rates. This subgroup was selected because this dose regime appeared to be the most effective in suppressing testosterone below 0.5 ng/mL. [Later it was shown in a larger cohort that a dose of 240 mg (40 mg/mL) resulted in testosterone suppression in a higher proportion of patients, and this dosage was chosen for marketing.] Among these 24 patients, the median age was 74 years (interquartile range, 70–79 years; range, 62–86 years), and the median body mass index was 25.3 kg/m^2 (interquartile range, 24.2–27.9 kg/m^2). The median time elapsed from prostate cancer diagnosis to study enrollment was 41.5 days (range, 14–1392 days). At enrollment two patients had localized disease, 15 locally advanced, and six metastatic disease, while one was incompletely classified. According to the reported cancer staging, all but one of the selected

subjects underwent bone scanning, and six of these patients (26%) were found to have skeletal metastasis (M1). Rectal examinations were carried out on 21 patients at the time of enrollment, and 17 of these patients (81%) were found to have extracapsular tumor extensions. Biopsies evaluated and graded according to Gleason showed a Gleason score of 2–4 in one patient (4%), of 5–6 in six patients (25%), and of 7–10 in 17 patients (71%). Three of the subjects had undergone radical prostatectomy, and one of these three patients had also received adjuvant radiotherapy. All patients were Caucasian.

Measurement of kallikreins

Free and total PSA were determined with the dual-label DELFIA immunofluorometric assay (Prostatus™ PSA Free/Total PSA from Perkin-Elmer Life Sciences, Turku, Finland). This assay determines free and complexed PSA in an equimolar fashion, and the cross-reaction for PSA-ACT in the fPSA assay is below 0.2% (24). The lower limit of detection for tPSA is 0.10 ng/mL (coefficient of variation of 5.0% at 2.32 ng/mL) and for fPSA 0.04 ng/mL (coefficient of variation of 5.9% at 0.25 ng/mL). For detection, the 1235 automatic immunoassay system from Perkin-Elmer Life Sciences was used. Levels of cPSA were calculated by subtracting fPSA from tPSA as reported previously (25). The assay for hK2 used further optimized inhouse research assay protocols (9) compared to earlier reported versions (26). We used a biotinylated capture monoclonal antibody (MAb; 6H10); this antibody has a 5% cross-reaction to PSA, which is eliminated (≤0.005%) by adding three PSA-blocking MAbs (5H6, 5F7, and 2E9). Finally, Eu-labeled tracer MAb (7G1) was added. The detection limit of the assay is 0.005 ng/mL with assay imprecision values (mean coefficients of variation) ranging from 5.7% to 11% for high and low hK2 controls, respectively. An assay based on a unique europium-labeled detection antibody (4D4 or 5C3) that recognizes only iPSA but not PSA that is internally cleaved at Lys_{145} or Lys_{146} (i.e., nicked PSA) was reported earlier (7, 27). The detection limit of this assay version is 0.035 ng/mL, with a coefficient of variation of 8.9% as previously reported (6).

Analysis was carried out on blood samples drawn 0, 1, 2, 3, 7, 14, 21, and 28 days following degarelix treatment.

Statistical analyses

The pre-dose biomarker levels and percentage change from pre-dose level at various time-points were summarized descriptively. Each patient's time to 50% reduction in biomarker levels was estimated using linear interpolation between measurements, and was reported descriptively using median and interquartile range.

The median percentage change in biomarker levels from pre-dose levels was plotted over time. To test for differences in elimination rates between markers, we compared the difference in percent change from pretreatment measurement at specific time-points (3, 7, 14, and 28 days post-treatment) using the Wilcoxon matched-pairs signed-ranks test. Post-treatment measurements that were taken in a non-castrate state (testosterone level >0.5 ng/mL) were excluded in all analyses. All statistical analyses were conducted using Stata 9.0 (Stata Corp., College Station, TX, USA).

Results

Testosterone levels were less than the castration cut-off of 0.5 ng/mL at 24 h after degarelix administration in 22 participants (92%), and in all participants after 72 h. The median levels of tPSA, cPSA, fPSA, iPSA, and hK2 at baseline were 23.4, 19.2, 3.68, 2.51, and 0.286 ng/mL, respectively (Table 1). At day 28, the respective median biomarker concentrations were 4.31, 3.85, 0.66, 0.25, and 0.054 ng/mL. The median time to 50% reduction in biomarker levels was 8 days for tPSA, 9 days for cPSA, 4 days for fPSA, 3 days for iPSA, and 2 days for hK2 (Table 1). The median percentage change in tPSA, cPSA, fPSA, iPSA, and hK2 from pre-dose level against time is plotted in Figure 1. For all analytes, levels declined in an exponential fashion after degarelix administration.

At day 3 and day 7, the percentage decline in hK2 from pretreatment levels was significantly greater than that of tPSA (both $p<0.001$); there were no important differences at day 14 and day 28. We observed similar results for the difference in percentage decline between fPSA and tPSA: the percent decline at day 3 and day 7 was significantly greater than that of tPSA ($p<0.001$ and 0.014, respectively). The elimination rates of cPSA and tPSA were similar (absolute differences <5% through day 28), while the elimination rates of iPSA were similar to those of fPSA and hK2.

Table 1 Summary of biomarker measurements and elimination times.

	Number of patients	Total PSA	Complexed PSA (calculated)	Free PSA	Intact PSA	Human kallikrein 2
Median biomarker level, ng/mL (interquartile range)						
Pre-dose	24	23.4 (15.8, 59.8)	19.2 (13, 51.5)	3.68 (1.91, 8.36)	2.51 (0.850, 5.87)	0.286 (0.192, 0.927)
Median percentage of pre-dose level, % (interquartile range)						
Day 1	22	96.7 (94.4, 101)	102 (94.9, 104)	81.4 (78.2, 88.3)	77.3 (67.6, 90.1)	78.2 (65.4, 87.4)
Day 3	23	81.7 (70.6, 86.1)	85.7 (74, 91.6)	48.6 (43.9, 57.8)	44.4 (30.2, 50.0)	37.3 (31.7, 43.7)
Day 7	24	51.3 (46.5, 62.4)	51.8 (47.6, 63.9)	40.0 (25.4, 59.8)	34.3 (20.6, 43.2)	28.2 (23.5, 40.2)
Day 14	23	31.2 (20.7, 45.8)	29.1 (20.9, 44.1)	28.0 (16.4, 57)	21.9 (14.8, 36.2)	21.7 (11.5, 37.1)
Day 21	22	20.2 (13.8, 37.2)	19.8 (12.5, 35.6)	30.9 (13.6, 52.2)	18.2 (9.22, 26.2)	17.3 (9.55, 31.5)
Day 28	23	15.4 (7.03, 25.9)	13.8 (7.08, 24.8)	25.4 (9.29, 38.3)	12.0 (7.28, 23.6)	15.4 (7.6, 23.4)
Median time, days (interquartile range)						
Days to 50% drop	23	8 (7, 12)	9 (7, 12)	4 (3, 17)	3 (2, 4)	2 (2, 4)

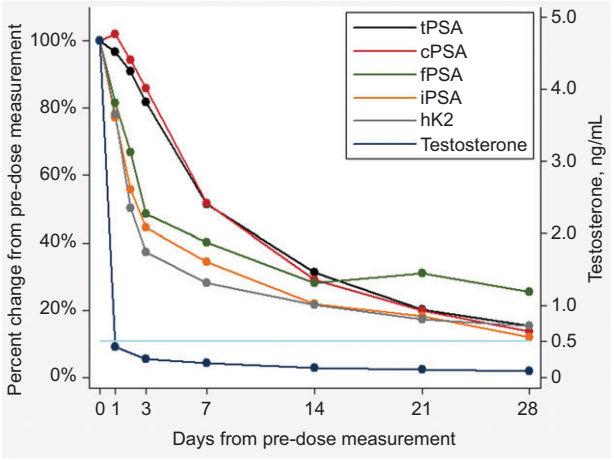

Figure 1 Median percent change in biomarkers over time.
Black line: tPSA; red line: cPSA (calculated); green line: fPSA; orange line: iPSA; gray line: hK2. The dark blue line shows median testosterone levels pre-treatment (day 0) and post-treatment at days 1, 3, 7, 14, 21, and 28; the light blue line represents the castration cut-off of 0.5 ng/mL. Confidence intervals are not shown in order to facilitate readability of the figure. There is an apparent rebound in free PSA levels at 21 days, while testosterone levels remained below the castration cut-off. Given the variability associated with these estimates due to the small sample size, we interpret these findings as consistent with these levels continuing to decline or leveling off past 14 days.

Discussion

We have demonstrated that, after rapid onset of medical castration with degarelix, PSA forms and hK2 decrease in an exponential fashion, with rates of elimination dropping significantly faster for either fPSA, iPSA or hK2 as compared with both cPSA and tPSA. In the 2-week period following degarelix administration, the median time to 50% reduction was within 2 to 4 days for fPSA, iPSA and hK2, compared to 8 days for tPSA or cPSA. Our data clearly support the hypothesis that the complexed and uncomplexed forms of prostate kallikreins follow separate pathways of elimination.

The elimination of fPSA, iPSA and hK2 induced by degarelix, a GnRH antagonist, was substantially faster than that reported after treatment with GnRH agonists. This more rapid elimination of uncomplexed biomarkers in our study may result from the more efficient castration induced by the GnRH antagonist than by GnRH agonists. In general, the various forms of PSA and hK2 displayed elimination profiles very similar to those seen after radical prostatectomy.

The elimination rates obtained in this study are unlikely to be importantly affected by variability in the assays, given the modest analytical imprecision of the assays for tPSA, fPSA, iPSA, and hK2 (coefficients of variation 2%–6%). Similarly, the results are unlikely to be importantly affected by biological variation in levels of these proteins. Intra-individual coefficients of variation, whether they be measured on a daily, weekly, or bi-annual basis, have consistently been found to amount to 11%–13% or less for tPSA, fPSA, iPSA, and hK2 (28). Hence, over the shorter time span of this study, intra-individual variation is not likely to not be greater.

Our study demonstrates that uncomplexed forms of prostate kallikreins provide additional information when monitoring the treatment effect of androgen blockade with a GnRH antagonist. Compared to tPSA, hK2 is more closely related to tumor volume (29), high-grade prostate cancer (30), and extracapsular tumor growth (29, 31). Similarly, free and complexed PSA have been shown to be differentially associated with benign and malignant prostate tissue (29, 30). Further, including measurements of iPSA and hK2 are now suggested to importantly improve the prediction of cancer risk at prostate biopsy (5). Currently, treatment effect is monitored by means of serial tPSA determinations. Due to the prolonged elimination kinetics of cPSA, the major component of tPSA, the effect of a given treatment cannot be fully evaluated until approximately 4–6 weeks after initiation. Based on our data, we reason that measurement of free forms of prostate kallikreins would provide the clinician not only with additional information on aggressiveness, but also with faster and more accurate feedback on treatment effect, due to the more rapid clearance from circulation. We note, however, that patients with renal disease have slower elimination rates for fPSA and hK2 (10) due to reduced glomerular filtration.

Conclusions

After rapid onset of medical castration with degarelix, fPSA, iPSA and hK2 are eliminated rapidly, whereas cPSA and tPSA are eliminated more slowly. Therefore, monitoring of fPSA, iPSA and hK2 can, theoretically, allow treatment effect, biochemical recurrence, and malignancies insensitive to hormonal treatment to be evaluated and diagnosed at an earlier phase. Although further studies are needed, fPSA, iPSA and hK2 could provide a more reliable monitoring option for patients undergoing intermittent treatment programs. In theory, this could reduce risks for long-term side effects caused by androgen deprivation treatment, including reduced quality of life and overall survival.

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Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article. The funders had no involvement in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.
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