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EFFECT OF TERAZOSIN ON TISSUE VASCULARITY AND APOPTOSIS IN TRANSITIONAL CELL CARCINOMA OF BLADDER

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Abstract

Objectives—To present a pilot study to determine whether the α_1 -adrenoceptor antagonist terazosin can induce apoptosis in transitional cell carcinoma (TCC) of the bladder, similar to the effect seen with prostate cancer. The α_1 -adrenoceptor antagonist terazosin has recently been shown to induce apoptosis in prostate cancer cells both in vitro and in vivo and to reduce prostatic tissue vascularity by potentially affecting endothelial cell adhesion.

Methods—The records of 24 men who underwent radical cystectomy for TCC of the bladder at the Lexington Veterans Affairs Medical Center were reviewed. The control group consisted of 15 men who were never exposed to terazosin. The study group consisted of 9 men who were treated with terazosin before cystectomy. Sections of the bladder tumor and normal trigone were subjected to immunohistochemical analysis for microvessel density, endothelial cell CD31 expression, and apoptosis detection (terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling), as well as high-molecular-weight cytokeratin staining.

Results—A significant reduction in tissue vascularity (14.0 versus 19.2, $P < 0.05$) and a significant increase in the apoptotic index (3.0% versus 1.7%, $P < 0.05$) was detected in terazosin-treated bladder tumors compared with untreated bladder tumors. Most TCC specimens (80%) exhibited strong and consistently uniform immunostaining for high-molecular-weight cytokeratin staining.

Conclusions—These results suggest that terazosin reduces tumor vascularity and induces apoptosis in TCC of the bladder. Additional studies with more patients are necessary to reach definitive conclusions. However, considering the proven apoptotic action of terazosin in prostatic tissue, this study may have implications for the use of terazosin in the treatment of bladder TCC.

Approximately 60,240 new cases of bladder cancer will have been diagnosed in 2004 in the United States, and an estimated 12,710 patients will die of the disease.¹ Manipulation of angiogenesis and apoptosis has become a target of modern chemotherapy. In bladder cancer, angiogenesis correlates with lower disease-free and overall survival rates, supporting the role of tumor vascularity as an independent prognostic marker.² Furthermore, angiogenesis is a potential target for therapy in bladder cancer, because tumors cannot grow beyond 2 to 3 mm without developing their own blood supply.^{3,4} Defects in the apoptosis pathway play a major role in tumor progression,⁵ enabling tumor cells to survive and grow beyond their normal lifespans and become resistant to cytotoxic chemotherapy.⁶ Major apoptosis regulators have recently emerged as attractive candidates for therapeutic targeting of bladder cancer, as well as potential biomarkers of tumor progression.⁷

The quinazoline-based α_1 -adrenoreceptor antagonists doxazosin and terazosin have been shown to induce apoptosis in prostate cancer cells both in vitro and in vivo⁸ and to reduce prostatic tissue vascularity by potentially affecting endothelial cell adhesion.^{9,10} Induction of apoptosis has only been identified in the quinazoline-based α_1 -adrenoceptor antagonists. Other subtypes of α_1 -adrenoceptor antagonists, such as the sulfonamide-based drug tamsulosin, have not been shown to induce apoptosis in prostate epithelial or stromal cells.^{11,12}

We hypothesized that the α_1 -adrenoceptor antagonist terazosin will induce apoptosis and inhibit angiogenesis in transitional cell carcinoma (TCC) of the bladder. Specimens from bladder tumors and normal bladder trigone were subjected to immunohistochemical analysis to determine the effect of terazosin on apoptosis and vascularity in TCC of the bladder.

MATERIAL AND METHODS

Patient Selection

Pathologic specimens were obtained from 24 male patients (age 50 to 80 years), who had undergone radical cystectomy for TCC of the bladder at the Lexington Veterans Affairs Medical Center from 1993 to 2003. Only those patients with TCC on the final pathologic examination were included in the study. In addition, adequate documentation concerning exposure to terazosin and the dose and duration of treatment was required before inclusion in the study. Patients with a history of 5- α -reductase inhibitor use (ie, finasteride) were excluded.

The control group consisted of tissue specimens from 15 patients with no documented exposure to terazosin. The treatment group consisted of 9 patients who had been treated with therapeutic doses of terazosin (2 to 10 mg/day) for lower urinary tract symptoms before surgery. The treatment period was 3 to 60 months (median 10). All patients in the study group reported using terazosin immediately up to the point of radical cystectomy. Because of the retrospective nature of the study, compliance with terazosin treatment was not known at specimen acquisition.

Specimens from bladder TCC and normal bladder trigone were obtained from permanent paraffin-embedded histologic sections for each patient. Normal trigone specimens were examined to determine whether a tissue-specific effect occurred or whether the effect was present throughout the bladder. The clinicopathologic characteristics of the patients were as follows: 1 had Stage Ta, 4 had Stage T1, 5 had Stage T2, 9 had Stage T3, and 4 had Stage T4 TCC; 2 patients had carcinoma in situ (CIS) only and 15 had carcinoma in situ in addition to bladder TCC. On histologic confirmation of bladder TCC and bladder trigone structure, serial sections (5 μ m) from each tissue type were subjected to immunohistochemical analysis. Normal bladder trigone specimens were not available from all patients.

Vascularity

Formalin-fixed, paraffin embedded-sections were incubated with the primary antibody (rabbit anti-human von Willebrand factor, Dako, Carpinteria, Calif), as previously described.⁹ Vessels were counted in three different fields at 200 $\times$ magnification by two independent observers (A.T. and L.Z.). The microvessel density (MVD) was defined as the average number of vessels per field.

For the evaluation of the number of single endothelial cells, antigen retrieval was performed using enzymatic digestion with Proteinase K (Dako). The primary antibody used was the mouse anti-human CD31 specific for endothelial cells from Dako (overnight incubation at 4°C). Two independent observers (A.T. and N.K.) counted CD31-positive cells under microscopic examination, and the CD31 score was calculated as the average number of single CD31-positive endothelial cells per 400 $\times$ field.

Apoptosis Evaluation

Apoptotic cells were detected using the Apoptag Peroxidase In Situ Apoptosis Detection Kit (Serologicals, Norcross, Ga), as previously described.¹³ In brief, paraffin-embedded sections were treated with Proteinase K (Dako) and were subsequently incubated with terminal deoxynucleotidyl transferase enzyme at 37°C. Apoptotic cells were counted in three different fields (400×) by three independent observers (A.T., L.Z., and N.K.). The apoptotic index was determined by counting the number of terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL)-positive cells and dividing this by the total number of cells.

High-Molecular-Weight Cytokeratin Staining

Slides were subjected to microwave antigen retrieval using the Dako target retrieval solution (10 minutes). The mouse anti-human high-molecular-weight cytokeratin antibody was used (clone 34β 12, Dako). The staining scoring system was as follows: 1, less than 10% of tumor cells positive for high-molecular-weight cytokeratin staining; 2, 11% to 25%; 3, 26% to 50%; 4, 51% to 75%; and 5, greater than 75% tumor cells positive.

Statistical Analysis

The numerical values are expressed as the mean ± standard error of the mean (SE). One-way analysis of variance was performed to determine the statistical differences between the different groups. A *P* value of less than 0.05 was considered statistically significant.

RESULTS

The characteristic appearance of apoptotic cells in two TCC specimens is shown in Figure 1, with TUNEL-positive cells scattered among the tumor cells and the surrounding stroma. Apoptosis was infrequent among the tumor cell populations in the untreated tissue. However, a statistically significant increase in the apoptotic index was observed in bladder tumors from terazosin-treated patients compared with the untreated control group (3.0% versus 1.7%, *P* < 0.05). No significant difference was found in the apoptotic index of the bladder trigone between the untreated and treated specimens (Table I).

Treatment with terazosin resulted in a statistically significant decrease in MVD, as determined by factor VIII immunostaining. The MVD of bladder tumor in patients treated with terazosin decreased by approximately 27% compared with untreated bladder tumor (14.0 versus 19.2, *P* < 0.05). Figure 2 presents a characteristic photomicrograph of the immunostaining pattern, showing increased MVD in TCC from an untreated patient. No significant difference was found in the MVD of the bladder trigone between the untreated and treated specimens (Table I).

Immunostaining for CD31 to identify single endothelial cells was subsequently performed to achieve more precision in the detection of changes in vascularity. CD31 staining resulted in identification of continuous endothelia in the arteries, veins, arterioles, venules, and capillaries in a similar manner to factor VIII staining.¹⁴ The quantitative data shown in Table I indicate a notable decrease (32%) in the number of single CD31-positive endothelial cells in TCC specimens treated with terazosin compared with the untreated controls. This difference, however, failed to reach statistical significance.

COMMENT

The treatment options for patients with superficial bladder TCC include transurethral resection followed by surveillance versus intravesical chemotherapy or immunotherapy. Patients with muscle-invasive tumor, frequent tumor recurrence, escalation in grade or stage, or recurrent

refractory carcinoma in situ are candidates for partial or radical cystectomy. Chemotherapy and radiotherapy regimens have not proved to be highly effective in treating TCC of the bladder.

Angiogenesis has become a target of modern chemotherapy in recent years. It has been shown that tumor growth can be effectively inhibited with antiangiogenic factors with minimal toxicity to the host.¹⁵ Recent studies have documented the antiangiogenic effect of the quinazoline-based α_1 -adrenoceptor antagonist terazosin on human prostatic tissue by demonstrating a significant decrease in tumor MVD.⁹ The current findings suggest that terazosin decreases the MVD in clinical TCC specimens. Terazosin had no significant effect on the tissue vascularity of the bladder trigone. The increased vasculature of the human prostate and bladder and the rather scarce vasculature characterizing the trigone might provide a basis for the organ-specific targeting observed in this study. In addition, these observations provide further support that terazosin's antiangiogenic effect is independent of its α_1 -adrenoceptor action, considering the high density of α_1 -adrenoceptors in trigonal smooth muscle.^{16,17}

Immunostaining for CD31 is generally considered to be more sensitive than factor VIII staining for the identification of small, immature vessels and single endothelial cells.¹⁴ Complementary staining for both factor VIII and CD31 to identify single endothelial cells was performed to provide an integrated evaluation of the neovascularization in the specimens. A consistent correlation was observed between the decrease in the number of single CD31-positive endothelial cells and the reduction in tissue vascularity based on the MVD evaluation in terazosin-treated TCC specimens, even though the decrease in CD31-positive cells was not statistically significant. No significant differences in the CD31 score were detected in the bladder trigone between the two groups, paralleling the results of factor VIII immunostaining and further supporting the seemingly tissue-specific effect of terazosin on vascularity.

Several apoptosis-based approaches are currently under investigation for the treatment of bladder cancer. Recombinant adenoviruses and antisense oligonucleotides have been used in an attempt to reintroduce tumor suppressor or pro-apoptotic genes and inhibit the production of antiapoptotic proteins, such as *bcl-2*.⁷ The significant increase in the apoptotic index of terazosin-treated TCC could translate into a significant therapeutic effect in the management of bladder cancer. These findings are similar to our previous observations of terazosin-induced apoptosis in prostate cancer.⁹ In addition, these findings in terazosin-treated TCC provide support for recent evidence suggesting an increased apoptotic index of human bladder myofibroblast and lamina propria cells in patients treated with α_1 -antagonists for benign prostatic hyperplasia symptoms.¹⁸ The molecular mechanisms underlying the apoptotic and potential antiangiogenic action of terazosin against bladder and prostate tumors have yet to be fully defined. Recent evidence has implicated transforming growth factor-beta signaling and transforming growth factor-beta ligand in the induction of apoptosis in doxazosin-treated prostate cancer cells.¹⁹ Downregulation of angiogenic factors, such as vascular endothelial growth factor, basic fibroblast growth factor, or cyclooxygenase-2, may account for the decline in tissue vascularity in terazosin-treated TCC. This concept gains firm support from recent reports implicating functional targeting of vascular endothelial growth factor during doxazosin-induced anoikis of prostate cancer epithelial cells, as well as vascular endothelial cells.^{20,21}

The high prevalence of high-molecular-weight cytokeratin positivity in TCC detected in the present analysis is in accord with previous reports.²² The staining profile for this marker was comparable between the two groups (treated and untreated patients; data not shown).

The results of this study represent an initiation point for additional exploration of the effect of terazosin (and other quinazoline-based α_1 -antagonists) on TCC of the bladder. The small

number of TCC specimens analyzed, in addition to the variable terazosin dose, treatment period, and treatment compliance are potential limitations of the study. Because of the small sample size, subclassification of tumors according to stage, grade, or presence of carcinoma in situ was not possible, and one has to consider that any or all of these factors could have a potential effect on the behavior of TCC exposed to terazosin.

More extensive evaluation of neovascularization based on the combined analysis of factor VIII and CD31 immunoreactivity, and sampling of multiple sections from a larger specimen pool, will enable a detailed dissection of the effect of terazosin on TCC vascularity and apoptosis. Insight into the temporal dynamics of parallel apoptosis induction/vascularity suppression is currently being pursued by ongoing retrospective studies involving a larger number of clinical specimens across the entire tumor grade spectrum and focused on defining the expression profile of key apoptosis (*bcl-2*) and angiogenesis regulators (vascular endothelial growth factor) in serial sections of TCC.

CONCLUSIONS

The results in this study provide the first evidence to suggest that terazosin induces apoptosis and reduces vascularity in TCC of the bladder. These findings may have potential therapeutic value in treating TCC of the bladder in the future with terazosin, either as a single chemotherapeutic regimen or as an adjunct to current strategies. As promising as this concept might appear, one should consider the limitations associated with this small pilot study. Future studies with larger sample/patient population sizes and prospective control of experimental variables are necessary to establish the clinical significance of the antitumor effect of terazosin on TCC of the bladder.

References

1. Jemal A, Tiwari RC, Murray T, et al. Cancer statistics, 2004. *CA Cancer J Clin* 2004;54:8–29. [PubMed: 14974761]
2. Bochner BH, Cote RJ, Weidner N, et al. Angiogenesis in bladder cancer: relationship between microvessel density and tumor prognosis. *J Natl Cancer Inst* 1995;87:1603–1612. [PubMed: 7563203]
3. Streeter EH, Harris AL. Angiogenesis in bladder cancer—prognostic marker and target for future therapy. *Surg Oncol* 2002;11:85–100. [PubMed: 12031871]
4. Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med* 1971;285:1182–1186. [PubMed: 4938153]
5. Reed JC. Dysregulation of apoptosis in cancer. *J Clin Oncol* 1999;17:2941–2953. [PubMed: 10561374]
6. Kerr JF, Winterford CM, Harmon BV. Apoptosis: its significance in cancer and cancer therapy. *Cancer* 1994;73:2013–2026. [PubMed: 8156506]
7. Duggan BJ, Kelly JD, Keane PF, et al. Molecular targets for the therapeutic manipulation of apoptosis in bladder cancer. *J Urol* 2001;165:946–954. [PubMed: 11176520]
8. Kyprianou N, Benning CM. Suppression of human prostate cancer cell growth by alpha₁-adrenoceptor antagonists doxazosin and terazosin via induction of apoptosis. *Cancer Res* 2000;60:4550–4555. [PubMed: 10969806]
9. Keledjian K, Borkowski A, Kim G, et al. Reduction of human prostate tumor vascularity by the alpha₁-adrenoceptor antagonist terazosin. *Prostate* 2001;48:71–78. [PubMed: 11433417]
10. Pan SL, Guh JH, Huang YW, et al. Identification of apoptotic and antiangiogenic activities of terazosin in human prostate cancer and endothelial cells. *J Urol* 2003;169:724–729. [PubMed: 12544352]
11. Kyprianou N, Litvak JP, Borkowski A, et al. Induction of prostate apoptosis by doxazosin in benign prostatic hyperplasia. *J Urol* 1998;159:1810–1815. [PubMed: 9598465]
12. Chon JK, Borkowski A, Partin AW, et al. Alpha₁-adrenoceptor antagonists terazosin and doxazosin induce prostate apoptosis without affecting cell proliferation in patients with benign prostatic hyperplasia. *J Urol* 1999;161:2002–2008. [PubMed: 10332490]

13. Kyprianou N, Tu H, Jacobs SC. Apoptotic versus proliferative activities in human benign prostatic hyperplasia. *Hum Pathol* 1996;27:668 – 675. [PubMed: 8698310]
14. Giatromanolaki A, Koukourakis MI, Theodossiou D, et al. Comparative evaluation of angiogenesis assessment with anti-factor-VIII and anti-CD31 immunostaining in non-small cell lung cancer. *Clin Cancer Res* 1997;3:2485–2492. [PubMed: 9815651]
15. Folkman J. Angiogenesis and apoptosis. *Semin Cancer Biol* 2003;13:159–167. [PubMed: 12654259]
16. Benning CM, Kyprianou N. Quinazoline-derived α_1 -adrenoceptor antagonists induce prostate cancer cell apoptosis via an α_1 -adrenoceptor-independent action. *Cancer Res* 2002;62:597– 602. [PubMed: 11809715]
17. Walden PD, Durkin MM, Lepor H, et al. Localization of mRNA and receptor binding sites for the α_1 adrenoceptor subtype in the rat, monkey and human urinary bladder and prostate. *J Urol* 1997;157:1032–1038. [PubMed: 9072538]
18. Erdogru T, Gulkesen KH, Kukul E, et al. Increased bladder apoptosis with α_1 -adrenoceptor antagonists in benign prostatic hyperplasia. *Scand J Urol Nephrol* 2002;36:188 –193. [PubMed: 12201933]
19. Partin JV, Anglin IE, Kyprianou N. Quinazoline-based α_1 -adrenoceptor antagonists induce prostate cancer cell apoptosis via TGF- β signaling and I kappa B α induction. *Br J Cancer* 2003;88:1615–1621. [PubMed: 12771931]
20. Keledjian K, Kyprianou N. Anoikis induction by quinazoline based α_1 -adrenoceptor antagonists in prostate cancer cells: antagonistic effect of bcl-2. *J Urol* 2003;169:1150 –1156. [PubMed: 12576871]
21. Keledjian K, Garrison JB, Kyprianou N. Doxazosin inhibits vascular endothelial cell adhesion, migration and invasion. *J Cell Biochem* 2005;94:374 –388. [PubMed: 15526277]
22. Varma M, Morgan M, Amin MB, et al. High molecular weight cytokeratin antibody (clone 34betaE12): a sensitive marker for differentiation of high-grade invasive urothelial carcinoma from prostate cancer. *Histopathology* 2003;42:167–170. [PubMed: 12558749]

FIGURE 1. Immunohistochemical evaluation of apoptosis in TCC specimens. TUNEL-positive cells detected (arrows) in grade III bladder tumor after terazosin treatment (4 months). Original magnification $\times 400$.

FIGURE 2.

Immunohistochemical detection of vascularity in TCC tissue using factor VIII staining. Several vessels (arrows) identified in untreated grade III TCC specimen. Original magnification $\times 200$.

TABLE 1

Effect of terazosin treatment on transitional cell carcinoma of bladder

Specimen	Apoptotic Index (%)	Microvessel Density	CD31 Score
Bladder TCC			
Controls (n = 15)	1.7 ± 0.2	19.2 ± 2.3	5.7 ± 0.7
Terazosin (n = 9)	3.0 ± 0.5*	14.0 ± 2.0*	3.9 ± 0.4
Trigone			
Controls (n = 11)	1.9 ± 0.2	15.5 ± 1.7	5.3 ± 0.6
Terazosin (n = 7)	2.2 ± 0.3	14.4 ± 1.6	4.4 ± 1.0

Data presented as mean ± SE.

* Statistical significance at $P < 0.05$.