Effect of the Synthetic Retinoid Fenretinide on Circulating Free Prostate-Specific Antigen, Insulin-Like Growth Factor-I, and Insulin-Like Growth Factor Binding Protein-3 Levels in Men with Superficial Bladder Cancer

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ABSTRACT

Purpose: **Fenretinide (4-HPR) is a synthetic retinoid that has shown a preventive activity in prostate cancer animal models.**

Experimental Design: **We measured the changes in total and free prostate-specific antigen (PSA) and its association with insulin-like growth factor I (IGF-I) and IGFBP-3 levels after 1 year of treatment in 24 subjects given 4-HPR and 24 control subjects enrolled in a randomized bladder cancer prevention trial.**

Results: **No significant effect of 4-HPR was observed on total and free fraction of PSA levels. The median percentage [95 confidence interval (95% CI)] change for % free PSA and total PSA in the 4-HPR and the control group were, respectively, 7.6 (95% CI, −4.0 to 69.3) versus 5.1 (95% CI, −21.4 to 59.8) and −7.8 (95% CI, −18.2 to 52.5) versus −12.3 (95% CI, −44.6 to 9.6). However, in patients ages <60 years, there was a trend to an increase of total free PSA and % free PSA after treatment with 4-HPR that was different from a trend to a decrease in the control group (P = 0.002 and 0.052, respectively). The interaction between age and treatment was statistically significant on free PSA (P = 0.001). A similar pattern was noted with smoking status (P = 0.011 for the interaction on free PSA). No association was observed between PSA levels and IGF-I or IGFBP-3 levels.**

Conclusions: **We conclude that 4-HPR has no significant effect on circulating PSA, but it increases significantly free PSA levels in subjects younger than 60 years and in nonsmokers. These effects might support an activity in prostate cancer prevention but further studies are required.**

INTRODUCTION

Prostate cancer is an increasing health problem in Western countries, with one man in 10 destined to develop this disease during his lifetime (1). Among control strategies, chemoprevention attempts to halt or delay the process of carcinogenesis in a preclinical phase with the use of natural and synthetic agents (2). For prostate cancer prevention, a large phase III trial of the 5-α reductase inhibitor finasteride has recently proven the efficacy of this approach, the drug being associated with a 25% relative reduction in the development of prostate cancer compared with the placebo arm (3). However, a higher proportion of high-grade tumors was noted in the finasteride arm, and the long-term effects of these findings are unknown (3). In another large phase III trial, the SELECT trial (4), the activity of selenomethionine or vitamin E is being assessed in a 2 × 2 design based on previous promising clinical data (5, 6).

Over the past years, vitamin A and its analogues (the retinoids) have received great attention as chemopreventive agents due to their ability to inhibit carcinogenesis in preclinical models (7). Fenretinide (4-HPR), a synthetic derivative of all-*trans*-retinoic acid, has growth inhibition, differentiation, and apoptosis effects in preclinical models. These activities can be receptor dependent or receptor independent (8, 9). Timing and dosage can also modulate differently the retinoid effects and the pathway involved (9). It has been shown that apoptosis can be mediated by caspase 8 activation (10, 11). Interestingly, this retinoid has shown potent antitumor activity in prostate cancer cell lines (12, 13) as well as in the treatment and prevention of prostate cancer in animal models (14–16). Moreover, 4-HPR lowers circulating insulin-like growth factor I (IGF-I) levels (17), which have been associated with a higher risk of prostate cancer in several cohort studies (18–20).

In prostate cancer prevention, prostate-specific antigen (PSA), which is being universally used as the clinical marker of disease (21, 22), holds promise as an intermediate end point biomarker to predict the activity of preventive agents in clinical trials (23, 24). Furthermore, PSA has IGFBP-3 protease activity, lowering the binding affinity to IGF-I, and this activity may be involved in the progression of prostate cancer in humans (25, 26). Recent observations indicate that PSA is also secreted in other reproductive organs under the modulation of different ligands of the steroid/thyroid/retinoid receptor superfamily, including estrogen, progesterone, and glucocorticoids (27, 28).

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Serum PSA is present in several molecular forms, one of which is a free, non complexed 33-kDa protein, which tends to be released at lower concentration in prostate cancer in comparison with benign disease. Evaluation of the free form is currently being exploited to improve the performance of PSA as a screening and diagnostic test (29–31). Although the biological basis of these observations are unclear, the unbound form of PSA has been shown to be up-regulated by several hormones, including estrogens or progestins (32–34), possibly reflecting an increased cellular differentiation, as for instance in benign prostatic hyperplasia (22) or in the mammary gland during lactation (34). Thus, an increase in the proportion of free PSA over total PSA (% free PSA) after drug intervention might be regarded as an index of biological activity of candidate preventive agents (23, 24). Furthermore, modulation of total PSA has been documented in prostate cancer cell lines treated with retinoids (35, 36). We therefore studied the effect of 4-HPR on PSA forms and its association with IGF-I and IGFBP-3 in a series of subjects with resected superficial bladder cancer, participating in a chemoprevention trial.

MATERIALS AND METHODS

Subjects. The study population was composed of a subgroup of 48 men recruited for a superficial bladder cancer (stage pTa, pT1) chemoprevention trial (oral 4-HPR, 200 mg/d versus no treatment). A detailed description of the original trial has been reported elsewhere (37). Participants with no symptoms related to the prostate or a past history thereof, in whom serum aliquots were available both at baseline and 1 year after randomization, were included in the analysis. No difference between the present cohort and the whole study population was noted on baseline characteristic (data not shown). The study was approved by the Institutional Review Board and all patients signed an informed consent.

Biomarkers Assay Methods and Measurements. To avoid any perturbation of PSA levels, blood samples were obtained before cystoscopy. Total and free PSA were measured using two commercially available kits. Both assays were solid-phase tests which used biotinylated antibodies in streptavidin coated polystyrene tubes as catcher and peroxidase-conjugated antibodies as tracer. ABTS was the substrate-chromogen used. All measurements were done blinded and in duplicate on serum aliquots frozen at −30°C in a multibatch analyzer ES 300 (Boehringer-Mannheim, Tutzing, Germany). The measuring range of free PSA was 0 to 20 ng/mL and the lower limit of detection was 0.05 ng/mL. The intra and interassay coefficients of variation were below 5% and 8%, respectively.

Plasma concentrations of total IGF-I were determined by a RIA system using commercially available kits purchased from Biosource Europe S.A. (Nivelles, Belgium). Serum IGFBP-3 was measured by RIA using commercially available kits provided by Bioclone Australia Pty Ltd. (Marrickville, New South Wales, Australia). A detailed description is reported elsewhere (38).

Statistical Analysis. The correlations between total and free PSA, IGF-I, IGFBP-3, and IGF-I/IGFBP-3 ratio were evaluated through Spearman's rank correlation coefficients. The associations between the baseline levels of all the biomarkers and age, body mass index (BMI), and smoking status were evaluated with linear regression after log-transforming the response variables to normalize their distribution. The Kruskall-Wallis test was used to assess differences between the 4-HPR and the control group in the percentage change of total and free PSA, IGF-I, IGFBP-3, and IGF-I/IGFBP-3 ratio from baseline to 1 year of intervention. In addition, we did an exploratory analysis to determine whether the effect of treatment was different across variables which have been shown to influence the effects of retinoids in previous studies, including age (17, 39), smoking (current versus former and never smokers; refs. 40–42), and total PSA. Both age and total PSA were categorized using the median values at baseline as cutoff points. To test for interactions between each baseline characteristic and treatment, we used a multiple regression analysis of each biomarker on the corresponding value at baseline, the characteristic under investigation, and a dummy variable for treatment and their interaction. Total and free PSA, IGF-I, IGFBP-3, and IGF-I/IGFBP-3 levels were log-transformed to achieve normality.

RESULTS

The clinical characteristics of the patients at the baseline were evenly distributed between the two groups. Mean (±SD) age (years) was 61.0 ± 9.0 (range: 46-78) and 59 ± 10.1 (range: 36-77) and mean (±SD) BMI (kg/m^2) was 27.2 ± 4.4 and 25.7 ± 2.5 in the 4-HPR and the control group, respectively. Current smokers were 55% in the 4-HPR and 45% in the control arm.

At baseline, total and free PSA levels were positively correlated ($\rho = 0.83$, $P < 0.001$) as well as IGF-I and IGFBP-3 ($\rho = 0.33$, $P = 0.030$). Conversely, levels of PSA and IGF-I or IGFBP-3 did not show any significant correlation (data not shown). Moreover, total PSA was significantly associated with age and BMI, presenting a 3% increase for each year of age ($P = 0.035$) and an 8% reduction for each BMI unit change ($P = 0.034$). Similar relationships were observed for free PSA, but not for % free PSA, which was independent of age and BMI. PSA levels were not associated with smoking status. A weak association was seen between age and IGF-I (1% reduction for each year, $P = 0.083$), and between age and IGFBP-3 (1% reduction for each year, $P = 0.055$), but not with their ratio; moreover, IGFBP-3 was 13% lower in smokers than in nonsmokers ($P = 0.043$).

Baseline levels of total PSA, free PSA, and % free PSA, are reported in Table 1 together with the percentage change from baseline to 1 year. There was no significant effect of 4-HPR treatment on total or free PSA nor on their % ratio. The greatest effect was observed on free PSA, where the control group exhibited an 8% reduction, whereas the 4-HPR group showed a 12% increase ($P = 0.108$). As previously reported (38), treatment with 4-HPR resulted in a statistically significant reduction of IGF-I. In the present analysis, also the IGF-I/IGFBP-3 ratio was reduced compared with the control group ($P = 0.005$), whereas it had no effect on IGFBP-3 levels ($P = 0.559$).

Treatment with the retinoid showed different effects depending on age, smoking status, and baseline PSA levels. Table 2 illustrates the effect of 4-HPR according to age. Subjects ages ≤60 years in the 4-HPR group exhibited a trend to an increase of free PSA and % free PSA which was statistically significantly different to a trend to a decrease in the control group ($P = 0.002$

Table 1 Levels of total PSA, free PSA, and % free PSA at baseline and median percentage change over 1 year of treatment

	Baseline		Percentage change from baseline to 1 year		
	4-HPR group (n = 24), geometric mean (95% CI)	Control group (n = 24), geometric mean (95% CI)	4-HPR group, median (IQ range)	Control group, median (IQ range)	P*
Total PSA, ng/mL	1.5 (1.0–2.4)	1.4 (1.0–1.9)	−7.8 (−18.2 to 52.5)	−12.3 (−44.6 to 9.6)	0.16
Free PSA, ng/mL	0.3 (0.2–0.4)	0.3 (0.2–0.4)	12.3 (−13.9 to 47.1)	−7.8 (−32.6 to 16.5)	0.10
% Free PSA	0.2 (0.1–0.2)	0.2 (0.2–0.3)	7.6 (−4.0 to 69.3)	5.1 (−21.4 to 59.8)	0.33

*Kruskall-Wallis test for between-group comparison.

and P = 0.052). At variance, no significant difference between treatment groups was observed in subjects ages >60 years. The interaction term between treatment and age was statistically significant for free PSA (P = 0.001). Overall, the effect of 4-HPR on IGF-I and IGF-I/IGFBP-3 ratio was significantly different from the effect of placebo in the younger group (P = 0.007 and P = 0.018), but no significant difference was observed between age groups. The effect of 4-HPR according to smoking status is summarized in Table 3. Among nonsmokers, subjects treated with 4-HPR showed a significant increase in total and free PSA levels as opposed to a decrease observed among control subjects (P = 0.012 and P = 0.005), but no difference was observed for % free PSA (P = 0.259). No significant effect of 4-HPR on PSA was observed among smokers. The interaction term between treatment and smoking status was significant for total and free PSA (P = 0.021 and P = 0.011, respectively). Because PSA may have proteolytic activity on the complex IGF-I - IGFBP-3 (25), we looked at the percentage changes of IGF-I, IGFBP-3 and IGF-I/IGFBP-3 ratio after 1 year of treatment in two subgroups of subjects. The analysis was done according to the baseline PSA level, using the median value of 1.275 ng/mL of total PSA as cutoff (Table 4). The reduction of IGF-I by treatment with 4-HPR was significantly greater than the reduction observed in the control group among subjects with lower baseline levels of total PSA (P = 0.005), but not in subjects with higher baseline levels (P = 0.284). The interaction between treatment and baseline levels of total PSA was not statistically significant.

DISCUSSION

Previous studies have documented a remarkable preventive activity of 4-HPR in several prostate cancer animal models (14–16). By contrast, the results of pilot clinical trials have been less encouraging (43, 44). Fenretinide can act through several pathways; one of these is mediated by its nuclear receptors, retinoic acid receptors (RAR), and retinoic X receptors (RXR). Each of these receptors has three subtypes α, β, and γ, and they are all expressed in the prostate gland. However, a differential phenotype has been described in normal, premalignant and neoplastic tissue with a reduction of RXRα, RXRβ, and RARβ expression (45). Moreover a gradient of retinoic receptor expression has been noted among distant, adjacent, and neoplastic areas, supporting the concept of field carcinogenesis (46). The lack of retinoic receptor expression may result in a functional retinoid deficiency. On the other hand, a retinoic acid deficiency may lead to a lower expression of receptors. This is supported by the observation that concentration of retinoic acid is five to eight times lower in prostatic cancer compared with benign prostatic hyperplasia and normal prostate gland (47). Thus, the retinoid administration can up-regulate RXRα and RARβ expression *in vitro*, and this observation was shown also in a small cohort of prostate cancer patients, but did not reach statistical significance (45).

Because recent data point to a role for PSA, IGF-I, and IGFBP-3 as putative surrogate biomarkers of prostate cancer prevention (20, 25, 26, 48), we analyzed their modulation by

Table 2 Percentage change of total PSA, free PSA, % free PSA, IGF-I, IGFBP-3, and IGF-I/IGFBP-3 from baseline to 1 year according to median age

	4-HPR group		Control group		
Age group	n	Median (IQ† range)	n	Median (IQ range)	P*
Total PSA, ng/mL					
≤60	11	−0.7 (−12.8 to 61.1)	13	−11 (−44.4 to 8.9)	0.060
>60	13	−12.5 (−37.5 to 42.4)	11	−13.6 (−39.1 to 15.1)	0.885
Free PSA, ng/mL					
≤60	11	35.7 (−0.8 to 61.4)	13	−20.0 (−43.8 to 0.0)	0.002
>60	13	−6.5 (−33.3 to 24.2)	11	15.7 (−9.9 to 37.2)	0.434
% Free PSA					
≤60	11	8.5 (−6.2 to 82.6)	13	−19.5 (−27.8 to 12.9)	0.052
>60	13	2.2 (−1.7 to 42.5)	11	9.3 (2.3 to 64.8)	0.469
IGF-I, nmol/L					
≤60	11	−13.2 (−30.1 to −9.3)	13	−2.8 (−8.5 to 2.6)	0.007
>60	13	−7.8 (−15.9 to 11.1)	11	8.1 (−4.0 to 26.6)	0.140
IGFBP-3, nmol/L					
≤60	10	−5.0 (−16.2 to 3.9)	12	−12.9 (−15.2 to 0.9)	0.668
>60	11	0.0 (−14.8 to 10.2)	10	−2.2 (−18.8 to 6.6)	0.597
IGF-I/IGFBP-3					
≤60	10	−19.7 (−28.8 to −4.4)	12	9.5 (−1.0 to 12.3)	0.018
>60	11	−3.3 (−18.7 to 5.5)	10	21.5 (−3.8 to 37.5)	0.078

*Kruskall-Wallis test for between-group comparison.
†IQ, interquartile range (25th and 75th percentiles).

Table 3 Percentage change of total PSA, free PSA, % free PSA, IGF-I, IGFBP-3, and IGF-I/IGFBP-3 from baseline to 1 year according to smoking status

	4-HPR group		Control group		
Smoking	*n*	Median (IQ† range)	*n*	Median (IQ range)	*P**
Total PSA, ng/mL					
No	13	23.9 (−12.5 to 71.7)	15	−18.7 (−48.2 to 1.1)	0.012
Yes	11	−14.9 (−46.8 to 10.9)	9	0.2 (−30.1 to 19.6)	0.425
Free PSA, ng/mL					
No	13	45.5 (14.6 to 184.6)	15	−8.3 (−30.6 to 10.5)	0.005
Yes	11	−10.9 (−44.7 to 8.8)	9	0.0 (−31.6 to 15.7)	0.621
% Free PSA					
No	13	30.9 (2.2 to 85.1)	15	12.9 (−7.4 to 61.1)	0.259
Yes	11	0.0 (−13.0 to 7.6)	9	−19.5 (−23.1 to 6.6)	0.382
IGF-I, nmol/L					
No	13	−12.4 (−23.9 to −7.8)	15	6.1 (−6.0 to 13.0)	0.002
Yes	11	−9.9 (−14.5 to 14.1)	9	−4.3 (−7.9 to 2.6)	0.470
IGFBP-3, nmol/L					
No	11	−10.0 (−18.2 to 4.2)	13	−12.5 (−17.9 to 0.0)	0.643
Yes	10	2.0 (−9.0 to 11.8)	9	6.2 (−14.3 to 11.1)	1.000
IGF-I/IGFBP-3					
No	11	−10.5 (−25.8 to −2.9)	13	12.0 (−0.8 to 21.3)	0.003
Yes	10	−8.2 (−25.0 to 7.6)	9	9.2 (−10.3 to 22.5)	0.414

*Kruskall-Wallis test for between-group comparison.
†IQ, interquartile range (25th and 75th percentiles).

4-HPR in men with previously resected superficial bladder cancer in an age-range cohort that is considered at risk for prostate cancer. We used circulating PSA isoforms and IGF-I and IGFBP-3 as potential surrogate biomarkers.

As expected from previous studies (49), we found an association between age and level of total and free PSA, and level of IGFBP-3. As previously reported by us (38), treatment with 4-HPR was associated with a significant down-regulation of circulating IGF-I. No substantial change in PSA forms and IGFBP-3 was observed during 4-HPR treatment. Compared with the untreated control group, an increase of free PSA was found in the 4-HPR group, but this was not statistically significant. However, an increase of free PSA and % free PSA reached statistical significance among subjects ages ≤60 years. PSA forms had no significant influence on the changes in IGF levels during 4-HPR.

Table 4 Percentage change of IGF-I, IGFBP-3, and IGF-I/IGFBP-3 from baseline to 1 year according to baseline PSA level

	4-HPR group		Control group		
Total PSA*	*n*	Median (IQ‡ range)	*n*	Median (IQ range)	*P*†
IGF-I, nmol/L					
≤1.275	11	−13.2 (−23.8 to −9.1)	13	−2.2 (−4.3 to 8.1)	0.005
>1.275	13	−7.8 (−23.3 to 11.1)	11	−0.8 (−9.7 to 20.1)	0.284
IGFBP-3, nmol/L					
≤1.275	10	−13.8 (−19.7 to 5.4)	11	−12.5 (−19.7 to 3.3)	0.972
>1.275	11	3.6 (−9.8 to 6.4)	11	0.0 (−13.8 to 4.9)	0.375
IGF-I/IGFBP-3					
≤1.275	10	−10.7 (−24.6 to −2.8)	11	13.4 (−0.6 to 21.5)	0.159
>1.275	11	−10.5 (−27.8 to −1.6)	11	11.4 (−1.2 to 19.1)	0.020

*The cut point corresponds to the median value.
†Kruskall-Wallis test for between-group comparison.
‡IQ, interquartile range (25th and 75th percentiles).

Our data might suggest an age-dependent role of 4-HPR on prostate carcinogenesis inhibition. The age-related effect is in line with a large breast cancer prevention trial, where 4-HPR exerted a statistically significantly different effect on second breast cancer depending on age, with a significant reduction of risk only in women ages ≤50 years and not in women ages > 51 years (39). Importantly, a stronger association between high IGF-I concentration and prostate cancer risk has been shown in men ages <59 years (50). Similarly, breast cancer risk was associated to high IGF-I plasma concentration only in women ages ≤50 years (51).

Although the effect of 4-HPR on PSA was limited, the increased free fraction of PSA could be considered as a beneficial effect as it may reflect a differentiation of the prostate gland (29, 52). High level of free PSA is associated with benign hyperplasia and not with prostate cancer (53). Moreover, the reduction of serum IGF-I level may be of potential benefit given the increasingly recognized role of IGF-I as a risk factor for prostate cancer (20, 26). However, recent pilot chemoprevention trials have shown the inability of 4-HPR and other retinoids to down-regulate the level of PSA (43, 44, 54). The results of these studies do not provide evidence for a potent preventive activity of this retinoid in prostate cancer in humans. *In vitro* data have shown that PSA may have a direct proliferative effect on prostate stromal cells, but also an indirect proliferative activity through proteolysis of the IGF-I-IGFBP-3 complex (55). Our results have shown a higher IGF-I reduction in subjects treated with 4-HPR with lower PSA level at baseline, but no correlation between the two biomarkers. The hypothesis that PSA can modulate IGF-I levels can be true within the prostate gland, since it loses the enzymatic activity whereas in the blood flow (56). It is worthy to mention that other authors have shown a positive correlation between PSA and IGF-I in the serum and this association was stronger in older

men (57). IGF-I level can increase prostate volume, which may in turn lead to a PSA elevation.

One possible explanation for the discrepancy between the results obtained in animal studies and the results of pilot clinical trials may be related to the insufficient dose used in humans (44, 58). *In vitro*, the induction of apoptosis by 4-HPR is evident at higher concentrations than those achieved *in vivo* at the oral dose 200 mg/d (12). Extrapolations from recent *in vitro* studies indicate that a daily dose of 200 mg may not achieve growth inhibitory (apoptotic) concentrations at the target tissue (i.e., >5 μmol/L; refs. 8, 59, 60), which are about five times those attained in the blood with 200 mg/d. Likewise, the doses that were effective in animal studies are much higher than the corresponding doses used in clinical trials. Another possible explanation for a lack of activity by 4-HPR is its poor bioavailability at the prostate tissue level, which has been documented in prostate biopsies compared with other organs (43, 44).

Interestingly, our data suggest that the effect of 4-HPR may be influenced by smoking status. In the treated group, nonsmokers showed an increase of total and free PSA, and a decrease of IGF-I and the IGF-I/IGFBP-3 ratio. These effects seem to suggest that only nonsmokers may benefit from 4-HPR. This observation is in line with the detrimental effect of vitamin A and its derivates or analogues in smokers, which was observed in prevention studies such as the ATBC, CARET, etc. (40–42). Several explanations have been advocated to explain these findings, including (a) increase of P450 enzymes which leads to β-carotene oxidative metabolites that further increases P450; (b) reduction of retinoic acid which may lead to a decrease expression of RARβ; and (c) reduction of RARβ which may results in an increase expression of c-fos and c-jun, inducing hyperproliferation and facilitate metaplasia insurgence (61, 62). These pathways seem to be enhanced only when β-carotene is given at high dose (63). Taken together, these findings point to a very complex interaction between retinoids and host or lifestyle characteristics, underlining the importance of selecting appropriate cohorts who may benefit from the retinoid intervention.

In conclusion, in this exploratory analysis treatment with 4-HPR for 1 year was associated with no change in total PSA and a modest increase in free PSA levels. However, men ages ≤60 years showed a statistically significant increase of total free PSA and % free PSA levels after retinoid treatment, suggesting a possible interaction of 4-HPR on prostate carcinogenesis. Our findings support a possible role of 4-HPR for prostate chemoprevention in younger men, where the role of the IGF system seems to be more important (50). Further studies are necessary to determine the possible role of 4-HPR or new generation retinoids in prostate cancer prevention, with particular attention for the cohort characteristics, timing and dosage.

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