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Combined effects of terazosin and genistein on a metastatic, hormone-independent human prostate cancer cell line

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ABSTRACT

Metastatic prostate cancer progresses from androgen-dependent to androgen-independent. Terazosin, a long-acting selective α1-adrenoreceptor antagonist, induces apoptosis of prostate cancer cells in an α1-adrenoreceptor-independent manner, while genistein, a major soy isoflavone, inhibits the growth of several types of cancer cells. The present study was designed to test the therapeutic potential of a combination of terazosin and genistein using a metastatic, hormone-independent prostatic cancer cell line, DU-145.

Terazosin or genistein treatment inhibited the growth of DU-145 cells in a dose-dependent manner, whereas had no effect on normal prostate epithelial cells. Addition of 1 μg/ml of terazosin, which was inactive alone, augmented the growth inhibitory effect of 5 μg/ml of genistein. Co-treatment with terazosin resulted in the genistein-induced arrest of DU-145 cells in G2/M phase being overridden and an increase in apoptotic cells, as evidenced by procaspase-3 activation and PARP cleavage. The combination also caused a greater decrease in the levels of the apoptosis-regulating protein, Bcl-X_L, and of $VEGF_{165}$ and $VEGF_{121}$ than genistein alone.

In conclusion, the terazosin/genistein combination was more effective in inhibiting cell growth and VEGF expression as well as inducing apoptosis of the metastatic, androgen-independent prostate cancer cell line, DU-145, than either alone. The doses used in this study are in lower and nontoxic anticancer dosage range, suggesting this combination has potential for therapeutic use.

1. Introduction

Prostate cancer is a leading cause of cancer-related deaths in men [1,2]. Mortality results from metastasis to the bone and lymph nodes and progression from androgen-dependent to androgen-independent prostatic growth [3]. Radiation therapy is curative for localized disease, but there is no treatment for metastatic prostate cancer [1]. Terazosin is a long-acting selective α1-adrenoceptor antagonist that is used clinically to provide acute relief of the obstructive symptoms associated with benign prostatic hypertrophy (BPH) [4–6] and recent studies have shown that it induces apoptosis of prostate epithelial and smooth muscle cells in patients with BPH [7–10]. It also induces apoptosis of prostate cancer cells via an α1-adrenoreceptor-independent mechanism [11–17] and has anti-angiogenic effects in the human prostate [18–21]. These findings provide the rationale for the development of an effective therapeutic strategy using terazosin for patients

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with androgen-dependent or androgen- independent prostate cancer.

Epidemiological studies have shown that, in Asia, the decreased occurrence of cancers, including prostate cancer, is associated with consumption of soy [22,23]. Soy isoflavones are natural chemoprotectors against cancer and are not toxic for normal cells [24]; genistein (5,7,4′-trihydroxyisoflavone) is one of the predominant compounds of soy isoflavones [24,25]. Genistein inhibits cell growth both in several types of cancer, including prostate [26,27], breast [28,29], lung [30], bladder [31,32], and liver [33,34] cancers, and in BPH [35] and inhibits angiogenesis in tumors [36].

In an attempt to reduce the therapeutic dosage of terazosin so as to decrease its toxicity in prostate cancer treatment, we tested the effect of combining it with genistein, using a hormone-independent prostate cancer cell line, DU-145. The anti-angiogenesis and apoptosis-related proteins are considered to be the common downstream effectors mediating the effects of terazosin and isoflavones in prostate cancer, those were examined to evaluate therapeutic efficacy.

2. Materials and methods

2.1. Cell culture and viability assay

The DU-145 cell line, an androgen-independent tumor cell type, derived from a human prostate carcinoma, was obtained from the American Type Culture Collection (Rockville, MD). The cells were maintained in MEM (GibcoBRL, Grand Island, NY) containing 10% fetal bovine serum (GibcoBRL, Grand Island, NY). Normal human prostate epithelial cell (PrEC) was obtained from Clonetics (San Diego, CA) and maintained according to the manufacturer's instructions using PrEGM medium. PrEC cells were used at passage 3–6.

Cells were seeded in each well of a 24-well culture plate (Corning, New York, USA) and grown at 37 °C in a 5% CO_2 incubator. After 24 h incubation, the cells were treated with terazosin (Sigma, St. Louis, MO) and/or genistein (GibcoBRL, Grand Island, NY) for 3 days, and then cell number was counted with crystal violet elution assay for viability, and expressed as a percentage of that of the corresponding control group. Terazosin was dissolved in distilled water. Genistein was dissolved in dimethyl sulfoxide (DMSO, Sigma, St. Louis, MO), the final DMSO concentration being less than 0.5% (v/v); the same concentration of DMSO was added to the controls.

2.2. Cell cycle analysis

For 48 h with or without terazosin and/or genistein treatment, the distribution of cells in different stages of the cell cycle was estimated by flow cytometric DNA analysis, as described previously [31]. A minimum of 1×10^4 cells per sample was evaluated by a Elite-Esp flow cytometry (Miami FL, US) in each case. The percentage of cells in each cell cycle phase (Sub-G1, G0/G1, S, or G2/M) was calculated using Cell FIT research software (Becton-Dickinson, Mountain View, CA).

2.3. Detection of apoptosis by flow cytometry and fluorescence microscopy

TUNEL staining was performed following the protocol recommended in the commercial kit (Boehringer, Mannheim, Germany). Apoptotic cells were also detected by fluorescence microscopy using Hoechst 33342 dye (Sigma, St. Louis, MO) to label the nuclei and propidium iodide to stain DNA as described previously [37].

2.4. Western blot analysis

Cytosolic extracts were prepared from cells and the protein in the supernatant was quantified using a protein assay kit (Bio-Rad Laboratories, Hercules, CA). A sample (60 μg) was electrophoresed on 12% SDS–polyacrylamide gels then transferred to nitrocellulose membranes. The rabbit polyclonal antibodies against human PARP,

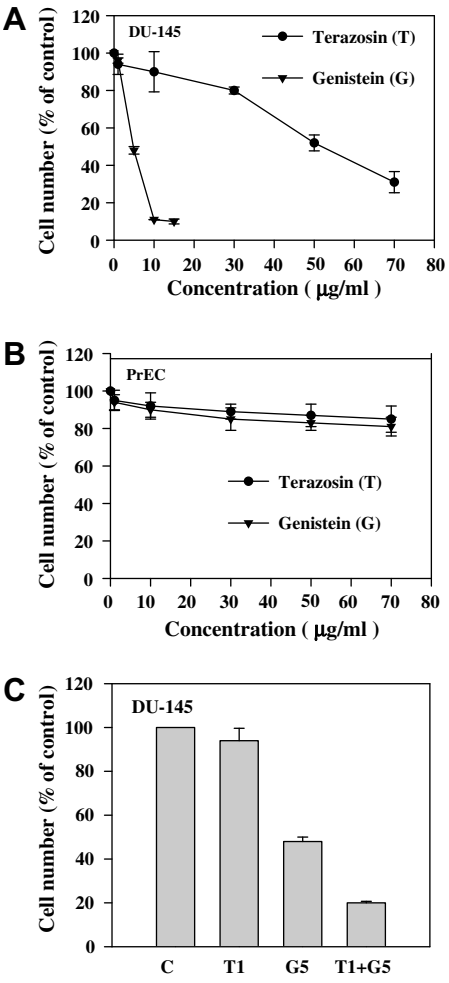

Fig. 1. Effects of genistein and terazosin on DU-145 and PrEC cell growth. Triplicate samples of cells were incubated for 3 days with different concentrations of genistein (G) or terazosin (T) alone (A and B) or in combination (C). $^{*}P < 0.05$ compared to the untreated group. $^{\#}P < 0.05$ compared to the genistein-treated group.