Phase II Study of Nitric Oxide Donor for Men With Increasing Prostate-specific Antigen Level After Surgery or Radiotherapy for Prostate Cancer

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OBJECTIVES To evaluate the effect of low-dose glyceryl trinitrate (GTN) on men with biochemical recurrence of prostate cancer after primary therapy. Preclinical, proof-of-principle studies have demonstrated that nitric oxide signaling plays a significant role in the hypoxia-induced progression of prostate cancer.

METHODS A prospective, open-label clinical trial of men with an increasing prostate-specific antigen (PSA) level after surgery or radiotherapy was conducted. Men with PSA recurrence were enrolled in a 24-month trial investigating the effect of a low-dose, slow-release transdermal GTN patch. The PSA doubling time (PSADT) was compared before and after treatment initiation, as well as with a matched control group that received no immediate treatment for their PSA recurrence.

RESULTS A total of 29 patients were enrolled in the study. Of the 29 patients, 62% completed the 24-month protocol, with 10% experiencing clinical disease progression. The calculated PSADT of the treatment group before initiating GTN was 13.3 months, not significantly different from that of the matched control group at 12.8 months. In an intention-to-treat analysis, the end-of-study PSADT for the treatment group was significantly different at 31.8 months ($P < .001$).

CONCLUSIONS We report the first clinical trial of a GTN patch in patients with prostate cancer. The prolongation of the PSADT and the safety of the drug, coupled with the corresponding preclinical in vitro and in vivo data documenting the ability of nitric oxide to attenuate hypoxia-induced progression of prostate cancer, warrant further testing in a placebo-controlled study. UROLOGY 74: 878–883, 2009. © 2009 Elsevier Inc.

Despite the stage migration afforded by the early detection of prostate cancer with serum prostate-specific antigen (PSA) testing and an apparent trend toward improved survival during the past several years,[1] prostate cancer remains a significant cause of morbidity and mortality.[2-4] Biochemical failure after primary therapy remains a significant healthcare burden, and strategies to delay clinical prostate cancer progression and prolong the interval from treatment failure to systemic therapy would be of significant clinical benefit for men with PSA recurrence.

Microenvironmental factors have been demonstrated to play a pivotal role in the selection of neoplastic cell subpopulations expressing more malignant phenotypes and contributing to the progression of localized and metastatic disease. Very low levels of oxygen (<10 mm Hg) have been well described in many solid tumors, and the extent of hypoxia has been demonstrated to represent an independent marker of poor prognosis for patients with various types of cancer.[5,6] Tumor hypoxia contributes to numerous adaptive phenotypes, including increased invasion and metastasis,[7,8] evasion of immune cell surveillance,[9,10] and increased resistance to radiotherapy[11,12] and chemotherapy.[13,14] Although cellular adaptive responses to hypoxia are likely mediated by various mechanisms, our previous studies have suggested that decreased nitric oxide (NO)-dependent signaling plays a significant role in this progression of a malignant phenotype.[7,14-16] We have shown that hypoxia-induced tumor cell invasiveness, metastatic ability, resistance to chemotherapeutic agents, and evasion of immune cell recognition are inhibited by molecules that activate the NO

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Table 1. Clinical and pathologic characteristics

Characteristic	Value
Primary therapy (n)	
Radical prostatectomy	19 (66)
Radiotherapy	10 (34)
Age (y)	
Mean	70
Range	61-76
Gleason score (n)	
≤6	6 (21)
7	21 (72)
8-10	2 (7)
PSA level before treatment (ng/mL)	
Mean	4.5
Median	2.9
Interval between primary therapy and trial (mo)	
Mean	72
Range	14-146

PSA = prostate-specific antigen.
Data in parentheses are percentages.

signaling pathway involving cyclic guanosine monophosphate generation and that pharmacologic inhibition of NO signaling results in phenotypes similar to those induced by exposure to hypoxia.

The present study served as a Phase II investigation to determine whether NO is a potentially useful biologic target by evaluating the efficacy of a low-dose, sustained delivery of glyceryl trinitrate (GTN) in PSA recurrent prostate cancer after definitive radiotherapy or radical prostatectomy.

MATERIAL AND METHODS

This study was a prospective Phase II, open-label clinical trial of men with PSA recurrence after primary treatment of prostate cancer. The patients were enrolled from May 2001 to June 2004. The institutional review board of Queen's University reviewed and approved the study, which was investigator initiated, without industry sponsorship.

The patients included in the present study were those who had undergone previous treatment with definitive radiotherapy (n = 10) or radical prostatectomy (n = 19) for clinically localized disease (clinical Stage T1 or T2). Table 1 lists the clinical and pathologic characteristics of the study cohort. All patients had evidence of biochemical failure after definitive treatment: (a) detectable and increasing PSA level ≥3 months after surgery (≥3 values greater than the detection limit of the assay ≥3 months apart), or (b) ≥3 increasing values at any level after radiotherapy. Patients were included in the study after extensive counseling regarding the management of their PSA recurrence, including radiotherapy or early androgen deprivation therapy, and they had either declined or had contraindications to these salvage therapies. Patients with clinical or radiographic evidence of metastatic disease were excluded from the present study. Patients could have received neoadjuvant hormonal therapy but not within 6 months of entry into the study. Patients receiving any other adjuvant therapies, including the use of nutritional supplements suspected of altering the growth of prostate cancer cells or affecting PSA serum levels (ie, saw palmetto, PC-SPES, vitamin E, selenium), were excluded from participation. Only 1 patient had received a short course of a nonsteroidal antiandrogen (2 weeks) before the initiation of radiotherapy. Because of reports of low testosterone levels in men after radiotherapy, the serum total testosterone levels were measured midway through the clinical trial. No patient had a significantly decreased testosterone level (mean 12.1 nmol/L, range 7.8-22.7). Patients with any contraindication to GTN (including concomitant use of nitroglycerin formulations or phosphodiesterase inhibitors) were excluded.

A total of 29 patients who had evidence of biochemical relapse after definitive radiotherapy or surgery were enrolled in the present study. These patients were treated with low-dose, sustained-release GTN supplied as the Minitran (nitroglycerin) transdermal delivery system (3M, St. Paul, MN) in an open-label, nonblinded fashion. GTN is a nitro donor that has been used in the management of angina for >100 years, has a well-documented safety and tolerability record, and has never been associated with carcinogenesis. Our previous preclinical studies demonstrated that very low molar concentrations of NO donors are required to attenuate hypoxia-induced malignant phenotypes. Because conventional therapeutic doses for cardiac ischemia can lead to vasodilatory side effects and continuous application can lead to tolerance, it was determined to use very low doses of GTN in the present trial. The smallest available Minitran patch, delivering 0.2 mg/h, was subsequently cut to one sixth the patch size to deliver 0.033 mg/h. The patients were asked to wear the patch continuously, changing the patch every 12 hours.

The clinic visits to assess for side effects and PSA levels were monthly for the first 6 months and then every 3 months for the rest of the 24-month trial. The patients were allowed to continue the therapy until (a) they exhibited evidence of concerning PSA progression (defined by a PSA doubling time [PSADT] calculated at <3 months), or (b) they had clinical or radiographic evidence of distant metastases. The patients underwent bone scans for any clinical suspicion of metastatic disease, including a concerning PSA level, as well as at the end of the study.

The main PSA outcome was evaluated as a change in the calculated PSADT before treatment compared with that at the end of the 24-month study. The PSADTs of all patients were included in this calculation, whether they had completed the entire study or had withdrawn. Additionally, the PSADT before treatment was compared with the PSADTs during treatment at 6, 12, and 24 months. The PSADT was calculated using the following formula: $PSADT = \log 2 \times t/[\log(\text{final PSA}) - \log(\text{initial PSA})]$. The PSADTs using this formula were also compared with those using 3 or 4 PSA readings. Because no statistically significant differences in the results were seen, the initial and final PSA readings were used to calculate the PSADT for the different periods of study drug use. These post-therapy PSADTs were also compared with the pretreatment values for each patient in a descriptive manner, categorizing patients by a fast (<6 months), intermediate (6-12 months), or slow (>12 months) PSADT, or as a stable/declining absolute value before and after treatment. Furthermore, the PSADTs of the treatment group were compared with those from a contemporary, matched control group (n = 14) of men with similarly defined PSA recurrence after primary treatment of prostate cancer who had not received any adjuvant or salvage systemic treatment during at least a 2-year period. This untreated cohort was identified from a surgical database at Queen's University and had a mean age of 69 years (range 56-74) at

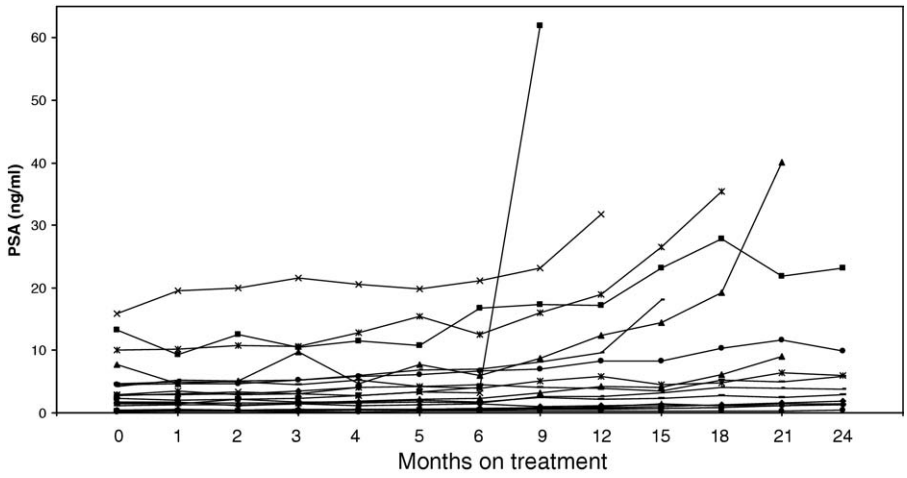

Figure 1. Plot of prostate-specific antigen (PSA) values for all patients enrolled in study during 24-month period.

initiation of the study, a similar disease stage and grade (median Gleason score 7/10, range 6-8), and a mean follow-up period of 4.6 years.

Statistical Analysis

In the present study, we used the nonparametric Friedman rank sum method to test for differences among the repeated values. The null hypothesis is that the mean of all samples will be equal. We used the nonparametric Wilcoxon signed rank test on the calculated paired difference scores, followed by Dunn's comparison post hoc test, to determine the significant differences. All statistical tests were 2-sided, and differences were considered statistically significant at $P < .05$. The GraphPad Prism 4 statistical software package (GraphPad Software, San Diego, CA) and Statistical Package for Social Sciences, version 9.1 (SPSS, Chicago, IL) were used for analysis.

RESULTS

Of the 29 patients enrolled in the study, 17 (58%) completed the entire 24 months of the study medication. Only 3 of the 29 patients (10%) had documented disease progression at 3, 9, and 21 months during the trial. Of these 3 patients, 2 had documented asymptomatic bony metastases on bone scan performed for suspicious PSADTs. One patient had bony metastases confirmed on magnetic resonance imaging 3 months after initiation of the study. Magnetic resonance imaging was performed for an equivocal bone scan obtained before study entry (PSA level 9 ng/mL). A total of 5 study patients withdrew from the trial between months 12 and 24 to pursue other treatment options (2 patients with slow PSADTs underwent salvage postoperative radiotherapy; 3 patients decided to initiate early androgen deprivation therapy). Four patients voluntarily withdrew from the trial for personal reasons (2 patients moved from the province and 2 had exacerbation of pre-existing health issues, not associated with prostate cancer or its treatment). The data of all of these patients were included in the main PSADT outcome. Compliance was excellent, with no cardiovascular toxicities or other serious adverse events encountered. Of the 17 patients completing the trial, 7 (44%) requested continuation of the trial medication, and these patients were subsequently followed up with serial PSA measurements.

Figure 1 presents all the PSA measurements for the entire treatment cohort during the 24-month trial. Before initiating treatment, the mean PSA level of the treatment group was 4.5 ng/mL (range 0.22-15.40), and no patient had a stable PSA level, with a mean PSADT before initiating treatment in the intermediate range of 13.2 months. Figure 2A presents the PSADT of the treatment cohort at the end of study compared with the PSADT of these patients before the initiation of the GTN patch and with the matched control group. No significant difference was found between the PSADT of the untreated control group (mean 12.8 months) and the pretreatment PSADT of the study cohort. The PSADTs of all patients were incorporated, including those of the patients who had discontinued therapy. For those who stopped therapy, the last PSA level available or the last PSA before initiating local or systemic therapy was used to calculate the PSADT. A statistically significant difference ($P < .001$, Wilcoxon signed rank test) was found between the end-of-study PSADT (mean 31.8 months) and that before treatment initiation, as well as compared with that of the matched control group. Figure 2B presents the PSADT of patients receiving GTN at 6, 12, and 24 months of the study as per the protocol and demonstrates the sustained changes in PSA response over time. Again, a statistically significant difference was found between the PSADT before treatment compared with the measured points (6 months, $P = .037$; 12 months, $P = .006$; and 24 months, $P = .002$; Wilcoxon signed rank test). We also examined the PSADT for the 3 points (6, 12, and 24 months) by categorizing the 29 patients by a fast (<6 months), intermediate (6-12 months), or slow (>12 months) PSADT. Table 2 lists the observed counts for each category. With time, we noted a significant shifting from the fast and intermediate categories to the slow

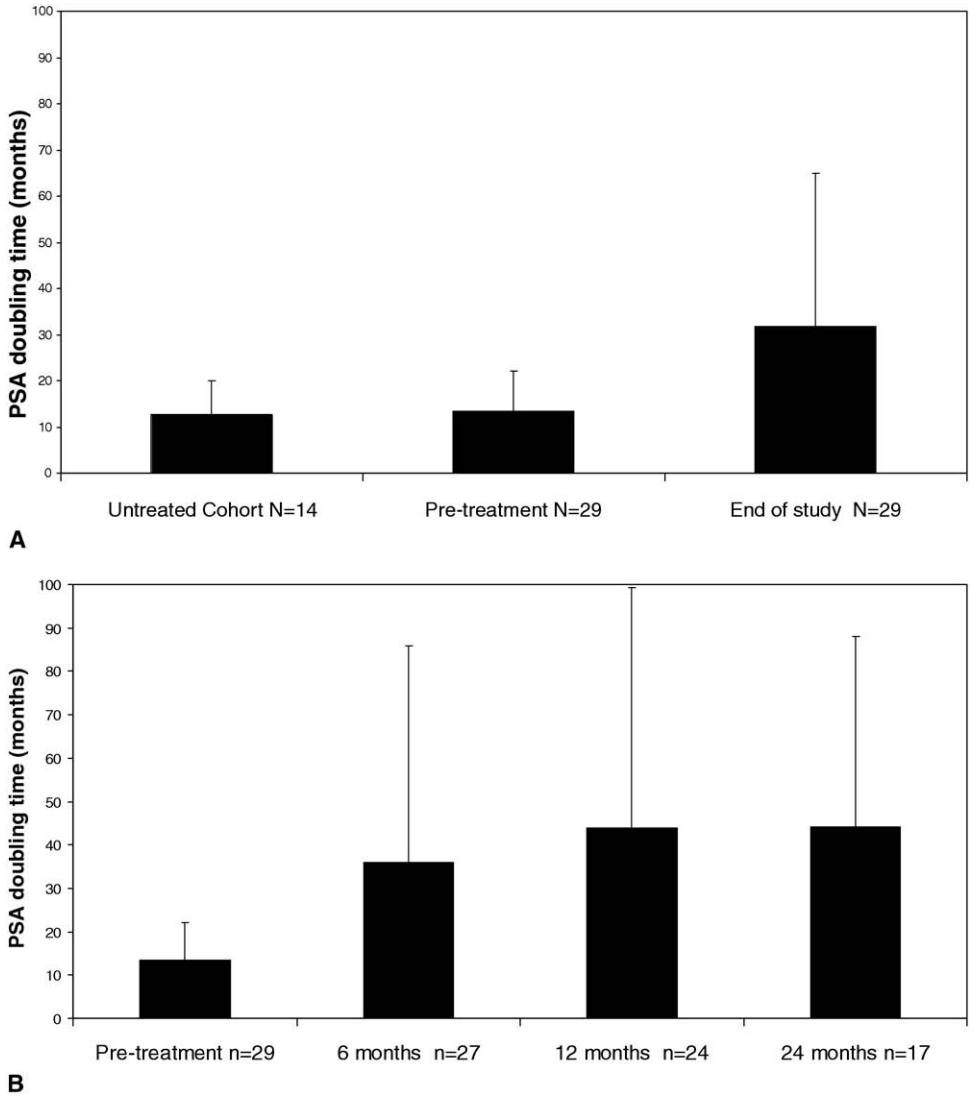

Figure 2. (A) Mean prostate-specific antigen (PSA) doubling time of study patients before initiating drug and at study end ($P < .001$, Wilcoxon rank sum test) as intention-to-treat analysis. Significant difference also shown for study group compared with untreated, nonrandomized control group ($P < .001$, Wilcoxon rank sum test). **(B)** Mean PSA doubling times for patients taking study drug at 6, 12, and 24 months, demonstrating persistent effect of PSA doubling time. Significant difference shown between pretreatment value and at each point (6 months, $P = .037$; 12 months, $P = .006$; and 24 months, $P = .002$; Wilcoxon rank sum test).

Table 2. PSA doubling times categorized as fast, intermediate, or slow or as stable/declining PSA value with time during GTN therapy

Treatment Duration	High (<6 mo)	Moderate (6-12 mo)	Slow (>12 mo)	Stable/Declining
Before treatment	4/29 (14)	13/29 (45)	12/29 (41)	0/29 (0)
6 mo	0/27 (0)	11/27 (41)	12/27 (44)	4/27 (15)
12 mo	0/24 (0)	7/24 (29)	13/24 (54)	4/24 (17)
24 mo	0/17 (0)	1/17 (6)	14/17 (82)	2/17 (12)

PSA = prostate-specific antigen; GTN = glyceryl trinitrate.

Friedman test was significant ($P = .001$) implying difference between PSA categories from beginning to end of study for 17 patients who completed protocol.

category, with several patients having a stable or decreasing PSA level. Of the 17 patients who completed the 24-month trial with medication, all except for 1 had a PSADT that was categorized as slow or stable/declining. The Friedman test was significant ($P = .001$), implying a difference between PSA categories from the beginning to the end of the study for the 17 patients who completed the protocol. No evidence was found of asymptomatic metastases on the end-of-study bone scans. After trial completion, 7 patients requested continuation of the

GTN patch. At trial completion, these patients had no evidence of disease clinically, and the mean PSA level was 6.3 ng/mL (range 0.69-17.7). All of these patients demonstrated continued stable PSA levels. Because of the relatively small sample size, no correlations could be observed between the response to treatment and the clinical variables, such as initial therapy modality (ie, surgery vs radiotherapy), disease stage, or disease grade.

COMMENT

PSA is widely accepted as the most useful prognostic marker of prostate cancer progression, particularly after primary therapy with radical surgery or radiotherapy.[17] Despite the improved cancer control rates with definitive management of early-stage prostate cancer, PSA recurrence is common (25%-50%) in most large case series.[2-4] However, given the generally prolonged natural history of prostate cancer, this biochemical recurrence predates radiologic or clinical evidence of disease by a significant period.[17] With the increasing age of our population and the earlier detection of clinically localized disease, the prevalence of this asymptomatic cohort of patients with biochemical evidence of prostate cancer recurrence is substantial and growing. Furthermore, the options for patients who have undergone primary radical therapy with curative intent and who develop subsequent PSA recurrence without documented evidence of metastatic disease remain limited and controversial. Nontoxic and economical therapeutic alternatives that could further prolong the interval from primary treatment failure to clinically demonstrable disease progression would clearly be beneficial in improving the quality of life and, possibly, cancer-specific survival for these patients.

In the present Phase II study, low-dose, sustained delivery of GTN was used to treat patients after failure of primary therapy for their clinically localized prostate cancer. The preclinical in vitro and in vivo evidence supporting the use of such low doses of NO donors confirmed the absence of cytotoxic activity against neoplastic cells, but revealed that its use decreased the emergence of a more malignant phenotype, including invasion and metastases, induced by a hostile tumor microenvironment. Our results suggest a significant inhibition of progressive disease given the effect on the PSADT with GTN treatment compared with the PSADT before initiating the trial. Although 5 of the patients had progression of their PSADT, the rest of the cohort experienced stabilization of their PSA levels by 6 months. Within 12 months of the trial, 17 of 24 patients had PSADTs in the slow category or even stable/declining PSA levels. The mean PSADT of the entire cohort increased to 31.8 months from 13.2 months before starting treatment. Compared with the matched control group of patients with PSA recurrence who did not receive any treatment, a similar significant difference in PSADT was observed. Although this was not a randomized control group, they were similar to the treatment group in all respects, including the initial PSADT, disease grade and stage, and follow-up period.

During the trial, 3 patients had radiographic evidence of new bony metastases. One of these patients underwent magnetic resonance imaging to confirm metastases after suspicious bone scan findings after the patient had received the study medication for only 3 months. This patient likely had metastatic disease before initiating therapy, although his PSA level was relatively low at 9 ng/mL. The other 2 patients had concerning increases in their PSADT and overall PSA level, suggesting that the GTN treatment was not falsely inhibiting PSA secretion independent of prostate cancer progression. This concept was confirmed with in vitro experiments with LNCaP cells that demonstrated no effect on PSA secretion with GTN treatment of varying concentrations (unpublished data).

This is the first report of the clinical use of NO donors in the treatment of prostate cancer. The role of NO in malignant progression has been the subject of controversy, with studies showing either tumor-promoting[18] or tumor-inhibitory roles.[19,20] These apparently contradictory effects of NO can be explained because this molecule can regulate phenotypes through a variety of mechanisms, depending on the local concentrations and the redox state of the cell. On the basis of our previous findings, we propose that the observed effects of GTN on the PSA of our patient cohort was related to the "low concentration" effects of NO.[10,14] Recently, Goluboff et al.[21] demonstrated that exisulind, a selective, pro-apoptotic agent whose mechanism of action may involve activation of the cyclic guanosine monophosphate-protein kinase G pathway, also had significant inhibitory effects on PSA in high-risk patients. Furthermore, Yasuda et al.[22] recently published a Phase II trial demonstrating the benefit of GTN as an adjuvant to chemotherapy for nonsmall cell lung cancer.

The prediction of whether PSA recurrence will result in clinical or radiologic evidence of disease is determined from multiple factors, including the PSA level, interval to PSA recurrence after treatment, PSADT, and Gleason score. Increases in PSA levels after radical treatment follow an exponential growth curve,[17,23] and it appears that the PSADT is the most robust predictor of the probability and interval to clinical disease progression, including metastases and death.[24] Several small Phase II studies have been published of novel agents in prostate cancer using PSADT as an endpoint in placebo-controlled trials.[25-28] Despite the significant effects on the PSADT and the lack of any adverse events with the low-dose GTN patch in our patients with recurrent prostate cancer, these preliminary results should be interpreted with caution. The ability of a treatment to affect PSA endpoints such as the doubling time has not been demonstrated to necessarily result in long-term clinical benefit to patients with recurrent prostate cancer. Furthermore, it is important to acknowledge that our study

was not a randomized trial and the comparison of PSADT before and after starting GTN, or with untreated control group, could have been biased by patient selection, as well as physiologic PSA variability, resulting in a "placebo effect,"[28] and highlighting the potential limitations of PSA variables as an outcome and reiterating the need for blinded, prospective studies.

CONCLUSIONS

The results of our study suggest that low-dose GTN has a consistent, inhibitory effect on PSA progression in men with recurrent prostate cancer after primary treatment failure. These significant effects on the slowing, and even stabilization, of the PSADT suggest a non-random drug effect of GTN. Implementation of strategies that could prolong the PSADT, thereby delaying the time to treatment with hormonal therapy and to death, would be of great benefit to patients. Our findings appear to justify additional investigation in a larger cohort of patients and with longer biochemical and clinical follow-up.

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