Suppression of Human Prostate Cancer Cell Growth By \( \alpha_1 \)-Adrenoceptor Antagonists Doxazosin and Terazosin via Induction of Apoptosis

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ABSTRACT

Recent evidence from our laboratory has demonstrated that \( \alpha_1 \)-adrenoceptor antagonists doxazosin and terazosin induced apoptosis in prostate epithelial and smooth muscle cells in patients with benign prostatic hypertrophy (BPH; J. Urol., 159: 1810–1815, 1998; J. Urol., 161: 2002–2007, 1999). In this study, we investigated the biological action of three \( \alpha_1 \)-adrenoceptor antagonists, doxazosin, terazosin, and tamsulosin, against prostate cancer cell growth. The antitumor effect of the three \( \alpha_1 \)-adrenoceptor antagonists was examined in two human prostate cancer cell lines, PC-3 and DU-145, and a prostate smooth muscle cell primary culture, SMC-1, on the basis of: (a) cell viability assay; (b) rate of DNA synthesis; and (c) induction of apoptosis. Our results indicate that treatment of prostate cancer cells with doxazosin or terazosin results in a significant loss of cell viability, via induction of apoptosis in a dose-dependent manner, whereas tamsulosin had no effect on prostate cell growth. Neither doxazosin nor terazosin exerted a significant effect on the percentage of apoptotic cells (14, 15). This marked antitumor effect against oncogene-induced prostate growth, without affecting their proliferative capacity (14, 15). This marked antitumor effect against oncogene-induced prostate growth, without affecting their proliferative capacity (14, 15).

INTRODUCTION

Prostate cancer threatens to become an American epidemic. It is the leading male malignancy in the United States, surpassing the once prevalent lung cancer status and a major contributor to cancer mortality among United States males, resulting in >40,000 annual deaths (1). Prostate cancer mortality results from metastasis to the bone and lymph nodes and progression from androgen-dependent to androgen-independent disease (2). Radical prostatectomy is considered curative for localized disease; however, no treatment for metastatic prostate cancer is available that effectively increases survival (3). A large percentage of prostate cancer patients present at an advanced stage of disease with only temporary, noncurative options available. Androgen ablation, the major therapeutic modality for advanced prostate cancer, is rarely curative because it is exclusively targeted against the androgen-dependent prostate cancer cell populations, whereas the androgen-independent cells survive this therapy and continue to grow (2). Combination regimes of hormonal ablation and chemotherapy failed to provide clinically convincing evidence of improvement in the therapeutic response (4). Considerable efforts have been directed recently toward drug design to selectively target apoptotic components that will have a significant impact on the therapeutic response while minimizing toxicity.

\( \alpha_1 \)-Adrenoceptors are members of the superfamily of G protein-coupled adrenergic receptors, which mediate actions of the endogenous catecholamines (norepinephrine and epinephrine), in a variety of target cells (5). Adrenoceptors coupled through G proteins modulate diverse intracellular processes, including activation of vascular smooth muscle contraction (6), promoting proliferative responses, such as DNA synthesis, probably through activation of mitogen-activating protein kinases in vascular smooth muscle cells (7) and modulation of cytoskeletal proteins in prostate smooth muscle cells (8). Currently, four native \( \alpha_1 \)-adrenoceptor subtypes have been identified, \( \alpha_1 \text{a}, \alpha_1 \text{b}, \alpha_1 \text{d}, \) and \( \alpha_1 \text{L} \). \( \alpha_1 \)-Adrenoceptors predominate in the prostate and bladder trigone (9, 10) and are believed to be functionally important in mediating prostate smooth muscle contraction (6, 10).

The therapeutic benefit of \( \alpha_1 \)-adrenoceptor antagonists in the medical treatment of BPH (11) is believed to be attributable to a direct action on \( \alpha_1 \)-adrenoceptors present in prostatic smooth muscle (11, 12). The documented durability of clinical response to \( \alpha_1 \) blockade in the face of ongoing hyperplasia (13), however, implies the existence of several loci on which \( \alpha_1 \)-antagonists could act. Recent studies from this laboratory have demonstrated that \( \alpha_1 \)-adrenoceptor antagonists may affect prostate pathophysiology by inducing apoptosis via mechanisms that transcend smooth muscle relaxation. Treatment of patients with BPH, with either of the two \( \alpha_1 \)-adrenoceptor antagonists doxazosin and terazosin, resulted in a significant induction of apoptosis among the epithelial and smooth muscle cells in the prostate gland without affecting their proliferative capacity (14, 15).

It is our hypothesis in this study that \( \alpha_1 \)-adrenoceptor antagonists suppress prostate cancer cell growth via induction of apoptosis. To test this hypothesis, we investigated the antitumor action of three clinically used \( \alpha_1 \)-adrenoceptor antagonists, doxazosin, terazosin, and tamsulosin, against human prostate tumor epithelial and smooth muscle cells. Our findings demonstrated that doxazosin and terazosin, but not tamsulosin, to induce apoptosis in human prostate cancer cells (within the clinically relevant therapeutic dose range), potentially via \( \alpha_1 \)-adrenoceptor-independent actions. These results may have significant therapeutic implications in identifying both doxazosin and
terazosin as potential antitumor agents in the treatment of advanced prostate cancer.

MATERIALS AND METHODS

Cell Culture. The following cell lines were used: (a) human prostate cancer cells PC-3 and DU-145 and human colon cancer cells SW-480 were obtained from American Type Culture Collection (Rockville, MD); (b) the human breast cancer cells MCF-7 were obtained from Dr. Angela Brodie (Department of Pharmacology, University of Maryland School of Medicine); (c) human bladder cancer cells HTB1 were generously provided by Dr. Michael Freeman (Children’s Hospital, Harvard Medical School); (d) human prostate smooth muscle cells SMC-1 (primary cultures) was a generous gift from Dr. Paul Walden (New York University; Ref. 17).

Drugs. The three clinically used α1-adrenoceptor antagonists used in this study were kindly donated by the following pharmaceutical companies: doxazosin (Cardura) was obtained from Pfizer (New York, NY); terazosin (Hytrin) was obtained from Abbott Labs (Chicago, IL); and tamsulosin (FLOMAX) from Yamanouchi Pharmaceuticals (Tokyo, Japan). The inhibitor, phenoxybenzamine HCl, was obtained from RBI (Research Biochemicals International, Natick, MA).

Cell Viability Assay. Subconfluent cultures of prostate cancer and smooth muscle cells (in six-well plates) were exposed (in triplicate) to increasing concentrations of doxazosin, terazosin, or tamsulosin (1–100 μM), and the number of viable cells was assessed after various treatment periods using the trypan blue exclusion assay. For the experiment using the irreversible inhibitor, cells were plated in six-well plates and at 60% density were treated with phenoxybenzamine (4 h), prior to exposure to increasing doses of α1-adrenoceptor antagonists. Values are expressed as the percentage of mean cell viability relative to the untreated cultures.

Rate of DNA Synthesis. The effect of α1-adrenoceptors on the rate of DNA synthesis in human prostate cells was evaluated using the [3H]thymidine uptake assay. Cells were exposed to increasing concentrations (1–100 μM) of doxazosin or terazosin for 2 days and were subsequently pulsed with [3H]thymidine (7 μCi/ml) for 4 h. After DNA precipitation with 10% trichloroacetic acid, the amount of [3H]thymidine incorporated was analyzed by liquid scintillation counting. Values were expressed as the percentage of inhibition of DNA synthesis in the treated, relative to the untreated, cultures.

Apoptosis Detection. Prostate cells were treated with doxazosin or terazosin (15 μM) for 1–3 days, and apoptotic cells were detected using the TUNEL assay (ApoTag fluorescein kit; Intergen, Purchase, NY). Cells are visualized using a fluorescence microscope (Axiovert-10 Zeiss model) and standard fluorescein excitation and emission filters. Quantitative analysis was performed by counting the green fluorescence-positive (FITC) cells under ×400 magnification.

PARP Assay. The PARP cleavage assay was used in cell lysates from cell cultures treated with doxazosin for various treatment periods. Western blot analysis was performed using a rabbit polyclonal anti-PARP antibody (Boehringer Mannheim, Indianapolis, IN) and an alkaline phosphatase detection kit according to the manufacturer’s instructions.

RT-PCR Analysis. RNA was extracted from prostate cancer cells and human prostate tissue, and RT-PCR was performed using 1 μg of total cellular RNA and the Superscript cDNA Preamplification System (Life Technologies, Inc., Gaithersburg, MD) in a Perkin-Elmer amplification cycler (Wellesley, MA) as described previously (17). The following primers, used for α1a-adrenoceptor expression, were obtained from Dr. Paul Walden (New York University Medical Center, NY): sense, 5’-ATATACCCCATGCTCAGGC-3’; antisense, 5’-GCTTTTACTTCTTACCCCG-3’. The primers for the human glyceraldehyde-3-phosphate dehydrogenase were obtained from Clontech (Palo Alto, CA) and the sequences were as follows: sense, 5’-TGAAGGRCGGAGTCAACGGATTGT-GT-3’; antisense, 5’-CATGTTGGGGCATGAGTCC-CACCAC-3’. The cycling conditions were the following: 94°C for 5 min; 94°C for 30 s; 52°C for 1 min; 72°C for 2 min (35 cycles); and 72°C for 7 min (1 cycle) for final extension. The amplified RT-PCR products were electrophoretically analyzed through 1% agarose gels and were visualized by ethidium bromide staining and photographed under UV illumination.

Tumorigenicity Studies. PC-3 prostate cells were inoculated (105 cells/site) in the flank of male immunodeficient mice (SCID), 4–6 weeks of age, and mice were maintained in a pathogen-free environment. Tumors were measured twice weekly using a digital caliper, and tumor volumes were calculated using the formula length × (width)2/2. At 7 days after implantation, mice were stratified into treatment groups of five mice/treatment, and treatment began on day 7. Doxazosin mesylate was administered in the following doses: 0, 3, 10, 100 mg/kg in sterile water, by oral gavage using a 22-gauge, 1.5-inch gavage needle. Animals were sacrificed after 2 weeks of treatment.
Statistical Analysis. *In vivo* data were analyzed by one-way ANOVA, followed by pairwise comparison using Fisher’s, Tukey’s, and Dunnett’s tests and using the nonparametric test, Kruskal Wallis. Data from *in vitro* experiments were analyzed using the Student’s *t* test. Values were expressed as the mean ± SE, and differences were considered statistically significant at *P* < 0.05.

RESULTS

The effect of doxazosin, terazosin, and tamsulosin on prostate cell growth was evaluated *in vitro* using two highly aggressive, androgen-independent human prostate cancer cell lines, PC-3 and DU-145, and a primary culture of human prostate smooth muscle cells, SMC-1. The dose response of cell viability of PC-3 prostate cells to three drugs is shown in Fig. 1A. The data indicate a significant loss of cell viability of human prostate cancer cells after 2 days of treatment with doxazosin and terazosin at concentrations ≥10 μM. Tamsulosin had no effect on prostate cancer cell viability (Fig. 1A). Fig. 1B documents the dose response of DU-145 prostate cancer cell viability to the three drugs. Interestingly enough, only doxazosin exerted a significant cell death effect (70% loss of cell viability at 25 μM), whereas terazosin and doxazosin had minimal antigrowth activity (at similar dose) in this prostate cell line (*P* > 0.05). Comparative analysis of the cellular response of the SMC-1 human prostate smooth muscle cells to doxazosin and terazosin revealed a relatively high sensitivity of these cells to the antigrowth effects of both α1-adrenoceptor antagonists in a concentration-dependent manner (Fig. 1C).

The effect of the three drugs doxazosin, terazosin, and tamsulosin on the rate of DNA synthesis of prostate cancer cells was also examined using the thymidine uptake assay. As shown in Fig. 2, doxazosin treatment resulted in a significant decrease in the rate of DNA synthesis at high concentrations in a dose-dependent manner in both cell lines PC-3 and DU-145. After 2 days of exposure to the drug (at doses of 50 and 100 μM), there was an ~40% inhibition of the rate of DNA synthesis in both PC-3 and DU-145 cells. Terazosin and tamsulosin, on the other hand, had no significant effect on the rate of DNA synthesis in either of the two prostate cancer cell lines examined at any of the tested doses (Fig. 2). A comparable profile, indicating no change in the rate of DNA synthesis in response to all three α1-

Table 1. Doxazosin-induced apoptosis in human prostate cells

Quantitative analysis of the apoptosis after treatment with doxazosin was performed by counting the TUNEL-positive cells within a field of 200 cells under ×400 magnification. Values represent mean percentage of the number of apoptotic cells in three different fields ± SE.

<table>
<thead>
<tr>
<th>Doxazosin treatment</th>
<th>% of TUNEL-positive cells</th>
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<tr>
<td></td>
<td>PC-3 cancer cells</td>
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<tr>
<td>Control (15 μM)</td>
<td>1.1 ± 0.3</td>
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<tr>
<td>1 day</td>
<td>2.2 ± 0.4</td>
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<tr>
<td>2 day</td>
<td>11.6 ± 1.6*</td>
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<tr>
<td>3 day</td>
<td>19.4 ± 2.5*</td>
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* Statistically different from the untreated control cells (*P* < 0.01).
adrenoceptor antagonists, was obtained for the prostate smooth muscle cells SMC-1 (data not shown).

Induction of apoptosis in doxazosin-treated prostate cells was assessed on the basis of the TUNEL assay (Fig. 3A). Table 1 summarizes the results from the quantitative evaluation of apoptosis induction in response to doxazosin treatment. A significant increase in the number of TUNEL-positive apoptotic cells was observed for both prostate cancer cells and smooth muscle cells after 2 days of treatment with doxazosin compared with the untreated control cultures. The apoptotic nature of the effect of doxazosin against prostate cancer cells was further confirmed by PARP cleavage assay. As shown in Fig. 3B, exposure of PC-3 prostate cancer cells to doxazosin (15 μM) was associated with cleavage of the PARP substrate (M, 115,000 protein) after 24 h of treatment, an effect that became more prominent with longer treatment periods.

The expression of the α1a-adrenoceptor mRNA in normal human prostate tissue and prostate cells was examined by RT-PCR analysis. As shown on Fig. 4, there is no detectable mRNA expression for α1a-adrenoceptor in the two prostate cancer cell lines PC-3 and DU-145; however, α1a-adrenoceptor mRNA is expressed in the normal human prostate and cardiac myocytes (used as a positive control; Fig. 4). Primary cultures of human smooth muscle cells apparently lose the expression of α1a-adrenoceptor after several passages in culture.4

The catecholamine neurotransmitter norepinephrine binds to α1-adrenoceptors located on the cell membrane and activates phospholipase, generating a second messenger that ultimately results in smooth muscle contraction (5). To determine whether the apoptotic effects of doxazosin and terazosin were an α1-mediated phenomenon, we subsequently tested the ability of an irreversible α1-adrenoceptor inhibitor, phenoxybenzamine, to interfere with the antitumor action of the two drugs. Exposure to phenoxybenzamine prior to treatment did not inhibit the apoptotic effect of doxazosin against human prostate cancer cells PC-3 (Fig. 5). Similar observations were obtained for the effect of α1-inhibitor in terazosin-treated prostate cancer cells (data not shown).

To establish that apoptosis induction in response to doxazosin and terazosin was specifically targeted against prostate tumor cells, in subsequent experiments we examined the effect of the two drugs against human cancer cell lines including breast cancer MCF-7 cells, colon cancer SW-480 cells, and bladder cancer HTB1 cells. The results shown in Fig. 6A indicate that breast cancer cells are very sensitive to the cell death effects of doxazosin, as shown by the significant loss of cell viability. The magnitude of the cytotoxic effect in these cells was comparable with that observed in the prostate cancer cell lines. In contrast, the bladder cancer cells and colon cancer cells exhibited a moderate sensitivity to both drugs (Fig. 6A). A similar cytotoxic profile of the various human cancer cell lines was observed in response to terazosin treatment (Fig. 6B), whereas tamsulosin exerted no detectable effect against any of the cell lines examined (Fig. 6C).

We subsequently performed an in vivo efficacy study to test whether doxazosin-mediated apoptosis in malignant prostate cells leads to significant suppression of prostate tumorigenicity at pharmacologically relevant doses. The PC-3 derived prostate cancer xenografts growing in SCID mice were used as a model of tumorigenicity. As shown in Fig. 7, doxazosin administration in tumor-bearing hosts (via oral gavage; at a dose of 3 mg/kg) resulted in a notable decrease in the tumor volume of prostate tumor xenografts compared with the vehicle control-treated animals (P < 0.5). Interestingly enough, administration of doxazosin at higher concentrations (10–100 mg/kg) did not have any further effect on tumor suppression.

**DISCUSSION**

Doxazosin and terazosin are long-acting selective α1-adrenoceptor antagonists that are clinically used to provide acute relief of the
obstructive symptoms associated with BPH (19–22) and for the treatment of hypertension (23). The pharmacology of both drugs has been well-characterized in humans (24, 25), and as a result of their prolonged use as first-line, once-a-day antihypertensive agents, their safety profiles have been established. The adverse-effect profile of these α1-adrenoceptor antagonists is acceptable: by virtue of the functional involvement of α1-adrenoceptors in the maintenance of vascular tone, the most frequent side effects include dizziness and hypotension (26).

Targeting apoptosis in an attempt to control prostatic growth emerges as a potentially powerful therapeutic approach for the effective treatment of advanced prostate cancer (27). The present study demonstrates that the α1-adrenoceptor antagonists doxazosin and terazosin (quinazoline derivatives) lead to induction of apoptosis of human prostate cancer cells and smooth muscle cells in vitro at intracellular concentrations comparable with the therapeutic doses. Characteristically, the drugs did significantly affect the rate of cell proliferation. These findings are consistent with our recent clinical data indicating the ability of doxazosin and terazosin to induce prostate apoptosis in situ without affecting the proliferative capacity of prostate cells in BPH patients (14, 15).

Three points of experimental evidence from the present study support that an α1-adrenoceptor-independent mechanism is involved in this apoptotic activity: (a) the observation that the recently introduced, relatively uroselective, α1-blocker tamsulosin (which is a methoxybenzene sulfonamide) had no effect against prostate cancer cell growth compared with the other two α1-blockers; (b) the irreversible α1-adrenoceptor inhibitor phenoxybenzamine does not inhibit the apoptotic effect of doxazosin or terazosin against prostate cancer cells; and (c) expression of α1-adrenoceptors in human prostate cancer cells is not required, because the cells lack it, and yet they exhibited sensitivity to the apoptotic killing of doxazosin and terazosin. The concept that doxazosin and terazosin suppress prostate growth potentially via α1-adrenoceptor-independent actions gains further support from another study documenting that doxazosin inhibits proliferation of human vascular smooth muscle cells independently of an antagonistic effect on α1-adrenoceptors (28). Moreover, our observations that the apoptotic effect of doxazosin and terazosin was not exclusively targeted at prostate cells indirectly support this concept. Interestingly enough, both drugs exerted a similar antigrowth effect against human MCF-7 breast cancer cells (similar to that observed in prostate cells) but not against human bladder or colon cancer cells, implying an action that may selectively be targeted at hormone-dependent cells.

The in vivo efficacy studies revealed that doxazosin treatment resulted in a significant suppression of tumorigenic growth of the androgen-independent PC-3 prostate cancer xenografts in SCID mice. In accord with these results, the in vivo functional significance of the action of doxazosin has been documented in the mouse reconstitution model of prostate hyperplasia (16), as well as in long-term reduction of intimal hyperplasia in a rabbit model (29).

In conclusion, the present study provides the first evidence that α1-adrenoceptor antagonists terazosin and doxazosin, but not tamsulosin, exert a negative effect on prostatic growth by inducing apoptosis in both prostate tumor epithelial cells and smooth muscle cells, an action that is independent of their capacity to antagonize α1-adrenoceptors. On the basis of this evidence and our previous clinical findings (14, 15), we promote the concept that induction of prostate apoptosis in response to terazosin and doxazosin is one of the molecular mechanisms contributing to the overall long-term clinical profile of both α1-adrenoceptor antagonists in BPH patients (13). Although

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Fig. 6. Effect of doxazosin (A), terazosin (B), and tamsulosin (C) on cell viability of different human cancer cell lines. Subconfluent cultures of MCF-7 breast cancer cells, SW-480 colon cancer cells, and HTB1 bladder cancer cells were treated with increasing concentrations of the drugs (as indicated), and cell viability was assessed (as described in “Materials and Methods”) after 2 days of treatment. Data represent average values from two independent experiments. •, DU-145; □, PC-3; ▲, MCF-7; X, HTB1; —, SW-480.

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5 N. Kyriianou and M. Wyllie, unpublished data.
transduction mechanisms (30). Doxazosin and terazosin may suppress and G-coupled receptor agonists exhibit substantial overlap in signal factor–

the potential underlying mechanism is presently unknown, one may consider that polypeptide growth factors such as transforming growth factor-β, platelet-derived growth factor, epidermal growth factor, and G-coupled receptor agonists exhibit substantial overlap in signal transduction mechanisms (30). Doxazosin and terazosin may suppress prostate growth through deregulation of signal transduction pathways, potentially involving transforming growth factor-β signaling or alternatively perturbations in cell attachment to the extracellular matrix. Studies are in progress to investigate the mechanistic aspects of the antigrowth effect of both drugs against prostatic tumors. The present studies provide a strong rationale for targeting doxazosin and terazosin against prostatic tumors in vivo. Considering that apoptosis induction in prostate biopsies from BPH patients was observed over the normal dose range (14, 15), effective clinical application of these drugs, with established safety profiles and already in clinical use for BPH treatment, may result in decreased morbidity in patients with advanced prostate cancer. Long-term, randomized studies involving a large patient population are required to establish the therapeutic significance of doxazosin/terazosin-induced apoptosis in prostate cancer. Once the anti-growth effects of the two medications against advanced human prostate tumors are firmly established, one would expect to demonstrate that in treated patients, there are significant changes of cellular content/prostate size that correlates with increased apoptosis.

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