Quinazoline-derived α1-Adrenoceptor Antagonists Induce Prostate Cancer Cell Apoptosis Via an α1-Adrenoceptor-independent Action

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ABSTRACT

Recent evidence suggests that the quinazoline-based α1-adrenoceptor antagonists, doxazosin and terazosin, exhibit a potent apoptotic effect against prostate tumor epithelial cells, whereas tamsulosin, a sulfonamide-based α1-adrenoceptor antagonist, was ineffective in inducing a similar apoptotic effect against prostate cells (Cancer Res., 60: 4550–4555, 2000). In this study, to identify the precise molecular mechanism underlying this apoptosis induction, we examined whether doxazosin and terazosin (both piperazinyl quinazolines) affect prostate growth via an α1-adrenoceptor-independent action. Transfection-mediated overexpression of α1-adrenoceptor in human prostate cancer cells, DU-145 (that lack α1-adrenoceptor), did not alter the ability of prostate cancer cells to undergo apoptosis in response to quinazolines. Significantly enough, there was no modification of the apoptotic threshold of the androgen-sensitive prostate cancer cells, LNCaP, to either quinazoline-based α1-agonist by androgens. Furthermore, human normal prostate epithelial cells exhibited a very low sensitivity to the apoptotic effects of doxazosin compared with that observed for the malignant prostate cells. These findings provide the first evidence that the apoptotic activity of the quinazoline-based α1-adrenoceptor antagonists (doxazosin and terazosin) against prostate cancer cells is independent of: (a) their capacity to antagonize α1-adrenoceptors; and (b) the hormone sensitivity status of the cells. This may have potential therapeutic significance in the use of quinazoline-based α1-adrenoceptor antagonists (already in clinical use for the treatment of hypertension and benign prostate hyperplasia) for the treatment of androgen-independent human prostate cancer.

INTRODUCTION

Prostate cancer has become the most prominent contributor to cancer mortality among American males, resulting in >40,000 deaths every year (1). Prostate cancer mortality results from metastasis to the bone and lymph nodes and progression from androgen-dependent to androgen-independent prostatic growth (2). Common treatment regimens for patients with localized disease involves surgical interventions (radical prostatectomy) or nonsurgical approaches (hormone ablation or radiotherapy). A large percentage of prostate cancer patients, however, present an advanced stage disease with 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 cells, whereas the androgen-independent cells survive (3). Attempts at combination regimes of hormonal ablation and chemotherapy failed to provide clinically convincing evidence of significant therapeutic success (4), primarily attributable to the slow rate of cell proliferation that characterizes prostatic tumors (5, 6).

Within the normal prostate gland, homeostasis is maintained by a unique balance between the rates of proliferation and apoptosis, such that neither overgrowth nor involution of the gland occurs (7). Compelling evidence suggests that the tumorigenic growth of the prostate depends on the evasion of normal homeostatic control mechanisms, because of an increase in cell proliferation and a decrease in apoptotic death (5, 6). Thus, enhancing the apoptotic process emerges as a significant therapeutic target for the effective elimination of androgen-dependent and androgen-independent prostate cancer cells (3). Characteristically, because increased bcl-2 expression correlates with development of androgen-independent prostate cancer, an apoptotic defect that renders prostatic tumors resistant to therapy (8, 9), recent efforts have targeted key apoptosis regulators to enhance the therapeutic response with minimal toxicity (3). Modulation of molecular pathways of apoptosis execution will have significant effects on prostate tumor vascularity and sensitivity to other therapeutic modalities (such as radiotherapy).

With continued intensified search for the identification of apoptosis modifiers as therapeutic targets for prostate cancer treatment, attention has recently been directed toward a group of existing drugs: α1-adrenoceptor antagonists that are members of the quinazoline chemical class. 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 (10–12) and for the treatment of hypertension via reduction of total peripheral resistance by selective postsynaptic α1-blockade (13). Tamsulosin, a recently developed member of this class of α1-adrenoceptor antagonists, is selective for the predominant α1a-adrenoceptor subtype but has a different sulfonamide-related structure, and its potential therapeutic efficacy has been demonstrated in clinical trials (14). The therapeutic benefit of α1-adrenoceptor antagonists in the treatment of BPH has been historically attributed to a change in the periurethral tone of the prostate (15) via a direct action on α1-adrenoceptors present in the prostate smooth muscle (16).

A provocative challenge for the existing concept on α1-blocker action, stemmed from evidence gathered in this laboratory, suggests that two quinazoline-derived α1-adrenoceptor antagonists, doxazosin and terazosin, have additional effects against cellular growth, which transcend smooth muscle relaxation: both doxazosin and terazosin at pharmacologically relevant doses exert an apoptotic activity in stroma smooth muscle and epithelial cell populations in prostate tissue from BPH patients without affecting their proliferative rate (17, 18). These clinical findings were in full accord with experimental studies using a mouse reconstitution model of prostate hyperplasia, in which doxazosin demonstrated a potent apoptotic effect against oncogene-induced prostate growth (19). Furthermore, more recent in vitro studies documented a potent antigrowth effect of the two quinazolines (doxazosin and terazosin), but not tamsulosin, the sulfonamide-based α1-adrenoceptor antagonist, against two highly aggressive, androgen-independent human prostate cancer cell lines, PC-3 and DU-145, via induction of apoptosis (20–22).

Considering the ability of the quinazoline-derived α1-adrenoceptor antagonists, but not the uroselective, high affinity (sulfonamide-based antagonist) tamsulosin, to induce prostate tumor cell apoptosis, in this study, we examined whether the apoptotic effect of terazosin and doxazosin is: (a) an α1-adrenoceptor-mediated mechanism; and (b) dependent on androgens. Our results demonstrate that the quinazoline-
driven apoptosis is independent of α1-adrenoceptor action, and DHT does not affect the sensitivity of prostate cancer cells to the apoptotic effects of doxazosin and terazosin. Moreover, there was a minimal cell death effect (by quinazolines) in the normal prostate epithelial cells. The present findings may provide a rationale for advancing these long-acting, quinazoline-based α1-adrenoceptor antagonists toward the development of an effective therapeutic strategy for patients with androgen-independent prostate cancer.

**MATERIALS AND METHODS**

**Cell Culture**

The human prostate cancer cell lines, LNCaP and DU-145, were obtained from the American Type Tissue Culture Collection (Rockville, MD). Normal human prostate epithelial cells, PrEC, were purchased from Clonetics (Walkersville, MD). LNCaP cells were maintained in DMEM (Life Technologies, Inc., Gaithersburg, MD), supplemented with 10% fetal bovine serum/2 mm L-glutamine (Collaborative Biomedical Products, Bedford, MA) and 100 units of penicillin/100 mg/ml streptomycin. DU-145 cells were maintained in RPMI (Life Technologies, Inc.), containing 10% fetal bovine serum (Collaborative Biomedical Products, Bedford, MA) and 100 units of penicillin/100 mg/ml streptomycin. LNCaP cells were maintained in RPMI (Life Technologies, Inc.), containing 10% fetal bovine serum (Collaborative Biomedical Products) and antibiotics. PrEC cells were maintained in PrEGM media from Clonetics.

**Drugs**

The three α1a-adrenoceptor antagonists used in this study were generously provided by the following pharmaceutical companies that manufacture them, respectively: Doxazosin (Cardura; Doxazosin Mesylate) was provided by Pfizer Pharmaceuticals (New York, NY); Terazosin (Hytrin; Terazosin Hydrochloride) was obtained from Abbott Laboratories (Abbott Park, IL); and tamsulosin (FLOMAX; Tamsulosin Hydrochloride) was provided by Yamanouchi Pharmaceuticals (Tokyo, Japan). DHT (5-androstan-17β-OL-3-one) was obtained from Sigma Chemical Co. (St. Louis, MO).

**Transfection**

DU-145 cells were seeded at 1 × 10^6 cells/100-mm tissue culture dish and after 24 h, were cotransfected with the pcDNA3 plasmid encoding the human α1a-adrenoceptor (a generous gift from Dr. P. Walden, New York University, New York, NY) and the neomycin resistance-encoding plasmid via the calcium phosphate precipitation procedure as described previously (23). Neomycin-resistant colonies were selected in the presence of G418 (500 μg/ml; Life Technologies, Inc.), cloned, and expanded into cell lines.

**Cell Viability Assay**

Subconfluent cultures of cells in six-well plates were exposed in duplicate to increasing concentrations of doxazosin, terazosin, or tamsulosin (1-50 μM). The number of viable cells was assessed 2 days posttreatment using the trypan blue exclusion assay. For the experiments involving treatment with DHT, before treatment with doxazosin, LNCaP cells were grown in charcoal-stripped media from Clonetics.

**Cell Proliferation Assays**

Rate of DNA Synthesis. The effect of α1a-adrenoceptor antagonists on the rate of DNA synthesis in normal and malignant human prostate epithelial cells was evaluated using the [3H]thymidine uptake assay, as described previously (20).

**Cell Cycle Analysis**

Subconfluent cultures of the androgen-sensitive prostate cancer cells LNCaP were treated with doxazosin or terazosin (25 μM) for 48 h. Cells were subsequently harvested and washed with PBS (pH 7.4), and pellets were resuspended with PBS and 70% ethanol. They were stored at −20°C overnight and resuspended (10^6 cells/ml), at which time 1 mg/ml DNase-free RNase was added. The cell suspension was incubated at 37°C (30 min) and stained overnight at 4°C with 50 μg/ml propidium iodide (Sigma Chemical Co.). Cells were filtered through a 35 μM nylon filter and analyzed using Beckman-Coulter fluorescence-activated cell sorter analyzer. G1, S, and G2/M populations were quantitated using the Multicycle software program (Phoenix Flow Systems). Results are expressed as a percentage of cells in each stage of cell cycle.

**Apoptosis Evaluation**

The morphological appearance of apoptosis was assessed using the Hoechst staining as described previously (24). Briefly, cells were seeded in six-well plates (in triplicate) at 10^3 cells/well, and at subconfluence, they were treated with one of the three antagonists, doxazosin, terazosin, or tamsulosin (25 μM), for 48 h. After treatment, cells were fixed in 4% paraformaldehyde/PBS and stained with 10 μg/ml Hoechst 33342 dye (Sigma Chemical Co.)/0.1% Triton X-100. Fixed cells were incubated overnight at 4°C and visualized under a 365 nm UV light microscope (Zeiss Axiosvert 10). Quantitative analysis was performed by counting the green fluorescent (apoptosis positive) cells under ×400 magnification from three independent fields. Values are expressed as the percentage of apoptotic cells relative to the total number of cells per field (the average number of cells that were positive for apoptosis varied from 2–40/field, whereas the total number of cells counted per field was ~50–100).

**Protein Analysis**

**Western Blot Analysis.** LNCaP cells were treated with either doxazosin or terazosin (25 μM) for 1, 2, or 3 days. Cells from treated and control untreated cultures were lysed in 150 mM NaCl, 50 mM Tris (pH 8.0), 0.5% deoxycholic acid, and 1% NP40 with 1 mM phenylmethyl sulfonyl fluoride. Total cell lysates were electrophoretically analyzed through a 15% (w/v) polyacrylamide gel, and proteins were subsequently transferred onto a Hybond-P membrane (Amersham Pharmacia Biotech, Piscataway, NJ). Western blot analysis was performed using the rabbit polyclonal antibody against VEGF (Santa Cruz Biotechnology, Santa Cruz, CA) and the rabbit polyclonal antibody against PSA (DAKO, Carpenteria, CA). α-Actin expression was determined using the monoclonal-actin antibody (Oncogene Research, Cambridge, MA) as a loading and normalizing control. Protein detection was achieved using the Enhanced Chemilluminescence System (Amersham International, Arlington Heights, IL). Values for specific protein expression are expressed as fold-change, relative to α-actin expression (loading control) using the SCION Image analysis.

**ELISA Assay for PSA.** LNCaP cells were treated with doxazosin, terazosin, or tamsulosin (at 25 μM) for 24 h as described above. Supernatants from treated and nontreated LNCaP cells were assayed for PSA expression using an ELISA assay. PSA monoclonal antibody ( Fitzgerald Industries, Concord, MA) was used as the capture antibody (5 μg/ml). Supernatants were then added after blocking in 1% BSA/PBS (1 h at room temperature). The antirabbit PSA (1 μg/ml; Fitzgerald Industries) and the Goat F(ab')2 antirabbit IgG-biotin conjugate (1 μg/ml; Biosource International, Camarillo, CA) were subsequently applied (1 h). After washing, ExtrAvidin Alkaline Phosphatase Conjugate 1:1000 (Sigma Chemical Co.) was applied, and PSA protein was detected using p-nitrophenyl phosphate (Sigma Chemical Co.) according to the manufacturer’s instructions. Values represent the percentage of PSA expression relative to the untreated control samples.

**Statistical Analysis**

Statistical analyses of the numerical data were performed using ANOVA in the Graph Pad Prism program. All values are represented as averages ±SE. Values were considered statistically significant at P < 0.05.

**RESULTS**

To elucidate whether the mechanism underlying the apoptotic effect of doxazosin and terazosin in prostate cancer cells is dependent on their ability to antagonize the α1a-adrenoceptor, human prostate cancer cells DU-145, which totally lack α1a-adrenoceptor expression (20), were engineered to overexpress α1a-adrenoceptor, and cloned transfectants were evaluated in vitro. Fig. 1 represents the Western blot analysis of the representative α1a-adrenoceptor transfectant clones. Clone-32 was selected for additional experiments as it expressed a band at Mr 30,000, indicative of α1a-AR reactivity (Fig. 1; Ref. 16). The binding affinity of the transfected α1a-adrenoceptor to
Fig. 1. Expression of α1-adrenoceptor in DU-145 prostate cancer cells. Transfection of DU-145 cells with a α1-adrenoceptor-encoding plasmid expression vector, as well as the neomycin expression vector, was performed as described in “Materials and Methods.” Western blot analysis of selected cloned G418-resistant transfectants and neomycin control transfectant cells was performed using an antibody from Santa Cruz Biotechnology. C-49, C-39, C-36, and C-32 represent individual α1-adrenoceptor/neomycin transfectant clones. PC, the positive control (rat brain); Neo, the neomycin-only control transfectant. The size (M.W.) of the exogenously introduced α1-adrenoceptor subtypes is shown on the right.

![Western blot image](image_url)

Fig. 2. Effect of α1-adrenoceptor overexpression on the apoptotic response of DU-145 prostate cancer cells to quinazoline-based α1-adrenoceptor antagonist doxazosin. Parental prostate cancer cells DU-145, neo control transfectants, and clone-32 α1-adrenoceptor transfectants were treated with increasing concentrations of doxazosin as shown (1 500 M). Cell viability was evaluated after 48 h of treatment on the basis of trypan blue exclusion assay. Results represent the average of three experiments performed in triplicate (mean ±SE).

![Cell viability graph](image_url)

Fig. 3. Effect of doxazosin, terazosin, and tamsulosin on apoptosis in LNCaP prostate cancer cells. A, cell viability dose response of the three α1-adrenoceptor antagonists after 48 h of treatment. B, characteristic results of the Hoechst staining for the detection of LNCaP cells with the apoptotic morphology after the various treatments. LNCaP cells were treated with doxazosin, terazosin, or tamsulosin for 48 h, and cells were subsequently fixed and stained with Hoechst 33342. The fragmented nuclei of apoptotic cells were observed using a UV filter and quantitated (summarized on Table 1). Arrows, cells that are undergoing apoptosis.

![Apoptosis images](image_url)

Table 1 Quantitative analysis of apoptosis induction in normal and malignant prostate epithelial cells in response to α1-adrenoceptor antagonists

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LNCaP (% apoptotic cells)</th>
<th>Normal prostate cells (PrEC) (% apoptotic cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.1 ± 0.2%</td>
<td>2 ± 0.7%</td>
</tr>
<tr>
<td>Doxazosin (25 μM)</td>
<td>14 ± 0.8%</td>
<td>5 ± 1.1%</td>
</tr>
<tr>
<td>Terazosin (25 μM)</td>
<td>13 ± 1.2%</td>
<td>1 ± 0.3%</td>
</tr>
<tr>
<td>Tamsulosin (25 μM)</td>
<td>1.2 ± 0.3%</td>
<td>ND</td>
</tr>
</tbody>
</table>

Prostate cancer cells LNCaP and normal prostate epithelial cells (PrEC) were treated with doxazosin, terazosin, or tamsulosin (25 μM) for 2 days, and cells exhibiting the apoptotic morphology were assessed using the Hoechst staining as described in the legend for Fig. 3B. Values represent the average percentage of apoptotic cells of three independent experiments performed in duplicate (mean ±SE).
Doses, DHT had no effect on the temporal profile of cell death (as determined by loss of cell viability; Fig. 4A). To determine whether DHT could modify the apoptotic response of LNCaP cells to the quinazoline-derived \(\alpha_1\)-adrenoceptor antagonists, cells were treated with doxazosin alone (25 \(\mu\)M), doxazosin in the presence of DHT, or DHT alone (1 nM). As shown in Fig. 4B, the level of doxazosin-mediated apoptosis induction was similar to that obtained in the presence of DHT, whereas DHT (alone) treatment resulted in basal apoptosis levels, comparable with the values observed for the control, untreated cultures. Thus, DHT (at physiologically relevant doses) did not exert a significant protective effect on doxazosin-mediated apoptosis induction in LNCaP prostate cancer cells (Fig. 4B).

Doxazosin treatment at high concentrations resulted in a decrease in the rate of DNA synthesis in LNCaP prostate cancer cells (data not shown). In contrast, terazosin and tamsulosin had no significant effect on the rate of DNA synthesis in this cell line at any of the doses tested. The effect of doxazosin and terazosin on LNCaP cell cycle progression was determined by flow cytometry. After 2 days of treatment with either drug at 25 \(\mu\)M, the percentage of relative distribution of the prostate cancer cells in each phase of the cell cycle remained very similar (Table 2). There was not a significant increase in the number of cells in the G2-M phase, which supports the concept that the prostate antigrowth effect of the quinazolines results from targeting the apoptotic pathway without an antiproliferative activity (Table 2).

The effect of doxazosin and terazosin on the growth of normal prostatic epithelial cells was evaluated \(in vitro\) in the normal cell line, PrEC. Treatment with doxazosin (2 days) resulted in a notable loss of cell viability at higher concentrations, whereas terazosin had no significant effect against normal prostate cells (Fig. 5A). Comparative analysis of the effect of doxazosin on the rate of DNA synthesis in the normal and cancer epithelial cell lines, PrEC and LNCaP, demonstrated that whereas there was no significant effect in the normal prostate cells, a moderate dose-dependent decrease in the rate of DNA synthesis was detected in the LNCaP cells (data not shown). Induction of apoptosis in normal prostatic epithelial cells, PrEC, was evaluated by Hoechst staining. The results from the quantitative evaluation of apoptosis induction in response to all three \(\alpha_1\)-adrenoceptor antagonists in normal prostate epithelial cells are shown on Table 1. After 2 days of treatment with

\[
\begin{array}{ccc}
\text{Treatment} & \text{Control} & \text{Day-1 post-treatment} \\
\text{Doxazosin (25 \(\mu\)M)} & 74.5% (G_1) & 70.6% (G_1) \\
 & 17.8% (S) & 21.8% (S) \\
 & 7.7% (G_2) & 7.7% (G_2) \\
\text{Terazosin (25 \(\mu\)M)} & 74.4% (G_1) & 70.7% (G_1) \\
 & 17.8% (S) & 19.7% (S) \\
 & 7.7% (G_2) & 9.6% (G_2) \\
\end{array}
\]

* LNCaP cells were exposed to either doxazosin or terazosin for 1 and 2 days, and cell cycle analysis was performed after treatment as described in “Materials and Methods.” Values represent the mean percentage of cells in each phase of the cell cycle (shown in parentheses).
Current therapy for prostate cancer is limited by the propensity of the disease to progress from an androgen-dependent to an androgen-independent state. The present findings support the concept that the novel antigrowth activity of the quinazoline-based α1-adrenoceptor antagonists, doxazosin and terazosin, is selectively targeted at apoptosis in androgen-independent prostate cancer cells. Interestingly enough, normal prostate epithelial cells seem to be relatively protected from this quinazoline-mediated apoptotic effect. Our data established that neither of the two quinazoline-derived α1-adrenoceptor antagonists evaluated exerted a significant effect on cell cycle progression of prostate cancer cells. These observations are in accord with our previous clinically gathered data (17, 18, 27), as well as experimental studies by others (19), that doxazosin and terazosin inhibit benign and malignant prostate growth via induction of apoptosis (over the normal therapeutic dose range) without affecting the rate of cell proliferation. However, this is in contrast with a recent report demonstrating that doxazosin inhibits G1-S transition in human coronary smooth muscle cells (28), lending support to the possibility that cell type specificity exists with regards to the cellular process targeting of the α1-adrenoceptor antagonist antigrowth function.

Table 3 Effect of α1a-adrenoceptor antagonists on PSA secretion by human prostate cancer cells, LNCaP

<table>
<thead>
<tr>
<th>Dose (μM)</th>
<th>Doxazosin % PSA levels</th>
<th>Terazosin % PSA levels</th>
<th>Tamsulosin % PSA levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.1</td>
<td>86</td>
<td>82</td>
<td>100</td>
</tr>
<tr>
<td>0.5</td>
<td>59</td>
<td>80</td>
<td>100</td>
</tr>
<tr>
<td>1.0</td>
<td>63</td>
<td>64</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>52</td>
<td>55</td>
<td>75</td>
</tr>
<tr>
<td>25</td>
<td>14</td>
<td>27</td>
<td>75</td>
</tr>
<tr>
<td>50</td>
<td>ND</td>
<td>21</td>
<td>75</td>
</tr>
</tbody>
</table>

*LNCaP cells were treated with increasing concentrations of the α1-adrenoceptor antagonists (as shown), and PSA secretion in the culture medium was assessed after 2 days of treatment using an ELISA assay as described in “Materials and Methods.” Values are expressed as the percentage of secreted PSA levels relative to the untreated controls. Data are representative of three independent experiments, performed in triplicate.

DISCUSSION

Current therapy for prostate cancer is limited by the propensity of the disease to progress from an androgen-dependent to an androgen-independence state. The present findings support the concept that the novel antigrowth activity of the quinazoline-based α1-adrenoceptor antagonists, doxazosin and terazosin, is selectively targeted at apoptosis in androgen-independent and androgen-sensitive prostate cancer cells. Interestingly enough, normal prostate epithelial cells seem to be relatively protected from this quinazoline-mediated apoptotic effect. Our data established that neither of the two quinazoline-derived α1-adrenoceptor antagonists evaluated exerted a significant effect on cell cycle progression of prostate cancer cells. These observations are in accord with our previous clinically gathered data (17, 18, 27), as well as experimental studies by others (19), that doxazosin and terazosin inhibit benign and malignant prostate growth via induction of apoptosis (over the normal therapeutic dose range) without affecting the rate of cell proliferation. However, this is in contrast with a recent report demonstrating that doxazosin inhibits G1-S transition in human coronary smooth muscle cells (28), lending support to the possibility that cell type specificity exists with regards to the cellular process targeting of the α1-adrenoceptor antagonist antigrowth function.

Of major mechanistic significance was the observation that this apoptotic effect against prostate cancer cells was independent of the ability of the two α1-adrenoceptor antagonists to antagonize the α1a-adrenoceptor binding in prostate cancer cells. Our results are consistent with previous studies in other cellular systems, demonstrating that doxazosin inhibits growth and migration of the human vascular smooth muscle cells (29) and human coronary smooth muscle cells (28), independent of an antagonistic effect on α1-adrenoceptor. Moreover, this action is in accord with our previous observations that documented that preincubation with phenoxybenzamine, an irreversible inhibitor of α1-adrenoceptor binding (that inactivates α1-adrenoceptors in vascular smooth muscle cells), did not affect the cellular response to doxazosin-induced apoptosis in prostate cancer cells (20). Whereas these data provide an intriguing mechanistic insight, the precise cellular pathway that signals this effect remains to be elucidated. One could speculate that the apoptotic activity of the quinazoline-derived α1-adrenoceptor antagonists could reflect either a direct effect on cellular dynamics or could arise secondarily to an action on cellular apoptotic factors, such as transforming growth factor-β.

The present findings suggest that the cellular sensitivity to quinazoline-induced apoptosis in the androgen-sensitive prostate cancer cells, LNCaP, was not modified by androgens (at physiological levels). Interestingly enough, both doxazosin and terazosin treatment reduced PSA expression and secretion. This is consistent with recent clinical studies from our center that documented the ability of terazosin to reduce tissue PSA expression in patients with prostate cancer (27). Because androgens had no effect on the apoptotic response of LNCaP cells, the data imply that doxazosin-mediated reduction in PSA expression represents a result of the cell killing, rather than being a molecular target for the quinazoline-apoptotic action at the transcriptional level. The effect of quinazoline on VEGF expression is supported by our earlier observations that terazosin inhibits prostate tissue vascularity and VEGF protein levels in clinical specimens of prostate cancer (27).

Fig. 6. Effect of treatment with quinazoline-derived α1-antagonists on VEGF and PSA protein expression in prostate cancer cells. LNCaP cells were treated with doxazosin for 1, 2, and 3 days, and cell lysates were subjected to Western blot analysis as described in “Materials and Methods.” Aliquots of 60 μg of protein were subjected to electrophoretic analysis through 12% (w/v) SDS-PAGE gels, transferred to nitrocellulose, and probed with specific antibodies. Changes in protein expression were determined by semiquantitation using the Scion Image program and normalized to actin expression. The sizes/molecular weights of the respective proteins (VEGF, PSA, and α-actin) are shown on the right. Data are representative of three independent experiments.
The \textit{in vivo} antitumor efficacy of the \(\alpha\)-1-adrenoceptor antagonists, against prostate cancer growth via apoptosis induction (20), resembles the \textit{in vivo} effect in the model of intimal hyperplasia (30). Considering this evidence, it is tempting to speculate on a potentially clinically relevant concept that there may be substantive analogies between the effects of \(\alpha\)-1-adrenoceptor antagonists on cell growth in the cardiovascular system and in the prostate gland. Attractive as this argument might be, one should also consider the differential effects of doxazosin (apoptotic versus antiproliferative) demonstrated in the two cell types (present findings and Refs. 28 and 29, respectively).

In conclusion, the present study indicates that the quinazoline-derived \(\alpha\)-1-antagonists, doxazosin and terazosin, but not the sulfonamide-based tamsulosin, activate apoptosis in prostate cancer cells via a mechanism independent of androgens and without interfering with cell cycle progression. More significantly, this potent apoptotic activity of doxazosin and terazosin occurs through a novel pathway independent of an action on the \(\alpha\)-1-adrenoceptor, assuming that the molecular mechanism can be unraveled (32), this could represent an exciting new starting point for quinazoline-based drug design for targeting prostate cancer cell apoptosis.

\textbf{REFERENCES}

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