Pharmacological Reports
2008, 60, 399–403
ISSN 1734-1140

Effect of luteinizing hormone-releasing hormone (LHRH) analogue treatment on a cytokine profile in prostate cancer patients

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Abstract:
The aim of the study was to test serum concentrations of the chosen cytokines in patients with prostate cancer (PCa) treated with an luteinizing hormone-releasing hormone (LHRH) analogue. We tested interleukin (IL)-2, IL-10, tumor necrosis factor (TNF)-α, interferon (INF)-γ in blood at three time points; I – before the injection, II – 10 days and III – 20 days after the injection in 14 men with PCa. Patients had one depot injection of the LHRH analogue monthly. The cytokine concentrations in serum samples were determined by ELISA method. Prostate specific antigen (PSA) level was examined before and after six months of the LHRH analogue treatment. After six months of the therapy, we observed normalization of serum PSA value from 16.48 ng/ml to 1.45 ng/ml. LHRH analogue injection resulted in a significant drop of the IL-2 concentration, and the value gradually returned to normal in the next 20 days. IL-10 concentration transiently increased and then was down-regulated. Serum TNF-α and INF-γ concentrations in PCa patients were significantly lower compared to controls and were not affected by the treatment. LHRH analogue treatment in PCa patients modulates concentrations of the chosen cytokines which may result both in antitumor and a transient immunosuppressive effect.

Key words:
steroid hormones, LHRH analogue, cytokines, prostate cancer

Introduction

The early observations on the link between sex and cancer development were described by Batchelor and Chapman [1]. Gonadal steroids play a role in etiology of the prostate cancer (PCa) which is the second leading cause of cancer-related deaths in men in Europe [12]. Risk of this cancer rises parallelly to the androstendion level in serum, although the rate of PCa increases with age while the level of androgens drops [20]. The principle for endocrine treatment of PCa is elimination of stimulatory effects of androgen on the tumor cells, therefore, a luteinizing hormone-releasing hormone (LHRH) analogue is widely used in the therapy [13]. The LHRH analogue eliminates production of testosterone leading to pharmacological castration [20]. Androgens, besides their direct stimulatory

effect on the tumor cells, are considered to be immunosuppressive affecting T cell function irrespective of the presence or absence of gonadotropin releasing hormone (GnRH) [4, 8]. Gonadal steroid feedback at hypothalamic and pituitary levels affects the release of gonadotropins, which suggests that the immunosuppressive androgen features may result from their negative feedback on GnRH production [13, 19]. The complicated relation between immunological and endocrine systems is still the matter of debate. The relation between GnRH and gonadal steroids play an important role in the immune system modulation and development [8]. Numerous studies proved direct immunostimulatory properties of GnRH [2, 14]. Analysis of the effect of the hormonal system on human immune response is additionally complicated because lymphocytes both produce GnRH and express its receptors suggesting an autocrine role for GnRH [18].

The aim of our study was to test the effect of LHRH analogue treatment on the chosen proinflammatory mediators and cytokines in patients with prostate cancer.

Materials and Methods

The study comprised 14 men (age 71–83 years, mean 77.4) with prostate cancer (9 men –Gleason score 5–7 and 5 men – Gleason score 2–4) treated with LHRH analogue in the out-patient urology clinic of Ministry of Internal Affairs and Administration Hospital. Patients with concomitant diseases, diabetes, severe inflammatory processes or taking immunosuppressive drugs were excluded from the study. The study was conducted in the period from October 2005 to February 2006. The control group comprised 10 men treated for nephrolithiasis or benign prostate hyperplasia (BPH), in similar age range. Our study has been approved by the Local Ethics Committee (certificate no. RNN/86/03KB). Patients had been treated for 6 months with one depot injection of LHRH analogue monthly (Goserelin 3.6 mg per injection, *sc*) before the study started.

Material for examination, i.e. venous blood samples were collected at three time points: I – before injection, II – 10 days after the injection, III – 20 days after the injection. Serum prostate specific antigen (PSA) concentration was tested using immunoenzymatic commercial kit (BPC, Poland) before the treatment and six months later. Serum concentrations of the cytokines: interleukin (IL)-2, IL-10, tumor necrosis factor-α (TNF-α), interferon-γ (INF-γ) were tested using commercial ELISA kit Quantikine (R&D Systems, Inc.). The test reading was done with the aid of optical density reading apparatus with software. Samples were assayed in duplicate.

Statistical analysis

The tested group and the controls were compared with the Mann-Whitney U test, and the paired observations with the Wilcoxon signed rank test. Statistical analysis was performed using the Statistica 6.0 (Stat Soft) and $p < 0.05$ was considered statistically significant.

Results

IL-2 concentration dropped significantly ($p < 0.05$) from 29.74 pg/ml before the LHRH analogue administration to 7.12 pg/ml on day 10 after the injection and then was up-regulated to 13.35 pg/ml on day 20 after the injection. IL-2 concentrations 10 and 20 days after LHRH injection were markedly lower ($p < 0.05$) compared to controls 31.92 pg/ml (Fig. 1).

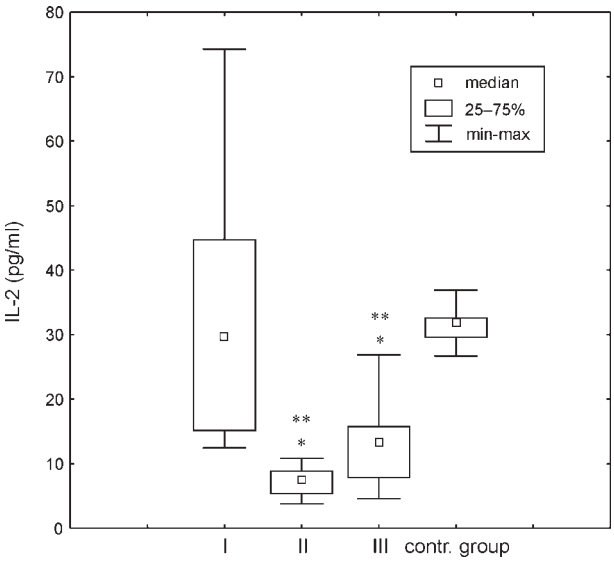

Fig. 1. IL-2 concentration in PCa patients. I – before the LHRH analogue injection, II – 10 days after the LHRH analogue injection, III – 20 days after the injection. * $p < 0.05$ *vs.* I, ** $p < 0.05$ *vs.* control group

IL-10 concentration increased significantly after the injection from 4.7 to 7.18 pg/ml ($p < 0.05$) and then 20 days later, significantly dropped to 3.68 pg/ml ($p < 0.05$). The second value was significantly different from the control group (4.75 pg/ml) (Fig. 2).

TNF-α concentrations at the respective time points were: 6.5 pg/ml 6.26 pg/ml and 6.01 pg/ml. All the values were significantly lower compared to the control group (9.4 pg/ml) (Fig. 3).

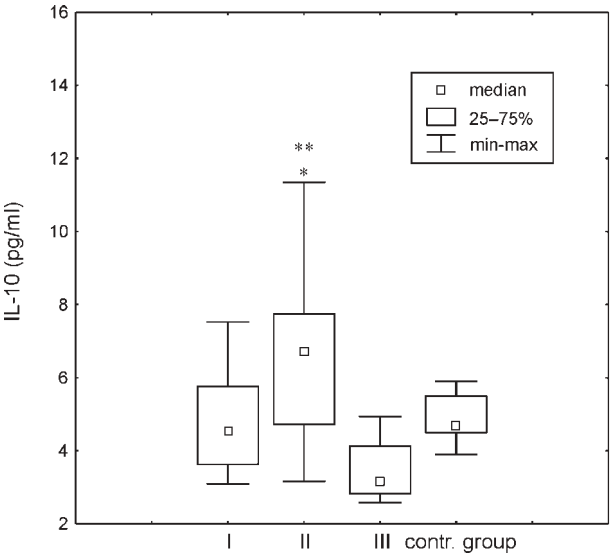

Fig. 2. IL-10 concentration in PCa patients. I – before the LHRH analogue injection, II – 10 days after the LHRH analogue injection, III – 20 days after the injection. * $p < 0.05$ *vs.* I, III, ** $p < 0.05$ *vs.* control group

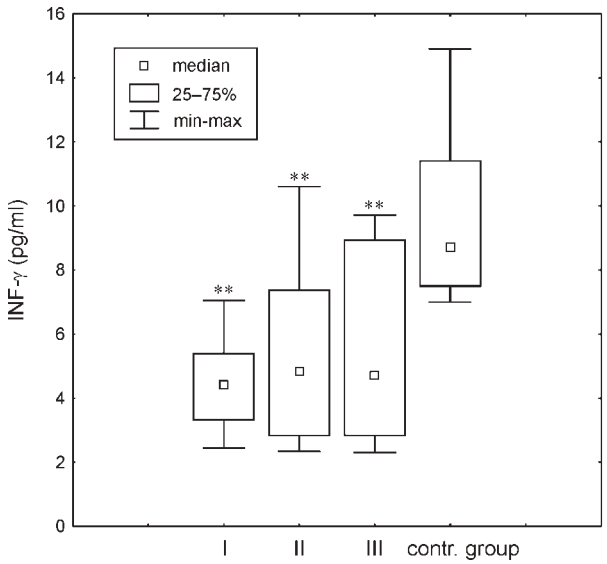

Fig. 4. INF-γ concentration in PCa patients. I – before the LHRH analogue injection, II – 10 days after the LHRH analogue injection, III – 20 days after the injection. ** $p < 0.05$ *vs.* control group

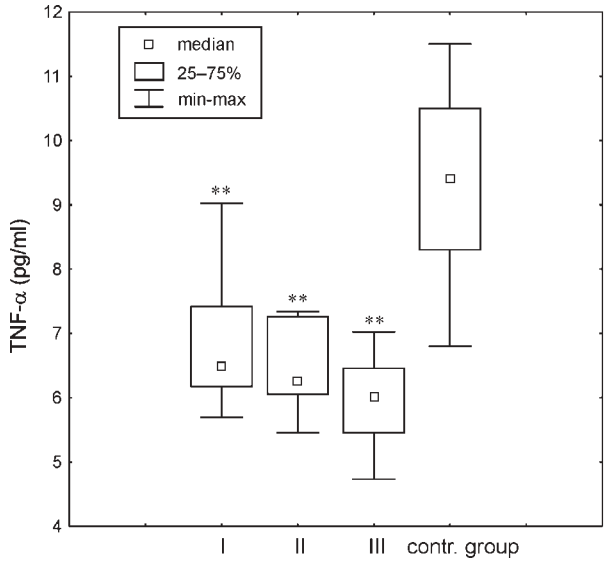

Fig. 3. TNF-α concentration in PCa patients. I – before the LHRH analogue injection, II – 10 days after the LHRH analogue injection, III – 20 days after the injection. ** $p < 0.05$ *vs.* control group

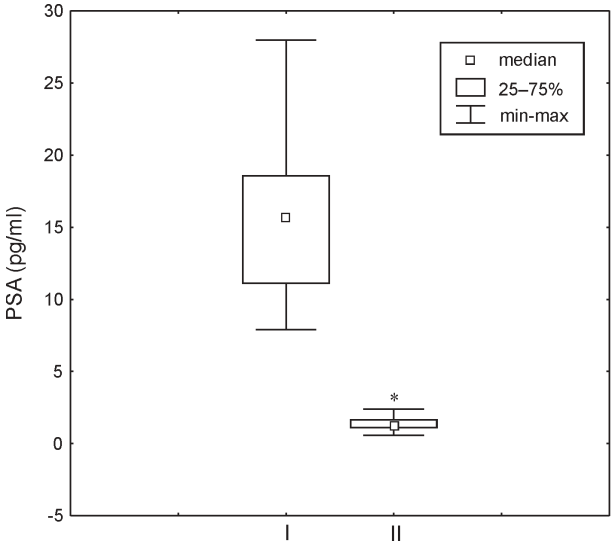

Fig. 5. PSA value after 6-month LHRH analogue treatment in PCa patients. I – before study, II – after 6-month treatment. * $p < 0.05$ *vs.* I

Serum INF-γ level was also not affected by the LHRH analogue treatment (4.43 pg/ml, 4.82 pg/ml and 4.71 pg/ml, respectively) and the values were significantly lower compared to the control (8.7 pg/ml) (Fig. 4).

PSA level was examined before and after 6-month LHRH analogue treatment. Its values dropped significantly from 16.48 ng/ml to 1.45 ng/ml (Fig. 5).

Discussion

The main antitumour activity of LHRH analogues in PCa cancer is based on pharmacological castration, although some beneficial effects of the LHRH on the immune system have also been suggested [5, 8]. Endocrine therapy of prostate cancer has been primarily reserved to the older patients with advanced stages of the disease. At present, it has been proven that an LHRH analogue in hormone-dependent PCa slows down progression of the disease or at least results in a transient remission [6, 9]. Significant drop of blood PSA level to a normal value after 6-month therapy was observed in all of our patients. The drop confirms suppression of the disease and proves androgen sensitivity of the tumors [20].

In our study, initial values of cytokines IL-2 and IL-10 did not differ from those in the control group whereas initial values of TNF-α and INF-γ were significantly lower.

In literature data PCa patients with different clinical picture do not present unequivocal results on the cytokine generation. According to Perambakam and colleagues, the cytokine profile in PCa patients depends on the tumor progression. Domination of the Th-1 cytokine pattern was noted in patients with low serum PSA compared to high-risk patients with high PSA and locally advanced disease [15].

Wise and colleagues presented data similar to ours on the baseline concentration of IL-10 in PCa patients and noted that the cytokine concentration was directly associated with the elevated PSA [20]. In other experiments testing the capacity of lymphocytes of the patients to produce type Th1 and type Th2 cytokines, a significant disequilibrium between Th1 and Th2 lymphocytes was noted. The basal expression of cytokines: IL-2, INF-γ, IL-10 in resting lymphocytes was very low and following stimulation, an IL-2 down-regulation was noted, and IL-10 was the only cytokine that increased markedly compared to the healthy controls [7]. Fillela suggested that the loss of balance between Th1/Th2 products may be implicated in cancer development [7]. The presence of IL-2 is necessary for proliferation and function of T helper, T cytotoxic, B and NK cells, thus the marked down-regulation of the cytokine concentration 10 days after the LHRH injection proves a transient suppression of the Th-1 cell function and worsening of the Th1/Th2 disequilibrium.

The observed changes in the tested cytokines in our PCa patients suggest adverse effect of the LHRH analogue on the Th1/Th2 balance. Very low INF-γ concentration throughout the whole treatment and a marked drop of IL-2 after the analog administration with transient up-regulation of the main Th2 cytokine (IL-10) suggest worsening of the antitumor response capacity [3]. On the other hand, Sterns et al. suggested that IL-10 played an important role in controlling the tumor growth and metastasis [17]. The results of experiments carried out on animal models confirm that IL-10 down-regulates expression of vascular endothelial growth factor (VEGF), TNF, matrix metalloproteinase 9 (MMP-9) and other proangiogenic agents in macrophages infiltrating tumors [10, 11, 16]. Thus, from this point of view, the temporal rise of cytokine 10 days after LHRH analogue administration could have beneficial effects. Taking together, the biological effects of IL-10 on tumor development range from facilitation of the tumor growth due to its suppressing effects on the immune system to inhibiting tumor angiogenesis and metastasis [3, 17].

Conclusions

LHRH analogue treatment in the androgen-sensitive PCa patients modulates concentrations of the chosen cytokines which may result both in antitumor and a transient immunosuppressive effect.

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Received:
May 19, 2007; in revised form: March 14, 2008.