Leptin receptor isoforms in benign prostatic hyperplasia (BPH). BPH and prostate cancer – no association between plasma concentrations of leptin and prostate specific antigen (PSA)

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KEY WORDS

benign prostatic hyperplasia ◗ prostate cancer ◗ leptin ◗ leptin receptors ◗ PSA

ABSTRACT

Introduction. Leptin (Ob) is an adipose tissue-secreted hormone. Out of six recognized isoforms of leptin receptor only the long form (Ob-Rb), in humans (huOb-R), activates full biological function of that cytokine. Recently, expression of leptin – leptin receptor system was demonstrated in the rat prostate, while data for human prostate are scarce and conflicting. Therefore present studies aimed to investigate expression of leptin and leptin receptor isoform genes in prostates of patients with BPH and prostate cancer (PC). Furthermore, in studied groups we looked for a possible interrelationship between plasma concentrations of leptin and PSA.

Material and methods. Conventional RT-PCR studies were performed on 5 glands removed surgically due to BPH. Leptin and PSA blood concentrations were evaluated in control patients (without LUTS) and patients with BPH and PC (qualified to radical prostatectomy or hormonal therapy).

Results. By means of classic RT-PCR we found expression of the long isoform of leptin receptor only in 2 out of 5 studied cases of BPH. On the contrary, nonfunctional human B219/OB receptor isoform HuB2191 was present in all studied cases and HuB2192 and HuB2193 isoforms were noted in 3 and 4 cases, respectively. Expression of both leptin gene and other nonfunctional leptin receptor splice variant form 132 could not be demonstrated. Leptin plasma levels were similar in control, BPH or PC groups qualified to radical prostatectomy. On the contrary, in patients qualified to LHRH analog and hormonal therapy plasma leptin levels were notably lower when compared with control.

Conclusions. results of present studies suggest that leptin is unlikely to substantially affect progression of either BPH or PC.

INTRODUCTION

The association between leptin and prostate cancer risk is a matter of wide discussion [1-21]. Leptin (Ob) is an adipose tissue-secreted hormone that decreases caloric intake and increases energy expenditure. This cytokine is also involved in the regulation of angiogenesis, hematopoiesis and neuroendocrine function, as well as in the stimulation of the proliferative activity of various cell types. Leptin acts via specific receptor (Ob-R), of which six isoforms are recognized at present (from Ob-Ra to Ob-Rf). Ob-Rb is the only isoform that is able to activate JAK-STAT and MAPK signaling cascade [22, 23, 24, 25].

Assuming that leptin may be involved in prostate cancer risk, it would be expected that human prostate will be provided with functional leptin receptor isoform and may be a place of leptin synthesis. In this regard, careful survey of literature gives uncertain knowledge. In human prostate expression of Ob-R was demonstrated by Northern blot [26]. Long and short isoforms of the leptin receptor were identified in human prostate cancer by immunocytochemistry [5] and in human prostate cancer cell lines by RT-PCR [27, 28]. In a preliminary report, expression of leptin gene at the mRNA level was described in one case of prostate cancer [29]. Furthermore, free immunoreactive leptin was described to be present in human seminal plasma [30]. In contrast to human prostate, in rat prostate expression of both leptin and leptin receptor isoforms were easily demonstrated at the level of both mRNA and protein [31, 32].

Inconsistent results of studies on association between leptin and normal and pathologically changed human prostate prompted us to look for possible associations between plasma leptin levels in relation to PSA in benign prostatic hyperplasia (BPH) and prostate cancer (PC) patients. Furthermore, we investigated expression of leptin and leptin receptor genes in adenomatous human prostate.

MATERIAL AND METHODS

Studies were performed on patients from the Department of Urology and Urooncology, Poznań University of Medical Sciences. Bioethical Committee of the University provided consent for the study protocol.

The following groups of patients were studied: (I) control group: patients without lower urinary tract symptoms (LUTS), with normal PSA blood levels, qualified during screening for PC (n=18); (II) patients with BPH qualified to transurethral resection of prostate (TUR-P) (n=11); (III) patients with PC qualified to radical prostatectomy (n=9); (IV) patients with disseminated PC qualified to LHRH analog and hormonal therapy, sampled before therapy initiation (n=7). Fasting blood was sampled in the presence of EDTA to establish plasma levels of leptin and PSA. Plasma was frozen at -80° C until leptin quantitation. Body weights and BMI were established in a routine way. Content of adipose tissue in the body (BF%) was calculated according to the formula of Deurenberg et al [33].

BPH and PC were diagnosed histopathologically. In all cases of BPH, prostate adenoma was found. In all patients with PC qualified to radical prostatectomy macro- and microtubular adenocarcinoma G2 was diagnosed and Gleason scores were from 5 to 7. Patients with disseminated PC qualified to LHRH analog and hormonal therapy combined: trabecular, microtubular and solid desmoplastic car-

Table 1. RT-PCR analyses of leptin and leptin receptor isoforms in benign prostatic hyperplasia (BPH). Oligonucleotide sequences for sense (S) and antisense (A) primers are shown. PBGD (porphobilinogen deaminase) – reference gene.

cDNA	Genbank Accession number	Primers	Primer sequence (5'–3')	Position	PCR product size (bp)
Human B219/OB receptor isoform HuB2191	HSU52912	S A	CAGAGTGATGCAGGTTTATATG CCCTGGGTACTTGAGATTAG	2509-2530 2716-2735	227
Human B219/OB receptor isoform HuB2192	HSU52913	S A	CAGAGTGATGCAGGTTTATATG CAACCTCCACCCAGTAGTT	2509-2530 2693-2711	203
Human B219/OB receptor isoform HuB2193	HSU52914	S A	CAGAGTGATGCAGGTTTATATG ACATTGGGTTCATCTGTAGTG	2509-2530 2802-2822	218
Human leptin receptor splice variant form 132	HSU66497	S A	TATGTAATTGTGCCAGTAA CTGATGCTGTATGCTTGATAA	2527-2545 2697-2716	190
Homo sapiens leptin receptor	BC131779	S A	TGTGCCTTAGAGGATTATGC ACAAAACCACACAGAATT	78-97 131-148	71
Homo sapiens leptin	NM_000230	S A	TTGTCACCAGGATCAATGACA TGAAGTCCAAACCGGTGACT	169-189 224-243	75
PBGD	NM_001024382	S A	GCAACAGCAGGTCCTACTATC GAGAGTGCAGTATCAAGAATC	25-45 245-265	241

Table 2. Basic clinical data of studied patients with benign prostatic hyperplasia (BPH), prostatic cancer (PC) and of controls. BMI - body mass index [kg/m2], BF% – body fat percentage. Results are means ± SE. Number of studied cases shown in brackets. Statistical evaluation of differences, in relation to control group – unpaired Student's t-test: *p<0.05; **p<0.02; ***p<0.001 from the respective control group.

Group	Age	Body mass	BMI	BF%
Control (18)	60.0 ±1.8	87.4 ±3.4	28.88 ±1.30	32.25 ±1.55
BPH -TUR-P (11)	69.6 ±2.5***	80.2 ±2.7	26.18 ±0.93	31.23 ±1.24
PC – Radical prostatectomy (9)	68.2 ±2.5**	77.8 ±2.9	26.86 ±0.83	31.59 ±1.05
PC – Patients qualified to LHRH treatment (7)	67.7 ±2.0*	81.7±5.7	27.73 ±1.94	32.65 ±2.6

cinoma (1 case) and microglandular prostate adenocarcinoma (6 patients), G3-G2 stages and their Gleason scores ranged from 7 to 9.

Leptin plasma levels were evaluated by means of Leptin EIA Kit (catalogue No 500600 Cayman Chemicals). Automated Chemiluminescence System ACS: 180 by Bayer Health Care was used to detect PSA blood levels.

Conventional RT-PCR studies were performed on 5 glands removed surgically due to BPH (adenectomy). All tissue samples were taken from the anterior periurethral area. Patients aged 66-75 years of age.

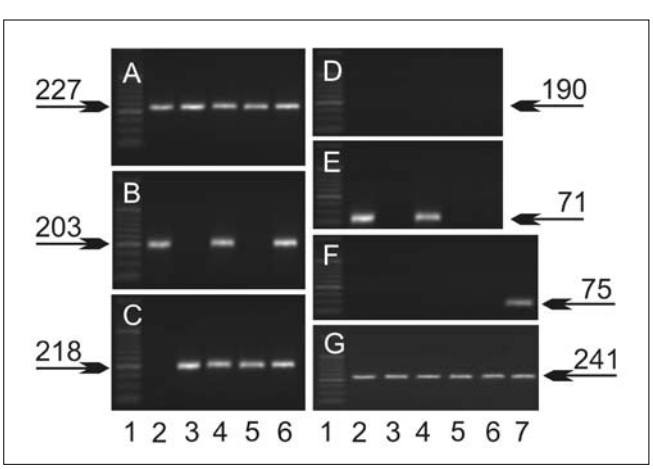

Fig. 1. Expression of leptin and isoforms of its receptor mRNAs in benign prostate hyperplasia (BPH). A – human B219/OB receptor isoform HuB2191; B – Isoform HuB2192; C - isoform HuB2193; D – receptor splice variant form 132; E – *Homo sapiens* leptin receptor; F - *Homo sapiens* leptin; G - PBGD (porphobilinogen deaminase; housekeeping gene). Lines: 1 – molecular size marker; 2-6 – studied cases; 7 – positive control (adipose tissue).

Total RNA was extracted from the gland using Tri Reagent (Sigma), as previously detailed [34, 35, 36, 37, 38]. The amount of total RNA was determined by measuring optical density at 260 nm and purity was estimated by 260/280 nm absorption ratio >1.8. From each sample equal amounts of RNA (0.5 µg) were taken to reverse transcription (RT). RT was performed using AMV Reverse Transcriptase (Promega, USA) with Oligo dT (PE Biosystems, Warrington, UK) primers. RT step was performed in 42° C for 60 min. Conventional RT-PCR was carried out in a Roche Light-Cycler 20 (Roche, Mannheim, Germany), as described earlier [39, 40], using the primers designed by means of Primer 3 Software (Whitehead Institute for Biomedical Research, Cambridge, MA, USA) (Tab. 1). They were purchased from the Laboratory of DNA Sequencing and Oligonucleotide Synthesis, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw.

The amplification program comprised: denaturation step (94°C for 10 min) and 35 cycles of three-step amplification (denaturation, 92° C for 30 s; annealing, 58° C for 30 s and extension, 72°C for 30 s). Then a final extension step was carried out at 68° C for 7 min. Detection of the PCR amplicons was performed by size fractionation in 2% agarose gel electrophoresis. All samples were amplified in duplicate.

The following control reactions were also performed: (I) positive internal control with porphobilinogen deaminase gene (PBGD, housekeeping gene); (II) positive control: with RNA extracted from adipose tissue – to confirm the good reaction conditions of RT-PCR for OB; (III) negative control without RNA – to exclude contamination of reagents for RNA isolation and RT-PCR reactions.

Leptin and isoforms of its receptor are commonly widespread in the human body, so it is difficult to find a reliable negative control, involving tissue without their expression. In this situation in each set of tissue RNA preparation one blind control was prepared, involving isolation procedure without addition of human material. This "prep without RNA" was then used as a negative control in RT-PCR reactions.

Statistics

Data were expressed as means ±SEM and their statistical comparison was done by the unpaired Student's t-test. Correlation coefficient between plasma concentrations of PSA and leptin was calculated according to Pearson, using Statistica R software.

RESULTS

Unavailability of normal human prostate forced us to study expression of leptin and leptin receptor genes in adenomatous prostate. As demonstrated in Fig 1, conventional RT-PCR revealed expression of human B219/OB receptor isoform HuB2191 in all studied cases, HuB2192 in 3 out of 5 cases and HuB2193 in 4 cases. In the studied material, receptor splice variant form 132 could not be demonstrated, while the long isoform of receptor (huOb-R) was found in 2 out of 5 studied adenomas. Expression of leptin gene could not be demonstrated in adenomatous prostates but was found in adipose tissue (positive control). In all studied cases expression of PBGD gene was detected and in all assays, reaction products were of the expected length.

In all studied groups of patients neither BMI nor BF% differed from those seen in the control group (Tab. 2). Leptin plasma levels were similar in the control group and in groups of patients with BPH or PC qualified to radical prostatectomy (Fig. 2). On the contrary, in patients qualified to LHRH analog and hormonal therapy plasma leptin levels were notably lower as compared to the control. Plasma PSA levels of patients with BPH were similar to those seen in controls, while in subjects with PC, PSA levels were notably elevated. For all studied cases there was no correlation between blood plasma PSA and leptin concentrations (Fig. 3). Pearson's correlation coefficient between plasma concentrations of PSA and leptin, calculated from data obtained from all 45 patients, was very low (-0.1802) and suggested lack of linear relationship between these two variables.

DISCUSSION

As mentioned in the introduction, data on the expression of leptin and leptin receptor isoforms in human normal and pathological prostates are sparse and conflicting. Because of unavailability of normal human prostate, we performed studies on prostate adenomas. Despite the small number of studied cases, the results that we obtained were rather unexpected. We found expression of the long isoform of leptin receptor (huOb-R) only in 2 out of 5 studied adenomas. On the contrary, nonfunctional human B219/OB receptor isoform HuB2191 was found to be present in all studied cases and HuB2192 and HuB2193 isoforms in 3 and 4 cases, respectively. Expression of both leptin gene and other nonfunctional leptin receptor splice variant form 132 could not be demonstrated. Earlier studies revealed that prostate cancer cell lines DU145, PC-3 (androgen independent cells), and LNCaP-FGC (androgen dependent cells) expressed huOB-R and huB2193 [27, 28]. Thus, the pattern of expression of functional leptin receptor isoforms in BPH differs markedly from that seen in human prostate cancer cell lines. To our knowledge, there are no data available on expression of these isoforms in human PC.

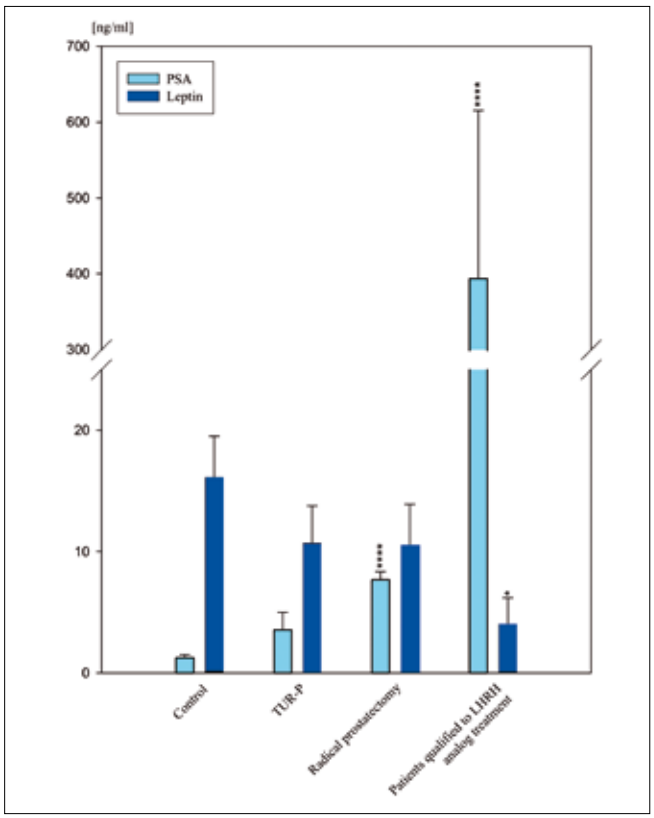

Fig. 2. Plasma PSA [ng/ml] and leptin [ng/ml] levels in studied patient groups. Bars are means ± SE. Statistical evaluation of differences, in relation to control group – unpaired Student's t-test: *p<0.05; **p<0.02; ***p<0.01; ****p<0.001 as compared to the respective control group.

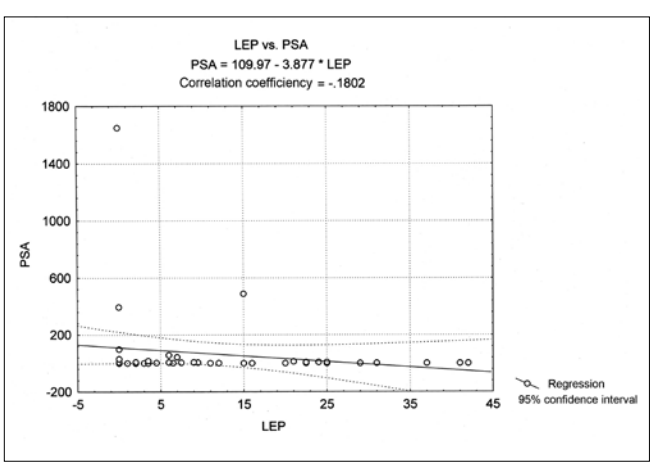

Fig. 3. Pearson`s correlation coefficient between plasma concentrations of PSA [ng/ml] and leptin [ng/ml]. Results of all studied patients (n=45) were subjected to analysis.

It should be emphasized that the data presented above differ markedly from those seen in the rat. In the rat prostate expression of the leptin gene is easily demonstrated both at the mRNA and protein level [31, 32]. Moreover, normal rat prostate is provided with all known splice variants of leptin receptor, among them that with the functional long isoform.

Since leptin is an adipose tissue-secreted hormone, association between obesity, leptin and prostate cancer risk is currently a highly debated topic. In this regard BMI is a reliable indicator of body fatness for humans, therefore numerous reports deal with the interrelationship between BMI and prostate cancer risk [for reviews see 9, 41]. Some epidemiological studies have found obesity to be

a risk factor for prostate cancer [42, 43, 44, 45, 46, 47], while there are also reports on lack of such a correlation [12, 13, 19, 48, 49, 50, 51, 52, 53].

Also results of studies on blood leptin levels in BPH and PC are inconsistent. In the earliest report Lagiou et al. [1] found no alterations in blood leptin concentrations in patients with BPH and PC and these findings were confirmed by others [4, 54, 55]. On the contrary, in infiltrating PC, however without metastases, elevated blood leptin levels were reported by other groups [2, 8, 16]. Present studies were performed on relatively small groups of patients and their BMI was similar and within the normal range values. In patients with BPH qualified to transurethral resection of the prostate and in patients with PC qualified to radical prostatectomy plasma leptin levels were comparable to those seen in the control group. On the contrary, in patients with disseminated PC, qualified to LHRH analog and hormonal therapy, leptin concentrations were notably lower in blood sampled before therapy initiation. In the available literature we did not find comparable data. This may reflect the fact that in developed countries prostate cancers of such advancement are very rarely diagnosed.

A possible interrelationship between blood levels of leptin and PSA in BPH and PC patients remains an open question. Only Freedland et al. [55] studied blood leptin and PSA levels in patients with inoperable PC at the pT3a stage and in patients at T1c-T2 stage, qualified to radical prostatectomy. In such patients they failed to demonstrate association between blood concentrations of leptin and PSA and present results confirm these observations. Recently, Fawke et al. [15] also reported lack of association between blood PSA and leptin levels in African-American and Caucasian men.

Numerous adipokines exert a variety of biologic effects on prostate cancer cells, modulating cellular differentiation, migration, apoptosis, proliferation, angiogenesis and secretion of growth factors [27, 56, 57, 58]. In this regard leptin is thought to stimulate cell proliferation specifically in androgen-independent DU145 and PC-3 prostate cancer cells but not in androgen-dependent LNCaP-FGC cells, although both cell types express functional leptin receptor isoform [27, 28, 58, 59]. However, the role of leptin in promoting prostate cancer growth and/or progression needs further clarification. To our knowledge, with the exception of one case of PC [29], there are no direct data on leptin gene expression in human BPH, PC or normal prostate, although immunoreactive leptin was identified in human seminal plasma [30]. Furthermore, our studies revealed expression of functional leptin receptor only in 2 out of 5 studied human prostate adenomas. This result clearly suggests an absence of associations between expression of functional leptin receptor and BPH. Moreover, unaltered or even lowered plasma concentrations of leptin in BPH and PC, as observed in present studies, seem to exclude the direct action of circulating leptin on proliferation of prostate cells in BPH and PC.

Results of our studies on leptin receptor isoforms expression in BPH casts doubts on the relevance of commonly used prostate cancer cell lines for characterization of biological activity of PC *in situ*. Those doubts arise from the fact of common expression of functional leptin receptors in established prostate cancer cell line, while in the material originating from adenomatous prostate expression of that leptin isoform is present only in some glands.

Thus, results of present studies suggest that leptin is unlikely to substantially affect progression of either BPH or PC.

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