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Original article

Do androgen deprivation drugs affect the immune cross-talk between mononuclear and prostate cancer cells?

Hertzel Salman [a,c,1], Michael Bergman [a,c,1], Naava Blumberger [a,c], Meir Djaldetti [b,c,*], Hanna Bessler [b,c]

[a] Department of Medicine C, Rabin Medical Center-Hasharon Hospital, Petah-Tiqva, Israel
[b] Laboratory for Immunology and Hematology Research, Rabin Medical Center-Hasharon Hospital, Petah-Tiqva, Israel
[c] Sackler School of Medicine, Tel-Aviv University, Ramat-Aviv, Israel

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ABSTRACT

The aim of the study was to examine the effect of androgen deprivation drugs, i.e. leuprolide and bicalutamide on the immune cross-talk between human peripheral blood mononuclear cells (PBMC) and cells from PC-3 and LNCaP human prostate cancer lines. PBMC, PC-3 and LNCaP were separately incubated without and with two androgen-deprivation drugs, i.e. leuprolide and bicalutamide, and the secretion of IL-1β, IL-6, IL-1ra and IL-10 was examined. In addition, the effect of both drugs on the production of those cytokines was carried out after 24 hours incubation of PBMC with both types of cancer cells. Leuprolide or bicalutamide did not affect the production of the cytokines by PBMC or by the prostate cancer cells from the two lines. Incubation of PBMC with PC-3 or LNCaP cells caused increased production of IL-1β, IL-6 and IL-10 as compared with PBMC incubated without malignant cells. While 10^{-7} M and 10^{-8} M of leuprolide caused a decreased secretion of IL-1β by PBMC previously incubated with prostate cancer cells without the drug, bicalutamide did not affect this PBMC activity at any drug concentration. This observation suggests the existence of an additional mechanism explaining the effect of androgen deprivation therapy in prostate cancer patients.

1. Introduction

It is generally accepted that prostate carcinoma is one of the most frequently malignancies in men. According to Sfanos et al. [1], a number of causes contribute to the development of prostate cancer, such as environmental, epidemiological, nutritional and habitual factors. However, chronic inflammation, either symptomatic or indolent, that is a common finding in adult prostate, presents a higher risk for tumor development. The linkage between chronic inflammation and carcinogenesis seems to be well documented [1–5]. Prostate cancer is one example of tumors that express the role of inflammation in the etiology of the disease. In an extensive review on the linkage between chronic inflammation and prostate cancer development, Haverkamp et al. [2] stress the role of a few pro-inflammatory cytokines such as IL-1β and IL-1α, IL-6, IL-8 and IL-17 as promoters of prostate cancer cells' proliferation, apoptosis and tumor environment angiogenesis. However, one has to keep in mind that studies dealing with cytokine secretion by prostate cancer cells have been carried out with lines that are either dependent or independent on androgen. Thus Chung et al. [6] have demonstrated that cells from three hormone-refractory prostate cancer lines secreted IL-6, whereas those from two hormone dependent lines did not secrete this cytokine at all. However, Salman et al. [5] have shown that cells from both PC-3 androgen-resistant and LNCaP androgen-dependent prostate cancer lines induced an increase secretion of IL-6 by peripheral blood mononuclear cells, whereas at the same incubation conditions only androgen-resistant cells caused an increased secretion of the pro-inflammatory cytokine IL-1β and the anti-inflammatory cytokine IL-10. Presently, hormone dependency that modulates prostate cancer development is the basis of androgen deprivation therapy, with or without radiation. Two drugs are currently in use for hormonal ablation in prostate cancer, i.e. leuprolide, which is a luteinizing hormone agonist and bicalutamide, an anti-androgen that acts by binding and blocking androgen receptors. Following our experience with the ability of prostate cancer cells from androgen-dependent and non-dependent lines to modulate cytokine production by immune cells [5], the present study was designed to examine the effect of these two drugs on cytokine secretion by human peripheral blood mononuclear cells (PBMC) induced by incubation with cells from PC-3 and LNCaP lines. Based on the observation that human PBMC incubated with both

* Corresponding author. Laboratory for Immunology and Hematology Research, Rabin Medical Center-Hasharon Hospital, 7, Keren Kayemet Street, Petah-Tiqva, Israel. Tel.: +972 3 9372397; fax: +972 3 9372398.
E-mail addresses: bermanm@clalit.org.il, meird@clalit.org.il (M. Djaldetti).
[1] The first and second authors contributed equally to the preparation of the manuscript.

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type of cancer cells responded by modulated production of IL-6, IL-1β and IL-10 [5], we decided to focus the present experiments on those cytokines only. The possibility that these anti-androgen drugs will modulate the secretion of inflammatory cytokines at those incubation conditions may suggest an additional way by which androgen deprivation therapy may benefit prostate cancer patients.

2. Materials and methods

2.1. Leuprolide and bicalutamide preparation

Leuprolide, a [D-Trp^6]-luteinizing-hormone-releasing-hormon agonist, and the non-steroidal androgen receptor antagonist bicalutamide were purchased from Sigma Israel. Stock solutions of 10^{-4}M of leuprolide in H_2O and 10^{-2}M of bicalutamide in DMSO were prepared and stored at −20 °C. Further dilutions were made in medium immediately before their addition to cell cultures. Leuprolide and bicalutamide were added at final concentrations of 10^{-6} M, 10^{-7} M and 10^{-8} M. Addition of bicalutamide at 10^{-5} M was found to be toxic to the cells. The concentration of DMSO used with the drug was 0.1%. Addition of DMSO to cell culture at this concentration had no significant effect on cytokine secretion.

2.2. Cell preparation

Peripheral blood mononuclear cells were separated from venous blood obtained from adult blood bank donors by gradient centrifugation. The cells were washed twice in phosphate buffered saline (PBS) and suspended in RPMI-1640 medium (Biological Industries, Beith Haemek, Israel) containing 2 mM L-glutamine and antibiotics (1% penicillin, streptomycin and nystatin, supplemented with 10% fetal calf serum [FCS], and designated as complete medium [CM]).

2.3. Prostate cancer cell lines

PC-3 and LNCaP human prostate cancer cell lines were obtained from American Type Culture Collection, (Rockville, MD, USA). PC-3 cells were maintained in CM, LNCaP cells were kept in CM supplemented with 5 μg/mL insulin (Sigma, Israel) and 10^{-9}M testosterone (New England Nuclear Company, England). The cells were grown in T-75 culture flasks at 37 °C in a humidified atmosphere containing 5% CO_2.

2.4. Effect of leuprolide and bicalutamide on cytokine secretion by non-stimulated and LPS-stimulated PBMC

0.1 mL aliquots (4×10^6/mL) of PBMC suspended in CM or in RPMI-1640 without or with 20 ng/mL lipopolysacchride (LPS, *E. coli*, Sigma) were added to each one of flat-bottomed 96-well plates (Nunc, Roskidle, Denmark). 0.1 mL of leuprolide or bicalutamide prepared as described above were added at the onset of the cultures to reach final concentrations of 10^{-8} M, 10^{-7} M and 10^{-6} M. Cultures incubated without the drugs served as controls. Plates were incubated for 24 h at 37 °C in a humidified atmosphere containing 5% CO_2. At the end of the incubation period the cells were removed by centrifugation at 1500 rpm for 10 min, the supernatants were collected and kept at −75 °C until assayed for cytokines content.

2.5. Effect of leuprolide and bicalutamide on cytokine secretion by PBMC induced by prostate cancer cells

0.5 mL of PBMC (4×10^6/mL of CM or RPMI-1640 only) was incubated with equal volume of each one of the prostate cancer cells suspended in the appropriate medium at 10^6/mL. Leuprolide or bicalutamide were added at the onset of the cultures at concentrations as indicated above. Cultures incubated without the drugs served as controls. The cultures were incubated for 24 h at 37 °C in a humidified atmosphere containing 5% CO_2. At the end of the incubation period the cells were removed by centrifugation at 1500 rpm for 10 min, the supernatants were collected and kept at −75 °C until assayed for cytokines content.

2.6. Cytokine content in the supernatants

Since incubation of prostate cancer cells with PBMC stimulated the production of IL-1β, IL-6 or IL-10 [5] the effect of leuprolide and that of bicalutamide on the interaction between malignant cells and PBMC was referred to these cytokines only. The level of the pro-inflammatory cytokines IL-1β, IL-6, and that of the anti-inflammatory cytokines IL-10 and IL-1ra in the supernatants was tested using ELISA kits specific for human cytokines (Biosource International, Camarillo, CA) as detailed in the guideline provided by the manufacturer. The detection level of these kits was 15 pg/mL for IL-6, and 30 pg/mL for IL-10.

2.7. Statistics

Data was analyzed using ANOVA with repeated measures for each cytokine and paired *t*-test to compare the difference between cytokine levels obtained without and with various concentrations of leuprolide and bicalutamide. P values < 0.05 were considered as statistically significant. The results are expressed as mean ± SEM.

3. Results

Supernatants obtained from malignant cells of both lines incubated in the appropriate CM for 24 h at concentrations used in this study did not contain detectable levels of any of the cytokines examined. The effect of the drugs on cytokine secretion was examined in the presence or absence of FCS. In the presence of FCS there was no effect of either of the drugs on spontaneous, LPS-induced or prostate cancer cells-induced cytokine secretion (data not shown). The results presented refer to cells incubated without serum.

3.1. Effect of leuprolide and bicalutamide on cytokine secretion by non-stimulated PBMC

Incubation of PBMC with increasing concentrations of either leuprolide or bicalutamide between 10^{-8} M and 10^{-6} M had no significant effect on IL-1β, IL-6, IL-1ra and IL-10 secretion ($P > 0.8$, $P > 0.8$, $P > 0.4$, $P > 0.3$, respectively) (Table 1).

3.2. Effect of leuprolide and bicalutamide on cytokine secretion by LPS-stimulated PBMC

LPS-stimulated PBMC secreted significantly higher concentrations of cytokines as compared with non-stimulated cells (Table 2).

Table 1
Effect of leuprolide and bicalutamide on cytokine secretion by non-stimulated PBMC.

	IL-1β, ng/mL *(n = 15)	IL-6 ng/mL *(n = 10)	IL-1ra, ng/mL *(n = 11)	IL-10, pg/mL *(n = 11)
0	0.91 ± 0.24	10.05 ± 3.34	1.29 ± 0.21	275 ± 33
Leuprolide 10^{-8} M	1.19 ± 0.27	12.84 ± 3.91	0.92 ± 0.04	240 ± 26
Leuprolide 10^{-7} M	0.91 ± 0.22	9.7 ± 3.21	1.4 ± 0.23	320 ± 54
Leuprolide 10^{-6} M	1.07 ± 0.28	12.01 ± 3.39	1.23 ± 0.19	302 ± 38
Bicalutamide 10^{-8} M	1.10 ± 0.27	11.2 ± 3.78	1.09 ± 0.17	278 ± 71
Bicalutamide 10^{-7} M	1.23 ± 0.28	11.34 ± 3.54	1.15 ± 0.2	266 ± 26
Bicalutamide 10^{-6} M	1.07 ± 0.27	12.2 ± 3.65	0.9 ± 0.25	237 ± 20

*n represents the number of experiments.