Glucocorticoids Suppress Tumor Angiogenesis and In vivo Growth of Prostate Cancer Cells

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Abstract
Purpose: Glucocorticoids, such as prednisone, hydrocortisone, and dexamethasone, are known to produce some clinical benefit for patients with hormone-refractory prostate cancer (HRPC). However, the underlying mechanisms by which glucocorticoids affect HRPC growth are not well established as yet. Here, we hypothesize that the therapeutic effect of glucocorticoids on HRPC can be attributed to a direct inhibition of angiogenesis through the glucocorticoid receptor by down-regulating two major angiogenic factors, vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8).

Experimental Design: The effects of dexamethasone on VEGF and IL-8 expression and cell proliferation were examined using DU145, which expresses glucocorticoid receptor. The effects of dexamethasone on DU145 xenografts were determined by analyzing VEGF and IL-8 gene expression, microvessel density, and tumor volume.

Results: Dexamethasone significantly down-regulated VEGF and IL-8 gene expression by 50% (P < 0.001) and 89% (P < 0.001), respectively, and decreased VEGF and IL-8 protein production by 55% (P < 0.001) and 74% (P < 0.001), respectively, under normoxic condition. Similarly, hydrocortisone down-regulated VEGF and IL-8 gene expression. The effects of dexamethasone were completely reversed by the glucocorticoid receptor antagonist RU486. Even under hypoxia-like conditions, dexamethasone inhibited VEGF and IL-8 expression. In DU145 xenografts, dexamethasone significantly decreased tumor volume and microvessel density and down-regulated VEGF and IL-8 gene expression, whereas dexamethasone did not affect the in vitro proliferation of the cells.

Conclusion: Glucocorticoids suppressed androgen-independent prostate cancer growth possibly due to the inhibition of tumor-associated angiogenesis by decreasing VEGF and IL-8 production directly through glucocorticoid receptor in vivo.

Prostate cancer is the most common noncutaneous cancer and the second leading cause of cancer-related deaths in men in the United States (1), and the number of prostate cancer patients has been rapidly increasing in Japan (2). Prostate cancer is proposed to change the initial property of androgen-dependent growth to androgen-independent and finally hormone-refractory growth through hormonal therapy (3). Among several treatment options, glucocorticoids (i.e., prednisone, hydrocortisone, or dexamethasone) are known to produce some benefit for hormone-refractory prostate cancer (HRPC) patients partly due to the inhibitory effects on adrenal androgen production (4, 5). Accumulating evidence of clinical effects of glucocorticoids on HRPC showed a >50% decline in prostate-specific antigen levels in 20% to 79% of patients with HRPC (6–9). Glucocorticoids are, therefore, recognized to be one of a limited number of treatment options for HRPC. However, the distinct mechanism of glucocorticoids for HRPC remained ill defined.

Angiogenesis is a fundamental event in the process of tumor growth and metastatic dissemination (10, 11). Therefore, to elucidate the molecular basis of tumor angiogenesis has been important in the field of cancer research. The vascular endothelial growth factor (VEGF) pathway is well established as one of the key regulators of tumor growth and metastasis (12, 13), and interleukin-8 (IL-8) has also been well documented as a tumor angiogenesis-related gene (14–16). Recently, glucocorticoid receptor–mediated gene regulation has received considerable attention due to the link with cell proliferation and angiogenesis (17). Glucocorticoids were shown to interfere with the transcriptional activity of several transcriptional factors, such as nuclear factor-κB (NF-κB; refs. 17, 18) and activator protein-1 (17, 19). In prostate cancer, it was shown that blockade of NF-κB activity by transfection with a mutated inhibitor of NF-κB in the human prostate cancer cell line PC-3M was associated with suppression of angiogenesis, invasion, and metastasis in vivo (20).
Dexamethasone, a synthetic glucocorticoid, has been reported to inhibit DU145 prostate cancer growth by acting through the glucocorticoid receptor to interfere with the transcriptional activity of NF-κB (21).

Our group recently showed that glucocorticoids suppress VEGF gene expression and protein production in renal cell carcinoma cells in vitro (22). In the present study, we now postulate that the therapeutic effects of glucocorticoids on HRPC can be attributed to the direct inhibition of angiogenesis through glucocorticoid receptors by down-regulating two major angiogenic factors, VEGF and IL-8, in vitro and in vivo. To elucidate the mechanism by which dexamethasone suppresses HRPC growth in addition to its inhibitory effects on adrenal androgen production is an important initial step for designing strategies and identifying therapeutic targets for HRPC. To confirm this hypothesis, we examined the gene expression and protein secretion of VEGF and IL-8 by dexamethasone-treated prostate cancer cells using the androgen-independent prostate cancer cell line DU145, which is known to express functional glucocorticoid receptors (21). Furthermore, we tested this hypothesis in vivo in a xenograft model of a DU145 prostate cancer tumor by assessing tumor volume and intratumor microvessel density, which is known to be closely associated with tumorigenesis and metastases of various neoplasms, including prostate cancer.

Materials and Methods

Cell culture and drug treatment. Three human prostate cancer cell lines, DU145, PC-3, and LNCaP, were obtained from the American Type Culture Collection (Manassas, VA) and maintained in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 μg/mL streptomycin at 37°C and 5% CO2. For treatment, cells were plated at 2 × 10^4 per well in 96-well assay plates with 100 μL medium with 10% dexamethasone-coated charcoal-stripped FBS, 2 × 10^4 per well in 24-well plates with 500 μL medium, or 4 × 10^5 per dish in 60-mm dishes with 3 mL medium. After 12- to 24-hour incubation, cells were treated with 2% dexamethasone-coated charcoal-stripped FBS with or without the test agents. Dexamethasone, hydrocortisone, 5α-dihydrotestosterone, and RU486 were purchased from Sigma (St. Louis, MO). RU486 was used as a glucocorticoid receptor antagonist.

Hypoxia-like conditions were chemically created by exposure of cells to 100 μmol/L cobalt chloride (CoCl2; Sigma) treated at 37°C and 5% CO2 for 2 hours. Cellular responses to either hypoxia or CoCl2 have been shown to share a common mechanism for oxygen sensing, signal transduction, and transcriptional regulation in several previous reports (23, 24).

Reverse transcription-PCR. Total RNA was extracted from the prostate cancer cells using Isogene according to the manufacturer's instructions (Wako, Osaka, Japan). cDNA was synthesized using a ThermoScript RT System (Invitrogen Corp., Carlsbad, CA) with 2 μg total RNA from each of the prostate cancer cell lines (DU145, PC-3, and LNCaP) and produced in final volumes of 20 μL cDNA (1 μL) was amplified by PCR in a 20 μL reaction mixture containing 0.2 units Takara Taq (Takara Bio, Inc., Shiga, Japan), and 10 pmol/L each of sense and antisense primers. The primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were sense 5'-ACCACATGCATTGCGATACTC-3' and antisense 5'-TCACTGGAGTTCCCTTATT-3' and the primers for glucocorticoid receptor were sense 5'-CAGCCATACTGCTCACTCCT-3' and antisense 5'-CACAGCAGTGGTGGGAGT-3'. The cycling conditions for both glucocorticoid receptor and GAPDH were as follows: initial denaturation at 95°C for 5 minutes followed by 25 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 30 seconds. All primer sequences were determined to be in different exons. A PTC-200 DNA Engine (MJ Research, Waltham, MA) was used for thermal cycling. The PCR products were subjected to gel electrophoresis in a 2% agarose gel, stained with ethidium bromide, and observed with an UV transilluminator. The GAPDH and glucocorticoid receptor PCR product lengths were 452 and 344 bp, respectively.

Real-time quantitative PCR. To evaluate the expression levels of angiogenesis-related genes [VEGF, IL-8, and basic fibroblast growth factor (bFGF)], the ratio of each mRNA to the GAPDH mRNA copy number was measured with real-time quantitative PCR using a LightCycler System and SYBR Green I dye (Roche Molecular Systems, Indianapolis, IN). The VEGF, IL-8, bFGF, and GAPDH primers and their standard cDNAs were obtained from Search-LC (Heidelberg, Germany). The reaction mixture contained 2 μL LC DNA Master SYBR Green I, 2 μL of each LightCycler Primer Set (Search-LC), and 5 μL cDNA, which was diluted form reverse transcription products with H2O in a ratio of 1:20. The final volume was adjusted with H2O to 20 μL. PCR conditions were programmed according to the primer supplier’s instructions. Fluorescent products were measured by a single acquisition mode after each cycle. The expression of VEGF, IL-8, bFGF, and GAPDH in each sample was quantified in separate tubes. To distinguish specific and nonspecific products and primer dimers, a melting curve was obtained.

VEGF and IL-8 protein assays. Prostate cancer cells were cultured in 60-mm dishes at 4 × 10^5 per dish in RPMI 1640 containing 10% dexamethasone-coated charcoal-stripped FBS for 24 hours, after which the medium was replaced with fresh medium containing 2% dexamethasone-coated charcoal-stripped FBS with or without test agents. The conditioned medium was collected after 24 hours, centrifuged for 10 minutes at 3,000 × g, and stored at –80°C. VEGF and IL-8 concentrations were measured using VEGF (Human VEGF Quantikine kit; R&D Systems, Inc., Minneapolis, MN) and IL-8 (BioSource Europe IL-8 ELISA, BioSource Europe S.A., Nivelles, Belgium) ELISA kits according to the manufacturers' instructions, respectively.

Cell proliferation assays. The effects of glucocorticoids on cell proliferation were estimated with a 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt assay using the CellTititer 96 Aqueous One Solution Cell Proliferation Assay (Promega, Madison, WI), which is a colorimetric method for determining the number of viable cells. For treatment, cells were plated in 96-well plates at 2 × 10^4 per well in RPMI 1640 containing 10% dexamethasone-coated charcoal-stripped FBS. From the following day, cells were grown in medium supplemented with 2% dexamethasone-coated charcoal-stripped FBS with or without 100 nmol/L dexamethasone for 48 hours. Subsequently, the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt assay reagent was added to each well, and absorbance was measured after 2-hour incubation at 37°C using a THERMOMax microplate reader (Molecular Devices, Sunnyvale, CA). To gain accuracy, four wells were used for each sample.

In vivo xenograft model. Animal studies compiled with the Animal Welfare Regulation of Tokyo Medical and Dental University. To establish a DU145 tumor xenograft model, DU145 cells were detached with trypsin, and a mixture of RPMI 1640 with 10% FBS containing DU145 cells and Matrigel basement membrane matrix (BD Biosciences, Bedford, MA; 1:1 v/v) was prepared immediately before inoculation. The 1 × 10^7 cells were placed in the dorsal s.c. space of 6-week-old male BALB/c nu/nu nude mice. When the average tumor volume reached 200 to 300 mm3, –2 weeks after inoculation, mice were randomly assigned to a control or experimental group (n = 9 mice each). In the experimental group, each mouse was given a s.c. pertumor injection thrice weekly of 1 μg dexamethasone, which had been dissolved in ethanol and diluted 1:2,000 in 100 μL sterile saline, whereas, in the control group, ethanol diluted 1:2,000 in 100 μL sterile saline was injected. The
tumor volumes were measured weekly and calculated according the following formula: length × width² / 2. The mice were sacrificed 3 weeks after treatment, and the tumors were removed and then either frozen for mRNA extraction or fixed in 10% buffered formalin for immunohistochemical analysis.

**Immunohistochemical analysis of microvessel density.** Paraffin-embedded sections (5 μm thick) from DU1145 xenograft tumors were placed on slides, deparaffinized, and rehydrated. Subsequently, the sections were microwaved thrice at 500 W for 5 minutes to improve antigen retrieval and incubated with 3% (v/v) hydrogen peroxide in PBS for 20 minutes at room temperature to inhibit endogenous peroxidase activity. After the sections were blocked with 10% goat serum albumin for 30 minutes at room temperature, they were incubated for 1 hour at room temperature with a rat monoclonal anti-mouse CD34 antibody (Abcam Ltd., Cambridge, United Kingdom) at a concentration of 10 μg/mL in PBS. The sections were then rinsed thrice with PBS, incubated with goat anti-rat secondary antibody diluted 1:100 in PBS (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) for 30 minutes at room temperature, and exposed to the avidin-biotin-peroxidase complex (R.T.U. Vectastain ABC Reagent, Vector Laboratories, Inc., Burlingame, CA) according to the manufacturer’s protocol. The sections were visualized by incubating the slides with 3,3′-diaminobenzidine (Histofine Simple Stain DAB solution, Nichirei, Tokyo, Japan) as chromogen and counterstained with Meyer’s hematoxylin (MUTO Pure Chemicals Co., Ltd., Tokyo, Japan). Negative controls were included by replacement of the primary antibody with PBS and showed no specific staining.

Microvessel density was assessed by procedures as reported previously (25, 26). Briefly, areas of highest neovascularization were found by scanning the tumor sections with light microscopy at low magnification (∼×40 and ×100). After areas of highest neovascularization were identified, individual microvessel counts were made on a ×200 field (∼×20 objective and ×10 ocular, 0.74 mm²/field). Any brown-stained endothelial cell or endothelial cluster, clearly separated from adjacent microvessels, tumor cells, and other connective-tissue elements, was considered a single, countable microvessel. The results were independently reviewed by two blinded authors (Y.F. and A.I.) and two areas of highest microvessel density identified within any single ×200 field were selected from each sample. Data were expressed as the average of these highest numbers.

**Statistical analysis.** For multiple comparisons, the significant difference was analyzed using one-way ANOVA followed by a multiple comparison Dunetti’s test. For single comparisons, the level of statistical significance was confirmed using Student’s t test. Data represent the mean and 95% confidence intervals (95% CI) in three independent experiments. All P < 0.05 were considered statistically significant. All statistical tests were two sided. Statistical analysis was done with JMP 5.0 software (SAS Institute, Cary, NC).

**Results**

**Glucocorticoid receptor expression profile and effects of dexamethasone on cell proliferation of human prostate cancer cell lines.** The hypothesis of this study was that the therapeutic effect of dexamethasone on HRPC can be directly attributed to the inhibition of angiogenesis. To confirm this, we analyzed the gene expression and protein secretion by dexamethasone-treated prostate cancer cells for VEGF and IL-8, which have been implicated in angiogenesis, tumorigenesis, and malignant potential of various neoplasms, including prostate cancer. We first examined glucocorticoid receptor mRNA expression in the human prostate cancer cell lines DU1145, PC-3, and LNCaP by reverse transcription-PCR (RT-PCR). DU1145 and PC-3 expressed glucocorticoid receptor mRNA, whereas LNCaP lacked the glucocorticoid receptor (data not shown), which was consistent with a previous study (21). In the next set of experiments, we analyzed the possible growth-inhibitory effect of dexamethasone on DU1145 cells
in vitro. 3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt assays revealed that dexamethasone did not affect any cell proliferation under these experimental conditions (Fig. 1). Similarly, in PC-3 and LNCaP cells, dexamethasone did not produce any effect on cell proliferation (data not shown).

**Effects of dexamethasone on angiogenesis-related gene expression and protein secretion by prostate cancer cells in vitro.** We next analyzed the inhibitory effects of dexamethasone on the expressions and protein productions in these prostate cancer cell lines of angiogenesis-related genes: VEGF, IL-8, and bFGF. VEGF and IL-8 mRNAs in these three prostate cancer cells treated with or without 100 nmol/L dexamethasone for 12 hours were quantified by real-time quantitative RT-PCR. The protein levels in the culture medium treated with or without 100 nmol/L dexamethasone for 24 hours were shown by ELISA. As shown in Fig. 2, in DU145 cells, the statistically significant down-regulation of VEGF mRNA expression by 50% (95% CI, 38-62; \( P < 0.001 \)) and protein production by 55% (95% CI, 54-5; \( P < 0.001 \)) were observed in dexamethasone-treated group compared with the control group. There was also a significant inhibitory effect on IL-8 mRNA expression by 89% (95% CI, 85-93; \( P < 0.001 \)) and protein production by 74% (95% CI, 71-77; \( P < 0.001 \)) compared with the control group. In PC-3 cells, a significant decrease in IL-8 mRNA expression by 35% (95% CI, 28-42; \( P = 0.001 \)) and protein production by 34% (95% CI, 32-36; \( P = 0.002 \)) were seen compared with the control group, whereas no significant differences were seen for VEGF. In addition, bFGF mRNA in these prostate cancer cells treated with or without 100 nmol/L dexamethasone for 12 hours was quantified by real-time quantitative RT-PCR. Dexamethasone down-regulated bFGF mRNA expression by 26% (95% CI, 9-43; \( P = 0.006 \)) in DU145 cells but not in PC-3 cells (data not shown). The DU145 cell line was chosen for subsequent experiments because it expresses the glucocorticoid receptor and dexamethasone exerted an inhibitory effect on all angiogenesis-related genes tested in DU145 cells. LNCaP lacked the glucocorticoid receptor, and neither VEGF nor IL-8 was suppressed by dexamethasone treatment in LNCaP cells. bFGF mRNA level in LNCaP cells was extremely low in dexamethasone-treated and the control groups.

Now, we examined in DU145 cells whether dexamethasone suppresses two major angiogenic factors, VEGF and IL-8 expression, in a concentration-dependent manner at the mRNA

**Fig. 3.** Effects of dexamethasone on VEGF and IL-8 mRNA expression and protein production in DU145 cells in a concentration- and time-dependent manner. DU145 cells were treated with dexamethasone at concentrations of 1 to 1,000 nmol/L for 12 hours for mRNA expression analysis and 24 hours for protein production analysis. mRNA and protein was quantified by real-time quantitative RT-PCR and ELISA, respectively. **A and B**, quantification of VEGF and IL-8 expression in a concentration-dependent manner, respectively. Columns, mean of three independent experiments; bars, 95% CI. *, \( P < 0.01 \), versus the control (0 nmol/L) group. **C and D**, time course of VEGF and IL-8 mRNA expression in DU145 cells after treatment with dexamethasone, respectively. DU145 cells were treated with 100 nmol/L dexamethasone for 2 to 24 hours. Columns, mean of three independent experiments; bars, 95% CI. *, \( P < 0.01 \), versus the control (0 hour) group.
and protein levels. DU145 cells were treated with dexamethasone at concentrations of 1 to 1,000 nmol/L for 12 hours for analysis of VEGF and IL-8 mRNA expression and for 24 hours for analysis of protein production in culture medium. The inhibitions on the VEGF and IL-8 mRNA level paralleled the inhibition on the protein level at concentrations of 10 to 1,000 or 1 to 1,000 nmol/L dexamethasone, respectively, in a concentration-dependent manner (Fig. 3A and B).

Subsequently, we tested whether dexamethasone exhibited a time-dependent inhibitory effect on VEGF and IL-8 expression. DU145 cells were treated with 100 nmol/L dexamethasone for 2 to 24 hours and mRNAs were measured by real-time quantitative RT-PCR. VEGF and IL-8 mRNA expression was significantly suppressed after 2 hours and VEGF expression was maximally suppressed at 24 hours by 61% (95% CI, 59-63; \( P < 0.001 \)) compared with the control group, respectively, as shown (Fig. 4A and B). The low dose of dexamethasone used in this study, 1 nmol/L, maximally suppressed at 24 hours by 61% (95% CI, 59-63; \( P < 0.001 \)) compared with the control group, respectively, as shown (Fig. 4A and B).

We next examined the effects of dexamethasone on the suppression of VEGF and IL-8 expression through the glucocorticoid receptor antagonist RU486 at concentration of 100 nmol/L reversed the inhibitory effects of dexamethasone on VEGF and IL-8 mRNA expression. RU486 completely reversed the suppression of VEGF and IL-8 mRNA levels by dexamethasone in DU145 cells. Without dexamethasone, RU486 did not affect VEGF or IL-8 expression in DU145 cells (data not shown).

Effects of dexamethasone on DU145 cells under hypoxia-like conditions. We next assessed the effects of dexamethasone on chemically induced hypoxia-like conditions by 100 nmol/L CoCl\(_2\). DU145 cells were treated with 100 nmol/L dexamethasone and/or 100 nmol/L CoCl\(_2\) for 12 hours. Even under hypoxia-like conditions, dexamethasone significantly suppressed VEGF mRNA expression by 45% (95% CI, 38-53; \( P < 0.001 \)) and IL-8 mRNA expression by 76% (95% CI, 71-82; \( P < 0.001 \)) compared with the control group, respectively, as shown (Fig. 4A and B). In addition, dexamethasone and hydrocortisone down-regulated IL-8 mRNA expression by 84% (95% CI, 81-87; \( P < 0.001 \)) and 76% (95% CI, 74-77; \( P < 0.001 \)) compared with the control group, respectively. By contrast, 5α-dihydrotestosterone exhibited no inhibitory effect on VEGF and IL-8 expression (data not shown).

Effects of dexamethasone on tumor angiogenesis and growth in DU145 xenograft models. In the last set of experiments, we examined whether dexamethasone could inhibit the in vivo growth and neovascularization of androgen-independent DU145 prostate tumors. The low dose of dexamethasone used in this study, 1 \( \mu \)g/mouse thrice weekly, was based on a previous report (21). The administration of low-dose dexamethasone is widely considered to be of clinical benefit to patients with HRPC.

To examine the effect of dexamethasone on the in vivo growth of DU145 tumors, we initiated the treatment ~2 weeks after the inoculation when the mean tumor volume reached 200 to 300 mm\(^3\) to confirm the accomplishments of appropriate inoculation. Three weeks after the initiation of treatment, the mean tumor volume in the dexamethasone-treated mice was 671 mm\(^3\) (95% CI, 467-875), significantly smaller (\( P = 0.005 \)) than that in the control mice (1278 mm\(^3\); 95% CI, 1,020-1,536; Fig. 5A and B).

We next analyzed angiogenesis-related gene expressions in tumors to confirm the decrease in transcription levels of VEGF and IL-8 in vivo. VEGF and IL-8 mRNA expression in the dexamethasone treatment group was significantly lower by 20% (95% CI, 9-31; \( P = 0.009 \)) and 19% (95% CI, 4-33; \( P = 0.04 \)) compared with the control group, respectively (Fig. 5C and D).

![Image](https://www.aacrjournals.org/clinics2006;12(10)/fig4a.png)

**Fig. 4.** Effects of dexamethasone on VEGF and IL-8 mRNA expression in DU145 cells under hypoxia-like conditions. Hypoxia-like conditions were chemically induced by exposure of cells to 100 \( \mu \)mol/L CoCl\(_2\). In addition to the treatment with or without CoCl\(_2\) (CoCl\(_2(+)/C0\)(-)), DU145 cells were treated with or without 100 nmol/L dexamethasone (DEX(+)/C0(+)) at 37°C and 5% CO\(_2\) for 12 hours. mRNA was quantified by real-time quantitative RT-PCR. A and B, quantification of VEGF and IL-8 mRNA expression, respectively. Columns, mean of three independent experiments; bars, 95% CI. * \( P < 0.01 \), versus the control [CoCl\(_2(-)/C0(-)]\) group; **, \( P < 0.005 \), versus the control [CoCl\(_2(-)/C0(-)]\) group.
Finally, we counted microvessel density in the tumors to determine whether in vivo administration of dexamethasone affected the intratumor neovascularization. We immunostained tumors excised from mice with an antibody against CD34 to detect vascular endothelial cells. Microvessel density in the dexamethasone treatment group was 30 microvessel counts (95% CI, 27-33), which was significantly lower ($P < 0.001$) than that in the control group (42 microvessel counts; 95% CI, 37-47) per 200 field (Fig. 6A and B).

Discussion

In this study, we have shown that the effects of glucocorticoids are directly through the glucocorticoid receptor and suppress two major angiogenic factors, VEGF and IL-8, in androgen-independent prostate cancer cells. Additionally, in the xenograft model, dexamethasone treatment suppressed intratumor VEGF and IL-8 gene expression, angiogenesis, and in vivo tumor growth. Previous reports have shown that these angiogenesis-related gene expressions are positively correlated with tumor angiogenesis, metastasis, and poor prognosis in various neoplasms, including prostate cancer (26–29). Our results, therefore, support the hypothesis that the therapeutic effect of glucocorticoids on HRPC can be directly attributed to the inhibition of angiogenesis by a reduction in both VEGF and IL-8. Tumor angiogenesis under hypoxic conditions is a critical step in tumor development (30, 31). Importantly, our results revealed that, even under hypoxia-like conditions, dexamethasone down-regulated both VEGF and IL-8 expression to a similar extent as seen under normoxic condition. The interplay between hypoxia and glucocorticoid-mediated signal pathways responsible for angiogenesis has been focused on in recent years (32–34). Various lines of evidence have shown that hypoxia up-regulates VEGF predominantly via hypoxia-inducible factor-1 (31, 35), whereas hypoxia up-regulates IL-8 via cooperation of NF-$kappa$B and activator protein-1 (36, 37). Interestingly, we have shown that in DU145 cells the exposure to hypoxia induced the...
up-regulation of VEGF but not IL-8. Therefore, it is likely that, in DU145 cells, hypoxia predominately mediates the hypoxia-inducible factor-1-dependent pathway rather than NF-κB and activator protein-1 signaling pathways. Additionally, hypoxia-inducible factor-1 activation seems not to play a direct role for IL-8 induction, as the hypoxia-inducible factor-1-binding motif can be found in the published sequence of the VEGF promoter region but not the IL-8 promoter region.

In the *in vitro* study, we have shown the growth-inhibitory effect of dexamethasone on glucocorticoid receptor–positive DU145 tumors in the xenograft model, whereas *in vivo* we did not observe a cell growth inhibition by dexamethasone. Recently, it was shown that dexamethasone directly suppressed the *in vitro* growth of DU145 and PC-3, both of which express glucocorticoid receptors (21). The discrepancy between our and their results might be due to the different phenotypes of cell lines used. Notably, in their study, the inhibitory effects of dexamethasone on DU145 cell growth were more evident *in vitro* (~50%) than *in vivo* (20%), suggesting the existence of other mechanisms in addition to the direct inhibitory effect on cell growth. Therefore, our results strongly support the mechanism to be at least in part a suppression of angiogenesis, which is known to be closely associated with tumor growth and metastasis in various tumors. However, prostate cancer is known to consist of heterogeneous population of cancer cells and may possess clinically distinct biological features from DU145 cells.

Glucocorticoids are not experimental agents but have long been clinically used in the treatment of numerous diseases, including rheumatoid arthritis, systemic lupus erythematosus, and arteriosclerosis (38). In addition, low-dose dexamethasone therapy was recently found to be beneficial in the treatment of HRPC (8, 9). Low doses of dexamethasone induced significant symptomatic improvements and decreased prostate-specific antigen levels with mild adverse effects. In this study, VEGF and IL-8 down-regulation was shown at concentrations achievable *in vivo* by oral administration of low-doses (1-2 mg/d) of dexamethasone.

In conclusion, the present study indicates that the growth-inhibitory effect of glucocorticoids is possibly through the reduction of tumor-associated angiogenesis by a down-regulation of VEGF and IL-8 directly through the glucocorticoid receptor pathway. The glucocorticoids-glucocorticoid receptor complex seems to be the most likely mediator of VEGF and IL-8 expression in tumor cells. The clinical use of glucocorticoids as an angiogenesis inhibitor in combination with anticancer agents, such as docetaxel, may enhance the therapeutic effect on HRPC.

References

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