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Rapid Communication

Apoptotic Activity of Doxazosin on Prostate Stroma In Vitro Is Mediated Through an Autocrine Expression of TGF-β1

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BACKGROUND. Doxazosin, an alpha-adrenergic antagonist, has been shown to induce apoptosis in prostatic stromal cells. The mechanism of this apoptotic action by Doxazosin remains undefined. The present study was carried out to demonstrate that the effect of Doxazosin on apoptosis of prostate stromal cells is mediated through an autocrine action of TGF-β1.

METHODS. Primary cultures of human prostate cells were treated with varying concentrations of Doxazosin (0, 0.1, 1, 10, and 100 μM) for a period up to 3 days. At the end of the 3-day culture, cell numbers were counted. Apoptosis was assessed by a colorimetric terminal deoxyribonucleotide transferase labeling technique. TGF-β1 was determined by enzyme-linked immunosorbent assay (ELISA).

RESULTS. Compared to control cultures, cell numbers were significantly decreased as much as 68.4% in cultures treated with 10 μM of Doxazosin after 3 days incubation, while apoptosis increased by 64.7% in cultures treated with the same concentration of Doxazosin after 24 h. This decrease in cell number was reversed when antibody to TGF-β1 was added to these cultures. Addition of TGF-β1 (0, 1.0, and 10 ng/mL) to the cultures also decreased the cell numbers. Quantitation of TGF-β1 in lysates of cells by ELISA revealed that the cells treated with Doxazosin (10 μM) produced as much as 62.5% more TGF-β1 than in that of untreated cells.

CONCLUSIONS. These results demonstrate that the apoptotic effect of Doxazosin on human prostatic stromal cells is mediated through an autocrine production of TGF-β1.
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KEY WORDS: **stromal cells; Doxazosin; TGF-β1; apoptosis**

INTRODUCTION

The prostate is richly innervated with autonomic nerves that include both adrenergic and cholinergic fibers [1]. These nerves play an important role in prostatic secretion and in smooth muscle contraction [2]. Denervation of the prostate results in a loss of functional and structural integrity of the gland [3,4]. The prostate expresses four native alpha-1-adrenoreceptors of which the alpha 1a subtype is related to smooth muscle contraction [5]. The use of alpha-adrenoreceptor antagonists in the treatment of benign prostatic hyperplasia (BPH) is based on their ability to

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prevent neural stimulation that induces prostate smooth muscle contraction, producing lower urinary tract symptoms. However, reports suggest that adrenoreceptors can mediate additional biological functions, such as mitogenesis and apoptosis [6–8].

Doxazosin is an alpha-adrenergic antagonist, which inhibits contraction of differentiated smooth muscle cells of the prostate and also modulates differentiation of stromal smooth muscle cell, thus rendering the prostate less responsive to sympathetic stimulation [9]. In parallel to inhibition of smooth muscle contraction, recent evidence has also indicated that Doxazosin induces apoptosis in both epithelial and stromal cells of the prostate with little effect on cell proliferation [10,11]. The mechanism underlying this observed apoptosis remains undefined. Results of the present study provide evidence to indicate that the apoptotic effect of Doxazosin is, at least in part, mediated through an autocrine production of TGF-β1, which in turn promotes apoptosis in prostatic cells.

MATERIALS AND METHODS

Prostatic Tissues and Cell Culture

Human prostate stromal cells were cultured according to the methods reported by Kassen et al. [12]. Briefly, prostatic tissue specimens were obtained from patients who underwent transurethral prostatectomy for relief of bladder neck obstruction secondary to BPH, following approval by the Institutional Review Board. The benign histology of the surgical specimens was confirmed following pathological evaluation. Prostatic tissues were cut with a sterile blade into 1.0 mm pieces, followed by enzymatic dissociation with DNAse and collagenase. Epithelial cells were separated from stromal cells by the discontinuous Percoll gradient centrifugation. The stromal cells obtained were then cultured in phenol-red-free RPMI-1640 supplemented with 10% fetal bovine serum (FBS).

Doxazosin Treatment

Stromal cells were plated in 48-well plates at a concentration of 20,000 cell/mL in phenol-red-free RPMI-1640 with 10% FBS. After 24 h, the medium was changed into serum-free RPMI-1640 supplemented with ITS (insulin, transferrin, and selenous acid) and Doxazosin (a gift from Pfizer Pharmaceuticals) at 0, 0.1, 1, 10, and 100 μM. After 3 days of incubation, the cells were harvested by trypsin digestion and counted in a Coulter counter. To determine the effect of Doxazosin on the autocrine production of TGF-β1 in these cells, cultures of prostatic stromal cells were treated with 10 μM of Doxazosin and with 10 μg/mL of specific antibody against TGF-β1 (Santa Cruz). In addition, cultures were also treated with TGF-β1 at different concentrations (0.01, 1.0, and 10 ng/mL) to determine its effect on growth of prostate stromal cells in culture.

Quantitation of Apoptosis

In addition to counting of cell number in each culture, the extent of apoptosis in the culture was evaluated. Stromal cells were plated in 96-well plate at a concentration of 20,000 cells/mL in phenol-red-free RPMI-1640 with 10% FBS. After 24 h, the cells were then treated with different concentrations of Doxazosin as before. To determine apoptosis in cells, a quantitative assay for the detection of DNA breakage, using the terminal deoxyribonucleotide transferase (TdT) color reaction assay (TiterTACS, Trevigen, Gaithersburg, MD), was employed. To study the effect of TGF-β1 on apoptosis of prostatic stromal cells, again, cells were plated at 20,000 cell/mL and treated with different concentrations of TGF-β1 for 24 h. Apoptosis was quantitated using the TiterTACS assay.

TGF-β1 Quantitation

To assess the amount of TGF-β1 produced by stromal cells with different treatment, the following procedure was carried out. Prostate stromal cells were seeded at 20,000 cells/mL in RPMI-1640 with 10% FBS in T-25 flasks and allowed to adhere for 24 h. The culture medium was then replaced with phenol-red-free RPMI-1640 supplemented with ITS with or without 10 μM Doxazosin. After 3 days of incubation, conditioned media and cell lysates were collected and assayed for TGF-β1 using an ELISA (enzyme-linked immunosorbent assay) kit (R and D Systems).

Statistical Analysis

All numerical data were expressed as mean ± standard error of the mean (SEM). Each experiment was conducted with at least four observations. One-way analysis of variance test (ANOVA) was carried out using the SigmaStat statistical package (Jandel Scientific). A P value of less than 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

The effect of Doxazosin on growth of human prostate stroma in culture is shown in Figure 1. Following a 3-day culture, Doxazosin inhibited growth of stromal cells. This effect of Doxazosin was at significant at 10 μM ($P < 0.05$). To determine if the decrease in cell number in Doxazosin-treated cells were due to programmed cell death, the TiterTACS

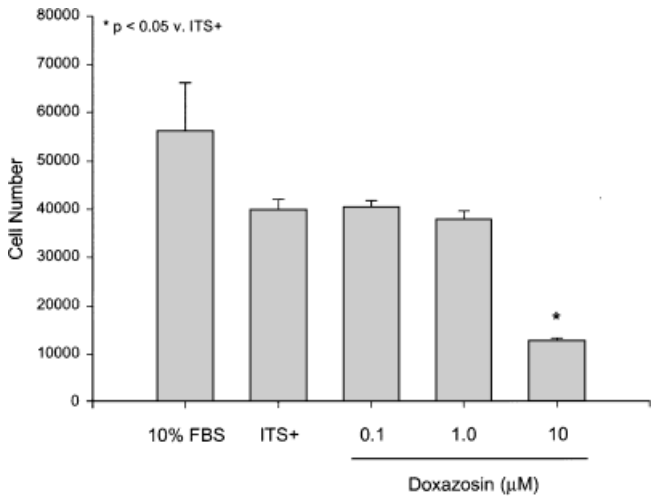

Fig. 1. Effect of Doxazosin on growth of human prostate stromal cells in culture. Primary cultures of human prostatic stromal cells were seeded into 48-well plates at a concentration of 20,000 cells/mL. Doxazosin was added into the serum-free culture medium at 0, 0.1, 1.0, 10, and 100 μM. After 3 days of incubation, cells were harvested and counted with a Coulter counter as described in the text. Vertical bars denote standard error of the mean. *Denotes that the value is significantly different from that of the ITS+ control ($P < 0.05$).

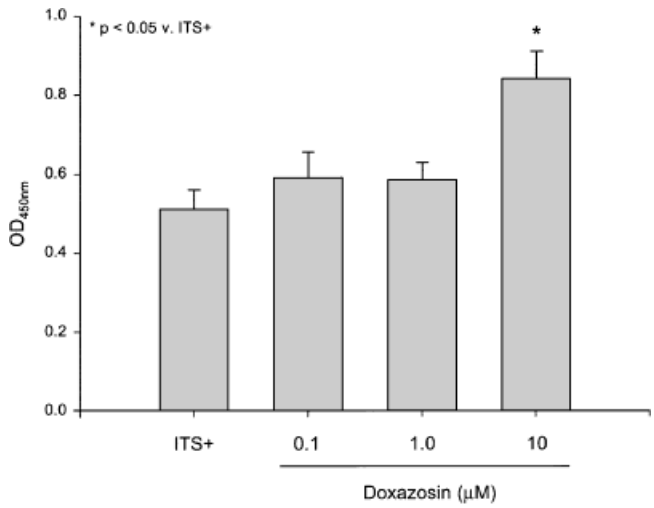

Fig. 2. Effect of Doxazosin on apoptosis of human prostate stromal cells in culture. Primary cultures of human prostatic stromal cells were seeded into 96-well plates at a concentration of 20,000 cells/mL. Doxazosin was added into the serum-free culture medium at 0, 0.1, 1.0, 10, and 100 μM. A quantitative assay for the detection of DNA breakage, using the terminal deoxyribonucleotide transferase (TdT) color reaction assay (TiterTACS, Trevigen), was employed to determine the extent of apoptosis in cells. Vertical bars denote standard error of the mean. *Denotes that the value is significantly different from that of the ITS+ control ($P < 0.05$).

assay was used to determine apoptosis quantitatively. The absorbance readings showed that cells treated with 10 μM Doxazosin for 24 h had the greatest number of cells that have undergone apoptosis (Fig. 2). These results confirm previous reports that Doxazosin has an inhibitory effect on prostatic stromal cells [10,11].

To test if this inhibitory effect of Doxazosin is mediated through an autocrine action of TGF-β1, a neutralizing antibody against TGF-β1 was added to cultures treated with Doxazosin. Figure 3 shows that the inhibition of prostatic stromal cells by Doxazosin could be reversed by addition of the specific antibody against TGF-β1. These findings support the concept that the Doxazosin-induced inhibition in prostatic stromal cells may be mediated through the action of TGF-β1. TGF-β1 is known to be inhibitory to growth of both prostatic epithelial and stromal cells [12–14]. In the present study, we verified that TGF-β1 treatment resulted in a decrease in cell number in cultures of human prostatic stromal cells (Fig. 4.). These observations are consistent with the recent report by Yang et al. [8] that Doxazosin and TGF-β1 had a synergistic effect on apoptosis of prostatic cells in their transgenic model.

Prostatic stromal cells are known to produce TGF-β1 [15]. However, it remains unclear if Doxazosin can induce these cells to produce this growth factor. Therefore, the following experiment was carried out to test if treatment with Doxazosin could lead to any

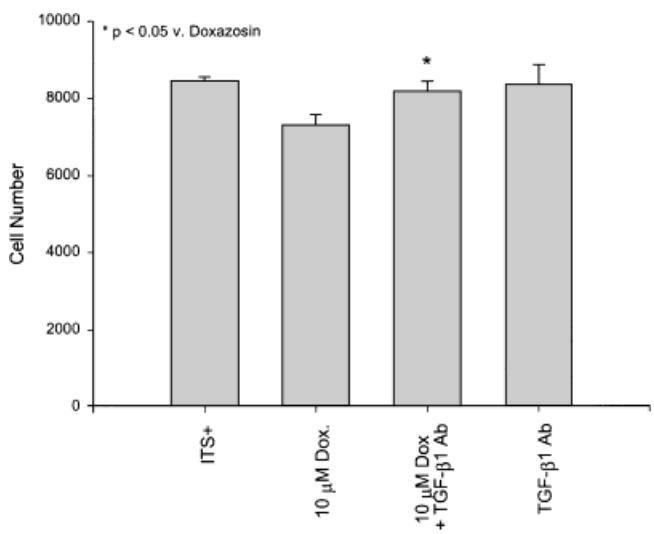

Fig. 3. Effect of Doxazosin and neutralizing antibody against TGF-β1 on prostate stromal cells in culture. Primary cultures of human prostatic stromal cells were seeded into 48-well plates at a concentration of 20,000 cells/mL. Doxazosin was added into the serum-free culture medium at 10 μM with or without the specific antibody against TGF-β1. Vertical bars denote standard error of the mean. *Denotes that the value is significantly different from that of the Doxazosin treatment alone ($P < 0.05$).

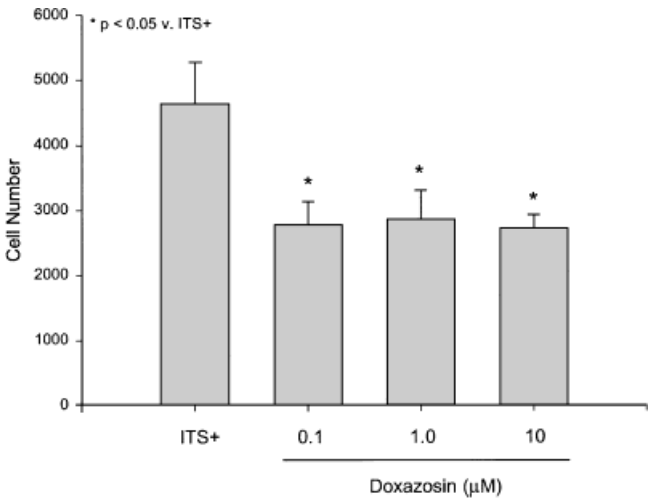

Fig. 4. Effect of TGF-β1 on growth of prostate stromal cells in culture. Primary cultures of human prostatic stromal cells were seeded into 48-well plates at a concentration of 20,000 cells/mL. TGF-β1 was added into the culture medium at 0, 0.1, 1.0, and 10 ng/mL. Twenty-four hours later, cells were harvested and counted. Vertical bars denote standard error of the mean. *Denotes that the value is significantly different from that of the ITS + control ($P < 0.05$).

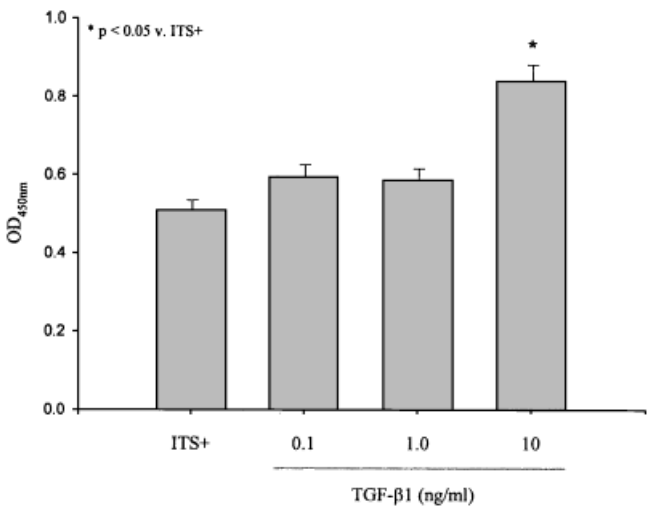

Fig. 5. Effect of TGF-β1 on apoptosis of prostate stromal cells in culture. Primary cultures of human prostatic stromal cells were seeded into 96-well plates at a concentration of 20,000 cells/mL. TGF-β1 was added into the culture medium at 0, 0.1, 1.0, and 10 ng/mL. Twenty-four hours later, cells were harvested for analysis of apoptosis. A quantitative assay for the detection of DNA breakage, using the terminal deoxyribonucleotide transferase (TdT) color reaction assay (TiterTACS, Trevigen) was employed to determine the extent of apoptosis in cells. Vertical bars denote standard error of the mean. *Denotes that the value is significantly different from that of the ITS + control ($P < 0.05$).

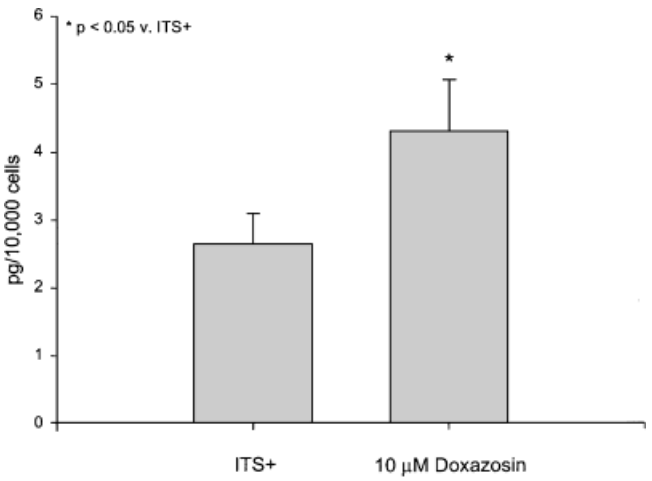

Fig. 6. Effect of Doxazosin on production of TGF-β1 in prostate stromal cells in culture. Primary prostatic stromal cells were seeded at 20,000 cells/mL in RPMI-1640 with 10% FBS in T-25 flasks and allowed to adhere for 24 h. The culture medium was then replaced with phenol-red-free RPMI-1640 supplemented with ITS, with and without Doxazosin at 10 μM. Three days later, conditioned media and cell lysates were assayed for TGF-β1 by ELISA. Vertical bars denote standard error of the mean. *Denotes that the value is significantly different from that of the ITS + control ($P < 0.05$)

increase in the production of TGF-β1 by human prostatic stromal cells. Figure 5 demonstrates that the level of TGF-β1 was increased by as much as 62.5% in cell lysates of cells treated with 10 μM Doxazosin (Fig. 5). To determine whether or not TGF-β1 can also induce apoptosis in prostatic stromal cells, this growth factor was added to cultures of primary stromal cells. Result showed significant apoptosis in wells treated with TGF-β1 at different concentrations (Fig. 6). Based on these results, it is conclude that the apoptotic effect of Doxazosin on prostate stromal cells in culture is mediated, at least, through an autocrine production of TGF-β1.

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